292-28464D
NOV. 2008

Microchip Electrophoresis System

for DNA/RNA Analysis

MCE-202 MultiNA

Instruction Manual

- Operating Procedure -

Read the instruction manual thoroughly before you use the product.

Keep this instruction manual for future reference.

SHIMADZU CORPORATION
KYOTO JAPAN

ANALYTICAL & MEASURING INSTRUMENTS DIVISION

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To ensure the safe use of this product:

- Follow all procedures described in these instruction manuals.
- Follow all WARNING and CAUTION instructions.
- Do NOT disassemble or modify this product without permission from Shimadzu Corporation.
- Please contact your Shimadzu representative for product service and repairs.

< Notice>

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- For the sake of improvement, the content of this manual is subject to modifications without notice.
- Every effort has been made to ensure that the content of this manual was correct at the time of creation. However, in the event that any mistakes or omissions are discovered, it may not be possible to correct them immediately.
- The contents of the hard disk in a PC can be lost due to an accident. Backup your data, to protect your important data from accidents.

Introduction

Thank you for purchasing the MCE-202 MultiNA microchip electrophoresis system for DNA/RNA analysis (hereafter “MultiNA”).

■ Important:

- Read this manual thoroughly prior to operating this product.
- Keep this manual near the product in a safe location for future reference.
- Operate this product in accordance with the instructions given in this manual.
- Follow all WARNING and CAUTION instructions.
- For information on the basic operation of the Windows® operating system, please refer to that product's Instruction Manuals.
- Do NOT disassemble or modify this product without permission from Shimadzu Corporation.
- Ensure that these instruction manuals are transferred if the user or site of use changes.
- Contact your Shimadzu representative if the following is required:
  - Replacement of this manual, or any WARNING or CAUTION labels
  - Product installation, adjustment, or re-installation after product movement, or for service and repairs

This product's Instruction Manual is provided in two separate volumes. Each volume contains the following sections:

Instruction Manual, Instrument and System

- Equipment Overview
- Parts Specifications
- Names and Functions of Each Part
- Operating Principles
- Installation Procedures
- Instrument Maintenance
- Moving and Storing the Instrument
- Specifications
- Error Diagnosis and Corrective Measures
- Maintenance Parts

Instruction Manual, Operating Procedure

- Outline of operation
- MultiNA Instrument Control Software Functions
- MultiNA Viewer Data Analysis Software Functions

Warranty

Shimadzu provides the following warranty for this product.

1. Period: Please contact your Shimadzu representative for information about the period of this warranty.

2. Description: If a product/part failure occurs for reasons attributable to Shimadzu during the warranty period, Shimadzu will repair or replace the product/part free of charge. However, in the case of products which are usually available on the market only for a short time, such as personal computers and their peripherals/parts, Shimadzu may not be able to provide identical replacement products.

3. Exceptions: Failures caused by the following are excluded from the warranty, even if they occur during the warranty period.

   1) Improper product handling

   2) Repairs or modifications performed by parties other than Shimadzu or Shimadzu designated companies

   3) Product use in combination with hardware or software other than that designated by Shimadzu

   4) Computer viruses leading to device failures and damage to data and software, including the product's basic software

   5) Power failures, including power outages and sudden voltage drops, leading to device failures and damage to data and software, including the product's basic software

   6) Turning OFF the product without following the proper shutdown procedure leading to device failures and damage to data and software, including the product's basic software

   7) Reasons unrelated to the product itself

   8) Product use in harsh environments, such as those subject to high temperature or humidity levels, corrosive gases, or strong vibrations

   9) Fires, earthquakes, or any other act of nature, contamination by radioactive or hazardous substances, or any other force majeure event, including wars, riots, and crimes

   10) Product movement or transportation after installation

   11) Consumable items
   Note: Recording media such as floppy disks and CD-ROMs are considered consumable items.

4. Non-Research Applications: This instrument should be used only for research applications. It should NOT be used for diagnosis or examination purposes. If this instrument is used for non-research applications, Shimadzu Corporation is in no way responsible for any problems related to the instrument, its data, claims from third parties, or any other problems that occur.

* If there is a document such as a warranty provided with the product, or there is a separate contract agreed upon that includes warranty conditions, the provisions of those documents shall apply.

* Warranty periods for products with special specifications and systems are provided separately.

After-Sales Service and Availability of Replacement Parts

After-Sales Service

If a problem arises, inspect the product and take the appropriate corrective action described in *-Instrument and System- "6 Instrument Maintenance"* and *"9 Error Diagnosis and Corrective Measures"*. If the problem persists or the symptoms are not covered in these chapters, contact your Shimadzu representative.

Replacement Parts Availability

Replacement parts for this product will be available for a period of seven (7) years after the product is discontinued. Thereafter, such parts may cease to be available. Note, however, that the availability of parts not manufactured by Shimadzu shall be determined by the relevant manufacturers.

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PLEASE READ THIS AGREEMENT CAREFULLY BEFORE USING THE SOFTWARE.

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8. GENERAL

a.This Agreement is the entire agreement. If any provision of this agreement is held invalid, the remainder of this agreement shall continue in full force and effect.

b.This Agreement shall be construed and governed in accordance with the laws of Japan, excluding its conflict of law rules.

c.The exclusive jurisdiction for any disputes arising out of or in connection with this Agreement shall be Kyoto District Court of Japan.

d.The invalidity or unenforceability of any provision of this Agreement shall not affect the validity or enforceability of any other provision.

Instruction Manuals

List of Instruction Manuals

The following manuals are provided.

Name	Media	Description
Instrument and System	Booklet + PDF	Describes the complete MultiNA system, including handling, operation, maintenance, and troubleshooting.
Operating Procedure	Booklet + PDF	Describes data acquisition and analysis procedures, including preparation of reagents and samples, software operation procedures, and others.
Help	Online Help	Click [Help] on the menu bar to access Online Help data (PDF versions of manuals).

Manual Notation

The following symbols are used in this manual.

Symbol	Meaning
⚠ **DANGER**	Indicates an imminently hazardous situation which, if not avoided, will result in serious injury or death.
⚠ **WARNING**	Indicates a potentially hazardous situation which, if not avoided, could result in serious injury or possibly death.
⚠ **CAUTION**	Indicates a potentially hazardous situation which, if not avoided, can result in minor to moderate injury or equipment damage.
NOTE	Provides additional information to ensure proper use of this product.
Reference	Indicates location of related information.
[ ]	Indicates text displayed on the screen, such as the names of buttons, menu items, settings, screens, and icons. Example: Click [OK] (where “OK” is the name of a button in a dialog box)
V1.05	Indicates a function added or changed in the software V.1.05 or later.

Safety Instructions

To ensure safe product operation, read these important safety instructions carefully before use and follow all WARNING and CAUTION instructions given in this section.

■ Product Applications

⚠ WARNING

- This is a microchip electrophoresis system for DNA/RNA analysis. Do NOT use it for any other application.
  Doing so may result in accidents.
- This instrument should be used only for research applications.
  It should NOT be used for diagnosis or examination purposes. If this instrument is used for non-research applications, Shimadzu Corporation is in no way responsible for any problems related to the instrument, its data, claims from third parties, or any other problems that occur.

■ Installation Site

⚠ WARNING

- Fire is prohibited in the vicinity of the instrument.
  Fire must not be used at the site where the instrument is installed. In addition, avoid installation in the same room with equipment that generates sparks. Do not use flammable sprays (such as hairsprays or aerosol insecticides) or flammable solvents in the vicinity of this instrument. Provide fire extinguishers in case of an emergency.
- Install a sink in the vicinity of this instrument.
  If reagent chemicals come in contact with the eyes or skin during operation, flush them away immediately with a large quantity of water. A sink should be installed in the vicinity of this equipment if at all possible.

⚠ CAUTION

- Installation of the instrument is prohibited at sites exposed to corrosive gases, or to significant debris and dust.

  To ensure the life of the instrument and to maintain proper operation, avoid installation sites that are exposed to corrosive gases or to significant dust or debris.

- Do NOT install the instrument near equipment that generates strong magnetic fields.

  To ensure normal operation of this instrument, avoid installation sites that are subject to strong magnetic fields. Add noise filters if there is significant noise on the power lines.

- To maintain performance, observe the following site conditions.
  - Rooms with an ambient temperature between 18°C and 28°C, and minimal daily temperature fluctuations (If the ambient temperature exceeds 28°C, a temperature control error can occur and instrument operation may stop.)
  - Sites where the instrument is protected from direct exposure to drafts from coolers, heaters or air conditioners
  - Sites protected from exposure to direct sunlight
  - Sites not exposed to vibrations
  - Sites where humidity is maintained between 40% and 80%
  - Sites where condensation does not occur

- During analysis, ensure that the drain tubing ports and the gap between the top cover and the instrument are not exposed to direct light sources (such as desktop fluorescent lamps, flashlights, and camera flashes).

  This may result in noise.

- Prior to installing and/or using the product in an industrial location:
  - Install the product away from devices generating strong electromagnetic fields.
  - Supply power from a separate power source.
  - Take countermeasures to prevent static electricity buildup.

■ Installation

⚠ WARNING

- For safe instrument operation, after the instrument is moved to a different location, contact your Shimadzu representative for instrument installation, adjustment, or reinstallation.
- The instrument's power supply voltage and power consumption are as follows. The power supply voltage is indicated on the label on the power connector on the back of the instrument. Connect the instrument to a suitable power source.

  Connecting it to an improper power source may result in fires or shocks.

  The intended performance may not be obtained if the power supply voltage is unstable or the power capacity is insufficient. Check the power requirements for the entire system before arranging a suitable power source.

Part No.	Voltage (indicated on the instrument faceplate)	Power Consumption	Frequency
292-28000-31	100-120/220-240 V	300 VA	50/60 Hz

- Ground the instrument.

  If malfunctions or leakages occur, shocks may result if the equipment is not properly grounded. Grounding is also important to ensure stable instrument operations.
- Do NOT insert or touch the adaptor ground lead to the power outlet.

  This may result in fires or electric shocks.
- Handle the power cable carefully.

  If the following cautions are not observed, the cable may be damaged, resulting in fires, electric shocks, or malfunctions. If the cord does become damaged, contact your Shimadzu service representative immediately.
  - Do NOT place it under heavy objects.
  - Do NOT place it near heating equipment.
  - Do NOT modify the cord.
  - Do NOT forcibly bend or stretch the cord.
  - Hold the plug when inserting and removing the cord.

⚠ CAUTION

- This instrument weighs 43 kg. When installing the system, consider the total system weight including the PC and other system components.
  Install the instrument on a flat, stable desk or stand, capable of supporting the total system weight.
- Install on a flat desk or stand.
  If the instrument is significantly inclined, the analysis performance will be adversely affected.
- Installation space for the instrument, PC, and glass rinse water bottle must be at least 1,015 mm to 1,165 mm W (if a desktop PC is used) by 600 mm D by 980 mm H.
  (See *-Instrument and System- " Example Installation 1: Waste container positioned in front of the instrument"*.)
  This does not include the waste container which is placed on the floor. The size displays the maximum height with the cover open.
- When installing the instrument against a wall, leave a gap of at least 50 mm between the rear of the instrument and the wall.
  If this condition is not met, fan-driven air cooling will not be sufficient and the instrument may overheat and reduce performance. Also the top cover will not fully open.
- Install the instrument at a site where the rear of the instrument can be accessed easily.
  The syringe cover on the rear of the instrument must be opened when replacing the syringe or plunger.
- Allow at least a 100 mm of space in front of the instrument.
  The front cover must be opened to replace the pump cartridges.
- When installing the instrument, leave enough space to operate the power switch on the bottom right side of the instrument near the rear.
  The power must be turned OFF quickly in the event of an instrument or other emergencies.The power must be turned OFF quickly in the event of a problem with the instrument or other emergency.
- Place the waste fluid container on the floor.
  Waste fluid is conveyed to the waste container by gravity. Place the container at a position lower than the instrument.
- Note the following cautions regarding the waste tubing. Cut the tubing as necessary to suit the installation site.
  - The tubing should not be bent.
  - The tubing should not be elevated.
  - The tip of the tubing should not be immersed in the waste fluid inside the waste container.
- If necessary, install safety fasteners and other earthquake measures.
  Recommended product: Part No. 037-62401-03, FASTENER, RT-400 GRAY (set of two)

■ Operation

⚠ WARNING

- Wear protective glasses and gloves and a lab coat or work clothes.
  Use of this instrument involves the handling of reagents that contain irritating or hazardous chemical substances. If a chemical substance, such as a reagent, gets into eyes or contacts skin, immediately wash the area with a large amount of water and consult a medical professional.
  If a chemical substance is accidentally swallowed or chemical vapors are inhaled, immediately consult a medical professional.

■ Top Cover Usage

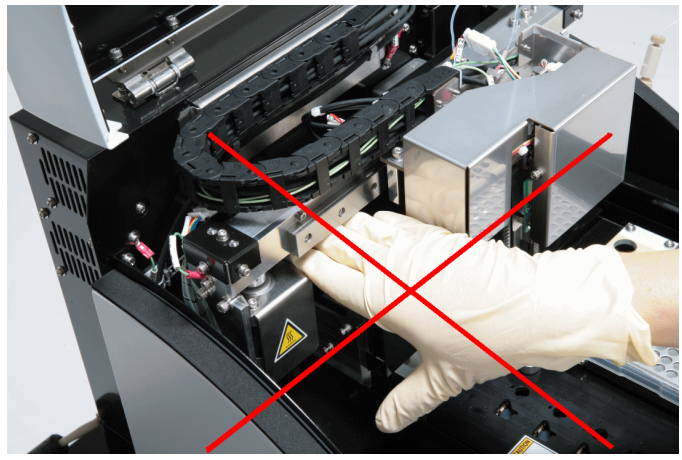
⚠ WARNING

- Do NOT forcibly open the top cover during instrument operation (blinking green LED on front indicator).
  During operation the top cover is locked and cannot be opened.
- Do NOT apply lateral force to the top cover when opening or closing.
  This may deform the top cover and result in damage.
- Do NOT remove the top cover.
  Injuries or accidents may occur.
- If the top cover does not open or close properly, contact your Shimadzu service representative immediately.
  A technician from Shimadzu will perform repairs.

■ Drive Unit

⚠ WARNING

- Do NOT touch any parts other than those specified in these manuals.



■ Replacing Parts and Daily Maintenance

⚠ WARNING

- Turn the instrument OFF before maintenance or parts replacement.
  Shocks or accidents may occur.
- Internal repairs are performed by certified Shimadzu technicians.
  Contact your Shimadzu service representative.
- Do NOT perform any disassembly or modification procedures that are not described in these manuals.
  Injuries or accidents may occur.

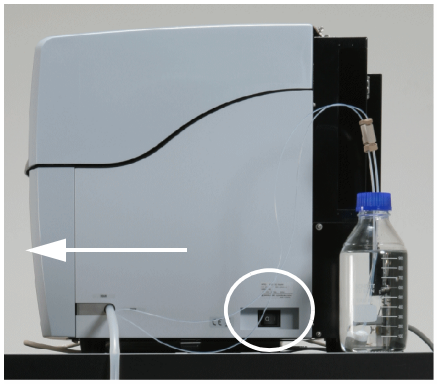
⚠ CAUTION

- Use only the parts described in *-Instrument and System- "2 Parts Specifications"* or *"10 Maintenance Parts"*.
  Normal operations are not ensured if other parts are used.
- Do NOT leave the instrument wet.
  This may result in rust or discoloration. To clean the instrument, wipe it with a soft cloth moistened slightly with water. Then remove any moisture with a soft dry cloth.
- Do NOT wipe it with alcohol, paint thinner or other organic solvents.
  These solvents may damage the paint on the instrument cover.
- Dispose of the waste fluid in accordance with guidelines from the applicable management departments.
  In addition to rinse water, the waste liquid contains separation buffer, marker solution, and dyes. It will also contain formamide from RNA analysis.

■ Emergency Measures

⚠ WARNING

- If a strange noise or smell emanates from the instrument, or some other irregularity is noticed, stop instrument operation using the following procedure, and contact your Shimadzu service representative.
  - Switch OFF the power switch on the lower right side of the product.
  - Detach the power cable.



Power switch

■ During a Power Outage

⚠ CAUTION

- If an electric power outage occurs during analysis, the instrument stops. If separation buffer is left remaining in a microchip or sample probe when the instrument stops it may dry out and obstruct the microchip or sample probe. To avoid such a condition, take the action described below.
  1. Turn OFF the power of the instrument.
  2. When the electric power comes back on, turn ON the instrument. If the power of the PC is OFF, turn it ON.
  3. Rinse the microchip with water according to the procedure described in *-Instrument and System- "6.2.7 Microchip Reservoirs"* and reinstall it in the instrument.
  4. Rinse the sample probe according to the procedure described in *-Instrument and System- "6.2.2 Sample Probe"*.
  5. Check if the sample probe is obstructed according to the procedure described in *-Instrument and System- "6.4.4 Plunger Replacement Inspection"*.
  6. Rinse all microchips installed in the instrument according to the procedure described in "All Chip Washing" in *"2.5.8 Wash" P.126*.

  Carrying out the procedure above enables regular operation. In case of any abnormal condition, see *-Instrument and System- "9 Error Diagnosis and Corrective Measures"* and take actions accordingly.
  In MultiNA, results of analysis for each sample are saved in data files during analysis of multiple samples. Therefore, even if analysis is stopped due to a power outage during analysis, results of analyses up to right before the power outage are saved in files.

NOTE

- Files may not be normally created on rare occasions, for example, when the PC stops during a process to save data in a file.

■ Microchip Use

⚠ CAUTION

- Do NOT scrub the reservoir on the microchip when wiping away moisture.
  Lint may clog the microchip channels.
- Do NOT touch the surfaces of the microchip with your bare hands.
- Do NOT scratch the microchip electrodes during handling.
  If salt or other material has hardened on the electrodes, apply water to dissolve the salt and then gently wipe it away.
- Do NOT clean the microchip using an ultrasonic cleaner.
  This will damage the microchip.

■ Reagent Kit Use

⚠ WARNING

- The reagent kit provided with this instrument is only for experimental and research purposes.
  It is not authorized for the diagnosis and treatment of human or animal illnesses. Do NOT use it for any applications that directly affect human or animal bodies, such as with medical products, cosmetics, or foods.
- If reagent gets into the eyes or contacts the skin, immediately wash the applicable area with copious amounts of water and consult a medical professional. If reagent is accidentally swallowed or the vapor is inhaled, immediately consult a medical professional.
  Refer to the MSDS for details.
- Use only the reagents specified in these instruction manuals.

■ Relocating the Product

⚠ CAUTION

- Before moving the instrument, the drives must be fastened with the transportation fixtures.
  Contact your Shimadzu service representative.
- Unplug the LAN cable connected to the PC.
- Do NOT lift the instrument by the top cover.
- Do NOT pinch fingers or hands in gaps inside the product.
- Do NOT bump the instrument or subject it to excessive vibrations.

■ If MultiNA is idle in a cold environment for an extended period

⚠ CAUTION

- If this instrument is idle for an extended period of time in a cold environment, a robotics drive-system error may occur (home position detection error).
  This error can be prevented by first warming the robotics drive-system before starting operation.
  Follow the procedures described below if the instrument has been idle in a cold environment. If the error continues, contact your Shimadzu service representative.
- Warm procedure
  1. Turn ON the instrument.
  2. Start the control software, and confirm that the instrument and PC are connected.
  3. Select [Move All Axes to Home Position] on the [Instrument] pull-down menu.
  4. Wait for 30 minutes, with the top cover closed.
  5. After 30 minutes, operate the instrument as usual.

  If the error continues, wait another 30 minutes before starting the instrument.

Warning Labels

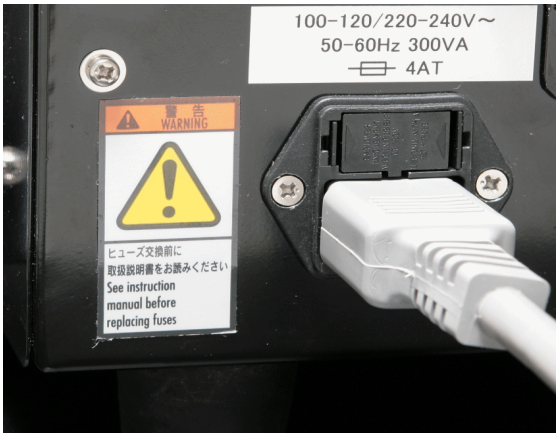
For safety, warning labels are attached in locations where special attention is required.
Should any of these labels peel off or become damaged, contact your Shimadzu representative immediately for a replacement.

• Indicates a high temperature area.

• Refer to the Instruction Manual before performing operations in this area.

• Fuses

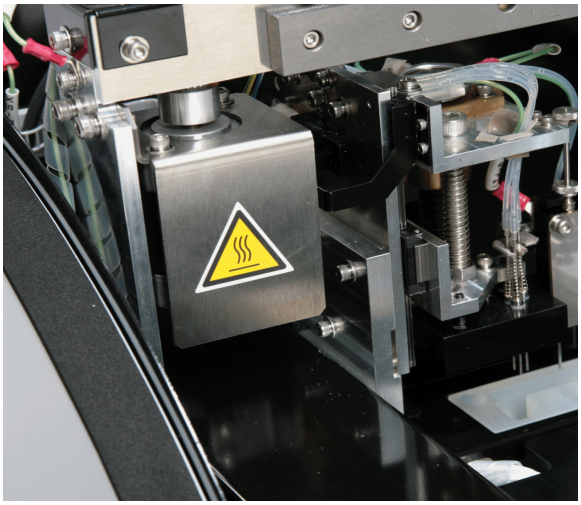
Before fuse replacement, refer to *-Instrument and System- "6.4 Part Replacement and Inspection"*.
Warning label: Part No. 228-42603



- Autosampler Motor Unit

  CAUTION: HOT! Touching while hot can result in burn.

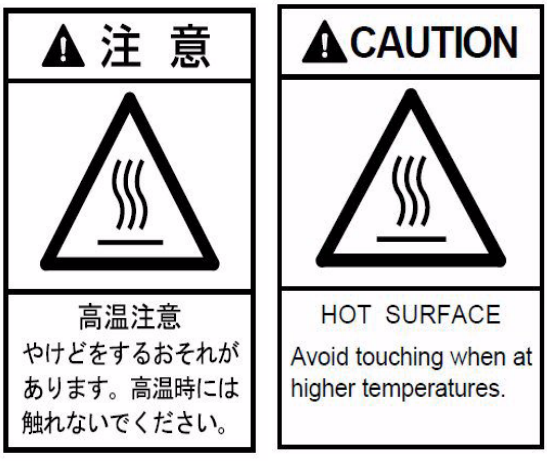
  Warning label: Part No. 037-72999-12

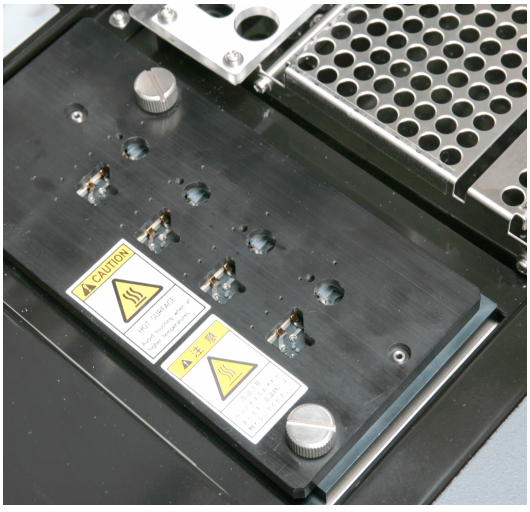


- Chip Stage

  The chip stage is kept at 37°C. During microchip replacement, prolonged contact with chip stage can result in a low-temperature burn.

  Warning label: Part No. 037-72123-00 (Japanese)/037-72126-02 (English)

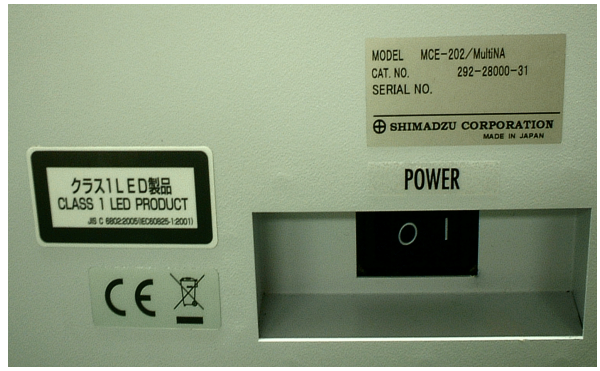




- Class 1 LED Product

  This is a Class 1 LED Product.

  Warning label: Part No. 292-27990

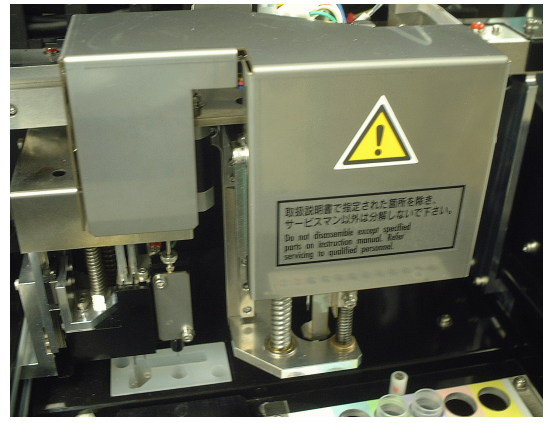


- Autosampler Cover

  Do NOT remove or disassemble any part of the cover, except where specified in the Instruction Manual.

  Warning label: Part No. 037-72999-02, 292-27778-07

取扱説明書で指定された箇所を除き、
サービスマン以外は分解しないで下さい。
Do not disassemble except specified parts on instruction manual. Refer servicing to qualified personnel.



Electromagnetic Compatibility

Descriptions in this section apply only to the following models:

- 292-28000-38 MULTINA SYSTEM ASSY (230VAC, English)

This product complies with European standard EN61326: 1997 + amendment 1: 1998 + amendment 2: 2001 + amendment 3: 2003, class B for electromagnetic interference (Emissions) and minimum requirement for electromagnetic susceptibility (Immunity).

■ EN61326-1 Immunity (Electromagnetic Susceptibility)

Test conditions are as follows.

- IEC 61000-4-2 Electrostatic Discharge:
  Air: 2/ 4/ 8 kV, Contact: 2/ 4 kV
- IEC 61000-4-3 Radiated, Radio-Frequency, Electromagnetic Field:
  3 V/m
- IEC 61000-4-4 Transient/Burst (Electrical Fast Transients):
  2 kV on AC Power Port, 0.5/ 1 kV on Signal and Control Lines
- IEC 61000-4-5 Voltage Surge:
  0.5/ 1 kV line to line, 0.5/ 1/ 2 kV line to ground
- IEC 61000-4-6 Conducted RF Immunity:
  3 V on AC Power Port, 3 V on Signal and Control Lines
- IEC 61000-4-8 Power Frequency Magnetic Field:
  30 A/m, 50 Hz
- IEC 61000-4-11 Voltage Variations/Dips/Interrupts:
  100% drop for 0.5/ 1 cycle

Compliance with these standards does not ensure that the product can operate at a level of electromagnetic interference that is stronger than the level tested. Interference stronger than the values specified above may cause the product to malfunction.

When installing or using this product, especially in an industrial location:

Locate the product away from any device emitting strong levels of electromagnetic noise.

Use a power source that is separated from the power source of any device emitting strong levels of electromagnetic noise.

To prevent static electricity:

Prior to touching the product, the operator should be sure to discharge the static electricity stored in their body by first touching a grounded metallic structure.

Do not touch any terminals or connectors that are not connected to cables while the product is turned ON.

Regulatory Information

For Europe:
The product complies with the following requirements.

EMC Directive 89/336/EEC
Low Voltage Directive 2006/95/EC

Product Name	Microchip Electrophoresis System for DNA/RNA Analysis
Model Name	MCE-202 MultiNA
Manufacturer	SHIMADZU CORPORATION ANALYTICAL & MEASURING INSTRUMENTS DIVISION
Address	1 NISHINOKYO-KUWABARACHO NAKAGYO-KU KYOTO 604-8511 JAPAN
Authorized Representative in EU	Shimadzu Europa GmbH
Address	Albert-Hahn-Strasse 6-10, 47269 Duisburg, F.R. Germany

Action for Environment

To all users of Shimadzu equipment in the European Union:

WEEE Mark

Equipment marked with this symbol was sold on or after 13th August 2005, and should not be disposed of with general household waste.

Our equipment is for industrial/professional use only.

Contact a Shimadzu service representative when the equipment is ready for disposal. They will advise you regarding the equipment take-back procedures.

With your co-operation we are aiming to reduce contamination from electronic waste and preserve natural resources through re-use and recycling.

Do not hesitate to ask a Shimadzu service representative, if you require further information.

Contents

1 Outline of Operation

4 Appendix

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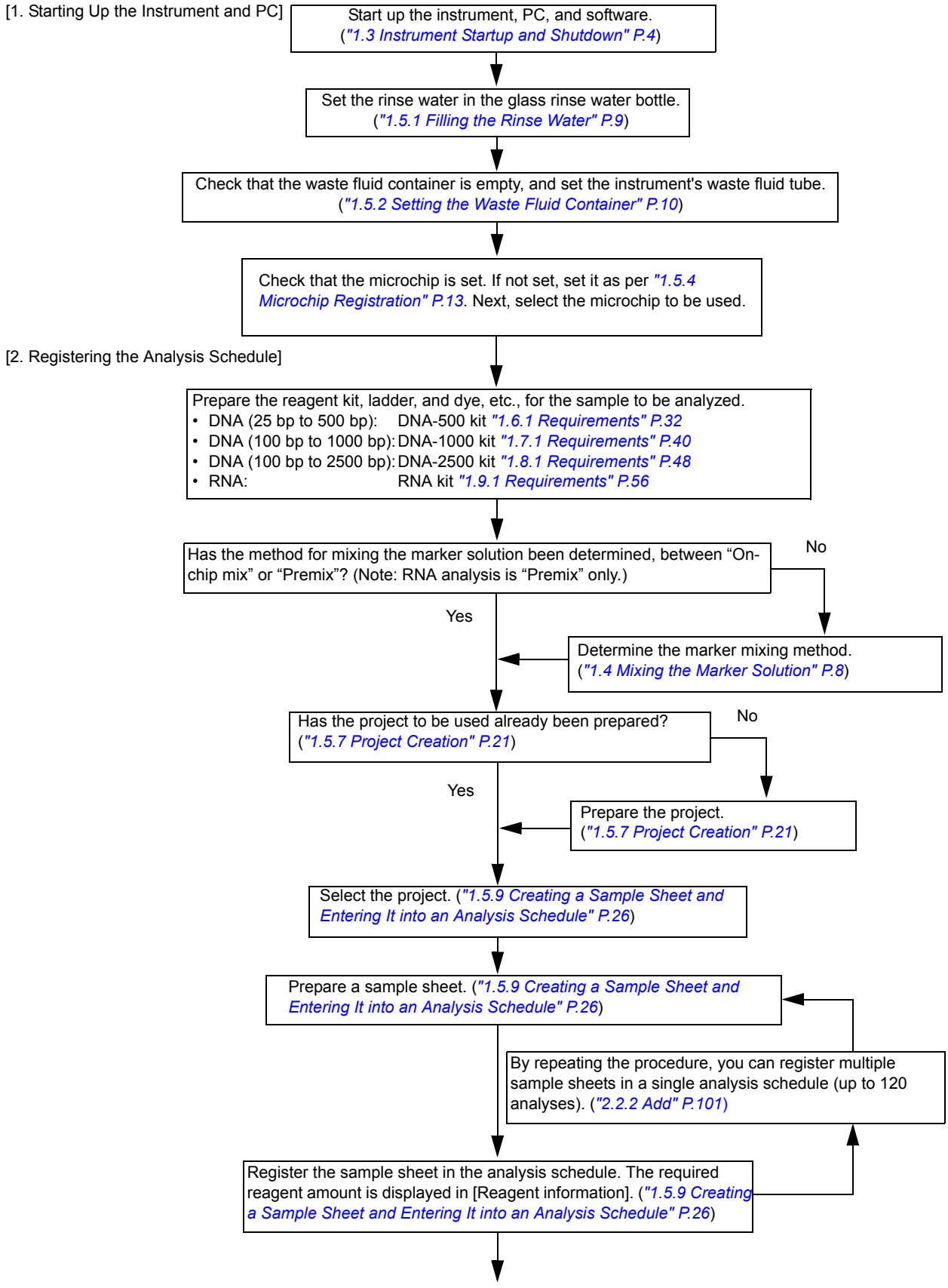
1 Outline of Operation

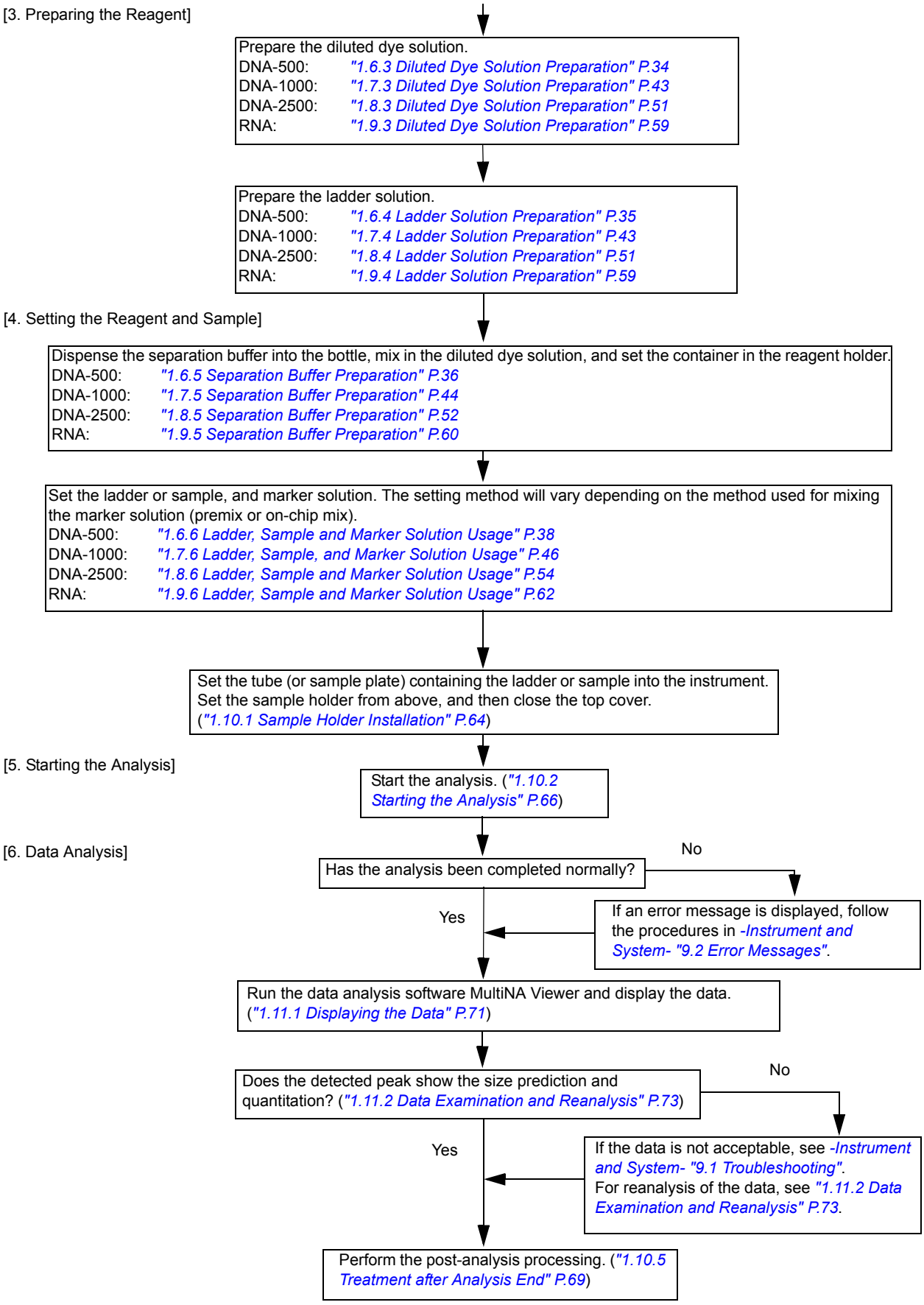
1.1 Operations Flow

This section describes the normal analysis operation flow. See the corresponding pages in this manual for details of each operations step.

Use the Analysis Operation Flow *(P.2)* as a “quick manual”. We recommend copying it to both sides of a sheet of paper.

Analysis Operation Flow (For details, see the MultiNA Instruction Manual, Operating Procedure pages.)




[3. Preparing the Reagent]
Prepare the diluted dye solution.
DNA-500: "1.6.3 Diluted Dye Solution Preparation" P.34
DNA-1000: "1.7.3 Diluted Dye Solution Preparation" P.43
DNA-2500: "1.8.3 Diluted Dye Solution Preparation" P.51
RNA: "1.9.3 Diluted Dye Solution Preparation" P.59
Prepare the ladder solution.
DNA-500: "1.6.4 Ladder Solution Preparation" P.35
DNA-1000: "1.7.4 Ladder Solution Preparation" P.43
DNA-2500: "1.8.4 Ladder Solution Preparation" P.51
RNA: "1.9.4 Ladder Solution Preparation" P.59
[4. Setting the Reagent and Sample]
Dispense the separation buffer into the bottle, mix in the diluted dye solution, and set the container in the reagent holder.
DNA-500: "1.6.5 Separation Buffer Preparation" P.36
DNA-1000: "1.7.5 Separation Buffer Preparation" P.44
DNA-2500: "1.8.5 Separation Buffer Preparation" P.52
RNA: "1.9.5 Separation Buffer Preparation" P.60
Set the ladder or sample, and marker solution. The setting method will vary depending on the method used for mixing the marker solution (premix or on-chip mix).
DNA-500: "1.6.6 Ladder, Sample and Marker Solution Usage" P.38
DNA-1000: "1.7.6 Ladder, Sample, and Marker Solution Usage" P.46
DNA-2500: "1.8.6 Ladder, Sample and Marker Solution Usage" P.54
RNA: "1.9.6 Ladder, Sample and Marker Solution Usage" P.62
Set the tube (or sample plate) containing the ladder or sample into the instrument. Set the sample holder from above, and then close the top cover. ("1.10.1 Sample Holder Installation" P.64)
[5. Starting the Analysis]
Start the analysis. ("1.10.2 Starting the Analysis" P.66)
[6. Data Analysis]
Has the analysis been completed normally?
No
If an error message is displayed, follow the procedures in -Instrument and System- "9.2 Error Messages".
Yes
Run the data analysis software MultiNA Viewer and display the data. ("1.11.1 Displaying the Data" P.71)
Does the detected peak show the size prediction and quantitation? ("1.11.2 Data Examination and Reanalysis" P.73)
No
If the data is not acceptable, see -Instrument and System- "9.1 Troubleshooting". For reanalysis of the data, see "1.11.2 Data Examination and Reanalysis" P.73.
Yes
Perform the post-analysis processing. ("1.10.5 Treatment after Analysis End" P.69)

1.2 Software Related to MultiNA

PCs prepared as shown in *-Instrument and System- "5.5 Software Installation"* will have two kinds of MultiNA-related software installed.

“MultiNA Control Software” is used to control the instrument. For details about using this software, see *"2 MultiNA Control Software Functions" P.83*.

“MultiNA Viewer” is used to view, print, and reanalyze the acquired data. For details about using this software, see *"3 MultiNA Viewer Functions" P.131*.

1.3 Instrument Startup and Shutdown

1.3.1 Instrument and PC Startup

Follow these steps to turn on the instrument and PC.

1 **Verify that the instrument and PC are properly connected using a LAN cable before turning ON the power.**

Reference

-Instrument and System- "5.4 Connecting the Instrument to a Computer (PC)"

2 **Turn ON the instrument, and wait for initialization to complete.**

During initialization the green LED blinks. When initialization ends, the green LED stops blinking and is steady lit and a buzzer sounds.

3 **Turn ON the PC.**

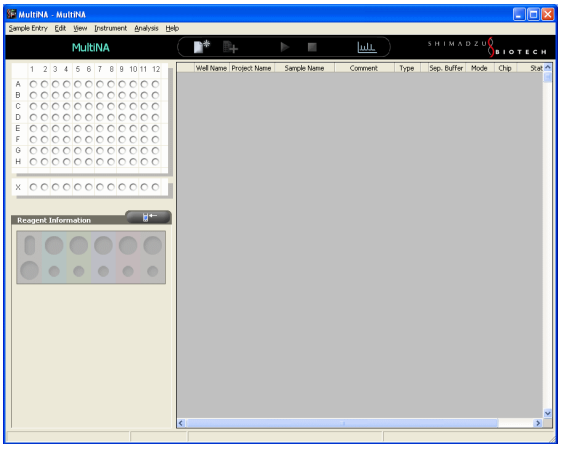
4 **Enter your user ID and password to log into Windows.**

NOTE

Use the same user ID as when the MultiNA software was installed.

5 **Click the** 

[MultiNA Control Software] icon on the desktop to open the instrument control software.



6 **Verify that the instrument name at the left end of the toolbar is shown in light blue.**

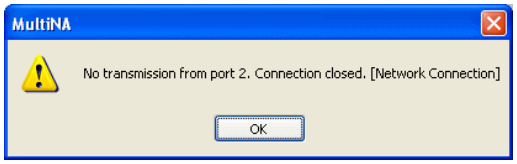
It will be shown in light blue if the instrument and PC are connected. It will be shown in light orange if they are disconnected.

In the default setting, the PC automatically connects with the instrument at startup and the name appears as light blue.

Reference

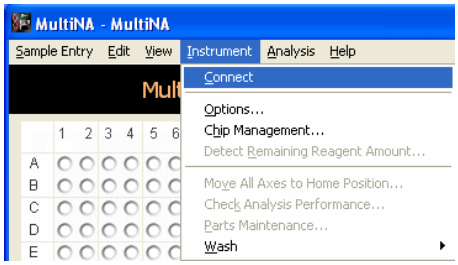
"2.5.2 Options" P.114

If the automatic connection fails, the following message is displayed. Proceed to 7.



7 **7If the instrument and PC are not connected after step 6, select [Connect] on the [Instrument] pull-down menu.**

Verify that the instrument name on the toolbar has changed to light blue.



1.3.2 Instrument Startup (PC Already Running)

Follow the procedures below to start the instrument if the PC has already been started, and the instrument was OFF.

1 **Verify that the instrument and PC are properly connected using a LAN cable before turning ON the power.**

Reference

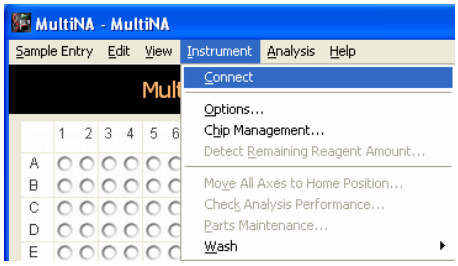
-Instrument and System- "5.4 Connecting the Instrument to a Computer (PC)"

2 **Turn ON the instrument, and wait for initialization to complete.**

During initialization the green LED blinks. When initialization ends, the green LED stops blinking and is steady lit and a buzzer sounds.

3 **Select [Connect] on the MultiNA Control Software [Instrument] pull-down menu.**

The instrument name on the toolbar will change from light orange (instrument and PC disconnected) to light blue (connected).



Reference

"2.5.1 Connect" P.113

1.3.3 Instrument and PC Shutdown

1 **Click the [Close] button at the top right corner of the MultiNA Control Software window to close the program. Alternatively, select [Exit] on the [Sample Entry] pull-down menu.**

This procedure is not required if the control software MultiNA was not started.

NOTE

The program cannot be closed if the instrument is in the middle of analysis, rinsing, or other operations.

2 **Click the [Close] button at the top right corner of the data analysis software MultiNA Viewer window to close the program. Alternatively, select [Exit] on the [File] pull-down menu.**

This procedure is not required if MultiNA Viewer was not started.

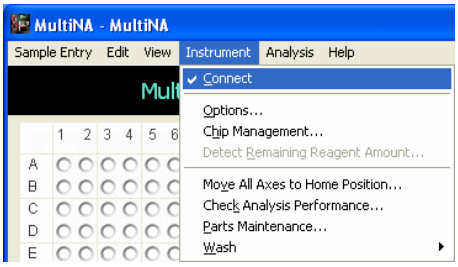
3 **Turn the instrument OFF.**

4 **Close all open programs on the PC, shut down Windows and turn OFF the PC.**

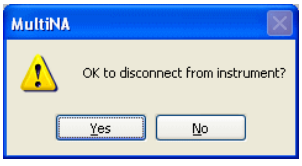
1.3.4 Instrument Shutdown (Leave the PC Running)

Follow the procedures below to turn the instrument OFF, leaving the PC ON.

1 **Select [Connect] on the control software MultiNA [Instrument] pull-down menu.**



2 **The message in the figure below will be displayed. Click [Yes].**



Check that the instrument name on the toolbar changes to light orange (disconnected).

3 **Turn the instrument OFF.**

1.3.5 Precaution for Stable PC Operation

Depending on the PC operating environment, memory leaks or memory fragmentation may occur if the system is run continuously for long periods of time, resulting in unstable PC operation. To avoid this kind of unexpected Windows-derived problem, either turn OFF the PC or restart the PC at regular intervals (about once a week) to ensure use in a clean state.

1.4 Mixing the Marker Solution

In this instrument, the marker solution which contains an internal standard substance is mixed with a ladder or sample and analyzed. The peak of the marker is detected and corrected, for the purpose of enhancing the accuracy of size prediction and concentration quantitation. The marker solution in the DNA analysis kit includes two types of markers, a lower marker and an upper marker. The marker solution in the RNA analysis kit includes only the lower marker.

There are two methods for mixing the marker solution into the ladder or sample.

- In “on-chip mix mode,” the ladder or sample is placed into a sample tube (or sample plate) and the marker solution is placed into a dedicated vial, then they are placed into the instrument and automatically mixed in a microchip reservoir.
- In “premix mode,” the sample and marker solution are manually mixed then placed into the instrument.

Characteristics of the two modes are shown below. As the procedures and amounts required for the samples and marker solutions to be prepared differ between the two methods, select the one that is most appropriate for the sample to be analyzed, and create the project (*"1.5.7 Project Creation" P.21*).

NOTE

In RNA analysis only the premix mode is used.

Mixing mode	On-chip Mix	Premix
Mixing method	Automatic (Mixed inside microchip reservoir No.1)	Manual mixing
Minimum required sample or ladder solution volume	5 µL to 30 µL (sample/ladder solution only)	DNA: 2 µL to 10 µL (after mixing in marker solution: 6 µL to 30 µL) RNA: 4 µL to 20 µL (after mixing in marker solution: 6 µL to 30 µL)
Sample or ladder preparation	Only the sample or ladder solution is added to the sample tube or sample plate, and then it is placed in the instrument.	Marker solution is added to the sample tube or sample plate that contains the sample or ladder solution. Use a micro pipette to mix, and then place it in the instrument.
Marker solution preparation	Only the marker solution is added to a dedicated vial (0.6 mL tube), and then it is placed in the instrument reagent holder.	-
Features	After a PCR reaction has ended, the sample tube or sample plate can be placed directly into the instrument and analyzed.	Can perform analysis with a small amount of sample.

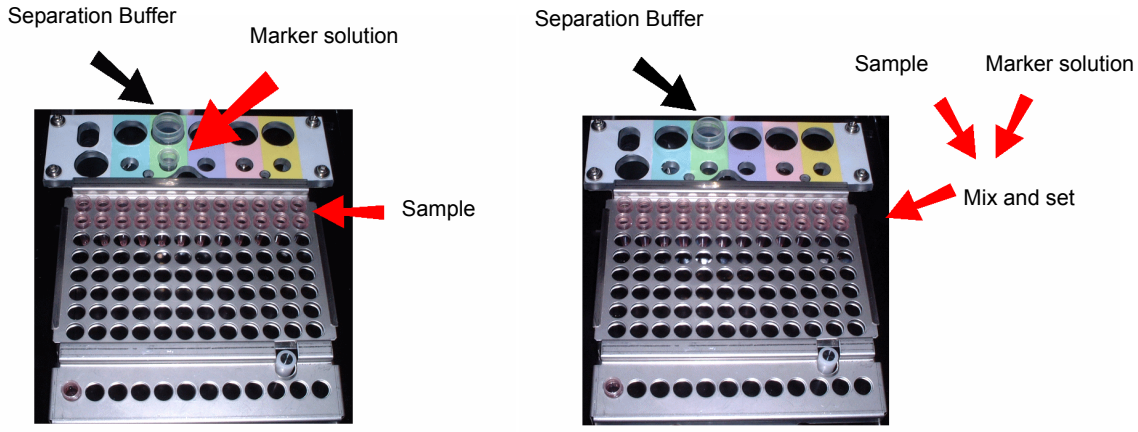


Fig.1-1 On-Chip Mix (Left) and Premix (Right)

1.5 Preparation Before Analyses

This section describes preparation of the instrument before analysis. For preparation of reagents and other items required for analysis, refer to the type of kit and the corresponding pages.

- *"1.6 Preparation for Analysis with the DNA-500 Kit" P.32*
- *"1.7 Preparation for Analysis with the DNA-1000 Kit" P.40*
- *"1.8 Preparation for Analysis with the DNA-2500 Kit" P.48*
- *"1.9 Preparation for Analysis with the RNA Kit" P.56*

1.5.1 Filling the Rinse Water

Put Milli-Q ultrapure water into the glass bottle for the rinse water. Normally 1000 mL of rinse water is required for 120 analyses, however, variations that occur from analysis to analysis may result in use of more than 1000 mL. Therefore, use of a 2 L rinse water bottle is recommended.

As shown in *"Fig.1-2 Glass Rinse Water Bottle" P.9*, confirm that the 2 suction filters have sunk to the bottom of the glass bottle, and that they do not float up again.

⚠ WARNING

- Remove the cap from the rinse bottle and fill the bottle away from the instrument.
  Spilled water on the instrument risks electrical leaks or electrical shocks.

⚠ CAUTION

- Before starting analysis, replenish the rinse water bottle with Milli Q ultrapure water and verify that the amount of rinse water is sufficient.
  Insufficient rinse water during analysis could disable the migration result, clog the microchip so that it becomes unusable, or cause other problems.

⚠ CAUTION

- Do NOT use anything other than Milli-Q ultrapure water for the rinse water.
  Use of other types of water could result in defective rinsing or equipment malfunction.

Fig.1-2 Glass Rinse Water Bottle

1.5.2 Setting the Waste Fluid Container

1 **Insert the waste tubing into the drain hook provided as a standard accessory.**

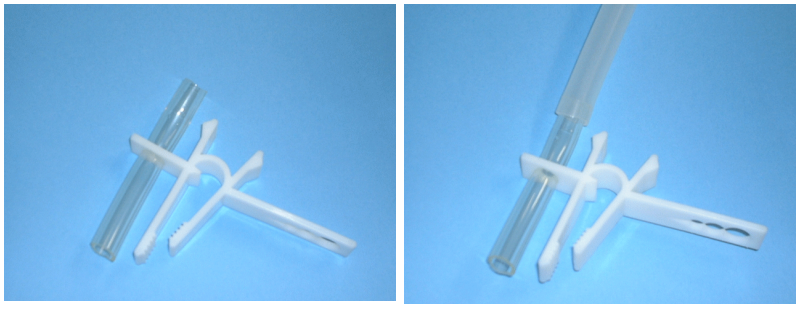


Fig.1-3 Drain Hook (Left) and Drain Hook with Waste Fluid Tube Attached (Right)

2 **Use the drain hook to attach the waste tubing to the waste container.**

⚠ CAUTION

- Before starting analysis, verify that the tip of the waste tubing is not immersed in the waste fluid inside the waste container, and verify that there is a sufficient empty space inside the container.
- Check that the waste tubing is not bent, or elevated.
  If the waste tubing becomes clogged, waste fluid may leak from inside the instrument.

Fig.1-4 Attaching the Drain Hook to the Waste Fluid Container.

1.5.3 Drive Positions

1 **Turn the instrument ON.**

When initialization is completed, the green LED on the instrument indicator lights up. If the instrument or PC is not turned ON, refer to *"1.3.1 Instrument and PC Startup" P.4* or *"1.3.2 Instrument Startup (PC Already Running)" P.5*.

2 **Open the top cover.**

Grip the front of the top cover and lift it.

⚠ WARNING

- Do NOT forcibly open the top cover during instrument operation (blinking green LED on front indicator).
  During operation the top cover is locked and cannot be opened.
- Do Not apply lateral force to the top cover when opening or closing.
  This may deform the top cover and result in damage.
- Do NOT remove the top cover.
  Injuries or accidents may occur.
- If the top cover does not open or close properly, contact your Shimadzu service representative immediately.
  A technician from Shimadzu will perform repairs.

Fig.1-5 Opening the Top Cover (Left: Gripped, Right: Lifted)

- The top cover uses a "auto-stop" mechanism, to stops the cover when it reaches an opening angle of approximately 60°.
- When the top cover is fully open, the instrument height is 98 cm.
- The top cover cannot be opened while the instrument is initializing or operating. It can be opened during normal standby status or when an error occurs.

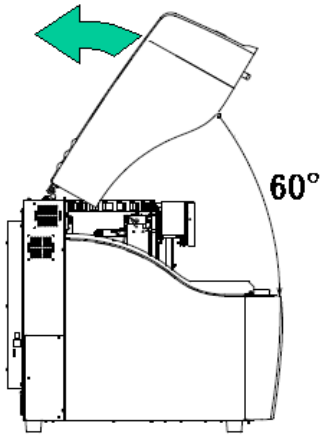


Fig.1-6 Top Cover Stop Position

3 **Ensure that the drives (autosampler and pneumatic unit) are at the back of the instrument (home position) as shown in the figure below.**

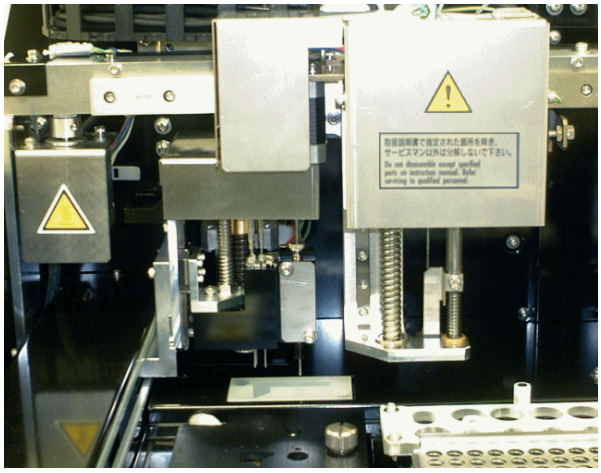


Fig.1-7 Home Positions of the Drives

NOTE

Usually, the drives will be left in the home position when analysis ends normally. However, if a power outage or error during analysis causes the instrument to stop, the drives may not have returned to the home position. In this case, see *"2.5.5 Move All Axes to Home Position" P.119*, and return the drives to the home position. The drives will not move to the home position during initialization after the instrument is turned ON.

1.5.4 Microchip Registration

- Up to four microchips can be inserted into this instrument.
- A record of the number of runs is managed by the serial number (chip ID) in the software.
- The chip ID is stamped on the surface of the microchip, and is also listed in the instruction manual that comes with the microchip (indicated on the right side of S/N. In the figure below, the chip ID is NA0001.)
- The microchip is composed of a quartz microchip and a plastic protective frame (chip frame).
- In this manual and in the software, it is sometimes expressed as “chip” (Ex.: Chip ID, chip position).

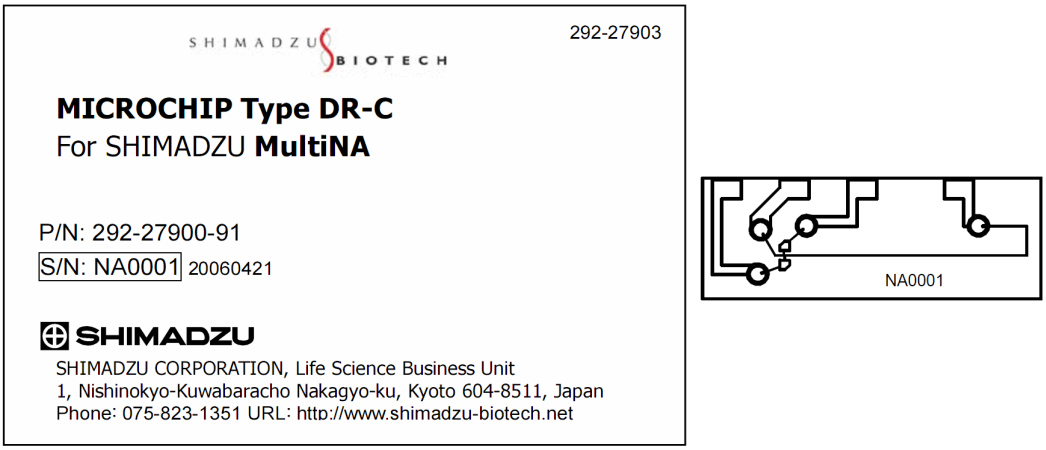


Fig.1-8 Cover of Microchip Manual (Left) and Microchip with Chip Frame Removed (Right)

NOTE

It is recommended that separate microchips be used for DNA and RNA analysis,

- Since the dye used in DNA analysis and RNA analysis differs, use of a microchip for DNA analysis followed immediately RNA analysis (or for RNA analysis followed immediately by DNA analysis) could have an adverse effect on the results.
- If the same microchip must be used for both types of analysis, use one of the procedures below to rinse the microchip between analyses.

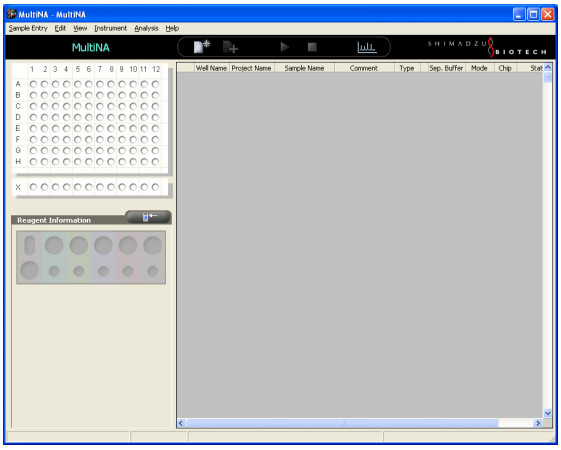
  Microchip used for DNA analysis and then used for RNA analysis:
  "4.8 Using a Chip for RNA Analysis after Using It for DNA Analysis" P.203

  Microchip used for RNA analysis and then used for DNA analysis:
  "4.9 Using a Chip for DNA Analysis after Using It for RNA Analysis" P.204

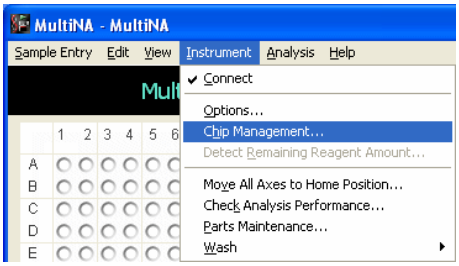
When using a new microchip, use the following procedure to register it. If the microchip is already installed, proceed to *"7" P.16*.

	Following Procedures
Using a new microchip	Proceed to *"1" P.14*.
Using an installed microchip	Proceed to *"7" P.16*.

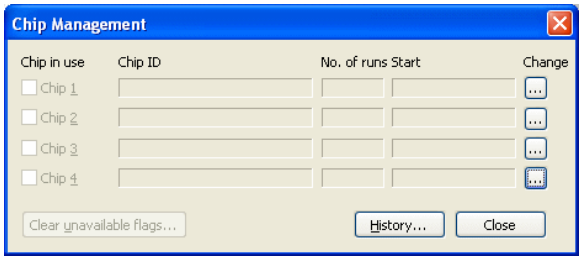
1 Start the MultiNA Control Software. Verify that the instrument and PC are connected.



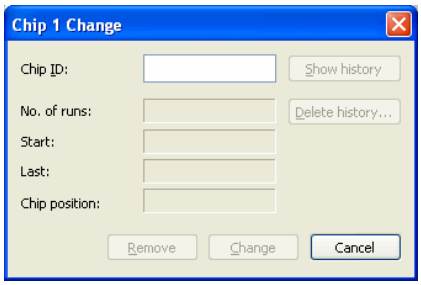
2 Select [Chip Management] on the [Instrument] pull-down menu.



The [Chip Management] window below is displayed.



3 Click the [...] [Change] button corresponding to the position of the chip to be inserted (chips 1 to 4).



☞ Reference

For details, see *"2.5.3 Chip Management" P.115*.

NOTE

The chip positions are arranged in 1, 2, 3 order from the back, with the front-most position being No.4.

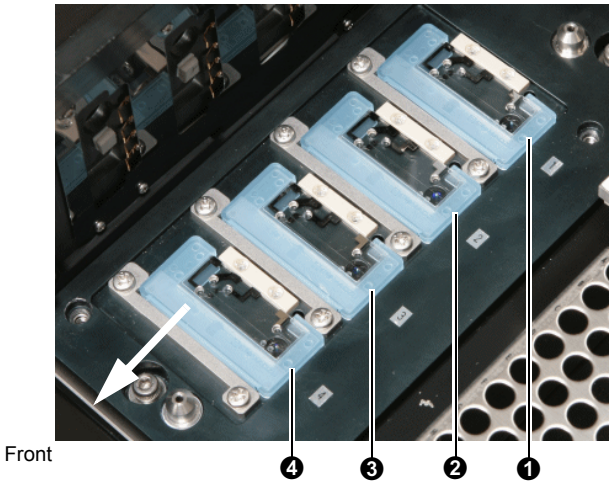
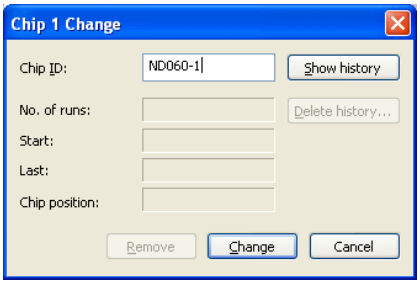


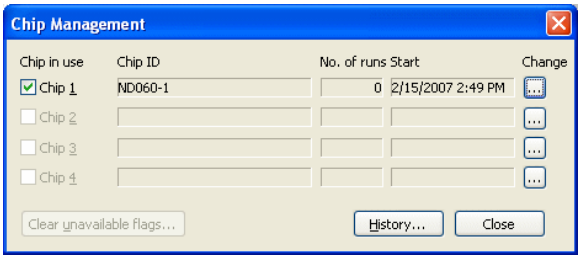
Fig.1-9 Chip Stage (Number Shows Chip Position No., and Arrow Shows Direction to Front of Instrument)

4 **Enter the chip ID (Ex,: ND060-1) into the chip ID column.**



5 **Click the [Change] button. The microchip is registered.**

A check mark appears next to the chip to be used.



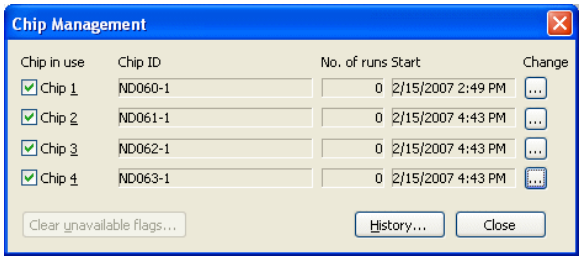
6 **Repeat steps 3 to 5 for all of the microchips that will be used.**

7 **Select the microchips to be used in analysis.**

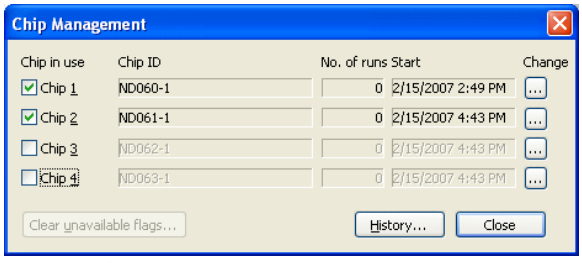
In the [Chip Management] window, verify that all of the microchips to be used have been selected.

NOTE

A microchip without a check mark will not be used in analysis even if it is installed in the instrument.



All four microchips are in use



Only chips at positions 1 and 2, are in use

1.5.5 Microchip Installation

1 **Loosen the knurled screws (2) on the chip cover.**

Fig.1-10 Opening the Chip Cover

2 **Lift the chip cover and place it to the side as shown in the figure below.**

Fig.1-11 Placement of the Chip Cover

⚠ CAUTION

- Do NOT touch the electrode pins in the back of the chip cover.
  Deformed electrode pins may cause defective contact with electrodes on the microchip surface and result in improper voltage application.

3 **Insert the microchip on the chip stage by pressing lightly on the upper surface of the microchip with both hands.**

Push the upper edge of the microchip onto the surface indicated by the arrows in the figure below.

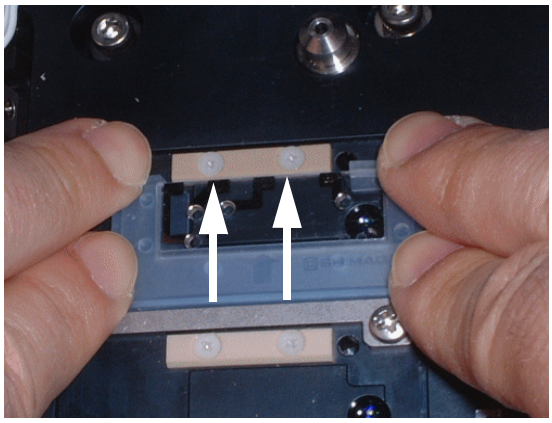


Fig.1-12 Inserting the Microchip into the Chip Stage

⚠ CAUTION

- Verify that the chip ID matches the installed chip position.

4 **Align the chip cover with the guide pins and close the cover. Use both hands to hold the chip cover in place and tighten the knurled screws.**

Fig.1-13 Closing the Chip Cover

1.5.6 Work Flow from Project Creation to Data Acquisition

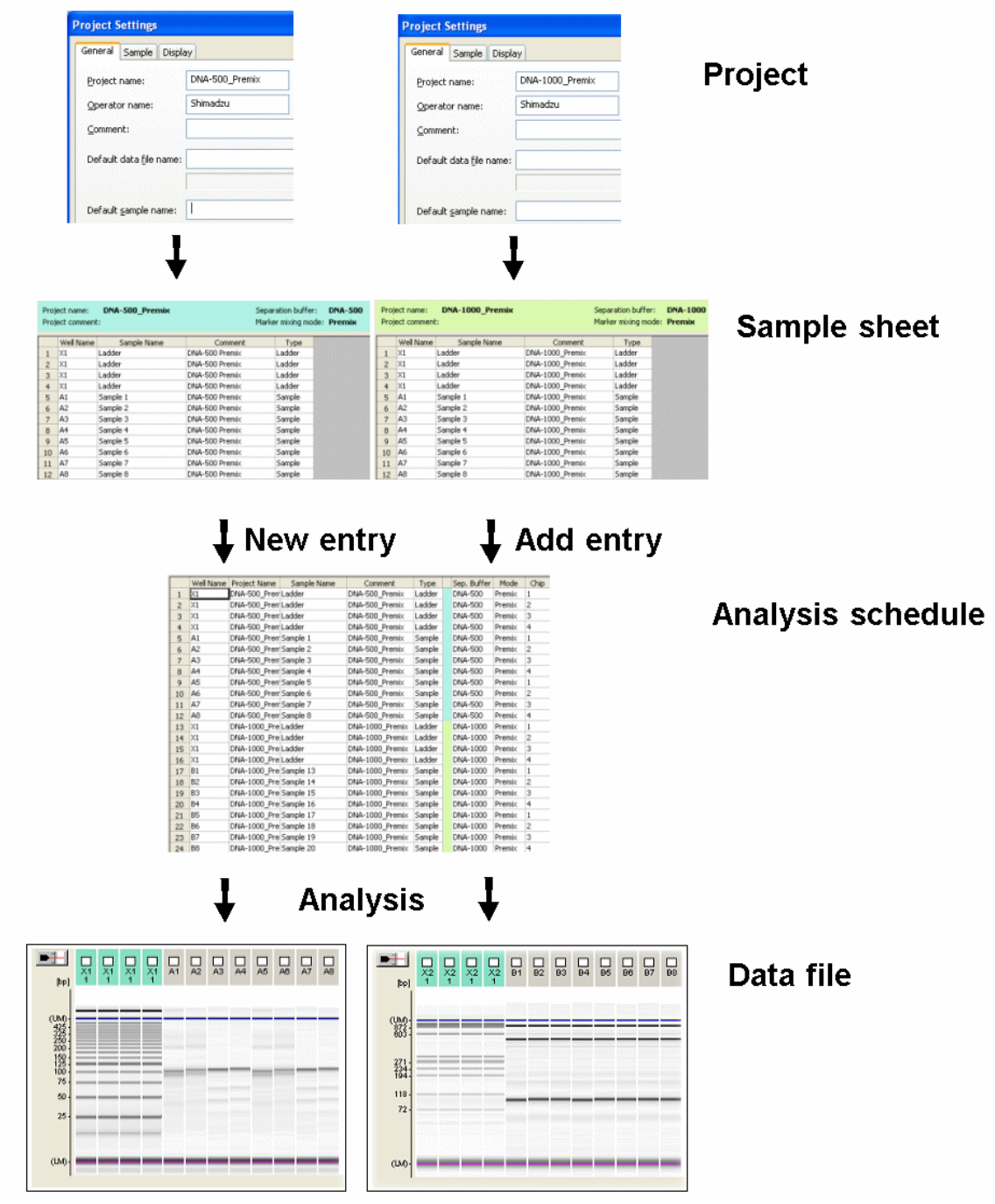


Fig.1-14 Work Flow from Project Creation to Data Acquisition

■ Project

The project is the user environment containing information required for analysis, including the reagent kit (separation buffer), marker solution mixture method (on-chip, premix), default data file name, and graphic display scale. A sample sheet is created based on the project information, and registered in the analysis schedule.

☞ Reference

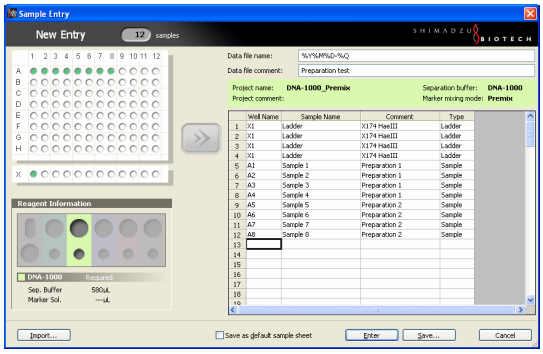
"1.5.7 Project Creation" P.21

■ Sample Sheet

The sample sheet is where information related to the targeted ladder or sample (well name, sample name, comment, and type) is entered. A sample sheet can be created in the [Sample Entry] window and saved in a file for each project (".SSH" is used for the file extension).

☞ Reference

"1.5.8 Rules for Sample Sheet Creation and Entering into an Analysis Schedule" P.24

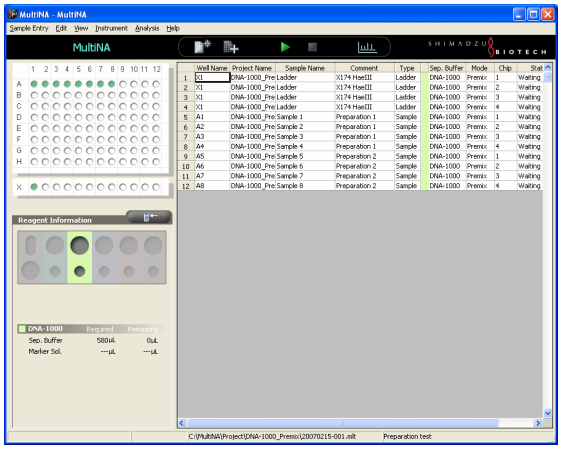


■ Analysis Schedule

The analysis schedule displays the order for ladder or sample analysis. When the sample sheet is created and entered in the analysis schedule, the schedule is displayed in the main window.

☞ Reference

"1.5.9 Creating a Sample Sheet and Entering It into an Analysis Schedule" P.26



Multiple sample sheets can be entered into a single analysis schedule.

☞ Reference

"2.2.2 Add" P.101

■ Data file

The data file saves the data obtained in the analysis, including both raw data and analysis data.

One data file is created for each sample sheet in the analysis schedule, and is saved in the project folder.

☞ Reference

"1.11.1 Displaying the Data" P.71

1.5.7 Project Creation

A project must be created before a sample sheet can be entered into an analysis schedule.

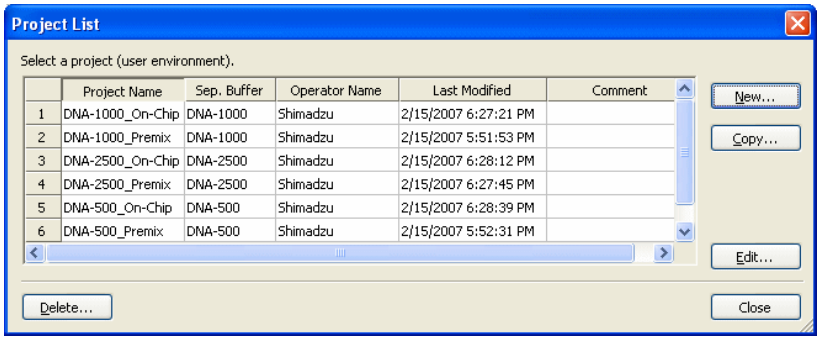
☞ Reference

"1.5.9 Creating a Sample Sheet and Entering It into an Analysis Schedule" P.26

The method for creating a new project is as shown below.

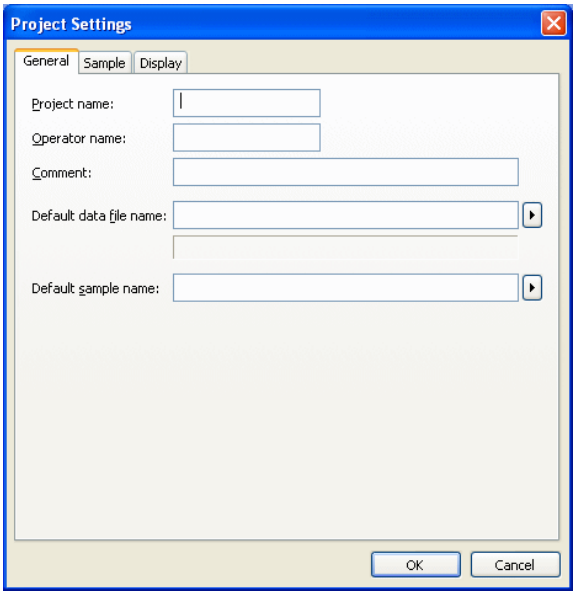
1 **Select [Edit] on the [Project Settings] pull-down menu.**

The [Project List] window is displayed.

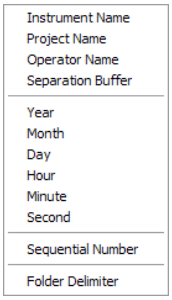
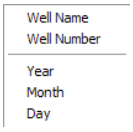


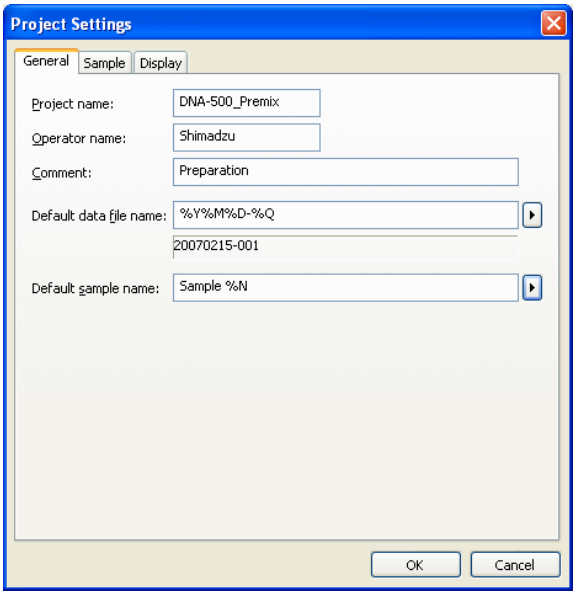
2 **Click the [New] button.**

The [Project Setting] window is displayed.



3 Enter the following items.

Item	Maximum number of characters	Explanation
Project name	30	Enter the project name (required). /\<>:"|?* and space cannot be used.
Operator name	20	Enter the operator name (required). /\<>:"|?* and space cannot be used.
Comment	50	Enter a comment about the project.
Default data file name	258 (Including path)	When the [Sample Entry] window is opened, specify the data file name to be automatically set. /\<>:"|?* and space cannot be used. In addition to normal characters, the following format characters can be used. %I: Instrument name (The name set in [Instrument] - [Option]) %J: Project name %O: Operator name %B: Separation buffer %Y: Year, %M: Month, %D: Day, %h: Hour, %m: Minute, %s: Sec. %Q: Sequential number (Reset in 3 digits at midnight) Click the ▸ button to select and enter a format.  Ex. In %Y%M%D-%Q, for analyses performed on January 30, 2007, the first data file name is 20070130-001 and the next one is 20070130-002.
Default sample name	30	In the sample entry window, select the sample name to be automatically entered. In addition to normal characters, the following format characters can be used. %W: Well name (A1, B1,...) %N: Well No. (1 to 108) %Y: Year, %M: Month, %D: Day Click the ▸ button to select and enter a format. 



4 Click the [Sample] tab, and select the items below.

Item	Explanation
Sample (Separation Buffer)	Select the separation buffer to be used in the project.
Total RNA/mRNA	Select RNA (RNA separation buffer) for RNA analysis. (The content of data analysis differs between Total RNA and mRNA. When calculating the ratio between 28S rRNA and 18S rRNA, select Total RNA.)
Marker mixing mode	Select on-chip or premix. For details about each mode, see *"1.4 Mixing the Marker Solution" P.8*.
Load default sample sheet while starting up	If this is selected, the default sample sheet (file name: default.ssh) is automatically read when the [Sample Entry] window is opened (*"2.2.1 New" P.89*).
Auto ladder entry / Well name	If the ladder is selected, the ladder is automatically registered when the sample entry window is opened. A well position (one from X1 to X12) for setting the ladder is specified at the same time.
V1.05 Ladder type	Select the standard ladder [Standard (STD)] normally. The optional ladder [Ladder1 (LD1)] may also be selected.
Analysis order	Select the analysis order (A1-B1-C1…, or A1-A2-A3…) for a sample sheet created from the wells selected in the sample entry window.

NOTE

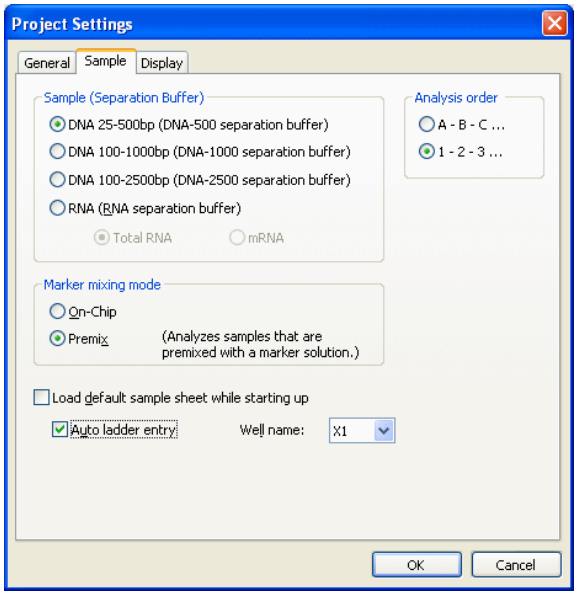
Ladder analysis is essential for size prediction, quantitation, and other data analysis (see-*Instrument and System- "4.2 Actual Analysis and Data Analysis"*).

It is recommended that [Auto ladder entry] be selected for well positions X1 to X12.

- The ladder is analyzed once at the start of the sample sheet for all microchips used in the analysis.
- Set the ladder into the appropriate well position (X1 to X12) on the extra sample stand.

V1.05

The basic performance specifications for this instrument (*-Instrument and System- "8 Specifications"*) are based on using a standard ladder. Performance may differ from the specifications if an optional ladder is selected.



5 **To set the [Display] tab items, see *"2.3.1 Project Settings"*, step *"3" P.104*.**

6 **Click [OK] to save the setting.**

7 **In the [Project List] window, click [Close].**

1.5.8 Rules for Sample Sheet Creation and Entering into an Analysis Schedule

Create a sample sheet and enter it into the analysis schedule using the [Sample Entry] window.

Rules for Creating a Sample Sheet

1. Create a sample sheet based on the selected project.
2. Enter the well name, sample name, comments, and type (ladder or sample).
3. A sample or ladder set in the sample stand (well position from A1 to H12) is analyzed only once. If it is set in the extra sample stand (well position from X1 to X12), analysis can be performed multiple times (assuming it is registered multiple times in the sample sheet).
4. A maximum of 120 ladders and sample analyses can be entered on a single sample sheet.
5. Ensure that the ladder is analyzed first for each of the microchips that is used.

NOTE

- For each microchip to be used, a size calibration curve is created based on the results of the first ladder analysis (see *"3.4.8 Size Calibration Curve" P.171*). All of the samples analyzed after the ladder are analyzed by using the size calibration curve.
- While analysis can be performed in sample sheets that do not include a ladder, analyzing a ladder first is recommended for obtaining analysis data with the highest accuracy.

6. Enter the created sample sheet into the analysis schedule.

The left side of *Fig.1-15* shows the case when four microchips are used, and the right side shows use of two microchips. For example, when four microchips are used, analysis is first performed on the four ladders (one analysis for each microchip) and then the samples are analyzed. When two microchips are used, analysis is first performed on the two ladders and then the samples are analyzed.

For the case of eight samples, the total number of analyses, including ladders, for four microchips is “4 + 8 = 12", and for two microchips is “2 + 8 = 10".

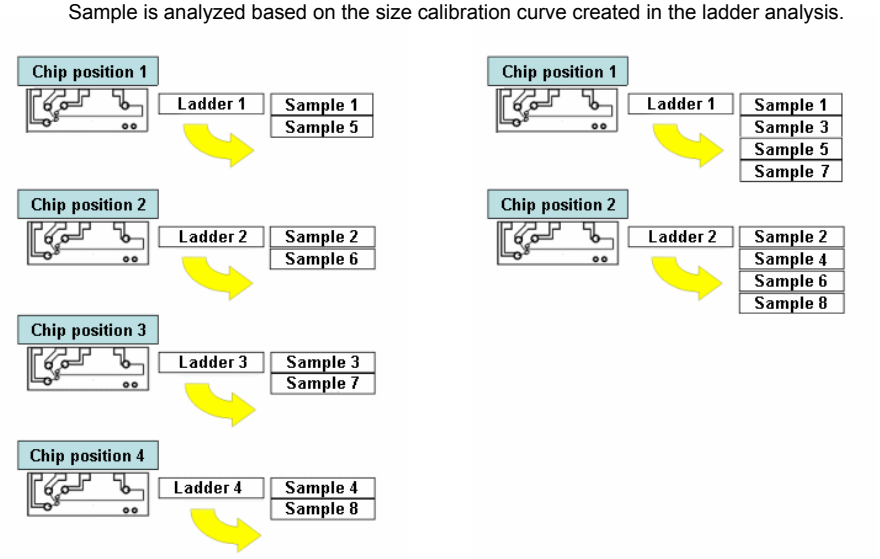


Fig.1-15 Relationship between Ladder and Sample Analysis (Left: Four Microchips Used, Right: Two Microchips Used)

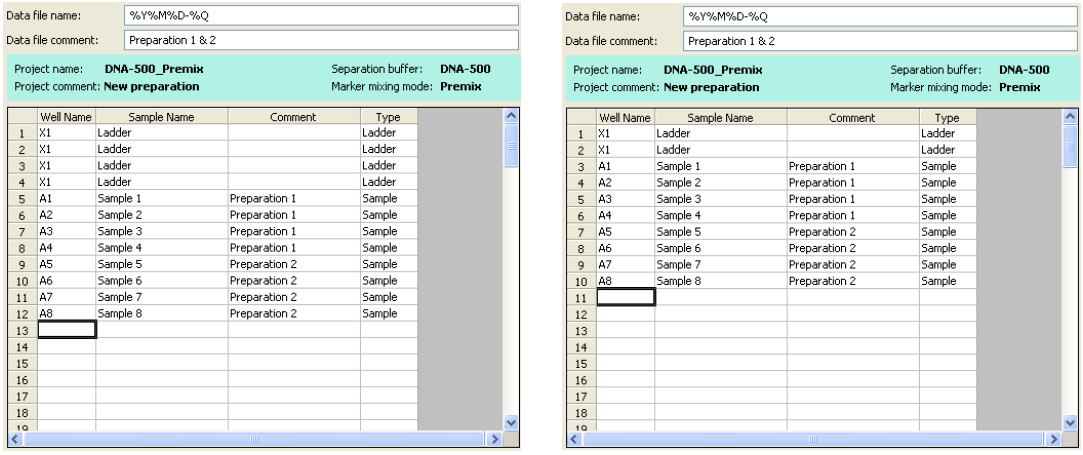


Fig.1-16 Sample Sheet Example (Left: Four Microchips Used, Right: Two Used)

Rules for Entry into an Analysis Schedule

1. A maximum of 120 analyses can be entered into a single schedule.
2. Analyses are performed in the order shown in the analysis schedule.
3. All registered microchips are used to execute the analysis schedule.

NOTE

An automatic check performed before analysis detects clogged microchips, and excludes any microchips judged to be unsuitable for analysis. The remaining microchips are then used to complete the analysis schedule.

⚠ CAUTION

- Insert a blank analysis at the beginning of each RNA sequence on each microchip.
  This is necessary for obtaining stable analysis results.
- For the blank analysis sample, use THE RNA Storage Solution.

⚠ CAUTION

- Use the table below as a guide for determining the number of samples that can be entered into a single RNA analysis.
  The sample stand does not have a cooling function. To maintain the denatured state of the RNA samples, use the table below as a guide for determining the number of samples in a single analysis, according to the number of microchips used.

Number of microchips used	Sample number	Ladder analysis	Blank analysis	Total number of analyses
1	1 to 6	1	1	8 max
2	2 to 12	2	2	16 max
3	3 to 16	3	3	22 max
4	4 to 14	4	4	22 max

1.5.9 Creating a Sample Sheet and Entering It into an Analysis Schedule

The method for specifying the ladder and sample positions using the well display window is described below.

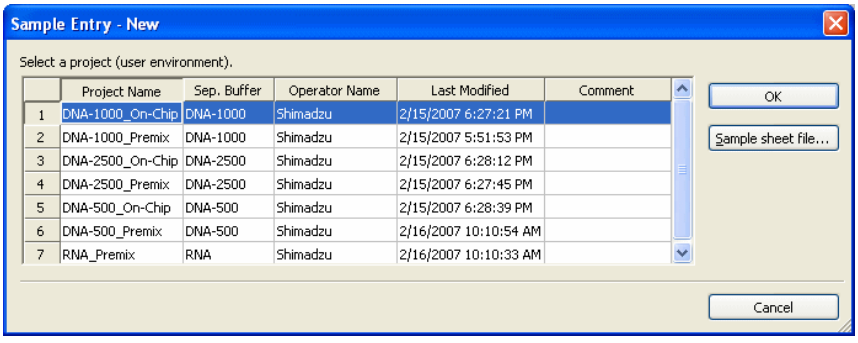
Other methods are also available, including direct entry of the well position, entry of only the sample number, and capture from Excel or CSV files. A sample sheet can also be created from the default sample sheet saved for each project.

☞ Reference

"2.2.1 New" P.89

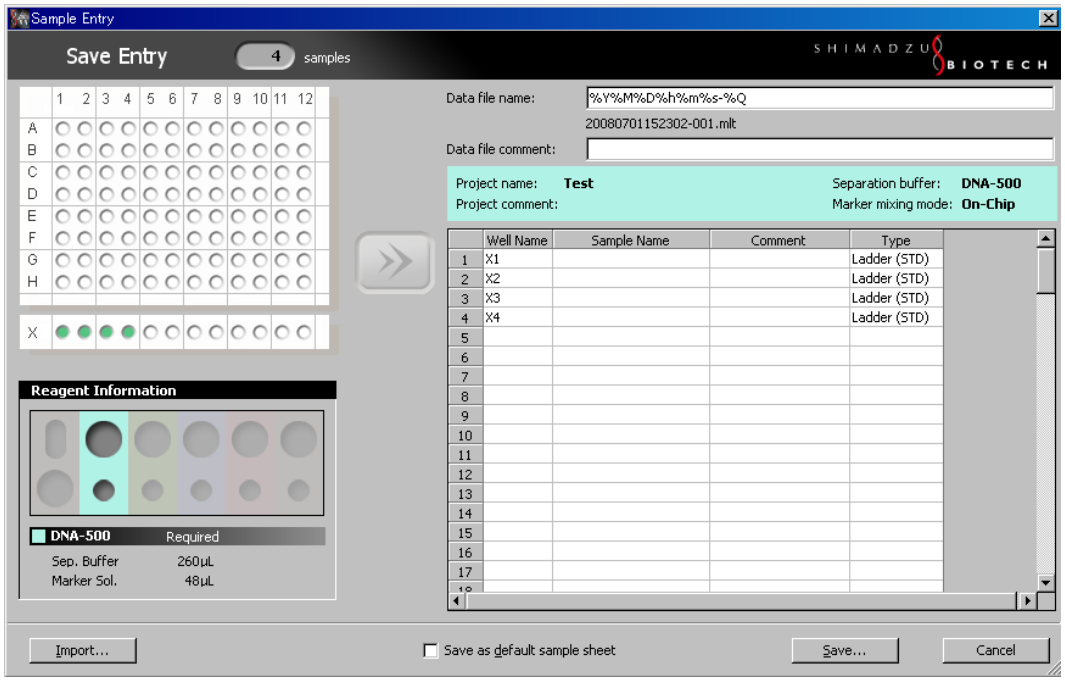
1 **Select [New] on the [Sample Entry] pull-down menu or click the [New Entry] button on the upper left side of the toolbar.**

The [Sample Entry - New] window below is displayed.



2 Select the project created in *"1.5.7 Project Creation" P.21*, and click [OK].

The [Sample Entry] window is displayed.
Four microchips are used in the example below, [Auto ladder entry] is selected (*P.23*), and the ladder well is X1.
In this case, for the first four analyses (four lines), the well name is X1 and "Ladder" is automatically displayed for [Type].



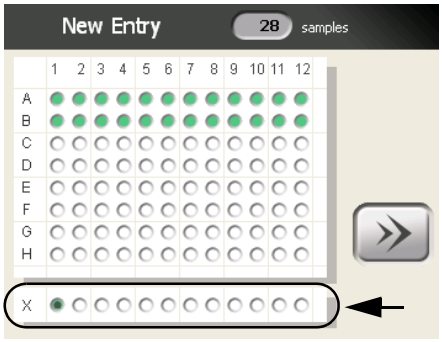
- In the [Data file name] column, the file name format is displayed in accordance with the project [default data file name]. For %Y%M%D-%Q, for example, the file name is automatically the year-month-day sequential number (Ex.: 20070130-001, first data file for January 30, 2007) (see *"1.5.7 Project Creation" P.21*).

V1.05

- The file name shown below the [Data File Name] column is the file name actually created. (Clicking the [Enter] button confirms the file name.)

3 Specify the ladder or sample wells in the upper left area of the well display window.

- To select the wells, either click the wells one by one, or drag the mouse over multiple wells.

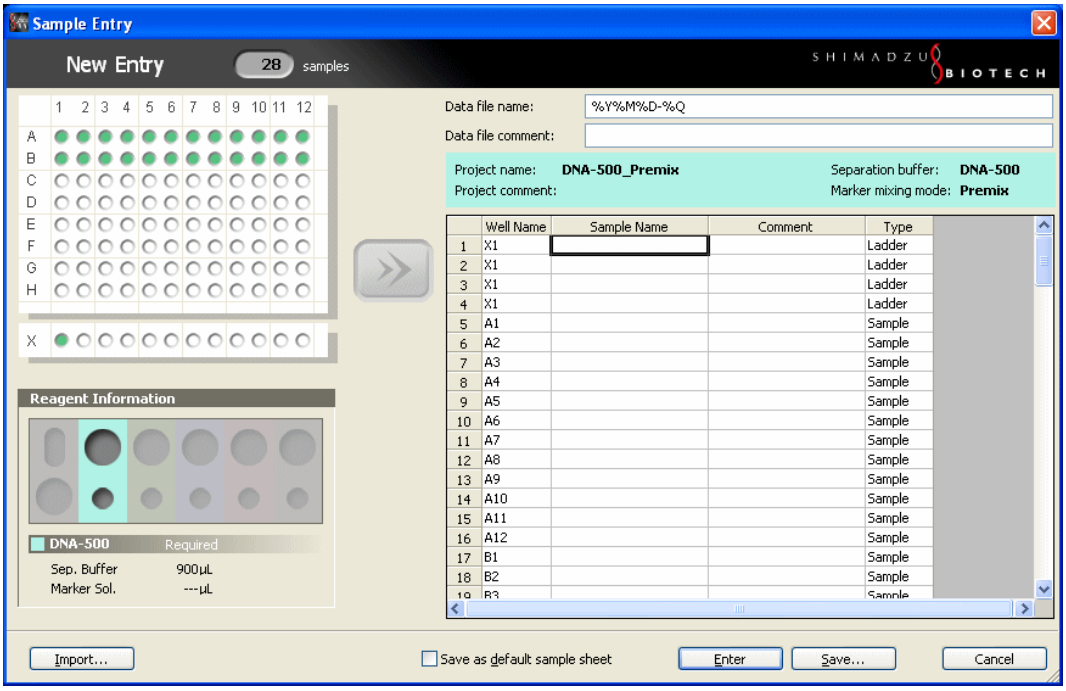


NOTE

Only one analysis can be performed from a single well in the A1 to H12 positions. Multiple analyses can be performed from the X1 to X12 positions in the extra sample stand. Choose the appropriate well positions according to the sample and number of analyses.

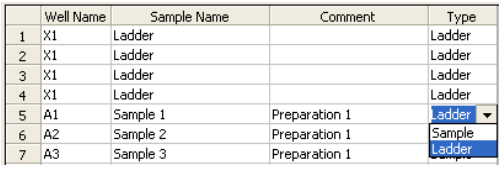
4 After specifying the wells, click the button.

The [Well name] and [Type] are automatically entered in the column on the right side. The [Type] is always “Sample”.



5 Enter the sample name and comment, and change the type.

- Clicking the [Type] cell displays a drop-down list. The type can be changed to [Ladder].

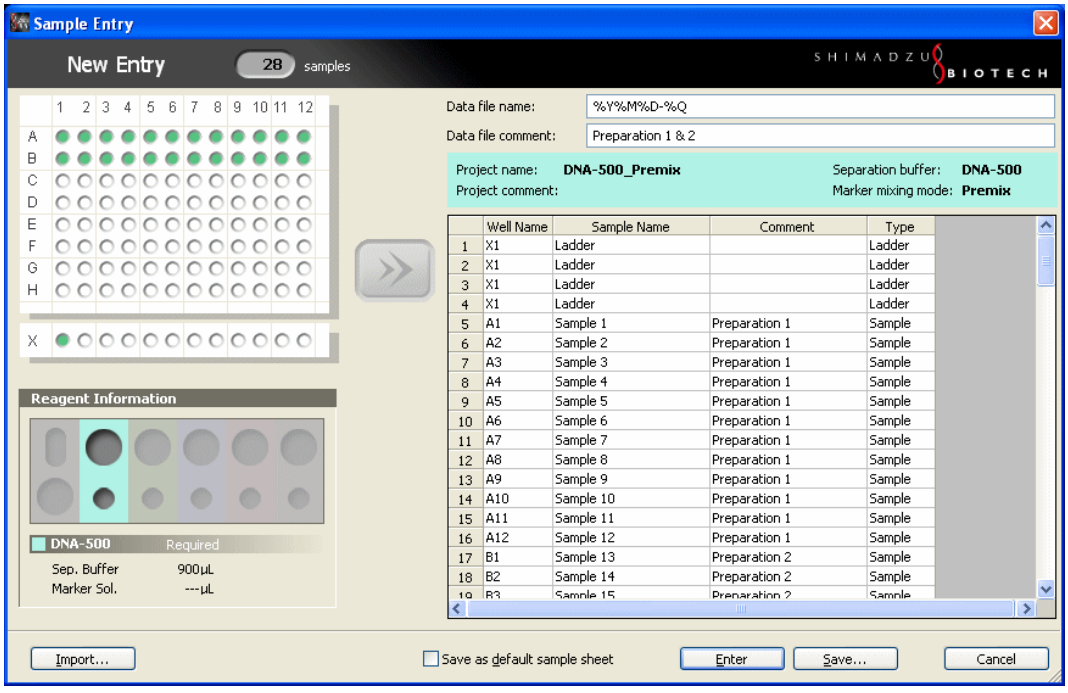


- Use either of the methods below to create multiple lines to perform multiple analyses from the X1 to X12 positions.
  - In the well display, re-select the wells in X1 to X12, and click the button.
  - Right-click the X1 to X12 lines that you want to copy, go to the pull-down menu to select [Copy], right-click the line in the vacant column, and select [Paste].
  - Enter X1 to X12 lines directly into the sample sheet.
- The sample name can be a maximum of 30 characters, and a comment can be up to 50 characters.

Reference

"2.2.1 New" P.89

6 **Repeat steps 4 to 5 as often as necessary to enter information for all of the ladders and samples to be analyzed.**

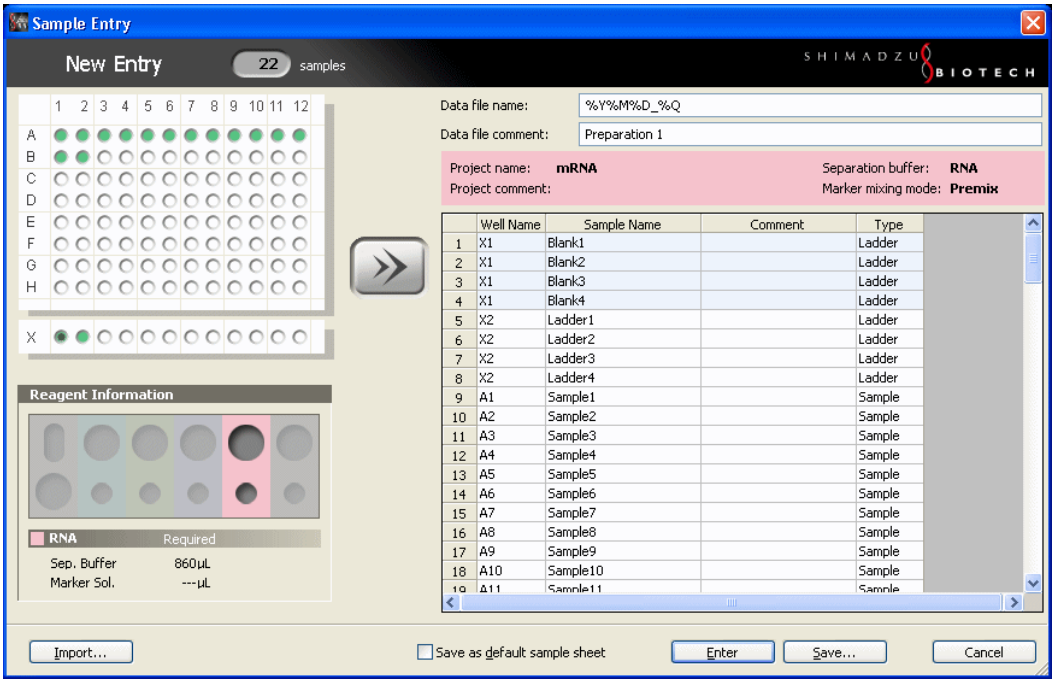


	Well Name	Sample Name	Comment	Type
1	X1	Ladder		Ladder
2	X1	Ladder		Ladder
3	X1	Ladder		Ladder
4	X1	Ladder		Ladder
5	A1	Sample 1	Preparation 1	Sample
6	A2	Sample 2	Preparation 1	Sample
7	A3	Sample 3	Preparation 1	Sample
8	A4	Sample 4	Preparation 1	Sample
9	A5	Sample 5	Preparation 1	Sample
10	A6	Sample 6	Preparation 1	Sample
11	A7	Sample 7	Preparation 1	Sample
12	A8	Sample 8	Preparation 1	Sample
13	A9	Sample 9	Preparation 1	Sample
14	A10	Sample 10	Preparation 1	Sample
15	A11	Sample 11	Preparation 1	Sample
16	A12	Sample 12	Preparation 1	Sample
17	B1	Sample 13	Preparation 2	Sample
18	B2	Sample 14	Preparation 2	Sample

NOTE

Enter a blank analysis for each microchip before starting the RNA ladder analysis.

Select [Ladder] as the [Type] for the blank analysis.



	Well Name	Sample Name	Comment	Type
1	X1	Blank1		Ladder
2	X1	Blank2		Ladder
3	X1	Blank3		Ladder
4	X1	Blank4		Ladder
5	X2	Ladder1		Ladder
6	X2	Ladder2		Ladder
7	X2	Ladder3		Ladder
8	X2	Ladder4		Ladder
9	A1	Sample1		Sample
10	A2	Sample2		Sample
11	A3	Sample3		Sample
12	A4	Sample4		Sample
13	A5	Sample5		Sample
14	A6	Sample6		Sample
15	A7	Sample7		Sample
16	A8	Sample8		Sample
17	A9	Sample9		Sample
18	A10	Sample10		Sample

7 To save the created sample sheet, click the [Save] button.

At this time, selecting [Save it as a default sample sheet] and then clicking the [Save] button will save it as a [Default sample sheet].

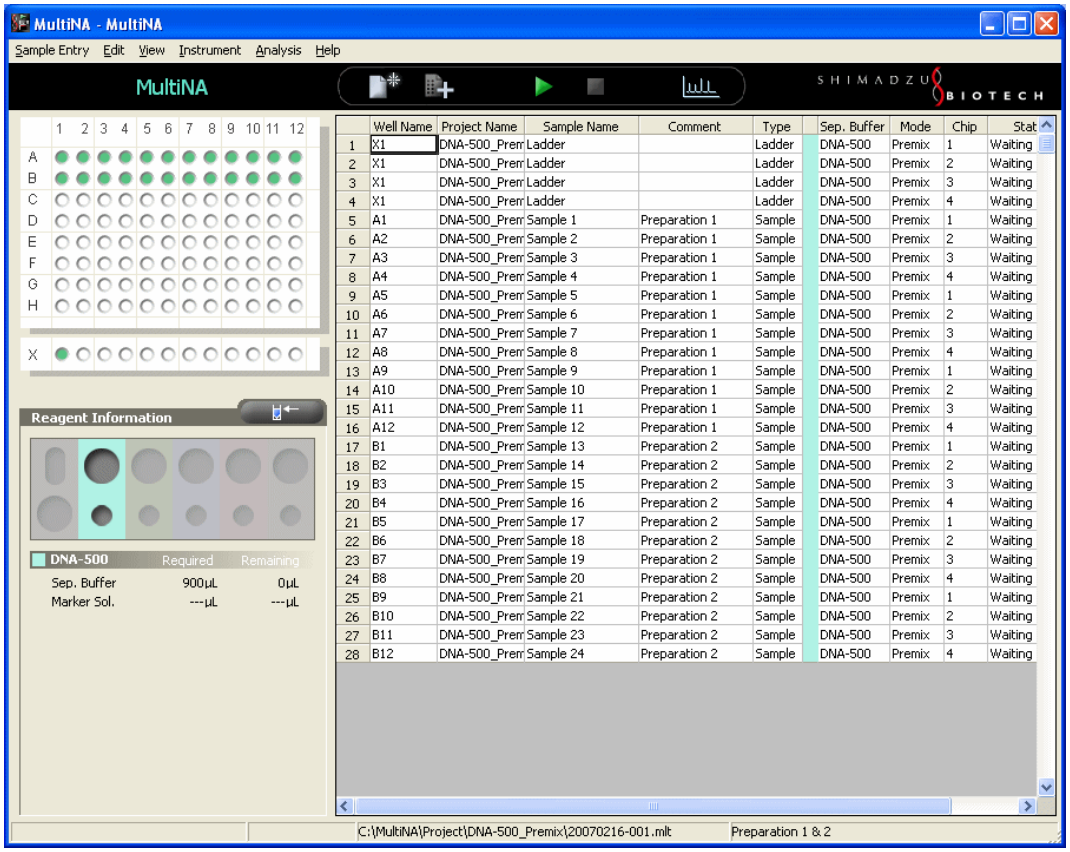
[Default sample sheet]
If [Loading default sample sheet while starting up] was selected in the project settings, the default sample sheet will automatically be displayed when a new [Sample Entry] window is opened.

Reference

"2.2.1 New" P.89

8 Click the [Enter] button.

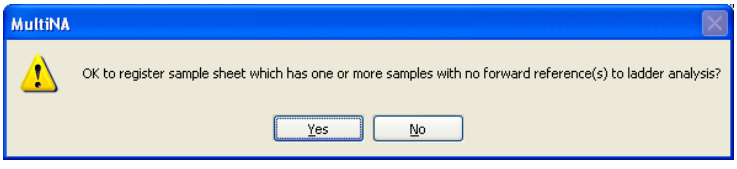
The created sample sheet is entered in the main analysis schedule window.



	Well Name	Project Name	Sample Name	Comment	Type	Sep. Buffer	Mode	Chip	Stat
1	X1	DNA-500_Prem	Ladder		Ladder	DNA-500	Premix	1	Waiting
2	X1	DNA-500_Prem	Ladder		Ladder	DNA-500	Premix	2	Waiting
3	X1	DNA-500_Prem	Ladder		Ladder	DNA-500	Premix	3	Waiting
4	X1	DNA-500_Prem	Ladder		Ladder	DNA-500	Premix	4	Waiting
5	A1	DNA-500_Prem	Sample 1	Preparation 1	Sample	DNA-500	Premix	1	Waiting
6	A2	DNA-500_Prem	Sample 2	Preparation 1	Sample	DNA-500	Premix	2	Waiting
7	A3	DNA-500_Prem	Sample 3	Preparation 1	Sample	DNA-500	Premix	3	Waiting
8	A4	DNA-500_Prem	Sample 4	Preparation 1	Sample	DNA-500	Premix	4	Waiting
9	A5	DNA-500_Prem	Sample 5	Preparation 1	Sample	DNA-500	Premix	1	Waiting
10	A6	DNA-500_Prem	Sample 6	Preparation 1	Sample	DNA-500	Premix	2	Waiting
11	A7	DNA-500_Prem	Sample 7	Preparation 1	Sample	DNA-500	Premix	3	Waiting
12	A8	DNA-500_Prem	Sample 8	Preparation 1	Sample	DNA-500	Premix	4	Waiting
13	A9	DNA-500_Prem	Sample 9	Preparation 1	Sample	DNA-500	Premix	1	Waiting
14	A10	DNA-500_Prem	Sample 10	Preparation 1	Sample	DNA-500	Premix	2	Waiting
15	A11	DNA-500_Prem	Sample 11	Preparation 1	Sample	DNA-500	Premix	3	Waiting
16	A12	DNA-500_Prem	Sample 12	Preparation 1	Sample	DNA-500	Premix	4	Waiting
17	B1	DNA-500_Prem	Sample 13	Preparation 2	Sample	DNA-500	Premix	1	Waiting
18	B2	DNA-500_Prem	Sample 14	Preparation 2	Sample	DNA-500	Premix	2	Waiting
19	B3	DNA-500_Prem	Sample 15	Preparation 2	Sample	DNA-500	Premix	3	Waiting
20	B4	DNA-500_Prem	Sample 16	Preparation 2	Sample	DNA-500	Premix	4	Waiting
21	B5	DNA-500_Prem	Sample 17	Preparation 2	Sample	DNA-500	Premix	1	Waiting
22	B6	DNA-500_Prem	Sample 18	Preparation 2	Sample	DNA-500	Premix	2	Waiting
23	B7	DNA-500_Prem	Sample 19	Preparation 2	Sample	DNA-500	Premix	3	Waiting
24	B8	DNA-500_Prem	Sample 20	Preparation 2	Sample	DNA-500	Premix	4	Waiting
25	B9	DNA-500_Prem	Sample 21	Preparation 2	Sample	DNA-500	Premix	1	Waiting
26	B10	DNA-500_Prem	Sample 22	Preparation 2	Sample	DNA-500	Premix	2	Waiting
27	B11	DNA-500_Prem	Sample 23	Preparation 2	Sample	DNA-500	Premix	3	Waiting
28	B12	DNA-500_Prem	Sample 24	Preparation 2	Sample	DNA-500	Premix	4	Waiting

NOTE

If ladder analysis is not included in the sample sheet, the following message is displayed.



Select [Yes] to enter the sample sheet without a ladder analysis into the analysis schedule. In this case, you cannot automatically obtain size prediction and quantitation results in the analysis data. However, you can import a ladder file created for other analysis data to perform a reanalysis and obtain the size prediction and quantitation.

Reference

"3.7.3 Change Ladder and Analyze" P.189

Selecting [No] stops registration in the analysis schedule, and the [Sample Entry] window remains open.

NOTE

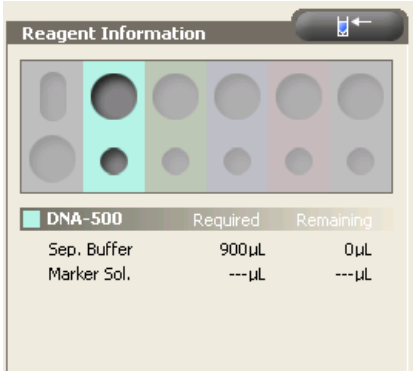
If a sample sheet is created during analysis, it can be saved but not entered into the analysis schedule. Click [Save] the [Enter] button is not displayed in the [Sample Entry] window.

9 **The separation buffer and marker solution volumes required for analysis are displayed in [Reagent Information].**

The example below is for DNA-500 premix mode. In premix mode, the amount of marker solution is not displayed.

Reference

"2.5.4 Detect Remaining Reagent Amount" P.118



NOTE

Separation buffer and marker solution placement is described in sections 1.6 to 1.9, for each reagent kit.

10 **Select [Add] on the [Sample Entry] pull-down menu to enter a different sample sheet into the analysis schedule.**

For details, see "2.2.2 Add" P.101.

1.6 Preparation for Analysis with the DNA-500 Kit

1.6.1 Requirements

Part Name	Sales origin	Part No. (Catalog No.)	Remarks
Microchip Type DR-C	Shimadzu Corporation	292-27900-91	-
Reagent kit DNA-500	Shimadzu Corporation	292-27910-91	-
SYBR® Gold	Invitrogen	S-11494	Diluted to 1/100
25 bp DNA ladder	Invitrogen	10597-011	Diluted to 1/50
TE buffer 10 MM Tris-HCl	-	-	pH 8.0
Micro tube	-	-	Volume from 200 µL to 1.5 mL For diluted dye solution and ladder solution
Sample tube or sample plate (recommended product)			Sample and ladder solution vessel (Note *1)
MicroAmp® reaction tubes	Applied Biosystems	N801-0533	1 tube
MicroAmp® strip tubes (without cap, 8 tubes/strip)		N801-0580	8-strip tubes
MicroAmp® Optical 96-well Reaction Plate		N801-0560	96-well PCR plate
Strips of 8 Thermo-Tubes	ABgene	AB-0452	8-strip tubes
Strips of 12 Thermo-Tubes		AB-1112	12-strip tubes
8 Low profile Thermo-strip		AB-0771	8-strip/12-strip tubes <Note> Since there are tabs on both ends of the tube, they cannot be used on the extra sample stand.
12 Low profile Thermo-strip		AB-0847	
Termo-Fast® 96 PCR Plate Non-Skirted		AB-0600	96-well PCR plate
Adhesive PCR Foil Seals (Aluminum seal)	ABgene	AB-0626	To avoid volatilization of the ladder and sample solutions, use this for analyzing a minimum of 13 samples with the 96-well PCR plate.
Buffer bottle Recommended product: Centrifugal tube, 5 mL, 1000/packing	ASSIST (Sarstedt)	60.558	Extra separation buffer container (Note *2)
Vial Recommended product: Vial made of PP, 0.6 mL and 1000/packing (without cap)	BIO MEDICAL EQUIPMENT	NC-502	Extra marker solution container (Note *2)
Micro pipet and tip	-	-	Designed for volume of 0.5 µL to 5 mL
Protective glasses, protective mask, and protective gloves	-	-	-
Vortex mixer	-	-	-

NOTE

*1) In this manual, the 0.2-mL PCR tube listed in the above table is called the sample tube, and the 96-well PCR plate is called the sample plate.

*2) One buffer bottle and two vials (marker solution bottles) are provided for each reagent kit. If more are required, order the recommended items separately.

⚠ CAUTION

- Use the sample plate (96 well plate) to analyze 13 or more samples, and attach an aluminum seal.
- Use only the aluminum seal recommended in the list above.
  If any other aluminum seal is used, the piercing needle may fail to penetrate, resulting in damage to the sample probe or other instrument problems.
  Do NOT use parafilm. It may adhere to the sample probe or cause other instrument problems.
- The aluminum seal can only be used with the sample plate recommended in the list above.
  Do NOT use the aluminum seal to cover sample tubes, it prevents the sample holder from being properly set in place.

⚠ CAUTION

- Use only the sample tube and sample plate recommended in the list above.
  Use of any other sample tube or sample plate could lead to dispensing failures of at least 5 µL.
- Do NOT cap a sample tube.
  Using a capped sample tube could result damage to the sample probe, or other instrument problems.

⚠ CAUTION

- The sample tube types shown below can be only used in the normal sample stand.
  There are tabs on both ends of the tube that prevent it from being properly positioned in the extra sample stand.
  - ABgene PCR 8-strip/12-strip tubes (low profile): Part No. AB-0771, AB-0847

⚠ CAUTION

- Dispense to sample tube not to exceed 30 µL. Otherwise, that may be interfered with analysis sequence.

1.6.2 Contents of the DNA-500 Kit

The DNA-500 kit (Part No. 292-27910-91) is composed of the following parts. When the kit is delivered, immediately open it, check the part names and quantity, and then store them at the temperatures specified for each part.

No.	Part Name	Capacity	Quantity	Remarks
❶	DNA-500 separation buffer	30 mL	1	Refrigerated storage at 2°C to 8°C (PP bottle) (Up to 1000 analyses)
❷	DNA-500 marker solution	1.2 mL	4	Frozen storage at -20°C (Aluminum pack) (Up to 1000 analyses)
❸	Buffer bottle	(5 mL)	1	Separation buffer dispensing container (with cap)
❹	Vial	(0.6 mL)	2	Marker solution dispensing tube (without cap)
❺	Manual	-	1	-

Fig.1-17 Contents of the DNA-500 Kit

- Expiration dates are noted on the labels. Use the reagents in refrigerated or frozen storage before the expiration dates.
- Observe the laws, regulations, and rules of the country, local governing authority, or resident facility when discarding reagents.

NOTE

To avoid repeated freezing and thawing of marker solutions, it is recommended that they be divided into small allotments for frozen storage.

1.6.3 Diluted Dye Solution Preparation

Prepare SYBR® Gold solution diluted to 1/100.

⚠ WARNING

- During operations, wear protective gloves, protective glasses, and a protective mask.
- See the MSDS for each reagent.

NOTE

- Restore each reagent to room temperature before use.
- Do NOT mix reagents from different lot numbers (the lot number is noted on labels of the buffer bottles or mark solution aluminum packs).
- For dye handling precautions and storage conditions, see the relevant Invitrogen Co. Product Information and MSDS.
- To avoid repeated freezing and thawing of SYBR® Gold for preparation of the diluted solution, it is recommended that the solution be divided into small allotments (about 10 µL) and placed into microtubes. It should be frozen and shielded from light.

■ Requirements

- SYBR® Gold
- TE buffer
- Microtube (volume from 200 µL to 1.5 mL)
- Micro pipette
- Vortex mixer

■ Procedures

1. **Use a micro pipette to dispense 99 µL of TE buffer to a microtube.**
2. **Add 1 µL of SYBR® Gold dye, and agitate the solution with the vortex mixer for at least of 10 seconds.minimum of 10 seconds.**

1.6.4 Ladder Solution Preparation

Dilute a 25 bp DNA ladder (Invitrogen Co., catalog No. 10597-011) to 1/50 to prepare the ladder solution.

V1.05

If using an optional ladder, prepare the ladder solution by referring to *"4.10 Using Optional Ladders" P.205*.

■ Requirements

- 25 bp DNA ladder
- TE buffer
- Microtube (volume from 200 µL to 1.5 mL)
- Micro pipette
- Vortex mixer

■ Procedures

1. **Use a micro pipette to dispense 49 µL of TE buffer to a microtube.**
2. **Add 1 µL of the 25 bp DNA ladder, and agitate the solution with the vortex mixer for at least 10 seconds.**

1.6.5 Separation Buffer Preparation

NOTE

The separation buffer mixed with diluted dye solution should be used on the day it is prepared.

■ Requirements

- DNA-500 separation buffer
- Diluted dye solution (*"1.6.3 Diluted Dye Solution Preparation" P.34*)
- Buffer bottle
- Micro pipette

■ Procedures

1 **Dispense the separation buffer included in the DNA-500 kit into the buffer bottle, and use the micro pipette to add the diluted dye solution.**

- Ensure that the volume of the mixed solution exceeds the required volume displayed in the [Reagent Information] window.
- Add the diluted dye solution until it reaches a volume ratio of 1/100 to the separation buffer transferred to the buffer bottle. For example, if the volume requirement is 1000 µL, add 10 µL of diluted dye solution to 990 µL of separation buffer.

Refer to the table below and use a micro pipette to dispense the separation buffer and diluted dye solution in the amounts required for the number of analyses (total ladders and samples) into the buffer bottle.

Number of analyses (Total ladders and samples)	Amount of separation buffer	Amount of diluted dye solution	Amount of mixed solution
8 analyses or less	495 µL	5 µL	500 µL
9 to 29 analyses	990 µL	10 µL	1000 µL
30 to 79 analyses	1980 µL	20 µL	2000 µL
80 to 120 analyses	2970 µL	30 µL	3000 µL

Guidelines for Required Amounts of Separation Buffer and Diluted Dye Solution

NOTE

The required amount of separation buffer is about 20 µL per analysis. The amounts described below are included in the required volumes indicated in [Reagent Information] and in the above guidelines.

- Amount needed for retries (up to 2 retries) if a problem is detected when automatically adding separation buffer to the microchip
- Dead volume of buffer bottle
- Minimum bottle amount detectable by a level sensor

NOTE

The reagent kit contains 30 mL of separation buffer (for 1000 analyses). The total number of analyses will be less than 1000 in situations where the number of analyses for each sequence is small, because the redundant amounts will be larger.

2 **Cap the bottle, and agitate the solution with the vortex mixer for at least 10 seconds.**

3 **Remove the cap and put the bottle in the blue reagent holder position (color code for DNA-500 kit).**

NOTE

Let the solution adhering to the inner sides of the buffer bottle settle, and use a micro pipette to remove any residual bubbles before placing the tube in the reagent holder.

NOTE

Move the bottle around to ensure that the bottom of the buffer bottle is in the correct position at the bottom of the reagent holder.

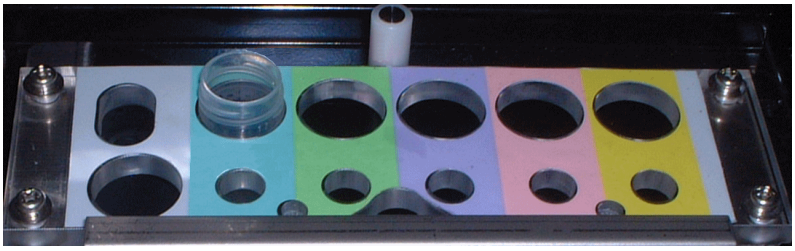
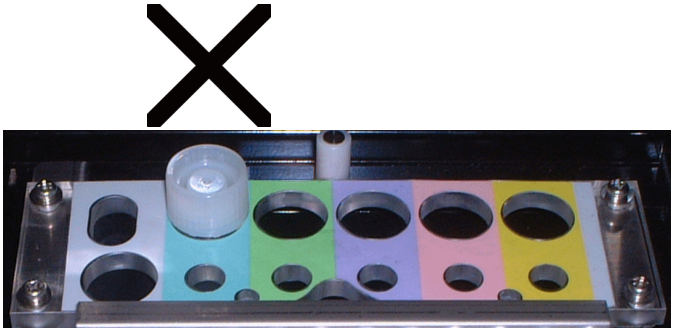


Fig.1-18 DNA-500 Separation Buffer in Reagent Holder

⚠ CAUTION

- Remove the cap from the buffer bottle before placing it in the reagent holder.
  The sample probe may be damaged.



1.6.6 Ladder, Sample and Marker Solution Usage

Insert the tubes into the positions specified in the analysis schedule.

NOTE

Follow these precautions to prevent volatilization of solution, and to confirm the minimum amount of dispensable solution.

- When total number of ladders or samples to be analyzed is 12 or less

  No aluminum seal is needed to prevent volatilization, and the recommended 8-strip or 12-strip sample tubes can be used. Dispense a minimum of 9 µL into each tube.

- When the number of ladders or samples is 13 or more

  Use the 96-well sample plate, and the recommended aluminum seal. Dispense a minimum of 6 µL (premix) or 5 µL (on-chip mix) into each well.

- When the same ladder or sample is analyzed multiple times

  Use the recommended 8-strip or 12-strip sample tubes without an aluminum seal, and place it in the extra sample stand (X1 to X12). Since the amount of solution dispensed to each tube differs between premix and on-chip mix, see the respective descriptions for those items.

NOTE

When the prepared sample is expected to exceed the quantitative range (0.5 ng/µL to 50 ng/µL) or the maximum salt concentration (KCl or NaCl concentration; maximum of 125 mM) for DNA samples use the TE buffer (pH 8.0) to dilute it prior to analysis.

■ For Premix

Mix the marker solution at a volume ratio of 2:1 into the ladder or sample, and place it in the instrument.

Nothing is placed in the marker solution position.

Sample stand (One analysis per well)				
Number of analyses (Total ladders and samples)	**Container**	**Amount of ladder or samples**	**Amount of marker solution**	**Amount of mixed solution**
12 analyses or less	Sample tube	3 µL	6 µL	9 µL
13 analyses or more	Sample plate with seal	2 µL	4 µL	6 µL

Extra sample stand (Multiple analyses per well)				
Number of analyses	**Container**	**Amount of ladder or sample**	**Amount of marker solution**	**Amount of mixed solution**
1 to 4 times	Sample tube	5 µL	10 µL	15 µL
5 to 9 times	Sample tube	1 x (Number of analyses + 1) µL	2 x (Number of analyses + 1) µL	3 x (Number of analyses + 1) µL

1 **Place the ladder solution and marker solution mix in the extra sample stand.**

Transfer the ladder solution prepared as per *"1.6.4 Ladder Solution Preparation" P.35* into the sample tube (or sample plate). Then, add the marker solution at a volume ratio of 2:1, and mix the solution thoroughly through pipetting.

- For example, to analyze a ladder solution four times from the extra sample stand requires a minimum of 15 µL (3 x (4 + 1) = 15 µL). Add 10 µL of marker solution to 5 µL of ladder solution.

2 **Place the sample and marker solution mix in the appropriate sample compartment.**

Transfer the sample to the sample tube (or sample plate). Then, add the marker solution at a volume ratio of 2:1, and mix the solution thoroughly through pipetting.

- For example, for analysis of up to 12 samples, mix 6 µL of marker solution with 3 µL of sample in a sample tube, and place the tube in the extra sample stand.
- To analyze 13 samples or more, mix 4 µL of marker solution with 2 µL of sample in a sample plate well, cover it with an aluminum seal, and place the sample plate on the sample stand.

■ For On-Chip Mix

In on-chip mixes, the ladder or sample, and the marker solution, are placed into the instrument separately. The ladder or sample is automatically mixed with the marker solution on the microchip.

Sample stand (One analysis per well)		
Number of analyses (Total ladders and samples)	**Container**	**Amount of ladder or sample**
12 analyses or less	Sample tube	9 µL
13 analyses or more	Sample plate with seal	5 µL

Extra sample stand (Multiple analyses per well)		
Number of analyses	**Container**	**Amount of ladder or sample**
1 to 3 times	Sample tube	9 µL
3 to 13 times	Sample tube	5 + 2 x (Number of analyses - 1) µL

1 Place the ladder solution in the extra sample stand.

Transfer the ladder solution prepared as per *"1.6.4 Ladder Solution Preparation" P.35* into the sample tube (or sample plate).

- For example, to analyze a ladder solution four times from the extra sample stand requires a minimum of 11 µL (5 + 2 x 3 = 11) of solution. Add 11 µL of ladder solution to the sample tube and place it in the extra sample stand.

2 Place the sample in the appropriate sample compartment.

Transfer the sample to the sample tube (or sample plate).

- For analysis of up to 12 samples, dispense 9 µL or more of sample into the sample tube, and place the tube in the extra sample stand.
- For analysis of 13 or more samples, dispense 5 µL or more of sample into the sample plate well, cover with an aluminum seal, and place the sample plate on the sample stand.

3 Dispense the marker solution.

Use a micro pipette to dispense marker solution in excess of the required amount shown in [Reagent Information] into a vial (PP 0.6-mL vial, no cap).

Or use the formula below to calculate the amount of marker solution required for the number of analyses (samples and ladders), and use a micro pipette to dispense it to a vial (PP 0.6-mL vial, no cap).

(Amount of marker solution required) = 2 x (Number of analyses) + 40 (µL)

NOTE

Since the minimum detectable amount using a level sensor is 40 µL, use a larger amount.

4 Put the bottle in the blue reagent holder position (color code for DNA-500 kit).

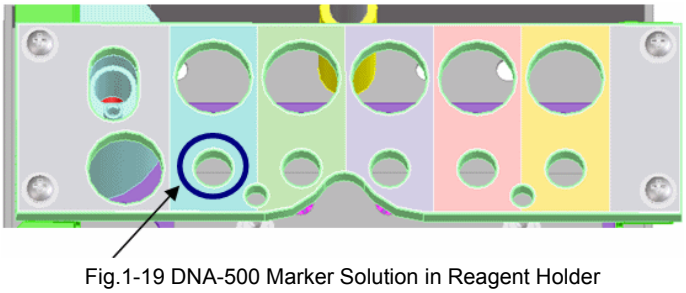


Fig.1-19 DNA-500 Marker Solution in Reagent Holder

1.7 Preparation for Analysis with the DNA-1000 Kit

1.7.1 Requirements

Part Name	Sales origin	Part No. (Catalog No.)	Remarks
Microchip Type DR-C	Shimadzu Corporation	292-27900-91	-
Reagent kit DNA-1000	Shimadzu Corporation	292-27911-91	-
SYBR® Gold	Invitrogen	S-11494	Diluted to 1/100
ϕX174 DNA/HaeIII Markers	Promega	G1761	Diluted to 1/100
TE buffer 10 MM Tris-HCl	-	-	pH 8.0
Micro tube	-	-	Volume from 200 µL to 1.5 mL For diluted dye solution and ladder solution
Sample tube or sample plate (recommended product)	-	-	Sample and ladder solution vessel (Note *1)
MicroAmp® reaction tubes	Applied Biosystems	N801-0533	1 tube
MicroAmp® strip tubes (without cap, 8 tubes/strip)		N801-0580	8-strip tubes
MicroAmp® Optical 96-well Reaction Plate		N801-0560	96-well PCR plate
Strips of 8 Thermo-Tubes	ABgene	AB-0452	8-strip tubes
Strips of 12 Thermo-Tubes		AB-1112	12-strip tubes
8 Low profile Thermo-strip		AB-0771	8-strip/12-strip tubes <Note> Since there are tabs on both ends of the tube, they cannot be used on the extra sample stand.
12 Low profile Thermo-strip		AB-0847	
Termo-Fast® 96 PCR Plate Non-Skirted		AB-0600	96-well PCR plate
Adhesive PCR Foil Seals (Aluminum seal)	ABgene	AB-0626	To avoid volatilization of the ladder and sample solutions, use this for analyzing a minimum of 13 samples with the 96-well PCR plate.
Buffer bottle Recommended product: Centrifugal tube, 5 mL, 1000/packing	ASSIST (Sarstedt)	60.558	Extra separation buffer container (Note *2)
Vial Recommended product: Vial made of PP, 0.6 mL and 1000/packing (without cap)	BIO MEDICAL EQUIPMENT	NC-502	Extra marker solution container (Note *2)
Macrobiotic and tip	-	-	Designed for volume of 0.5 µL to 5 mL
Protective glasses, protective mask, and protective gloves	-	-	-

NOTE

*1) In this manual, the 0.2-mL PCR tube listed in the above table is called the sample tube, and the 96-well PCR plate is called the sample plate.

*2) One buffer bottle and two vials (marker solution bottles) are provided for each reagent kit. If more are required, order the recommended items separately.

⚠ CAUTION

- Use the sample plate (96 well plate) to analyze 13 or more samples, and attach an aluminum seal.
- Use only the aluminum seal recommended in the list above.
  If any other aluminum seal is used, the piercing needle may fail to penetrate, resulting in damage to the sample probe or other instrument problems.
  Do NOT use parafilm. It may adhere to the sample probe or cause other instrument problems.
- The aluminum seal can only be used with the sample plate recommended in the list above.
  Do NOT use the aluminum seal to cover sample tubes, it prevents the sample holder from being properly set in place.

⚠ CAUTION

- Use only the sample tube and sample plate recommended in the list above.
  Use of any other sample tube or sample plate could lead to dispensing failures of at least 5 µL.
- Do NOT cap a sample tube.
  Using a capped sample tube could result damage to the sample probe, or other instrument problems.

⚠ CAUTION

- The sample tube types shown below can be only used in the normal sample stand.
  There are tabs on both ends of the tube that prevent it from being properly positioned in the extra sample stand.
  - ABgene PCR 8-strip/12-strip tubes (low profile): Part No. AB-0771, AB-0847

⚠ CAUTION

- Dispense to sample tube not to exceed 30 µL. Otherwise, that may be interfered with analysis sequence.

1.7.2 Contents of the DNA-1000 Kit

The DNA-1000 kit (Part No. 292-27911-91) is composed of the following parts. When the kit is delivered, immediately open it, check the part names and quantity, and then store them at the temperatures specified for each part.

No.	Part Name	Capacity	Quantity	Remarks
❶	DNA-1000 separation buffer	30 mL	1	Refrigerated storage at 2°C to 8°C (PP bottle) (Up to 1000 analyses)
❷	DNA-1000 marker solution	1.2 mL	4	Frozen storage at -20°C (Aluminum pack) (Up to 1000 analyses)
❸	Buffer bottle	(5 mL)	1	Separation buffer dispensing container (with cap)
❹	Vial	(0.6 mL)	2	Marker solution dispensing tube (without cap)
❺	Manual	-	1	-

Fig.1-20 Contents of the DNA-1000 Kit

- Expiration dates are noted on the labels. Use the reagents in refrigerated or frozen storage before the expiration dates.
- Observe the laws, regulations, and rules of the country, local governing authority, or resident facility when discarding reagents.

NOTE

To avoid repeated freezing and thawing of marker solutions, it is recommended that they be divided into small allotments for frozen storage.

1.7.3 Diluted Dye Solution Preparation

Prepare SYBR® Gold solution diluted to 1/100.

⚠ WARNING

- During operations, wear protective gloves, protective glasses, and a protective mask.
- See the MSDS for each reagent.

NOTE

- Restore each reagent to room temperature before use.
- Do NOT mix reagents from different lot numbers (the lot number is noted on labels of the buffer bottles or mark solution aluminum packs).
- For dye handling precautions and storage conditions, see the relevant Invitrogen Co. Product Information and MSDS.
- To avoid repeated freezing and thawing of SYBR® Gold for preparation of the diluted solution, it is recommended that the solution be divided into small allotments (about 10 µL) and placed into microtubes. It should be frozen and shielded from light.

■ Requirements

- SYBR® Gold
- TE buffer
- Microtube (volume from 200 µL to 1.5 mL)
- Micro pipette
- Vortex mixer

■ Procedures

1 **Use a micro pipette to dispense 99 µL of TE buffer to a microtube.**

2 **Add 1 µL of SYBR® Gold dye, and agitate the solution with the vortex mixer for at least 10 seconds.**

1.7.4 Ladder Solution Preparation

Dilute ϕX174 DNA/HaeIII Markers (Promega Co., catalog No. G1761) to 1/100 to prepare the ladder solution.

V1.05

If using an optional ladder, prepare the ladder solution by referring to *"4.10 Using Optional Ladders" P.205*.

■ Requirements

- ϕX174 DNA/HaeIII marker (Promega Co. catalog number G1761)
- TE buffer
- Microtube (volume from 200 µL to 1.5 mL)
- Micro pipette
- Vortex mixer

■ Procedure

1 **Use a micro pipette to dispense 99 µL of TE buffer to a microtube.**

2 **Add 1 µL of ϕX174 DNA/HaeIII Markers, and agitate the solution with the vortex mixer for at least 10 seconds.**

1.7.5 Separation Buffer Preparation

NOTE

The separation buffer mixed with diluted dye solution should be used on the day it is prepared.

■ Requirements

- DNA-1000 separation buffer
- Diluted dye solution (*"1.7.3 Diluted Dye Solution Preparation" P.43*)
- Buffer bottle
- Micro pipette

■ Procedures

1 **Dispense the separation buffer included in the DNA-1000 kit into the buffer bottle, and use the micro pipette to add the diluted dye solution.**

- Ensure that the volume of the mixed solution exceeds the required volume displayed in the [Reagent Information] window.
- Add the diluted dye solution until it reaches a volume ratio of 1/100 to the separation buffer transferred to the buffer bottle. For example, if the volume requirement is 1000 µL, add 10 µL of diluted dye solution to 990 µL of separation buffer.

Refer to the table below and use a micro pipette to dispense the separation buffer and diluted dye solution in the amounts required for the number of analyses (total ladders and samples) into the buffer bottle.

Number of analyses (Total ladders and samples)	Amount of separation buffer	Amount of diluted dye solution	Amount of mixed solution
8 analyses or less	495 µL	5 µL	500 µL
9 to 29 analyses	990 µL	10 µL	1000 µL
30 to 79 analyses	1980 µL	20 µL	2000 µL
80 to 120 analyses	2970 µL	30 µL	3000 µL

Guidelines for Required Amounts of Separation Buffer and Diluted Dye Solution

NOTE

The required amount of separation buffer is about 20 µL per analysis. The amounts described below are included in the required volumes indicated in [Reagent Information] and in the above guidelines.

- Amount needed for retries (up to 2 retries) if a problem is detected when automatically adding separation buffer to the microchip
- Dead volume of buffer bottle
- Minimum bottle amount detectable by a level sensor

NOTE

The reagent kit contains 30 mL of separation buffer (for 1000 analyses). The total number of analyses will be less than 1000 in situations where the number of analyses for each sequence is small, because the redundant amounts will be larger.

2 **Cap the bottle, and agitate the solution with the vortex mixer for at least of 10 seconds.**

3 **Remove the cap and put the bottle in the green reagent holder position (color code for DNA-1000 kit).**

NOTE

Let the solution adhering to the inner sides of the buffer bottle settle, and use a micro pipette to remove any residual bubbles before placing the tube in the reagent holder.

NOTE

Move the bottle around to ensure that the bottom of the buffer bottle is in the correct position at the bottom of the reagent holder.

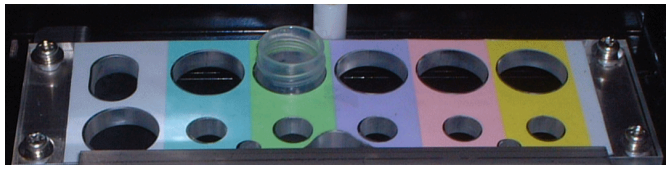
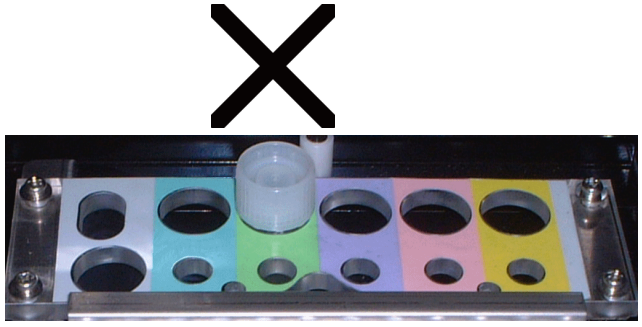


Fig.1-21 DNA-1000 Separation Buffer in Reagent Holder

⚠ CAUTION

- Remove the cap from the buffer bottle before placing it in the reagent holder.
  The sample probe may be damaged.



1.7.6 Ladder, Sample, and Marker Solution Usage

Insert the tubes into the positions specified in the analysis schedule.

NOTE

Follow these precautions to prevent volatilization of solution, and to confirm the minimum amount of dispensable solution.

- When total number of ladders or samples to be analyzed is 12 or less
  No aluminum seal is needed to prevent volatilization, and the recommended 8-strip or 12-strip sample tubes can be used. Dispense a minimum of 9 µL into each tube.
- When the number of ladders or samples is 13 or more
  Use the 96-well sample plate, and the recommended aluminum seal. Dispense a minimum of 6 µL (premix) or 5 µL (on-chip mix) into each well.
- When the same ladder or sample is analyzed multiple times
  Use the recommended 8-strip or 12-strip sample tubes without an aluminum seal, and place it in the extra sample stand (X1 to X12). Since the amount of solution dispensed to each tube differs between premix and on-chip mix, see the respective descriptions for those items.

NOTE

When the prepared sample is expected to exceed the quantitative range (0.5 ng/µL to 50 ng/µL) or the maximum salt concentration (KCl or NaCl concentration; maximum of 125 mM) for DNA samples use the TE buffer (pH 8.0) to dilute it prior to analysis.

■ For Premix

Mix the marker solution at a volume ratio of 2:1 into the ladder or sample, and place it in the instrument. Nothing is placed in the marker solution position.

Sample stand (One analysis per well)				
Number of analyses (Total ladders and samples)	**Container**	**Amount of ladder or samples**	**Amount of marker solution**	**Amount of mixed solution**
12 analyses or less	Sample tube	3 µL	6 µL	9 µL
13 analyses or more	Sample plate with seal	2 µL	4 µL	6 µL

Extra sample stand (Multiple analyses per well)				
Number of analyses	**Container**	**Amount of ladder or sample**	**Amount of marker solution**	**Amount of mixed solution**
1 to 4 times	Sample tube	5 µL	10 µL	15 µL
5 to 9 times	Sample tube	1 x (Number of analyses + 1) µL	2 x (Number of analyses + 1) µL	3 x (Number of analyses + 1) µL

1 **Place the ladder solution and marker solution mix in the extra sample stand.**

Transfer the ladder solution prepared as per *"1.7.4 Ladder Solution Preparation" P.43* into the sample tube (or sample plate). Then, add the marker solution at a volume ratio of 2:1, and mix the solution thoroughly through pipetting.

- For example, to analyze a ladder solution four times from the extra sample stand requires a minimum of 15 µL (3 x (4 + 1) = 15 µL). Add 10 µL of marker solution to 5 µL of ladder solution.

2 **Place the sample and marker solution mix in the appropriate sample compartment.**

Transfer the sample to the sample tube (or sample plate). Then, add the marker solution at a volume ratio of 2:1, and mix the solution thoroughly through pipetting.

- For example, for analysis of up to 12 samples, mix 6 µL of marker solution with 3 µL of sample in a sample tube, and place the tube in the extra sample stand.
- To analyze 13 samples or more, mix 4 µL of marker solution with 2 µL of sample in a sample plate well, cover it with an aluminum seal, and place the sample plate on the sample stand.

■ For On-Chip Mix

In on-chip mixes, the ladder or sample, and the marker solution, are placed into the instrument separately. The ladder or sample is automatically mixed with the marker solution on the microchip.

Sample stand (One analysis per well)		
Number of analyses (Total ladders and samples)	**Container**	**Amount of ladder or sample**
12 analyses or less	Sample tube	9 µL
13 analyses or more	Sample plate with seal	5 µL

Extra sample stand (Multiple analyses per well)		
Number of analyses	**Container**	**Amount of ladder or sample**
1 to 3 times	Sample tube	9 µL
3 to 13 times	Sample tube	5 + 2 x (Number of analyses - 1) µL

1 **Place the ladder solution in the extra sample stand.**

Transfer the ladder solution prepared as per *"1.7.4 Ladder Solution Preparation" P.43* into the sample tube (or sample plate).

- For example, to analyze a ladder solution four times from the extra sample stand requires a minimum of 11 µL (5 + 2 x 3 = 11) of solution. Add 11 µL of ladder solution to the sample tube and place it in the extra sample stand.

2 **Place the sample in the appropriate sample compartment.**

Transfer the sample to the sample tube (or sample plate).

- For analysis of up to 12 samples, dispense 9 µL or more of sample into the sample tube, and place the tube in the extra sample stand.
- For analysis of 13 or more samples, dispense 5 µL or more of sample into the sample plate well, cover with an aluminum seal, and place the sample plate on the sample stand.

3 **Dispense the marker solution.**

Use a micro pipette to dispense marker solution in excess of the required amount shown in [Reagent Information] into a vial (PP 0.6-mL vial, no cap).

Or use the formula below to calculate the amount of marker solution required for the number of analyses (samples and ladders), and use a micro pipette to dispense it to a vial (PP 0.6-mL vial, no cap).

(Amount of marker solution required) = 2 x (Number of analyses) + 40 (µL)

NOTE

Since the minimum detectable amount using a level sensor is 40 µL, use a larger amount.

4 **Put the bottle in the green reagent holder position (color code for DNA-1000 kit).**

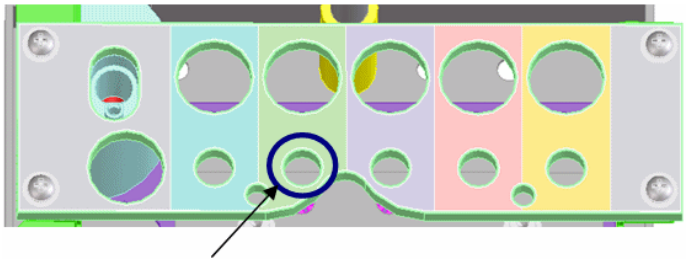


Fig.1-22 DNA-1000 Marker Solution in Reagent Holder

1.8 Preparation for Analysis with the DNA-2500 Kit

1.8.1 Requirements

Part Name	Sales origin	Part No. (Catalog No.)	Remarks
Microchip Type DR-C	Shimadzu Corporation	292-27900-91	-
Reagent kit DNA-2500	Shimadzu Corporation	292-27912-91	-
SYBR®Gold	Invitrogen	S-11494	Diluted to 1/100
pGEM® DNA Markers	Promega	G1741	Diluted to 1/100
TE buffer 10 MM Tris-HCl	-	-	pH 8.0
Micro tube	-	-	Volume from 200 µL to 1.5 mL For diluted dye solution and ladder solution
Sample tube or sample plate (recommended product)			Sample and ladder solution vessel (Note *1)
MicroAmp® reaction tubes	Applied Biosystems	N801-0533	1 tube
MicroAmp® strip tubes (without cap, 8 tubes/strip)		N801-0580	8-strip tubes
MicroAmp® Optical 96-well Reaction Plate		N801-0560	96-well PCR plate
Strips of 8 Thermo-Tubes	ABgene	AB-0452	8-strip tubes
Strips of 12 Thermo-Tubes		AB-1112	12-strip tubes
8 Low profile Thermo-strip		AB-0771	8-strip/12-strip tubes <Note> Since there are tabs on both ends of the tube, they cannot be used on the extra sample stand.
12 Low profile Thermo-strip		AB-0847	
Termo-Fast® 96 PCR Plate Non-Skirted		AB-0600	96-well PCR plate
Adhesive PCR Foil Seals (Aluminum seal)	ABgene	AB-0626	To avoid volatilization of the ladder and sample solutions, use this for analyzing a minimum of 13 samples with the 96-well PCR plate.
Buffer bottle Recommended product: Centrifugal tube, 5 mL, 1000/ packing	ASSIST (Sarstedt)	60.558	Extra separation buffer container (Note *2)
Vial Recommended product: Vial made of PP, 0.6 mL and 1000/packing (without cap)	BIO MEDICAL EQUIPMENT	NC-502	Extra marker solution container (Note *2)
Macrobiotic and tip	-	-	Designed for volume of 0.5 µL to 5 mL
Protective glasses, protective mask, and protective gloves	-	-	-
Vortex mixer	-	-	-

NOTE

*1) In this manual, the 0.2-mL PCR tube listed in the above table is called the sample tube, and the 96-well PCR plate is called the sample plate.

*2) One buffer bottle and two vials (marker solution bottles) are provided for each reagent kit. If more are required, order the recommended items separately.

⚠ CAUTION

- Use the sample plate (96 well plate) to analyze 13 or more samples, and attach an aluminum seal.
- Use only the aluminum seal recommended in the list above.
  If any other aluminum seal is used, the piercing needle may fail to penetrate, resulting in damage to the sample probe or other instrument problems.
  Do NOT use parafilm. It may adhere to the sample probe or cause other instrument problems.
- The aluminum seal can only be used with the sample plate recommended in the list above.
  Do NOT use the aluminum seal to cover sample tubes, it prevents the sample holder from being properly set in place.

⚠ CAUTION

- Use only the sample tube and sample plate recommended in the list above.
  Use of any other sample tube or sample plate could lead to dispensing failures of at least 5 µL.
- Do NOT cap a sample tube.
  Using a capped sample tube could result damage to the sample probe, or other instrument problems.

⚠ CAUTION

- The sample tube types shown below can be only used in the normal sample stand.
  There are tabs on both ends of the tube that prevent it from being properly positioned in the extra sample stand.
  - ABgene PCR 8-strip/12-strip tubes (low profile): Part No. AB-0771, AB-0847

⚠ CAUTION

- Dispense to sample tube not to exceed 30 µL. Otherwise, that may be interfered with analysis sequence.

1.8.2 Contents of the DNA-2500 Kit

The DNA-2500 kit (Part No. 292-27912-91) is composed of the following parts. When the kit is delivered, immediately open it, check the part names and quantity, and then store them at the temperatures specified for each part.

No.	Part Name	Capacity	Quantity	Remarks
❶	DNA-2500 separation buffer	30 mL	1	Refrigerated storage at 2°C to 8°C (PP bottle) (Up to 1000 analyses)
❷	DNA-2500 marker solution	1.2 mL	4	Frozen storage at -20°C (Aluminum pack) (Up to 1000 analyses)
❸	Buffer bottle	(5 mL)	1	Separation buffer dispensing container (with cap)
❹	Vial	(0.6 mL)	2	Marker solution dispensing tube (without cap)
❺	Manual	-	1	-

Fig.1-23 Contents of the DNA-2500 Kit

- Expiration dates are noted on the labels. Use the reagents in refrigerated or frozen storage before the expiration dates.
- Observe the laws, regulations, and rules of the country, local governing authority, or resident facility when discarding reagents.

NOTE

To avoid repeated freezing and thawing of marker solutions, it is recommended that they be divided into small allotments for frozen storage.

1.8.3 Diluted Dye Solution Preparation

Prepare SYBR® Gold solution diluted to 1/100.

⚠ WARNING

- During operations, wear protective gloves, protective glasses, and a protective mask.
- See the MSDS for each reagent.

NOTE

- Restore each reagent to room temperature before use.
- Do NOT mix reagents from different lot numbers (the lot number is noted on labels of the buffer bottles or mark solution aluminum packs).
- For dye handling precautions and storage conditions, see the relevant Invitrogen Co. Product Information and MSDS.
- To avoid repeated freezing and thawing of marker solutions, it is recommended that they be divided into small allotments for frozen storage.

■ Requirements

- SYBR® Gold
- TE buffer
- Microtube (volume from 200 µL to 1.5 mL)
- Micro pipette
- Vortex mixer

■ Procedures

1 **Use a micro pipette to dispense 99 µL of TE buffer to a microtube.**

2 **Add 1 µL of SYBR® Gold dye, and agitate the solution with the vortex mixer for at least 10 seconds.**

1.8.4 Ladder Solution Preparation

Dilute pGEM® DNA Markers (Promega Co., catalog No. G1741) to 1/100 to prepare the ladder solution.

V1.05

If using an optional ladder, prepare the ladder solution by referring to *"4.10 Using Optional Ladders" P.205*.

■ Requirements

- pGEM® DNA Markers
- TE buffer
- Microtube (volume from 200 µL to 1.5 mL)
- Micro pipette
- Vortex mixer

■ Procedures

1 **Use a micro pipette to dispense 99 µL of TE buffer to a microtube.**

2 **Add 1 µL of pGEM® DNA Markers, and agitate the solution with the vortex mixer for at least 10 seconds.**

1.8.5 Separation Buffer Preparation

NOTE

The separation buffer mixed with diluted dye solution should be used on the day it is prepared.

■ Requirements

- DNA-2500 separation buffer
- Diluted dye solution (*"1.8.3 Diluted Dye Solution Preparation" P.51*)
- Buffer bottle
- Micro pipette

■ Procedures

1 **Dispense the separation buffer included in the DNA-2500 kit into the buffer bottle, and use the micro pipette to add the diluted dye solution.**

- Ensure that the volume of the mixed solution exceeds the required volume displayed in the [Reagent Information] window.
- Add the diluted dye solution until it reaches a volume ratio of 1/100 to the separation buffer transferred to the buffer bottle. For example, if the volume requirement is 1000 µL, add 10 µL of diluted dye solution to 990 µL of separation buffer.

Refer to the table below and use a micro pipette to dispense the separation buffer and diluted dye solution in the amounts required for the number of analyses (total ladders and samples) into the buffer bottle.

Number of analyses (Total ladders and samples)	Amount of separation buffer	Amount of diluted dye solution	Amount of mixed solution
8 analyses or less	495 µL	5 µL	500 µL
9 to 29 analyses	990 µL	10 µL	1000 µL
30 to 79 analyses	1980 µL	20 µL	2000 µL
80 to 120 analyses	2970 µL	30 µL	3000 µL

Guidelines for Required Amounts of Separation Buffer and Diluted Dye Solution

NOTE

The required amount of separation buffer is about 20 µL per analysis. The amounts described below are included in the required volumes indicated in [Reagent Information] and in the above guidelines.

- Amount needed for retries (up to 2 retries) if a problem is detected when automatically adding separation buffer to the microchip
- Dead volume of buffer bottle
- Minimum bottle amount detectable by a level sensor

NOTE

The reagent kit contains 30 mL of separation buffer (for 1000 analyses). The total number of analyses will be less than 1000 in situations where the number of analyses for each sequence is small, because the redundant amounts will be larger.

2 **Cap the bottle, and agitate the solution with the vortex mixer for at least 10 seconds.**

3 **Remove the cap and put the bottle in the purple reagent holder position (color code for DNA-2500 kit).**

NOTE

Let the solution adhering to the inner sides of the buffer bottle settle, and use a micro pipette to remove any residual bubbles before placing the tube in the reagent holder.

NOTE

Move the bottle around to ensure that the bottom of the buffer bottle is in the correct position at the bottom of the reagent holder.

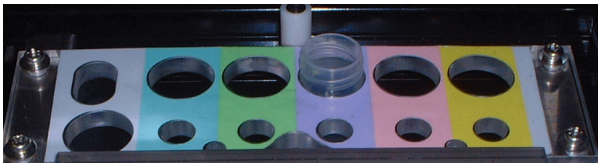
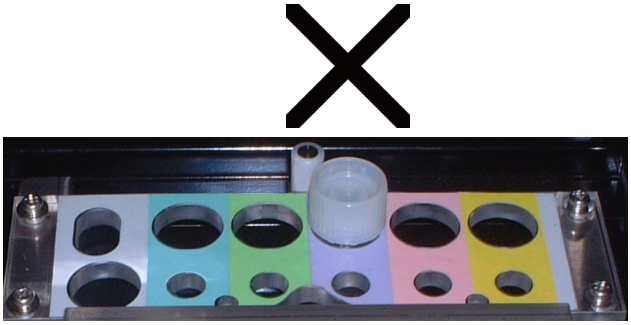


Fig.1-24 DNA-2500 Separation Buffer in Reagent Holder

⚠ CAUTION

- Remove the cap from the buffer bottle before placing it in the reagent holder.
  The sample probe may be damaged.



1.8.6 Ladder, Sample and Marker Solution Usage

Insert the tubes into the positions specified in the analysis schedule.

NOTE

Follow these precautions to prevent volatilization of solution, and to confirm the minimum amount of dispensable solution.

- When total number of ladders or samples to be analyzed is 12 or less

  No aluminum seal is needed to prevent volatilization, and the recommended 8-strip or 12-strip sample tubes can be used. Dispense a minimum of 9 µL into each tube.

- When the number of ladders or samples is 13 or more

  Use the 96-well sample plate, and the recommended aluminum seal. Dispense a minimum of 6 µL (premix) or 5 µL (on-chip mix) into each well.

- When the same ladder or sample is analyzed multiple times

  Use the recommended 8-strip or 12-strip sample tubes without an aluminum seal, and place it in the extra sample stand (X1 to X12). Since the amount of solution dispensed to each tube differs between premix and on-chip mix, see the respective descriptions for those items.

NOTE

When the prepared sample is expected to exceed the quantitative range (0.5 ng/µL to 50 ng/µL) or the maximum salt concentration (KCl or NaCl concentration; maximum of 125 mM) for DNA samples use the TE buffer (pH 8.0) to dilute it prior to analysis.

■ For Premix

Mix the marker solution at a volume ratio of 2:1 into the ladder or sample, and place it in the instrument. Nothing is placed in the marker solution position.

Sample stand (One analysis per well)				
Number of analyses (Total ladders and samples)	**Container**	**Amount of ladder or samples**	**Amount of marker solution**	**Amount of mixed solution**
12 analyses or less	Sample tube	3 µL	6 µL	9 µL
13 analyses or more	Sample plate with seal	2 µL	4 µL	6 µL

Extra sample stand (Multiple analyses per well)				
Number of analyses	**Container**	**Amount of ladder or sample**	**Amount of marker solution**	**Amount of mixed solution**
1 to 4 times	Sample tube	5 µL	10 µL	15 µL
5 to 9 times	Sample tube	1 x (Number of analyses + 1) µL	2 x (Number of analyses + 1) µL	3 x (Number of analyses + 1) µL

1 **Place the ladder solution and marker solution mix in the extra sample stand.**

Transfer the ladder solution prepared as per *"1.8.4 Ladder Solution Preparation" P.51* into the sample tube (or sample plate). Then, add the marker solution at a volume ratio of 2:1, and mix the solution thoroughly through pipetting.

- For example, to analyze a ladder solution four times from the extra sample stand requires a minimum of 15 µL (3 x (4 + 1) = 15 µL). Add 10 µL of marker solution to 5 µL of ladder solution.

2 **Place the sample and marker solution mix in the appropriate sample compartment.**

Transfer the sample to the sample tube (or sample plate). Then, add the marker solution at a volume ratio of 2:1, and mix the solution thoroughly through pipetting.

- For example, for analysis of up to 12 samples, mix 6 µL of marker solution with 3 µL of sample in a sample tube, and place the tube in the extra sample stand.
- To analyze 13 samples or more, mix 4 µL of marker solution with 2 µL of sample in a sample plate well, cover it with an aluminum seal, and place the sample plate on the sample stand.

■ For On-Chip Mix

In on-chip mixes, the ladder or sample, and the marker solution, are placed into the instrument separately. The ladder or sample is automatically mixed with the marker solution on the microchip.

Sample stand (One analysis per well)		
Number of analyses (Total ladders and samples)	**Container**	**Amount of ladder or sample**
12 analyses or less	Sample tube	9 µL
13 analyses or more	Sample plate with seal	5 µL

Extra sample stand (Multiple analyses per well)		
Number of analyses	**Container**	**Amount of ladder or sample**
1 to 3 times	Sample tube	9 µL
3 to 13 times	Sample tube	5 + 2 x (Number of analyses - 1) µL

1 Place the ladder solution in the extra sample stand.

Transfer the ladder solution prepared as per *"1.8.4 Ladder Solution Preparation" P.51* into the sample tube (or sample plate).

- For example, to analyze a ladder solution four times from the extra sample stand requires a minimum of 11 µL (5 + 2 x 3 = 11) of solution. Add 11 µL of ladder solution to the sample tube and place it in the extra sample stand.

2 Place the sample in the appropriate sample compartment.

Transfer the sample to the sample tube (or sample plate).

- For analysis of up to 12 samples, dispense 9 µL or more of sample into the sample tube, and place the tube in the extra sample stand.
- For analysis of 13 or more samples, dispense 5 µL or more of sample into the sample plate well, cover with an aluminum seal, and place the sample plate on the sample stand.

3 Dispense the marker solution.

Use a micro pipette to dispense marker solution in excess of the required amount shown in [Reagent Information] into a vial (PP 0.6-mL vial, no cap).

Or use the formula below to calculate the amount of marker solution required for the number of analyses (samples and ladders), and use a micro pipette to dispense it to a vial (PP 0.6-mL vial, no cap).

(Amount of marker solution required) = 2 x (Number of analyses) + 40 (µL)

NOTE

Since the minimum detectable amount using a level sensor is 40 µL, use a larger amount.

4 Put the bottle in the purple reagent holder position (color code for DNA-2500 kit).

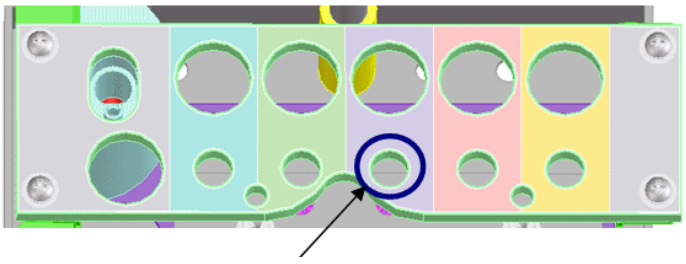


Fig.1-25 DNA-2500 Marker Solution in Reagent Holder

1.9 Preparation for Analysis with the RNA Kit

1.9.1 Requirements

Part Name	Sales origin	Part No. (Catalog No.)	Remarks
Microchip Type DR-C	Shimadzu Corporation	292-27900-91	-
Reagent kit RNA	Shimadzu Corporation	292-27913-91	-
SYBR® Green II (Note *2)	Invitrogen	S-7586	Diluted to 1/100
RNA6000 ladder	Ambion	7152	Diluted to 1/6
formamide Recommended product: 1)Biochemistry Deionized or 2)UltraPure® formamide	 Wako Invitrogen	 066-02301 15515-026	-
RNase ZAP®	Ambion	9780	Probe rinse solution
THE RNA Storage Solution	Ambion	7001	-
TE buffer 10mM Tris-HCl	-	-	pH 8.0
Micro tube	-	-	Volume from 200 µL to 1.5 mL For diluted dye solution and ladder solution
Sample tube or sample plate (recommended product)			Sample and ladder solution vessel (Note *1)
MicroAmp® reaction tubes	Applied Biosystems	N801-0533	1 tube
MicroAmp® strip tubes (without cap, 8 tubes/strip)		N801-0580	8-strip tubes
MicroAmp® Optical 96-well Reaction Plate		N801-0560	96-well PCR plate
Strips of 8 Thermo-Tubes	ABgene	AB-0452	8-strip tubes
Strips of 12 Thermo-Tubes		AB-1112	12-strip tubes
8 Low profile Thermo-strip		AB-0771	8-strip/12-strip tubes <Note> Since there are tabs on both ends of the tube, they cannot be used on the extra sample stand.
12 Low profile Thermo-strip		AB-0847	
Termo-Fast® 96 PCR Plate Non-Skirted		AB-0600	96-well PCR plate
MicroAmp® Caps, 8 Caps/Strip	Applied Biosystems	N801-0535	Cap for 1 tube and 8-strip tube (for prevention of volatilization of ladder and sample solution during thermal denaturation)
Adhesive PCR Foil Seals (Aluminum seal)	ABgene	AB-0626	Seal for 96-well PCR plate (to avoid volatilization of ladder and sample solutions)
Buffer bottle Recommended product: Centrifugal tube, 5 mL, 1000/packing	ASSIST (Sarstedt)	60.558	Extra separation buffer container (Note *3)
Vial Recommended product: Vial made of PP, 0.6 mL and 1000/packing (without cap)	BIO MEDICAL EQUIPMENT	NC-502	Extra marker solution container (Note *3)
Thermal cycler (oil free)	-	-	Item that can be heated to about 65°C for purposes of RNA denaturation, and then rapidly cooled
Macrobiotic and tip	-	-	Designed for volume of 0.5 µL to 5 mL
Protective glasses, protective mask, and protective gloves	-	-	-
Vortex mixer	-	-	-

NOTE

*1) In this manual, the 0.2-mL PCR tube listed in the above table is called the sample tube, and the 96-well PCR plate is called the sample plate.

*2) Use SYBR® Green II for the dye. This is different from SYBR® Gold used in DNA analysis.

*3) Each RNA reagent kit includes two buffer bottles and two vials (marker solution containers). One of the buffer bottles is used for the probe rinse solution. If more are required, order the recommended items separately.

Reference

"2.5.8 Wash" P.126

⚠ CAUTION

- Use only the formamide recommended in the list above.
  Since formamide decomposes easily, store it at -20°C. To avoid repeated freezing and thawing, it is recommended that the formamide be dispensed in 1 mL units in microtubes before being frozen.

⚠ CAUTION

- During thermal denaturation, use a cap for the sample tube (0.2 mL PCR tube) or an aluminum seal for the sample plate (96-well PCR plate).
  An oil-free thermal cycler is used to thermally denature the RNA sample before analysis. It is necessary to cap the vial or use the aluminium seal to prevent of evaporation of the sample solution.
- Use only the aluminum seal recommended in the list above.
  If another aluminum seal is used, the piercing needle may fail to penetrate, resulting in damage to the sample probe or in other instrument problems.
  Do NOT use parafilm. It may adhere to the sample probe or cause other instrument problems.
- The aluminum seal can only be used with the sample plate recommended in the list above.
  Do NOT use the aluminum seal to cover sample tubes. It prevents the sample holder from being properly placed over the sample tubes. The aluminum seal can only be used with the sample plate.

⚠ CAUTION

- Use only the sample tubes and plates recommended in the list above.
  Use of another sample tube or sample plate could lead to dispensing failures of at least 5 µL.

⚠ CAUTION

- The sample tube types shown below can be only used for setting samples into the normal sample stand.
  There are tabs on both ends of the tube, that prevent it from being properly placed in the extra sample stand.
  - ABgene PCR 8-strip/12-strip tubes (low profile): Part No. AB-0771, AB-0847

⚠ CAUTION

- Dispense to sample tube not to exceed 30 µL. Otherwise, that may be interfered with analysis sequence.

1.9.2 Contents of the RNA Kit

The RNA kit (Part No. 292-27913-91) is composed of the following items. When the kit is delivered, immediately open it, check the part names and quantity, and then store them at the temperatures specified for each part.

No.	Part Name	Capacity	Quantity	Remarks
❶	RNA separation buffer	30 mL	1	Refrigerated storage at 2°C to 8°C (PP bottle) (Up to 1000 analyses)
❷	RNA marker solution	1.2 mL	4	Frozen storage at -20°C (Aluminum pack) (Up to 1000 analyses)
❸	Buffer bottle	(5 mL)	2	Separation buffer dispensing container (with cap)
❹	Vial	(0.6 mL)	2	Marker solution dispensing tube (without cap)
❺	Manual	-	1	-

Fig.1-26 Contents of the RNA Kit

- Expiration dates are noted on the labels. Use the reagents in refrigerated or frozen storage before the expiration dates.
- Observe the laws, regulations, and rules of the country, local governing authority, or resident facility when discarding reagents.

NOTE

To avoid repeated freezing and thawing of marker solutions, it is recommended that they be divided into small allotments for frozen storage.

1.9.3 Diluted Dye Solution Preparation

Prepare SYBR® Green II solution diluted to 1/100.

> **⚠ WARNING**
>
> - During operations, wear protective gloves, protective glasses, and a protective mask.
> - See the MSDS for each reagent.

NOTE

- Restore each reagent to room temperature before use.
- Do NOT mix reagents from different lot numbers (the lot number is noted on labels of the buffer bottles or mark solution aluminum packs).
- For dye handling precautions and storage conditions, see the relevant Invitrogen Co. Product Information and MSDS.
- To avoid repeated freezing and thawing of SYBR® Green II, it is recommended that the solution be divided in small volumes (about 10 µL) and placed into microtubes. The microtubes should then be frozen and shielded from light.

■ Requirements

- SYBR® Green II
- TE buffer
- Microtube (volume from 200 µL to 1.5 mL)
- Micro pipette
- Vortex mixer

■ Procedures

1. **Use a micro pipette to dispense 99 µL of TE buffer to a microtube.**
2. **Add 1 µL of SYBR® Green II dye, and agitate the solution with the vortex mixer for at least 10 seconds.**

1.9.4 Ladder Solution Preparation

Dilute an RNA 6000 ladder (Applied Biosystems (formerly Ambion), catalog No. 7152) to 1/6 to prepare the ladder solution. For the amount to be prepared, see *"1.9.6 Ladder, Sample and Marker Solution Usage" P.62*.

The following procedure describes preparation of 6 µL of ladder solution.

■ Requirements

- RNA6000 ladder
- THE RNA storage solution
- Microtube (volume from 200 µL to 1.5 mL)
- Micro pipette
- Vortex mixer

■ Procedures

1 **Dispense 5 µL of THE RNA Storage Solution to a microtube.**

2 **Add 1 µL of the RNA 6000 ladder, use a micro pipette for repeating suction and discharge 5 times, and agitate the solution.**

1.9.5 Separation Buffer Preparation

NOTE

The separation buffer mixed with diluted dye solution should be used on the day it is prepared.

■ Requirements

- RNA separation buffer
- Formamide
- Diluted dye solution (*"1.9.3 Diluted Dye Solution Preparation" P.59*)
- Buffer bottle
- Micro pipette

■ Procedures

1 **Dispense the separation buffer included in the RNA kit into the buffer bottle, and add the diluted dye solution and formamide.**

1. Refer to the table below, and dispense the separation buffer into the buffer bottle in the required amounts based on the total number of analyses (ladder, sample, and blanks).
2. Add the diluted dye solution until it reaches a volume ratio of 1/80 to the separation buffer (see table below).
3. Add formamide until it reaches a volume ratio of 1:4 with the mixture of separation buffer and diluted dye solution (see table below).

Number of analyses (Total ladders, samples, and blank analyses)	Amount of separation buffer	Amount of diluted dye solution	Amount of formamide
6 analyses or less	395 µL	5 µL	100 µL
7 to 15 analyses	632 µL	8 µL	160 µL
16 to 22 analyses	790 µL	10 µL	200 µL

Guidelines for Required Amounts of Separation Buffer, Diluted Dye Solution, and Formamide

NOTE

When formamide is added, the volume of the mixed solution decreases by about 10%. Ensure that the mixed solution volume exceeds the required volume indicated in the [Reagent Information] window.

NOTE

The required amount of separation buffer is about 20 µL per analysis. The amounts described below are included in the required volumes indicated in [Reagent Information] and in the above guidelines.

- Amount needed for retries (up to 2 retries) if a problem is detected when automatically adding separation buffer to the microchip
- Dead volume of buffer bottle
- Minimum bottle amount detectable by a level sensor

NOTE

The reagent kit contains 30 mL of separation buffer (for 1000 analyses), the total number of analyses will be less than 1000 in situations where the number of analyses for each sequence is small, because the redundant amounts will be larger.

2 **Cap the bottle, and agitate the solution with the vortex mixer for at least 10 seconds.**

3 **Remove the cap and place the bottle in the peach reagent holder position (color code for RNA kit).**

NOTE

Let the solution adhering to the inner sides of the buffer bottle settle, and use a micro pipette to remove any residual bubbles before placing it in the reagent holder.

NOTE

Move the bottle around to verify that the bottom of the buffer bottle is in the correct position at the bottom of the reagent holder.

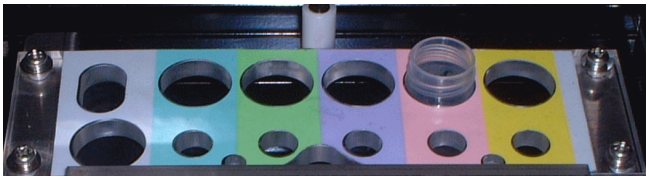
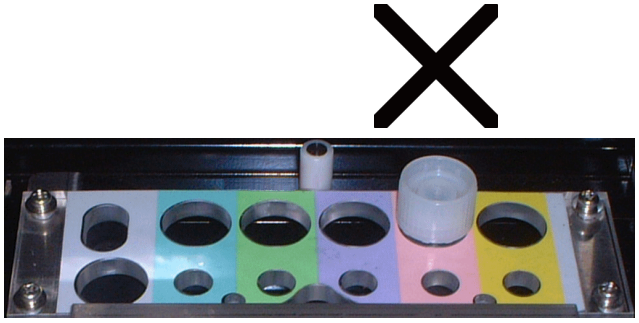


Fig.1-27 RNA Separation Buffer in Reagent Holder

⚠ CAUTION

- Remove the cap from the buffer bottle before placing it in the reagent holder.
  The sample probe may be damaged.



1.9.6 Ladder, Sample and Marker Solution Usage

⚠ CAUTION

- Use the table below as a guide for determining the number of RNA samples that can be entered into a single analysis.

  The sample stand has no cooling function. To maintain the denatured state of RNA samples, use the table below to determine the number of samples that can be entered into a single analysis based on the number of microchips used.

Number of microchips used	Number of samples	Ladder analysis	Blank analysis	Total number of analyses
1	1 to 6	1	1	8 max.
2	2 to 12	2	2	16 max.
3	3 to 16	3	3	22 max.
4	4 to 14	4	4	22 max.

- For RNA analysis, analyze a blank in each microtube before analyzing the ladder.

  ☞ Reference

  "1.5.9 Creating a Sample Sheet and Entering It into an Analysis Schedule", step *"6" P.29*

- For RNA analysis, only the premix mode is used for mixing the marker solution. Nothing has to be placed in the marker solution position.
- Add an equal amount of marker solution into each of the sample, ladder and THE RNA Storage Solution (blank) vials. Perform thermal denaturation before placing the sample tubes into the instrument. Cap the sample tube (aluminum seal on the sample plate) to prevent volatilization during thermal denaturation.
- Place the samples into the positions specified in the analysis schedule.

NOTE

Follow these precautions to prevent evaporation, and to confirm the minimum amount of dispensable solution.

- Normal samples

  Use the 96-well sample plate, and the recommended aluminum seal. Dispense a minimum of 6 µL into each well.

- Multiple analysis of ladders, samples, and blanks

  Use the recommended sample tube without an aluminum seal in the extra sample stand (X1 to X12). At least 3 x (Number of analyses + 1) µL of solution is required for each container.

NOTE

When the prepared sample is expected to exceed the quantitative range (Total RNA of 25 ng/µL to 500 ng/µL, mRNA of 25 ng/µL to 250 ng/µL) or the maximum salt concentration (Tris concentration 10 mM and EDTA concentration 1 mM) for RNA samples, use THE RNA Storage Solution to dilute it prior to analysis.

Sample stand (One analysis per well)			
Container	**Amount of ladder, sample, or blank**	**Amount of marker solution**	**Amount of mixed solution**
Sample tube	4.5 µL	4.5 µL	9 µL
Sample plate with seal	3 µL	3 µL	6 µL

Extra sample stand (Multiple analyses per well)				
Number of analyses	**Container**	**Amount of ladder or sample**	**Amount of marker solution**	**Amount of mixed solution**
1 to 4 times	Sample tube	7.5 µL	7.5 µL	15 µL
5 to 9 times	Sample tube	1.5 x (Number of analyses + 1) µL	1.5 x (Number of analyses + 1) µL	3 x (Number of analyses + 1) µL

1 **Mix the ladder solution and marker solution.**

Transfer the ladder solution prepared as per "1.9.4 Ladder Solution Preparation" P.59) into the sample tube (or sample plate). Then, add an equal amount of marker solution, and mix the solution thoroughly through pipetting.

- For example, to analyze a ladder solution four times from the extra sample stand requires a minimum of 15 µL 3 x (4 + 1) = 15. 7.5 µL of marker solution is added to 7.5 µL of ladder solution.

2 **Mix the sample and marker solution.**

Transfer the sample to the sample plate (or sample tube). Then, add an equal amount of marker solution, and mix the solution thoroughly through pipetting.

- For normal analysis, dispense 3 µL of marker solution and 3 µL of sample into a sample plate to mix them, and cover the sample plate with an aluminum seal.

3 **Mix the THE RNA Storage Solution and marker solution.**

Transfer THE RNA Storage Solution for blank analysis to the sample plate (or sample tube). Then, add an equal amount of marker solution, and mix the solution thoroughly through pipetting.

- For example, to analyze blank four times from the extra sample stand requires a minimum of 15 µL (3 x (4 + 1) = 15). 7.5 µL of marker solution is added to 7.5 µL of THE RNA Storage Solution.

4 **Immediately before analysis, use the oil-free thermal cycler to perform thermal denaturation.**

Use a cap on the sample tube (or aluminum seal on the sample plate) to prevent evaporation of the mixed solution, (see "1.9.1 Requirements" P.56).
The thermal cycler temperature conditions are as follows.
65°C, 5 minutes -> 4°C, 5 minutes

NOTE

After performing thermal denaturation at 65°C for 5 minutes, allow for cooling inside the thermal cycler at 4°C for 5 minutes. (Do NOT freeze the solution immediately after thermal denaturation at 65°C.)

5 **Place the sample plate (or sample tube with cap removed) into the sample stand (or extra sample stand).**

Use the positions specified in the analysis schedule.

⚠ CAUTION

- Remove the cap used for thermal denaturation before placing the sample tubes into the sample stand (or extra sample stand).
  Analysis with the cap attached may result in damage to the sample probe, or in other instrument problems.
  It is not necessary to remove the aluminum seal before placing the sample plate on the sample stand.

1.10 Analysis

1.10.1 Sample Holder Installation

The sample holder is placed over the sample tube (or sample plate) to secure them to the sample stand or extra sample stand.

⚠ CAUTION

- Always use a sample holder.
  Failure to use a sample holder will result in a stop error and analysis will not start.
- Install the sample holder so that it presses down on the sample tube (or sample plate).
  Starting the instrument when the sample holder is improperly installed (elevated) may cause instrument damage.

Use the following procedure to install the sample holder.

1 **Examine the orientation of the sample holder.**

Position the sample holder so that the holes in the sample holder match those of the sample stand (96-well) and extra sample stand (12-well).

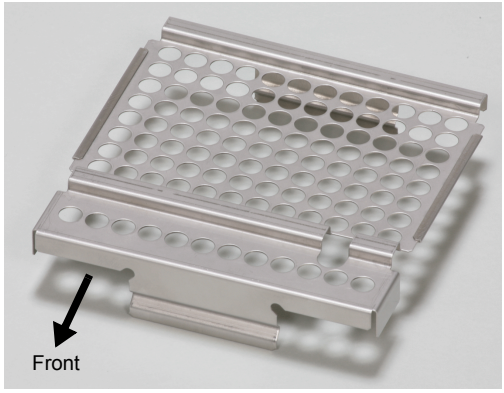


Fig.1-28 Sample Holder

2 **Insert the front side of the sample holder between the extra sample stand and the metal rod (behind the metal rod.)**

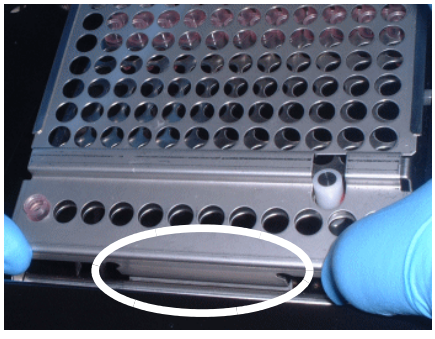


Fig.1-29 Front of the Sample Holder

3 **Insert the back side of the sample holder in front of the metal rod between the sample stand and reagent holder.**

Use the procedure shown in Steps 1 to 3 in the figure below.

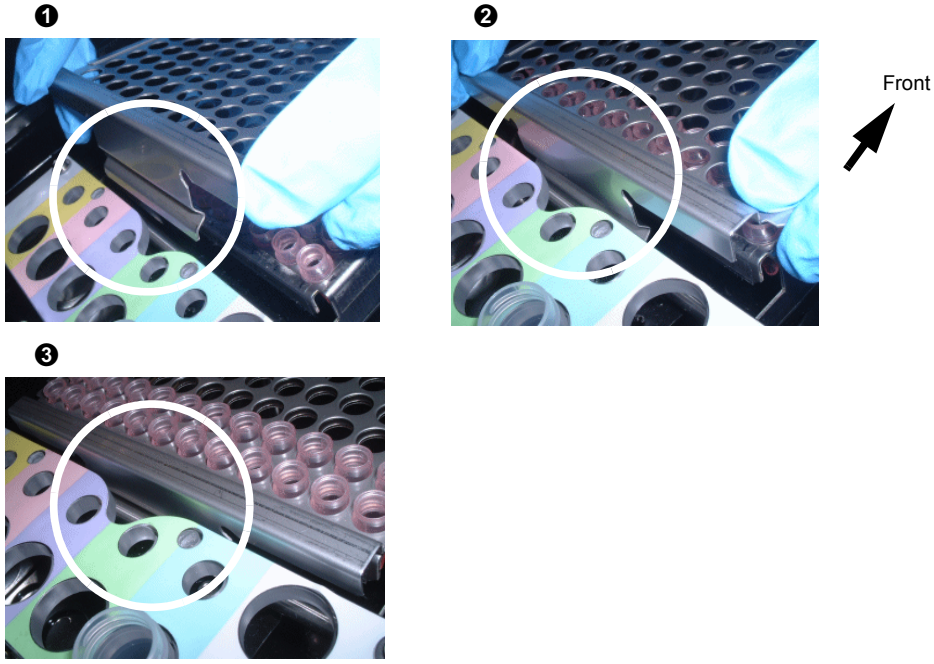


Fig.1-30 Installing the Sample Holder

4 **Apply downward pressure on the four corners of the sample holder, and verify that it is securely installed (not loose) over the sample stand and extra sample stand.**

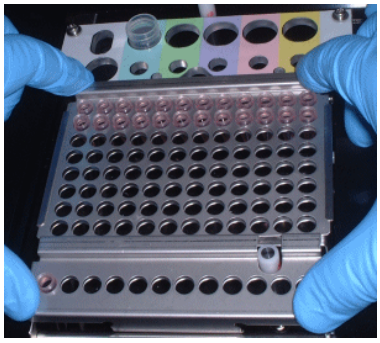


Fig.1-31 Checking the Sample Holder Installation

1.10.2 Starting the Analysis

1 **Verify that the chip cover is closed.**

☞ **Reference**

"1.5.5 Microchip Installation" P.16

2 **Ensure that the ladder or sample solution, separation buffer, marker solution (for on-chip mix), and rinse solution are all properly positioned in the instrument.**

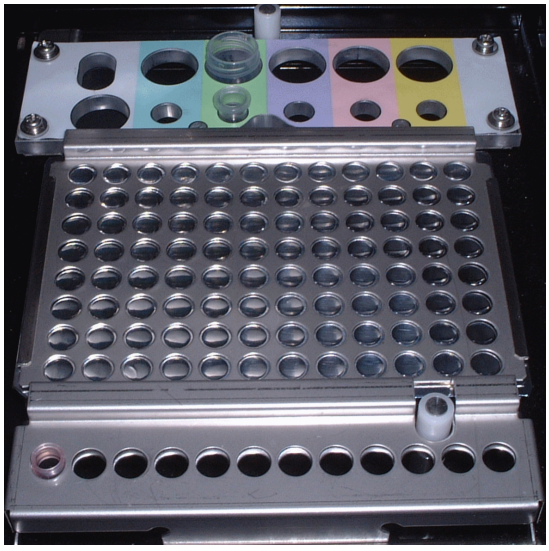


Fig.1-32 DNA-1000 Kit (On-Chip Mix Mode), Sample Plate with Aluminum Seal

3 **Close the top cover.**

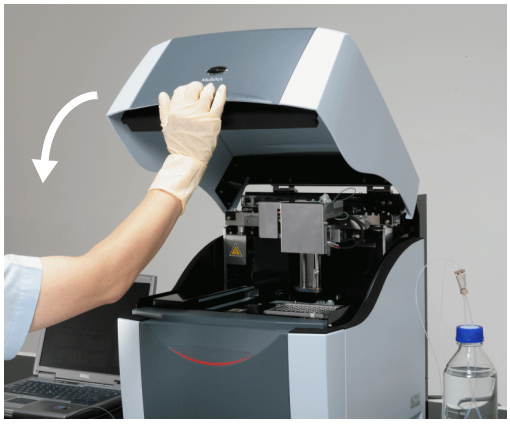


Fig.1-33 Closing the Top Cover

4 **Verify that the rinse water level is sufficient, especially for first time use or use after the instrument has remained idle for a long period. Initiate the Probe Rinse function before starting analysis.**

On the MultiNA Control Software [Instrument] pull-down menu, select [Probe Rinse] and then [Wash] to initiate the Probe rinse function.

Reference

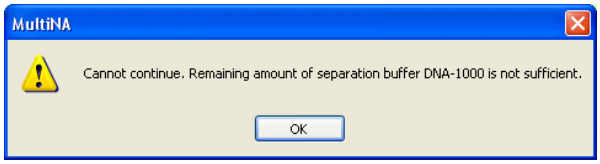
"2.5.8 Wash" P.126

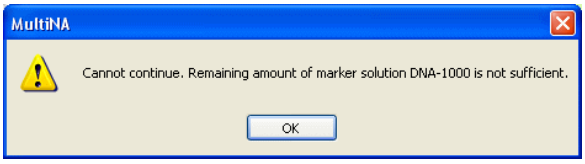
5 **Either select [Start] on the MultiNA Control Software [Analysis] pull-down menu, or click the [Start] button on the toolbar.**

Before starting analysis, the instrument automatically checks the following items. If no problems are found, the instrument proceeds to Step 6 and starts the analysis schedule. If a problem is detected, an error message is displayed and analysis is not started.

- Chip cover is closed.
- Top cover is closed.
- Sample holder is properly installed.
- Microchip is installed in the specified chip position.
- A sufficient volume of separation buffer and marker solution are correctly positioned in the reagent holder.
- The chip stage is at the set temperature.

For example, if the amount of separation buffer or marker solution (for on-chip mix only) is detected to be less than the necessary amount, the following message is displayed.
If this message is displayed, click [OK], add the necessary amount of reagent, and restart the analysis from Step 5.





NOTE

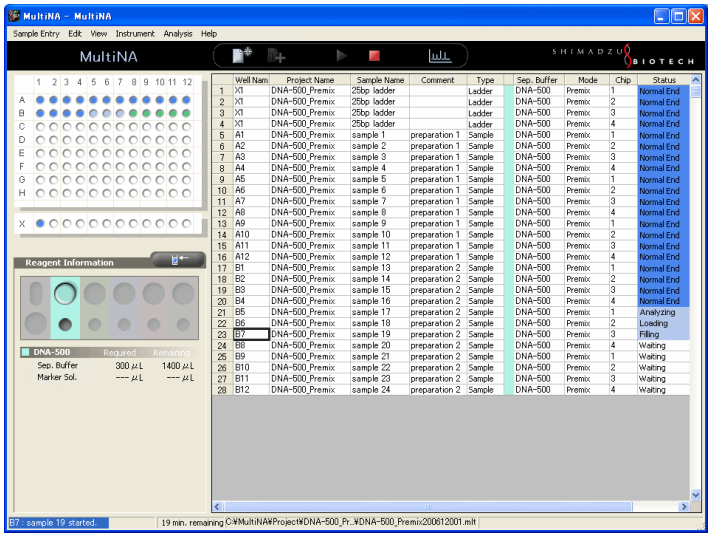
Do NOT step away from the instrument while these pre-analysis checks are in progress since an error message may be displayed.

6 **After analysis starts, you can check the instrument analysis status in the window.**

- The status of analysis, such as waiting, analyzing, normal end, and abnormal end, is shown in colors in the well display on the left and the schedule list on the right.
- The status bar at the bottom of the window shows the content of the latest instrument action, and the expected time remaining to the end of the analysis.

NOTE

The expected time remaining includes the automatic rinsing time after completion of analysis (see *"1.10.4 Ending Analysis" P.69*).



Well status display	Status display in analysis schedule list	State
Green	Waiting	State before pretreatment process on microchip (such as filling the separation buffer)
Light blue	Filling	Currently filling separation buffer into microchip
Blue (blinking)	Loading	Currently loading the sample
Blue (blinking)	Analyzing	Currently analyzing the sample (separation analysis)
Blue	Normal analysis end	Analysis has ended normally
Red	Abnormal end	An error occurred, or analysis was interrupted

Either select [Chip Status] on the [View] pull-down menu, or press the [F8] key to switch between the [Reagent Information] window (top figure) and [Chip Status] window (bottom figure).

V1.05

The window will automatically switch to the [Chip Status] window when starting to fill the first sample.

The voltage, current, and electropherogram are displayed in real-time for each microchip port (see *"2.1.8 Chip Status Window" P.88*).

1.10.3 Interrupting Analysis

To interrupt the analysis, either select [Stop] on the [Analysis] pull-down menu, or click the [Stop] button on the toolbar.

Reference

"2.6.2 Stop" P.128

1.10.4 Ending Analysis

The instrument automatically performs microchip rinsing and other posttreatment functions at the completion of the analysis sequence. Then the instrument stops.

NOTE

When RNA analysis ends or is interrupted, it is recommended that [RNase removal washing] also be performed on the sample probe as per *"2.5.8 Wash" P.126* after automatic rinsing is completed.

1.10.5 Treatment after Analysis End

■ Removing the current containers

Remove the buffer bottle, marker solution vial (on-chip mix), and ladders or samples from the instrument.

⚠ CAUTION

- During removal, avoid spilling any residual solution.
  Lift the buffer bottle from the reagent holder.

■ Discarding waste fluid

⚠ CAUTION

- Discard waste fluid on a regular basis to keep the bottle from overflowing.
- Treat the waste fluid appropriately in accordance with prescriptions or guidelines from the applicable management department.
  In addition to rinse water, waste fluid includes the separation buffer, marker solution, and dye. It will also contain formamide from RNA analysis.

Use the cartridge below for SYBR dye absorption in the waste container.

- boNd EX Starter Kit: Nippon Genetics (Catalog No. 740701)

■ Rinsing the microchips

The microchips are automatically rinsed and can remain installed in the instrument after the normal conclusion of analysis.

However, if the “Remove all chips and immediately wash them” error message is displayed, manually rinse the microchips as instructed in section *-Instrument and System- "6.2.7 Microchip Reservoirs"*.

(For details about error messages, see *-Instrument and System- "9.2 Error Messages"*.)

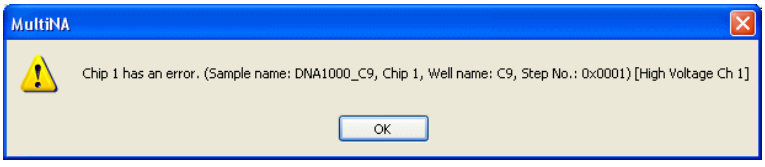
“Remove all chips and immediately wash them” message

- Analysis has ended without the microchips being rinsed because an error occurred during analysis.
- Immediately proceed to manual rinsing of the microchips to avoid clogs from dried separation buffer. Refer to section *-Instrument and System- "6.2.7 Microchip Reservoirs"*.

“Chip # has an error” message

Click [OK] to display the message “Error occurred. Chip # is now unavailable”.

- Automatic washing that includes the designated microchip has been completed in the instrument.
- Since the microchip of the displayed # (No.) is clogged or partially clogged, manually rinse it and check to see if it is clogged. If it is clogged, replace it with a new one.



☞ Reference

-Instrument and System- "6.2.7 Microchip Reservoirs"

■ Preparation for long idle periods

If the instrument is not to be used for a long period (two weeks or more), clean the rinse water tubing and the microchips, and properly store the microchips.

☞ Reference

-Instrument and System- "7.1 Moving or Storage Preparations", "7.3 Storage"

1.11 Data Analysis

This section describes the method for using the MultiNA Viewer software to display and reanalyze data after sample analysis.

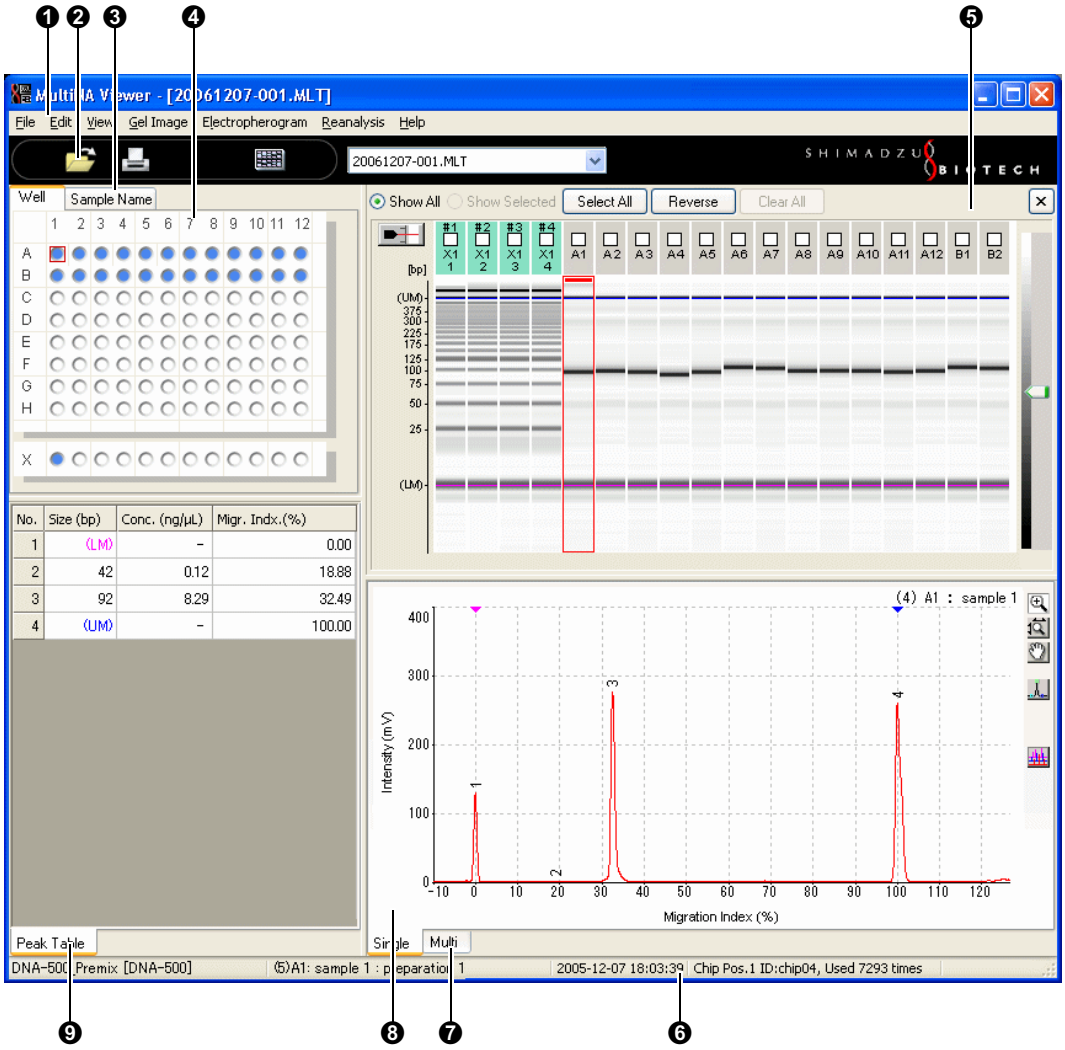
1.11.1 Displaying the Data

1 **Select [Data File] on the [View] pull-down menu of the MultiNA Control Software.**

(Or click the [Display Data File] button on the right end of the toolbar, or, press the [F12] key to perform the same operation.)
The MultiNA Viewer software starts and automatically displays the data file in the sample sheet.

NOTE

If the MultiNA Viewer [MultiNA Viewer] icon on the desktop is used to open the software, the data file is not automatically displayed. To open the data file, select [Open] on the MultiNA Viewer [File] pull-down menu, then specify the data file (see *"3.2.1 Open" P.143*).



No.	Name	Reference Page
❶	Pull-down menu	*"3.1.1 Pull-down Menu List" P.132*
❷	Toolbar	*"3.1.2 Toolbar" P.134*
❸	Sample Name tree	*"3.1.5 Sample Name Tree" P.135*
❹	Well display	*"3.1.3 Focused data and selected data" P.134*
❺	Gel Image	*"3.1.6 Gel Image" P.135*
❻	Status Bar	*"3.4.3 Status Bar" P.165*
❼	Electropherogram (Multi)	*"3.1.10 Electropherogram (Multi)" P.142*
❽	Electropherogram (Single)	*"3.1.9 Electropherogram (Single)" P.140*
❾	Peak Table	*"3.1.7 Peak Table" P.139*

2 Verify that the analysis ended normally.

In well display ❹, the analysis status determines the display colors, as shown below.

- Sample analyzed normally: Blue
- Sample with Warning: Yellow
- Sample with Error (and Fatal): Red

NOTE

- When Warning, and Error or Fatal, appear simultaneously, the status color is red.
- If multiple analyses were performed on the extra sample stand (X1 to X12), the yellow or red display color will appear if even one Warning, Error or Fatal, is generated.

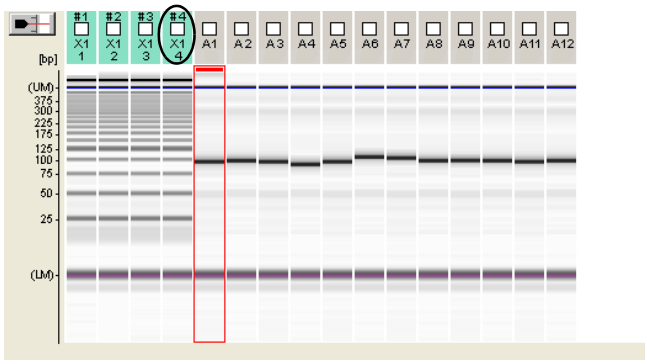
Reference

For information about Warning or Error, see *-Instrument and System- "9.2 Error Messages"*.

3 Display the ladder and sample data.

The ladder display color at the top of the gel image ❺ is light green, while the sample color is gray.

- For the ladder used in sample analysis, # mark (above the check box) and a chip position number are displayed. In the figure below, the circled #4 shows that the ladder was used for analysis at chip position 4.

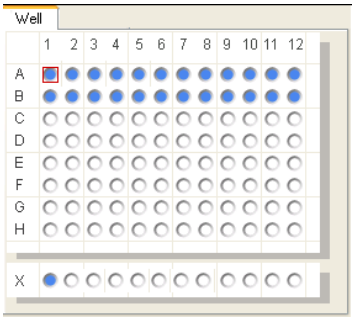


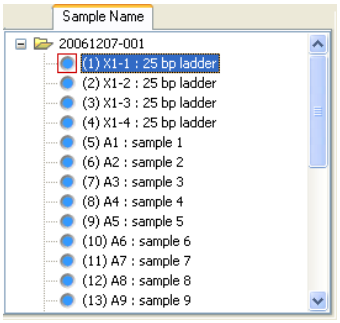
- Either click one well in the well display ❹, one sample name in the sample name tree ❸, or data in the gel image ❺ to focus on the data (becomes circled in red) and display the corresponding electropherogram (single).

Reference

"3.1.3 Focused data and selected data" P.134

- Information about each sample is displayed in the sample name tree. For example, [(1)X1-1: 25bp ladder] In the figure below indicates as follows: [(1)]: Order of analysis, [X1-1]: First analysis for well X1, [25bp ladder]: Sample name entered in sample sheet





- The peak table ❾ displays the peak detection and analysis results for the sample being focused on.

Reference

Items displayed in the peak table can be changed. See *"3.4.12 Options" P.174*.

1.11.2 Data Examination and Reanalysis

Inspect the electropherograms of all of the ladders and samples included in the data file.

1 Examine the detected peak numbers in the ladder and sample data.

Evaluate the electropherograms of all of the ladders and samples.

- The peak detection level in automatic analysis is classified into three categories, [Coarse], [Standard], and [Fine], according to the peak height and width at the lower detection limit. [Standard] analysis is performed immediately after the analysis completion.
- For detection for smaller peaks, use the [Reanalysis] pull-down menu and point to [Automatic] and then select [Fine] on the [Reanalysis] pull-down menu. Perform reanalysis. Select [Coarse] for detection of larger peaks, and perform reanalysis."

Reference

"3.7.1 Automatic" P.183

2 Check the ladder data.

NOTE

Accurate ladder analysis is necessary for obtaining highly precise results for size prediction and concentration quantitation. Use the procedure below to check all ladder data that has been obtained.

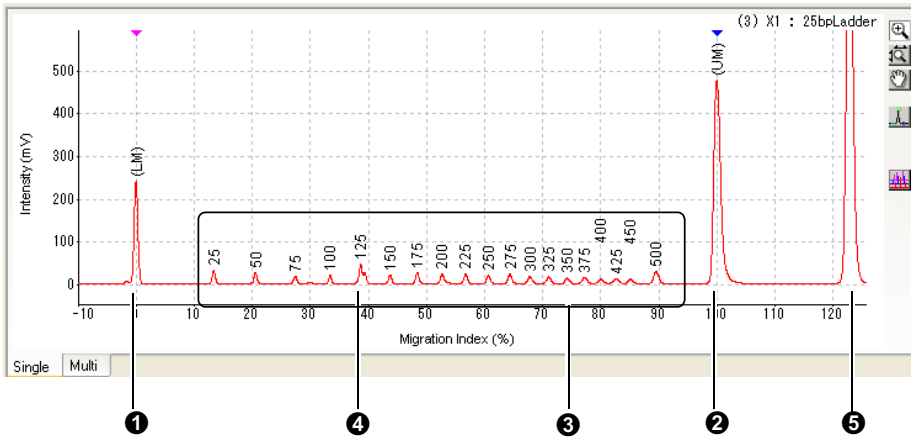
1 Refer to the recommended ladder data examples below to verify that ladder analysis has been performed correctly for each kit.

- Each fragment peak in the ladder must be separated as in the data examples.
- Each marker peak must be correctly detected.
- The size number must be displayed correctly for each fragment peak in the ladder.

NOTE

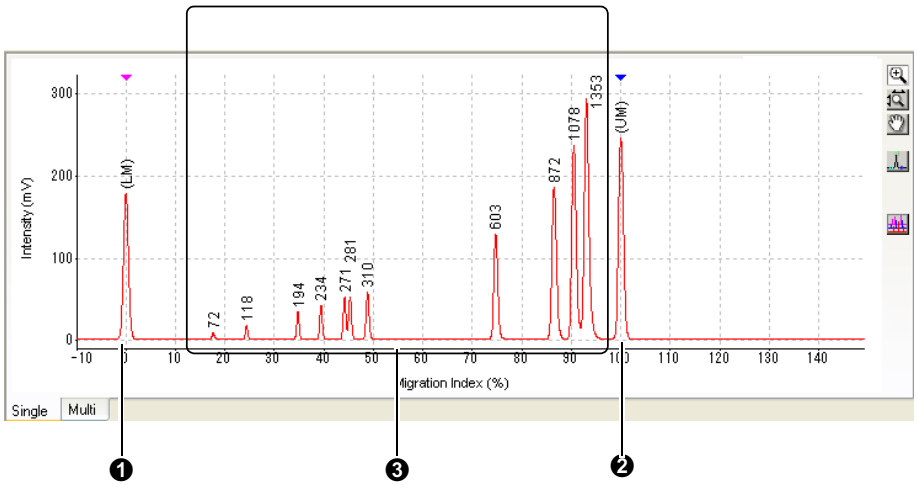
Lower markers are displayed as LM, while upper markers are displayed as UM.

DNA-500 kit



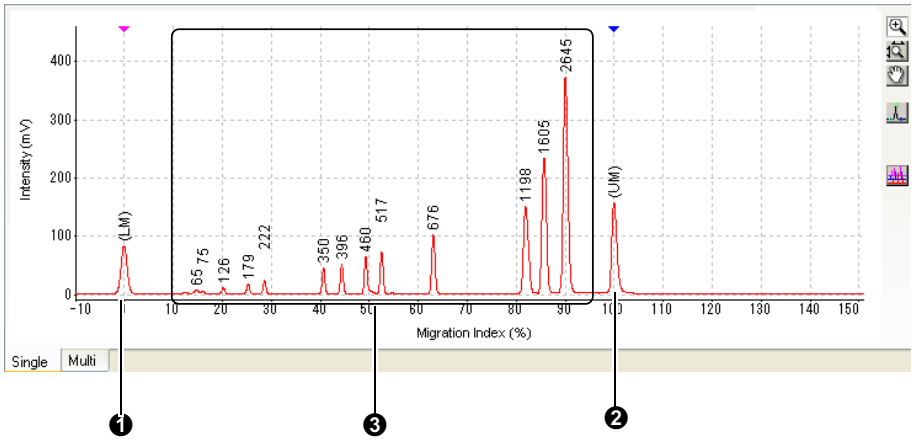
No.	Explanation
❶	Lower marker (Magenta arrow displayed on upper part)
❷	Upper marker (Blue arrow displayed on upper part)
❸	25 bp to 500 bp peaks included in ladder 25 bp, 50 bp, 75 bp, 100 bp, 125 bp, 150 bp, 175 bp, 200 bp, 225 bp, 250 bp, 275 bp, 300 bp, 325 bp, 350 bp, 375 bp, 400 bp, 425 bp, 450 bp and 500 bp; 19 peaks in total
❹	125 bp peak is divided into 2 at front edge. While this is derived from the 25 bp DNA ladder components, it is recognized as one peak in the data analysis.
❺	2652 bp peak detected after upper marker. It will cause no problem with the analysis.

DNA-1000 kit



No.	Explanation
❶	Lower marker (Magenta arrow displayed on upper part)
❷	Upper marker (Blue arrow displayed on upper part)
❸	72 bp to 1353 bp peaks included in ladder 72 bp, 118 bp, 194 bp, 234 bp, 271 bp, 281 bp, 310 bp, 603 bp, 872 bp, 1078 bp, and 1353 bp; 11 peaks in total

DNA-2500 kit

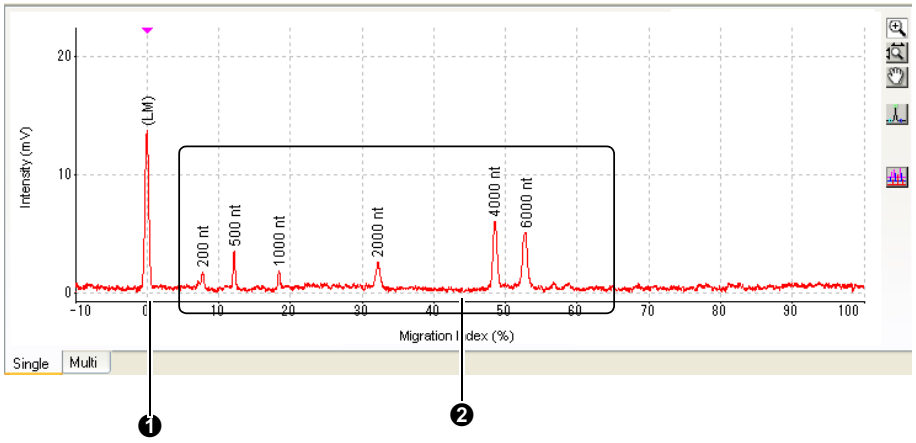


No.	Explanation
❶	Lower marker (Magenta arrow displayed on upper part)
❷	Upper marker (Blue arrow displayed on upper part)
❸	65 bp to 2645 bp peaks included in ladder 65 bp, 75 bp, 126 bp, 179 bp, 222 bp, 350 bp, 396 bp, 460 bp, 517 bp, 676 bp, 1198 bp, 1605 bp, and 2645 bp; 13 peaks in total

NOTE

While fragment peaks of less than 65 bp are detected, these are not used for preparation of size calibration curves.

RNA kit



No.	Explanation
❶	Lower marker (Magenta arrow displayed on upper part) (Note: The RNA marker solution does not include a upper marker)
❷	200 nt to 6000 nt peaks included in ladder 200 nt, 500 nt, 1000 nt, 2000 nt, 4000 nt and 6000 nt; 6 peaks in total

2 If a ladder has not been correctly analyzed, see the following page to perform reanalysis.

- When lower markers or upper markers are not being correctly detected
  Set the correct marker peak in [Manual Edit Mode] on the [Reanalysis] pull-down menu.

☞Reference

[Set to Lower Marker]/[Set to Upper Marker] in *"3.7.2 Manual Edit Mode" P.184*

- When fragment peaks are not being correctly detected
  Set the correct fragment in [Manual Edit Mode] on the [Reanalysis] pull-down menu.

☞Reference

[Add Peak]/[Delete Peak] in *"3.7.2 Manual Edit Mode" P.184*

- When the separation pattern is not normal
  Change the ladder, and proceed with analysis.

☞Reference

"3.7.3 Change Ladder and Analyze" P.189

3 When correct analysis data has been obtained for all samples, select [Save] on the [File] pull-down menu.

☞Reference

"3.2.3 Save" P.145

3 Check the sample data.

1 Click the sample data item one at a time, to focus.

2 Check that the marker and fragment peaks are correctly detected. If not correctly detected, use the following method to perform reanalysis.

- When lower makers or upper markers are not being correctly detected
  Set the correct marker peak in [Manual Edit Mode] on the [Reanalysis] pull-down menu.

☞Reference

[Set to Lower Marker]/[Set to Upper Marker] in *"3.7.2 Manual Edit Mode" P.184*

- When the necessary peak is not being detected
  On the [Reanalysis] pull-down menu, select [Manual Edit Mode] and point to [Add Peak] to add a peak.

☞Reference

[Add Peak] in *"3.7.2 Manual Edit Mode" P.184*

- When an unneeded peak should be deleted
  On the [Reanalysis] pull-down menu, select [Manual Edit Mode] and point to [Delete Peak] to delete the peak.

☞Reference

[Delete Peak] in *"3.7.2 Manual Edit Mode" P.184*

NOTE

In automatic analysis, peaks detected before the lower marker and peaks detected after the upper marker are not recognized as peaks. While such peaks can be recognized by selecting [Manual Edit Mode] and then [Add Peak] on the [Reanalysis] pull-down menu, the accuracy of analysis results cannot be guaranteed since these peaks fall outside of the markers in the size calibration curve.

3 If correct analysis data has been obtained for all samples, select [Save] on the [File] pull-down menu.

Reference

"3.2.3 Save" P.145

4 Check the peak table and RNA report (for RNA analysis only).

Items displayed in the peak table can be selected from among the following by selecting [Options] on the [View] pull-down menu.

Reference

"3.4.12 Options" P.174

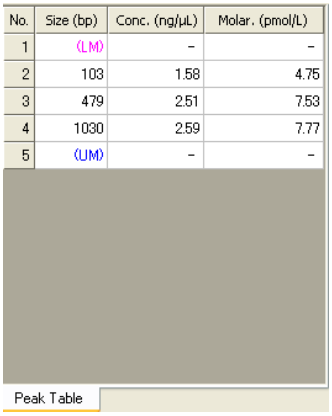
DNA data

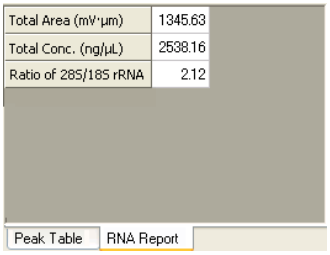
[Size], [Concentration], [Migration Index], [Height], [Area], and [Molarity]
(For the lower marker and upper marker, “LM” or “UM” is displayed respectively in the [Size] column. [Concentration] and [Molarity] are not displayed for them.)

Data of RNA

[Attribute], [Concentration], [Migration Index], [Height, Area], [Peak Start Index], and [Peak End Index]
(For the lower marker, [Attribute] (property) is displayed while [Concentration] is not displayed.)

In RNA analysis, the peak table and the RNA report tab are displayed.
The RNA report displays the calculated [Total Area] and [Total Concentration] results. In addition, the Total RNA analysis calculates and displays the [Ratio of 28S/18S rRNA].
The following shows examples of a peak table (DNA analysis) (left) and RNA report (right).

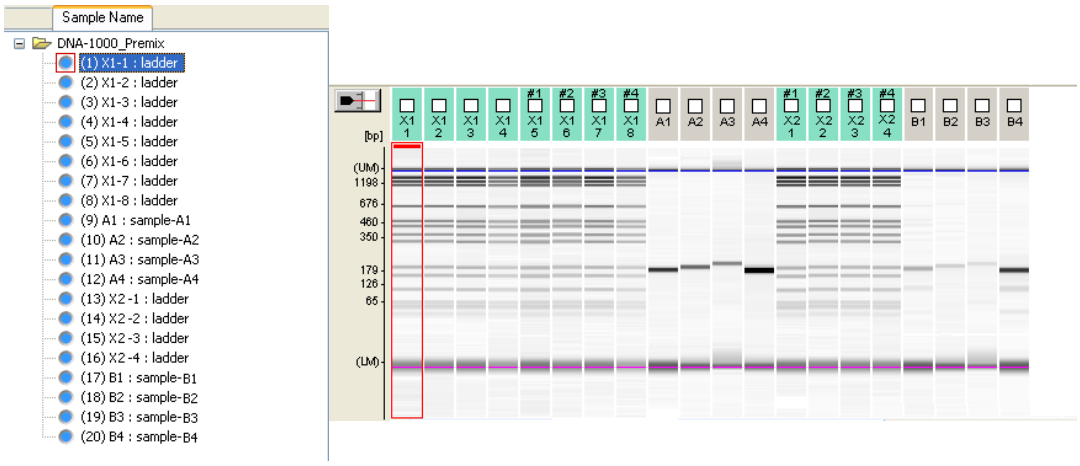




NOTE

If multiple ladders were analyzed on the same microchip, only the ladder data generated immediately before the sample will be used for data analysis (see the gel image in the figure below).

In the four microchip example below, 2 ladder analyses, 1 sample analysis, 1 ladder analysis and 1 sample analysis are performed. The relationship between the ladders and samples used in the analysis is as shown in the table below. The ladder data from (1) X1-1 to (4) X-4 is not used in the analysis. For samples (9) A1 to (13) A4, the ladder data immediately before their analyses is (5) X1-5 to (8) X1-8, and these are ladders used in the analysis. For samples (17) B1 to (20) B4, the ladder data immediately before their analyses is (13) X2-1 to (16) X2-4, and these are ladders used in the analysis.



Sample	Ladder used in analysis	Chip position
(9) A1	(5) X1-5	1
(10) A2	(6) X1-6	2
(11) A3	(7) X1-7	3
(12) A4	(8) X1-8	4
(17) B1	(13) X2-1	1
(18) B2	(14) X2-2	2
(19) B3	(15) X2-3	3
(20) B4	(16) X2-4	4

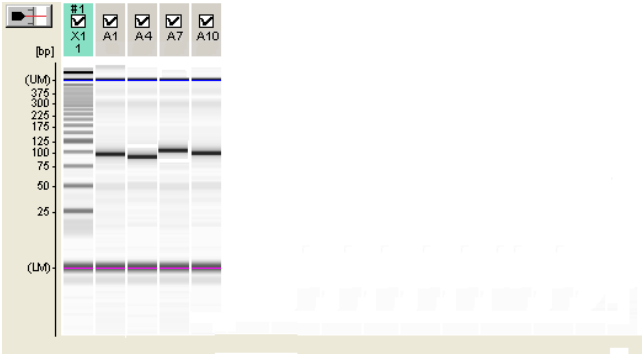
1.11.3 Printing the Data

Print the electropherogram, gel image, and analysis results.

1 **In the gel image, select the check boxes on the data to be printed.**

NOTE

If printing all of the data, this procedure is not necessary.



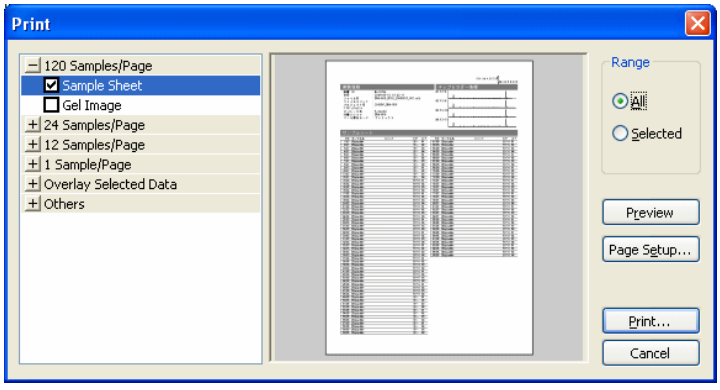
2 **Either select [Print] on the [File] pull-down menu, or click the [Print] button on the toolbar.**

3 **Use the [Print] dialogue box to select the layout to be printed.**

Multiple layouts may be selected.

4 **Select [All] or [Selected] on the [Range] menu.**

[Selected] indicates data that has been selected on the gel image or the electropherogram (multi).



5 **Click [Print] to start printing.**

Reference

For details about printing, see *"3.2.8 Print" P.152*.

1.11.4 Differences Between Analyzed Data and Raw Data

The MultiNA Viewer software can display two kinds of data, analyzed data and raw data (Refer to *"3.4.5 Analyzed Data/Raw Data" P.166*). Normally, when a data file is opened, the analyzed data is displayed.

- Raw data includes the detected signals saved without alteration. Analyzed data is based on the raw data. It includes the migration time index calculated based on the marker detection time, and the fragment size prediction value and concentration obtained after baseline correction.

The differences between analyzed data and raw data are detailed in the table below.

Item	Analyzed data	Raw data
Peak table	From the [Display] menu select [Option] and designate the desired display items. Peak width (value converted to distance axis, unit: µm) and height (mV) are used to calculate [Area].	Display items include time (sec), height (mV) and area (mV•µm) only. [Area] is calculated from peak width (sec) and height (mV).
Electropherogram horizontal axis	Migration index (Based on the marker detection time, it is expressed as a relative index value.) • In DNA analysis, the lower marker and upper marker detection times are set to 0% and 100%, respectively. • In RNA analysis, the lower marker detection time is set to 0%, and the electropherogram end point is set to 100%. You can also select [Migration Time] on the [Electropherogram] menu to display the migration time.	Migration time (sec)
Electropherogram vertical axis	Height (mV) obtained after baseline correction on the signal detected with PMT.	Signal strength (mV) detected with PMT.
Gel image	Distance image display (default) You can also select [Time Image] on the [Gel Image] menu to display the time image before conversion to the distance image. The vertical axis scale always indicates the size.	The vertical axis indicates the migration time (sec).
Reanalysis	All items on the [Reanalysis] menu can be executed.	Automatic reanalysis can be executed by selecting [Automatic] on the [Reanalysis]. [Change Ladder and Analyze] and [Manual Edit Mode] on the [Reanalysis] menu cannot be executed.

NOTE

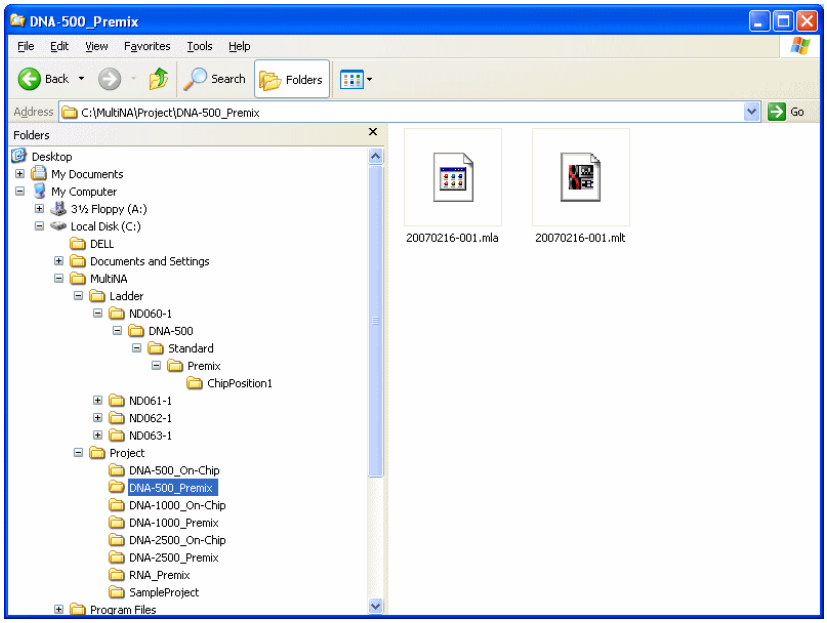
- The units for the calculation of area differs between the analyzed data and raw data. For analyzed data, the horizontal peak axis indicates the value converted to migration distance (unit: µm). For raw data it indicates the migration time (unit: sec). The peak height for both indicates signal strength (unit: mV).
- The migration index is a relative index value of the electropherogram time axis based on the marker detection time. In DNA analysis, the lower marker and upper marker detection times are set to 0% and 100%, respectively. In RNA analysis, the lower marker and electropherogram end point are set to 0% and 100%, respectively. If the lower marker was not detected, the electropherogram start point is set to 0%. In addition, in DNA analysis, if the upper marker was not detected, the electropherogram end point is 100%.
- When a data file is opened in the default display, the migration index is indicated on the horizontal axis in the electropherogram, while the distance image is indicated on the vertical axis in the gel image.

1.11.5 Saving the Data

Data files automatically created in the instrument are as follows.

Type	File name (Base name)	Extension	Folder saved
Raw data	Name specified at time of sample sheet creation	MLT	Folders are created for each project in the project folder located inside the data folder (normally, C:\MultiNA\). Raw data is saved within these folders.
Analyzed data	Same as raw data	MLA	Saved in the same folder as the raw data.
Ladder data	Number identifying date and time of analysis start	LDR	Folders are created for each chip ID used in the Ladder folder located inside the data folder (normally, C:\MultiNA\). Within these folders, folders are further saved according to the separation buffer, ladder, marker solution mix mode, and chip position.
Analytical performance check results	Same as raw data	LOG	Saved to the [(Reagent Kit Name)] folder in the Project folder located inside the data folder (Normally, C:\MultiNA\).

- To display the data in MultiNA Viewer, specify a raw data file (MLT file) (Refer to *"3.2.1 Open" P.143*). Analyzed data (MLA file) is automatically displayed at the same time.
- Files are created for each sample sheet and contain the analysis data for normal samples.
- Files are also created for each ladder analysis (ladder data: LDR file). This data is used for reanalyzing the data after the ladder has been changed (Refer to *"3.7.3 Change Ladder and Analyze" P.189*).
- Data folders are specified when the program is installed. The default location is C:\MultiNA (Refer to section *-Instrument and System- "5.5.1 Installation"*.



- In the MultiNA - Project folder, a folder is created for each created project, within which acquired data (extension MLT, MLA), etc., is saved.

- In the MultiNA - Ladder folder, ladder data that is automatically created when data was analyzed is saved (extracted to one file for each ladder). Folders are created as follows, based on the conditions where the ladder is acquired, and the ladders are categorized and saved in these folders.

  [Chip ID] (ND058-2, etc.)

  - [Kind of reagent kit] (DNA-500/DNA-1000/DNA-2500/RNA)
    - [Ladder type ID] (Normally, Standard only)
      - [Method of mixing the marker] (Premix/On-chip)
        - [Chip position] (ChipPosition1/ChipPosition2/ChipPosition3/ChipPosition4)

■ Data arrangement and backup

Guidelines for data file size are as follows.

- MLT file: 96 sample data, about 6 MB
- MLA file: 96 sample data, about 3 MB
- LDR file: 1 Ladder data, about 100 KB
- LOG file: 1 kit, 4 microchip data, about 3 KB

Therefore, since analysis for 96 samples requires a hard disk capacity of about 10 MB, periodically arrange and back up data to ensure that sufficient hard disk capacity is available during analysis.

NOTE

The MLT and MLA file for the each analysis should be copied and moved to the same folder.

- MLA files include automatically analyzed results and manually edited results.
- If no MLA file exists, select [Automatic] on the [Reanalysis] menu to re-create the automatic analysis data. However, if manually edited results exist for this file, the data is overwritten.

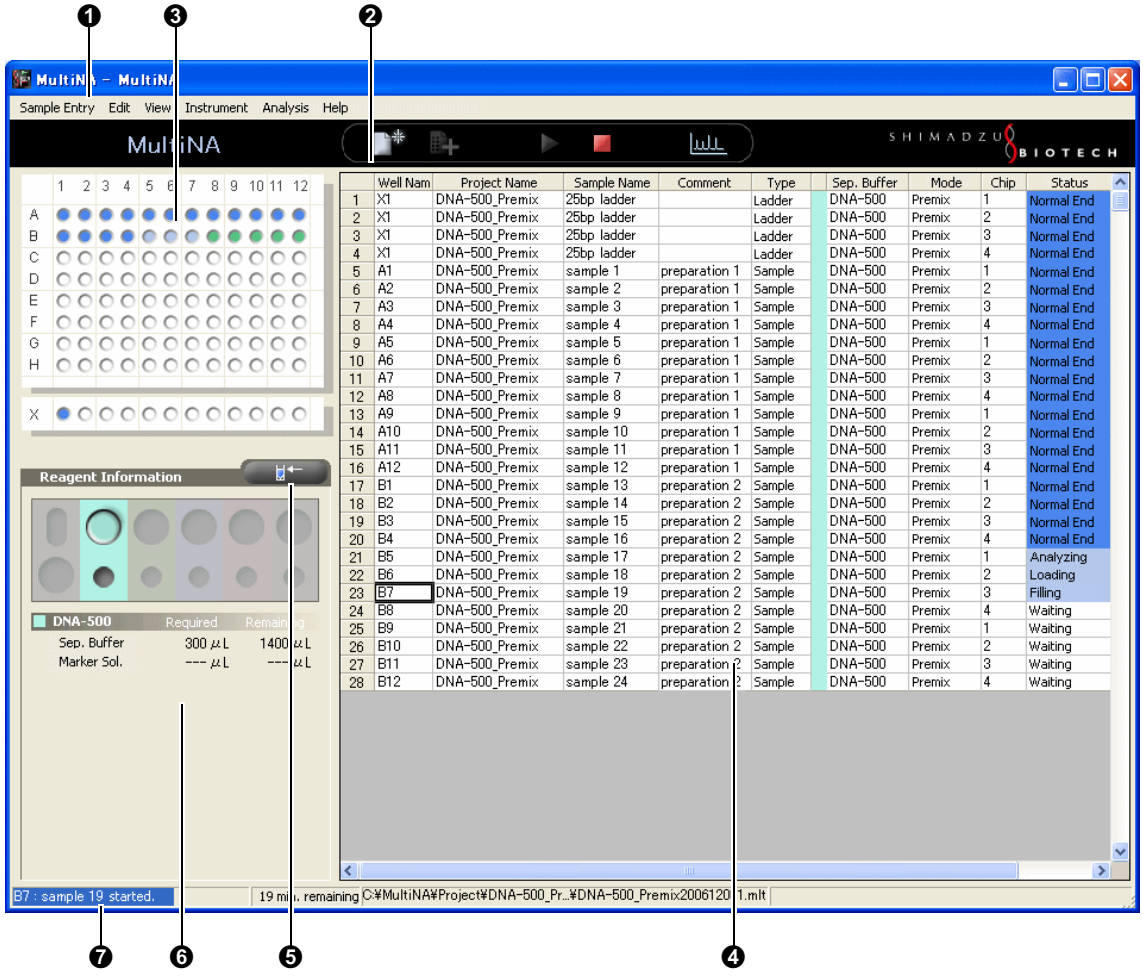
NOTE

Since the hierarchy of the folders into which LDR files are saved is complicated, and a considerable number of files are likely to be created, it is recommended that each ladder folder be compressed, copied and moved.

2 MultiNA Control Software Functions

2.1 Explanation of Windows

Main Window (Displayed during analysis)

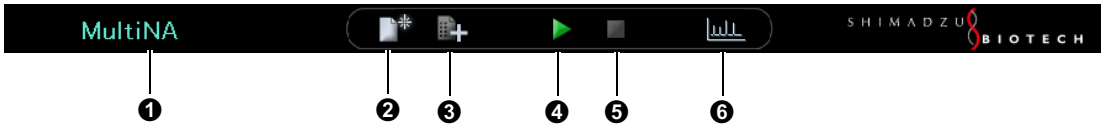


No.	Name	Reference
❶	Pull-down menu	*"2.1.1 Pull-down Menu List" P.84*
❷	Toolbar	*"2.1.2 Toolbar" P.85*
❸	Well Status Display	*"2.1.3 Well Status Display" P.85*
❹	Analysis Schedule List	*"2.1.4 Analysis Schedule List" P.86*
❺	Detect Remaining Reagent Amount button	*"2.1.5 Detect Remaining Reagent Amount Button" P.86*
❻	Reagent Information	*"2.1.6 Reagent Information" P.87*
❼	Status Bar	*"2.1.7 Status Bar" P.87*

2.1.1 Pull-down Menu List

Menu item		Functional overview	Shortcut key	Reference page
Sample Entry	New	Creates a new sample sheet to be analyzed	F5	P.89
	Add	Adds another sample sheet to an open analysis schedule.	F6	P.101
	Exit	Closes the MultiNA Control Software.		P.102
Edit	Project Settings	Create and edit a project	F7	P.103
	Edit Sample Sheet	Edits a sample sheet that is included in the open analysis schedule.		P.107
	Delete Sample Sheet	Deletes a sample sheet from an open analysis schedule		P.108
	Copy	Copy range selected from analysis schedule to clipboard	Ctrl + C	P.109
View	Chip Status	Displays the electropherogram and current voltage for each microchip	F8	P.110
	Data File	Opens the MultiNA Viewer software and displays the selected data file.	F12	P.111
	Log	Display event log		P.111
Instru-ment	Connect	Connect to or disconnect from instrument		P.113
	Options	Optional settings for instrument and displays		P.114
	Chip Management	Opens the [Chip Management] window where, microchips can be selected, deselected or changed.		P.115
	Detect Remaining Reagent Amount	Check residual amounts of separation buffer and marker		P.118
	Move All Axes to Home Position	Moves all of the drive axes to their home position.		P.119
	Check Analysis Performance	Closes the current analysis schedule and opens an inspection schedule.		P.120
	Parts Maintenance	Control replacement parts and execute check program after replacement		P.125
	Wash	Wash (rinse) microchips and probes Execute RNase removal and rinsing		P.126
	Periodic Maintenance	Peristalic pump maintenance when the instrument is out of use for an extended period.		P.126
Analysis	Start	Starts the analysis schedule.	F9	P.127
	Stop	Stop the analysis schedule	F10	P.128
Help	Quick Start Manual	Display the operations flow (Operating Procedure P2 to P3)	F1	P.128
	Instrument Manual	Display the Instrument Manual (Instrument and System)		
	Operation Manual	Display the Instruction Manual (Operating Procedure)		
	About MultiNA	Display the version information of the program		P.129

2.1.2 Toolbar



No.	Explanation
❶	Instrument name: Instrument name entered in option setting ([Instrument] - [Options] - [General]). The text color expresses the state, as follows. • Light blue: Connected to instrument • Pale orange: Disconnected from instrument • Light blue: Analyzing • Red: Error occurred
❷	[New] button ([Sample Entry] - [New])
❸	[Add] button ([Sample Entry] - [Add])
❹	[Start] button ([Analysis] - [Start])
❺	[Stop] button ([Analysis] - [Stop])
❻	[View Data File] button ([View] - [Data File]): Start MultiNA Viewer

2.1.3 Well Status Display

The well status is displayed as follows, depending on the status of the corresponding sample.

- White: Sample not registered
- Green: Sample registered, not yet analyzed
- Light blue: Separation buffer now filling
- Light blue/Blue blinking: Sample loading/Analyzing
- Blue: Analysis has ended normally
- Red: Analysis has ended abnormally

NOTE

When multiple analyses are performed on a sample in the extra sample stand, the status of the sample is displayed. The display becomes red if any one of the multiple analyses ends abnormally.

2.1.4 Analysis Schedule List

Displays the sample information entered in the analysis schedule and the sample status.

The column display can be changed by selecting [Options] on the [Instrument] menu and then selecting the [Column Selection] tab.

Column	Explanation	
Well name	Displays the information of sample registration. For details, see *"2.2 Sample Entry Menu" P.89*	
Project name		
Sample name		
Comment		
Type		
Sep. Buffer		
Mode		
Chip	Displays the chip No. to be used (or used) for the respective samples.	
Status	The status is displayed as follows, depending on the sample analysis state.	
	Waiting	Pre-analysis state before pretreatment of microchip.
	Filling	Pretreatment of microchip in progress (separation buffer filling, etc.).
	Loading	Loading sample onto microchip in progress.
	Analyzing	Analysis of sample in progress (separation in progress).
	Normal End	Analysis has ended normally.
	Abnormal End	An error occurred, or analysis was interrupted.
	Skipped	Because failure occurs to the microchip during analysis performance inspection, analysis was skipped.

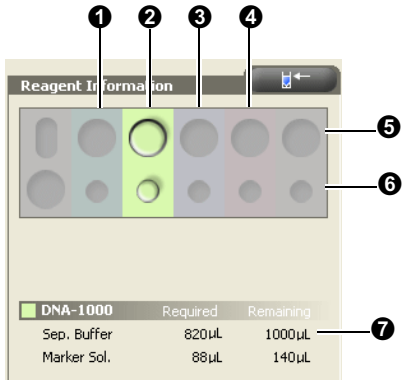
2.1.5 Detect Remaining Reagent Amount Button

Click this button before starting an analysis schedule to determine the residual amount of separation buffer or marker solution in the reagent holder.

This function can also be accessed by selecting [Detect Remaining Reagent Amount] from the [Instrument] pull-down menu).

2.1.6 Reagent Information

This window displays the amounts of separation buffer and marker solution (for on-chip mix only) required for analysis of the unanalyzed samples in the analysis schedule, and the remaining amounts of each reagent (after detection of the residual reagent amounts).



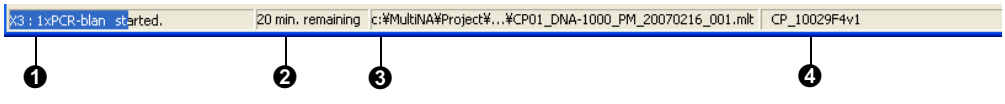
No.	Explanation
❶	DNA-500 kit
❷	DNA-1000 kit
❸	DNA-2500 kit
❹	RNA kit
❺	Displayed color indicates state of residual separation buffer amount. • Gray: Residual amount unconfirmed • Same color as background: Residual amount acceptable • Red: Residual amount insufficient
❻	Displayed color indicates state of residual marker solution amount. • Gray: Residual amount unconfirmed • Same color as background: Residual amount acceptable • Red: Residual amount insufficient
❼	Displays amounts of separation buffer and marker solution required for use (calculated from the sample sheet), and their residual amounts.

NOTE

Required amount displayed in [Reagent Information] includes the following redundant amounts.

- Amount needed for retries (up to 2) if a problem is detected when automatically adding separation buffer to the microchip
- Dead volume of the container
- Minimum bottle amount detectable by a level sensor

2.1.7 Status Bar



No.	Explanation	No.	Explanation
❶	Display of the latest instrument action	❸	Data file name
❷	The remaining time in the entire analysis schedule	❹	Data file comment

2.1.8 Chip Status Window

Select [Chip Status] on the [View] pull-down menu to display the electropherogram and current/voltage graphs for each microchip.

To change the graph display scale, point to [Project Settings] and select [Display] (see *"2.3.1 Project Settings" P.103*).

No.	Explanation
❶	Chip position 1
❷	Chip position 2
❸	Chip position 3
❹	Chip position 4
❺	Voltage status: Displays, in real-time, the voltage monitor value in each of microchip ports 1 to 4.
❻	Current status: Displays, in real-time, the current monitor value in each of microchip ports 1 to 4. (A negative value is indicated in red.)
❼	Electropherogram: Displays, in real-time, the photometry data detected for each microchip.

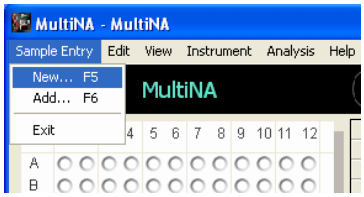
V1.05

The window will automatically switch to the [Chip Status] window when starting the analysis (when starting to fill the first sample).

NOTE

When the Windows color scheme is set to the Windows XP silver or a similar color, the electropherogram may be difficult to view. Use the procedure below to change the electropherogram display color.

2.2 Sample Entry Menu



2.2.1 New

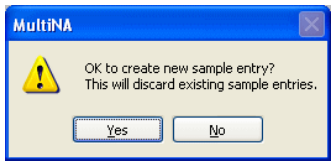
A new sample sheet is created and entered into the analysis schedule.

(Click the [New Entry] button on the toolbar to obtain the same result.)

NOTE

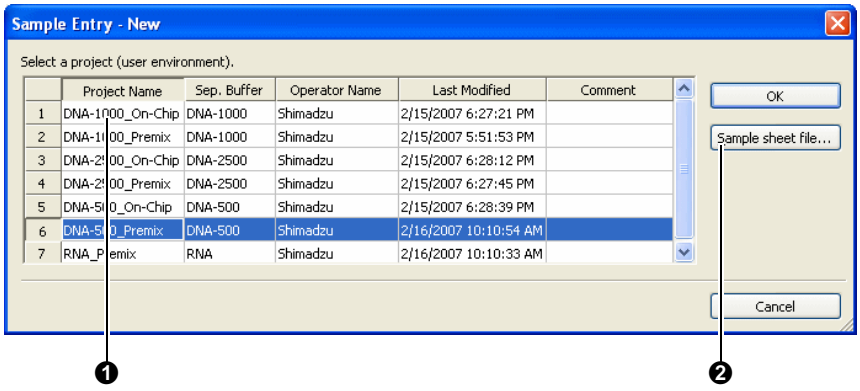
The following message is displayed if the analysis schedule already contains a sample sheet.

Click [Yes] to proceed with the new entry. The content of the existing sample sheet is discarded when the new sample sheet is entered.



1 **Select [New] on the [Sample Entry] pull-down menu.**

The [Sample Entry - New] window is displayed.



No.	Explanation
❶	List of projects already created
❷	Click this button to open a window for selecting the existing sample sheet file. Select the file name and click [Open] to display the sample sheet in the [Sample Sheet] window (for details, see *"Sample Sheet File" P.94*).

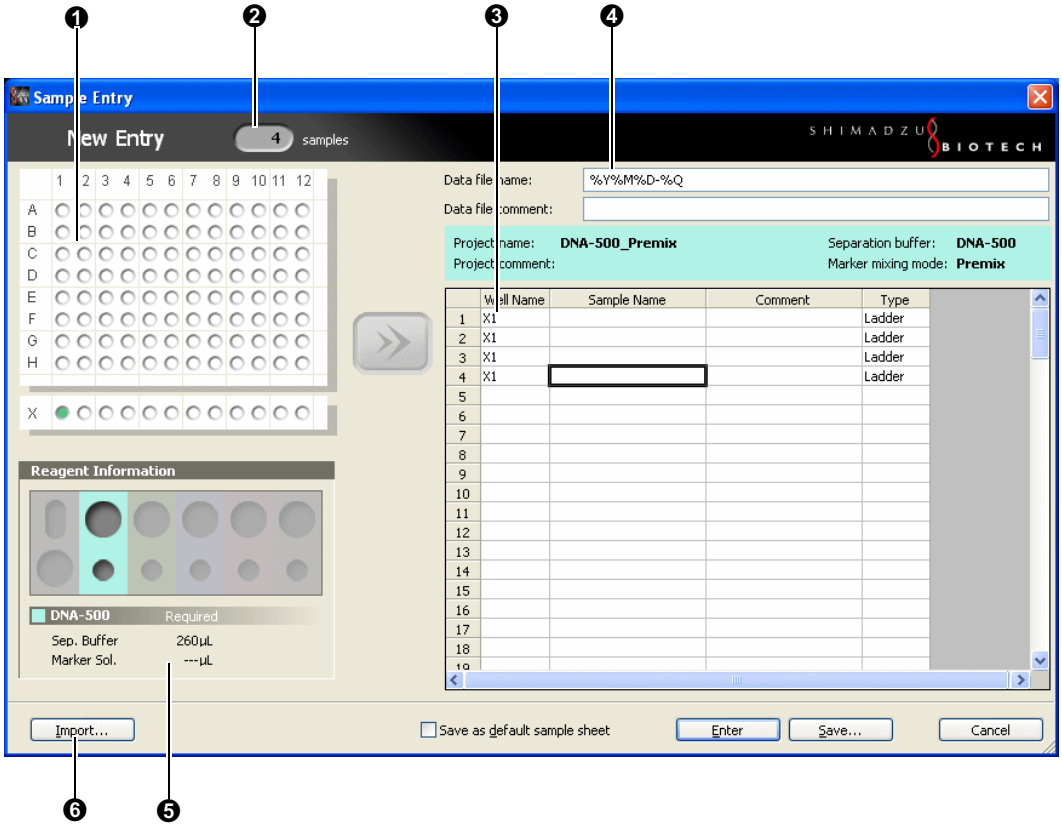
NOTE

If there is no project corresponding to the sample to be analyzed, cancel the window and create a new project.

Select [Project Setting] from the [Edit] pull-down menu to display the [Project list] window. Click the [New] button to create a new project (see *"2.3.1 Project Settings" P.103*).

2 In the [Sample Entry - New] window, select one project and click the [OK] button, or double-click the line.

The [New Entry] window is displayed.



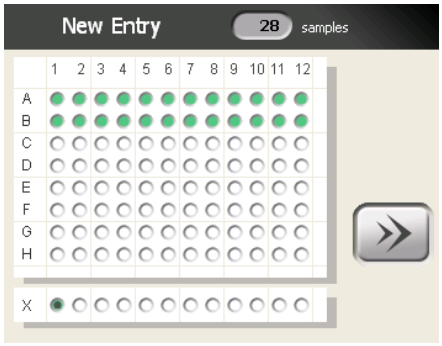
No.	Explanation
❶	Specifies the well position where the ladder solution or sample is located. Either click on the well, or drag the mouse over it to select it.
❷	Displays the number of the samples included in the sample sheet. If it is “0”, you can create a sample sheet by entering the total number of samples. The well positions are entered automatically based on the [Analysis Order] selected in the [Project Settings] window. In [Add Entry] (*"2.2.2 Add" P.101*), this number includes the samples in the current analysis schedule and the samples in the current sample sheet.
❸	When [Auto ladder entry] is selected on the [Edit] - [Project Settings] menu, the ladders are automatically entered at the top of the sample sheet according to the number of microchips being used (*"1.5.7 Project Creation" P.21*).
❹	The data file name is automatically entered according to the [Default data file name] selected on the [Edit] - [Project Settings] menu. The default format is displayed and is continued by clicking the [Enter] button. If the mouse cursor is moved over the data file name column, a tool tip is displayed, enabling reference to the actual full data file path. *V1.05* The file name shown below the column indicates the file name actually created. (Clicking the [Enter] button confirms the file name.)
❺	The required amounts of separation buffer and marker solution (on-chip mix only) are calculated and displayed in the [Reagent Information] window.
❻	Use the [Import] window (*"Import" P.96*) to extract sample information from an existing Excel or CSV file.

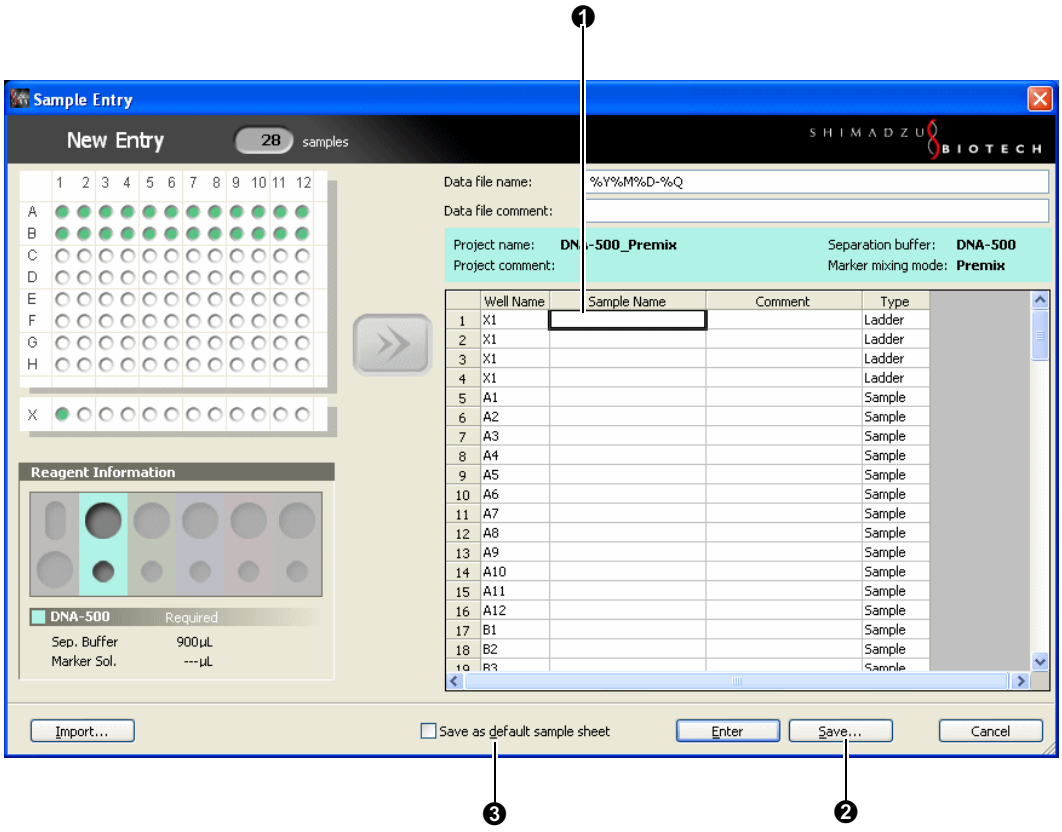
3 After selecting the sample well position, click the » button.

The sample information is entered into the appropriate columns. The content is automatically entered according to the [Project Settings].

Reference

"2.3.1 Project Settings" P.103





No.	Explanation
❶	Enter a [Sample Name] and [Comment]. Select ladder or sample for the [Type].
❷	Click the [Save] button to save the sample sheet content as a sample sheet file. Use the [Sample sheet file] button in either the [Sample Entry - New] window or [Sample Entry - Add] window to open the saved file (*"Sample Sheet File" P.94*).
❸	Clicking [Enter] or [Save] with this check box selected saves the content of the current [Sample Entry] window as the default sample sheet (file name: default.ssh). If [Load default sample sheet while starting up] is selected in the [Project Settings] window, this default sample sheet is displayed when the [Sample Entry] window is opened (*"1.5.7 Project Creation" P.21*).

4 **Click the [Enter] button, to add the set sample sheet to the analysis schedule.**

NOTE

In the following cases, the [Enter] button is not displayed and sample sheets cannot be added to the analysis schedule.

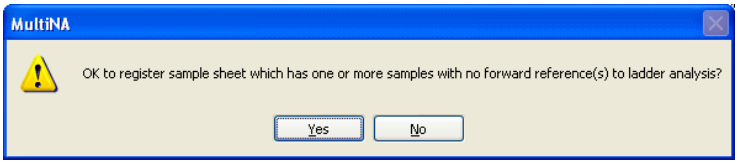
- When the instrument and PC are not connected (see *"1.3 Instrument Startup and Shutdown" P.4*).
- When the selected microchip is not installed or registered (see *"1.5.4 Microchip Registration" P.13*).

NOTE

The following message is displayed when the sample sheet does not contain a ladder analysis.

Usually, click [No], and edit the sample sheet to add the ladder analysis.

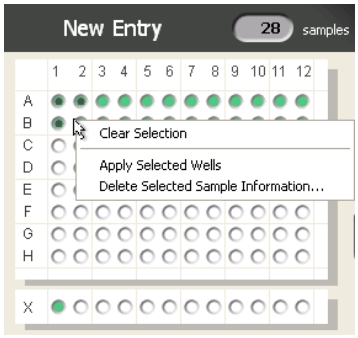
- The analysis schedule can be executed without a ladder analysis, however, the data obtained cannot include size prediction, quantitation, and other analysis results.
- Alternatively, use the [Reanalysis] menu to import a ladder from a separate ladder data file and perform the analysis (). There is a possibility that the analysis results will not satisfy the specifications because the ladder data being used is an “import ladder”.



NOTE

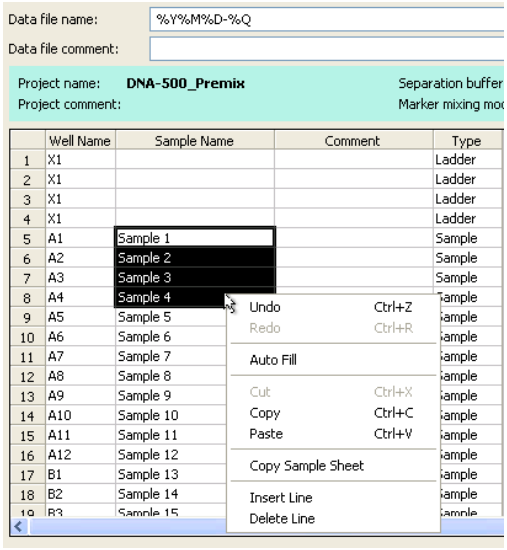
The maximum number of analyses (both ladders and samples) that can be entered into an analysis schedule is 120. The maximum number of analyses that can be entered into a sample sheet is also 120.

■ Well Image Right-Click Menu



Menu	Explanation
Clear Selection	Clears all well selections (black).
Apply Selected Wells	Enters sample information for the selected wells into the sample sheet.
Delete Selected Sample Information	Deletes information about the selected wells from the sample sheet.

■ Sample Sheet Right-Click Menu



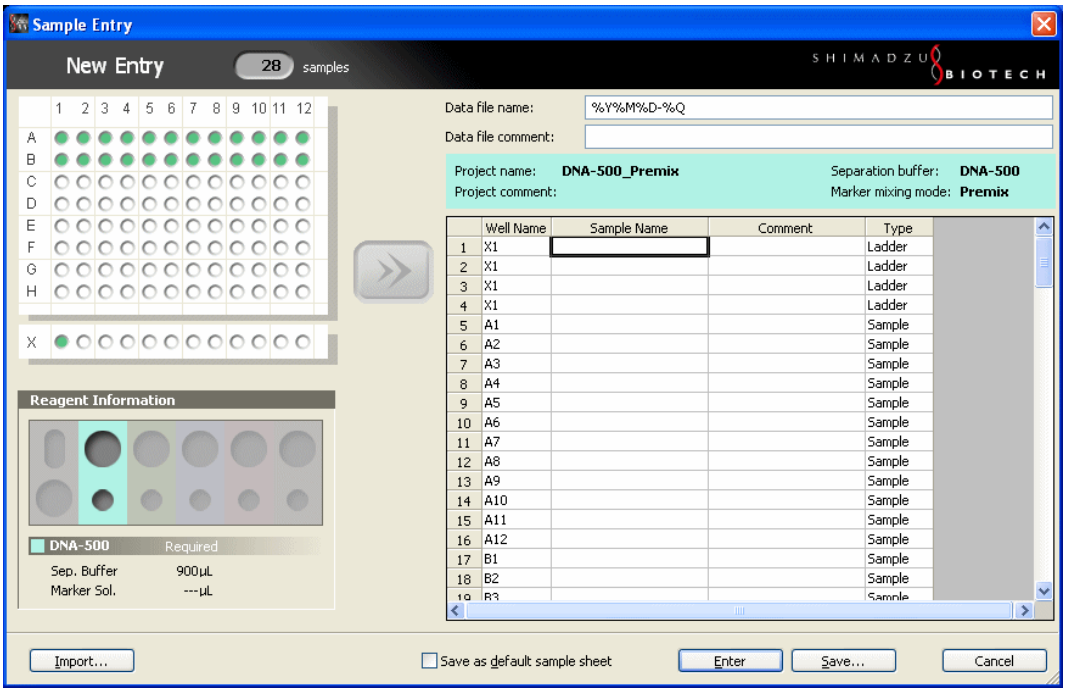
Menu	Explanation
Undo	Restores operation to the content before change.
Redo	Re-performs the just-canceled operation.
Auto Fill	Fills in the content of the selected cells. Enters the sample name, comment, and type, as follows. **Sample name and comment columns:** Enter a name or comment in the top cell. Select the range to be filled and select [Auto Fill]. The cells are filled with the sample name or comment and a sequential numeric value is added at the end of the sample name or comment. If the last character is a number the sequence initiates from that number. (Example: 002 is entered in the top cell, the sequence becomes 002,003,004...) **Type column:** Select the type in the top cell. Select the range to be set and select [Auto Fill], to copy the entered content from the top cell.
Cut	Copies the content of the selected cell to the clipboard, and then delete it.
Copy	Copies the content of the selected cell to the clipboard.
Paste	Pastes the content in the clipboard to the selected range.
Copy Sample Sheet	Copies all information in the sample sheet to the clipboard.
Insert Line	Inserts a line.
Delete Line	Deletes sample information in the selected line.
Hide/Show Columns (It is displayed if right-clicking on the top title line.)	Displays the window used for selecting items to be displayed in the column (see *"2.5.2 Options" P.114*).

■ Sample Sheet File

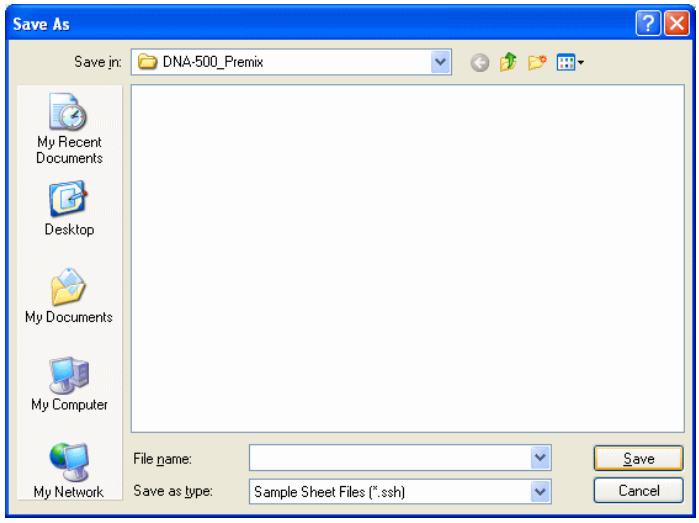
To perform repeated analyses with the same sample composition and save sample entry time, save the content entered in the [Sample Entry] window to a file, then open that file for the subsequent sample sheet entries.

Saving the Sample Sheet

1 **After creating the sample sheet, click the [Save] button.**



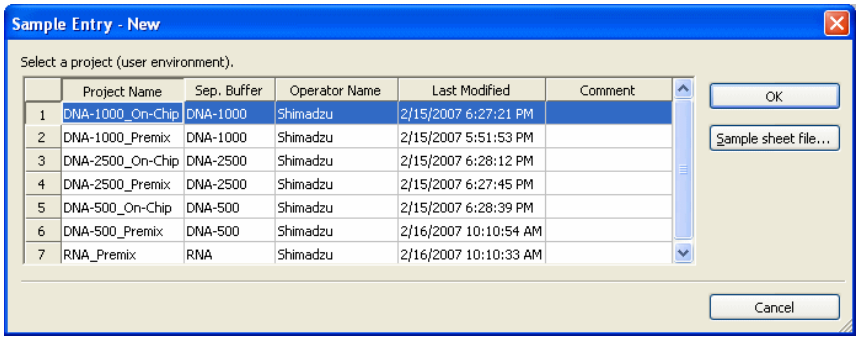
2 **In the [File name] column, enter the name of the file and click the [Save] button.**
A sample sheet file with extension .ssh is saved in the project folder.



Opening the Saved Sample Sheet

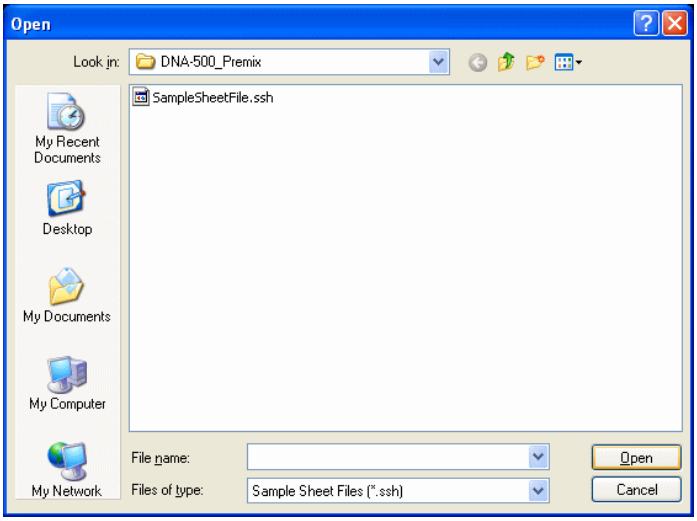
1 **Select [New] on the [Sample Entry] pull-down menu or click the [New Entry] button on the toolbar.**

The [Sample Entry - New] window is displayed.



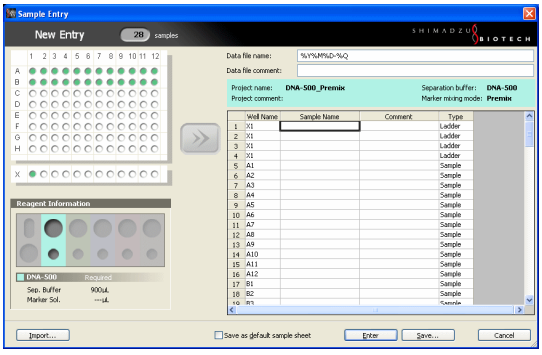
2 **Select the project, and then click the [Sample sheet file] button.**

A window for opening the file in the corresponding project folder is displayed.



3 **Select the sample sheet file, and then click the [Open] button.**

The selected sample sheet file is opened, and the [Sample Entry] window is displayed.



4 **Edit the data as necessary, and then click the [Enter] button to add it to the analysis schedule.**

■ Import

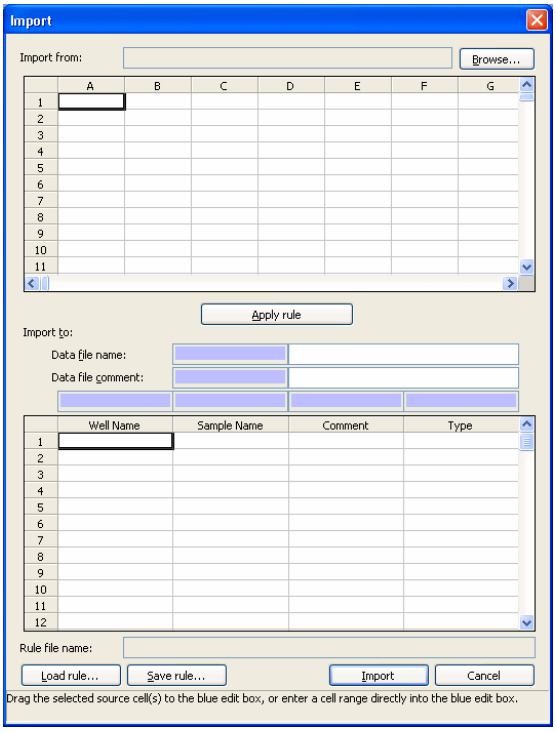
[Import] is a function for converting and inputting sample information from an existing Excel or CSV file to a MultiNA sample sheet.

[Import from]: import source

[Import to]: import destination

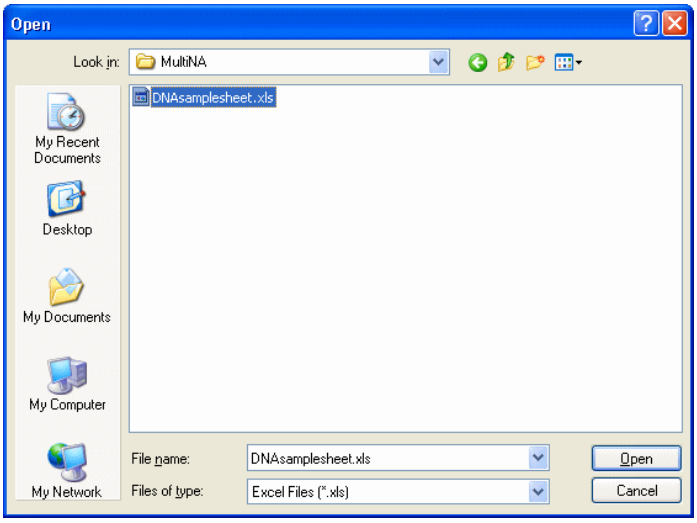
- [Data file name], [Data file comment], [Well name], [Sample name], [Comment] and [Type] information can all be imported.

1 **In the [Sample entry] window, clicking the [Import] button displays the window below.**



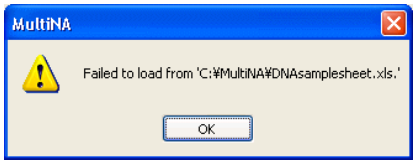
2 Click the [Browse] button to display the [Open] dialog box.

Select an Excel file or CSV file, and click the [Open] button.

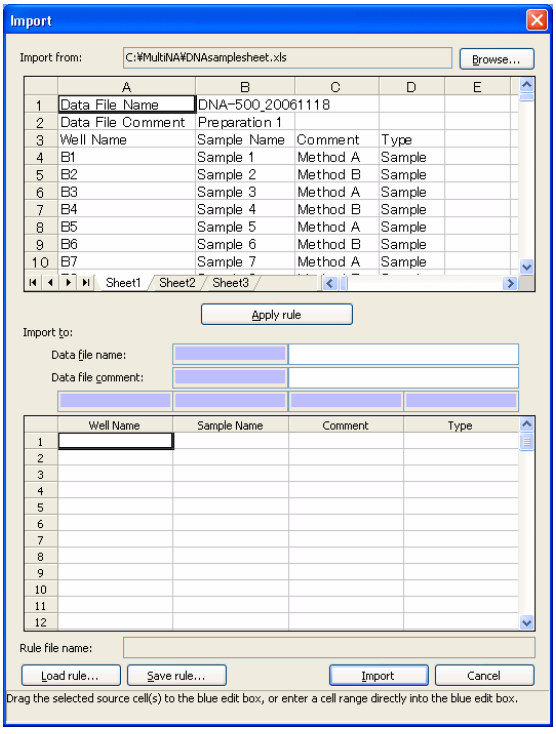


NOTE

f the Excel or CSV file is currently open in another program (Excel, etc.), the following message will be displayed. Close the file in other software, and then reopen the file in the MultiNA Control Software.



The Excel or CSV file is displayed in the [Import from] section of the [Import] window.



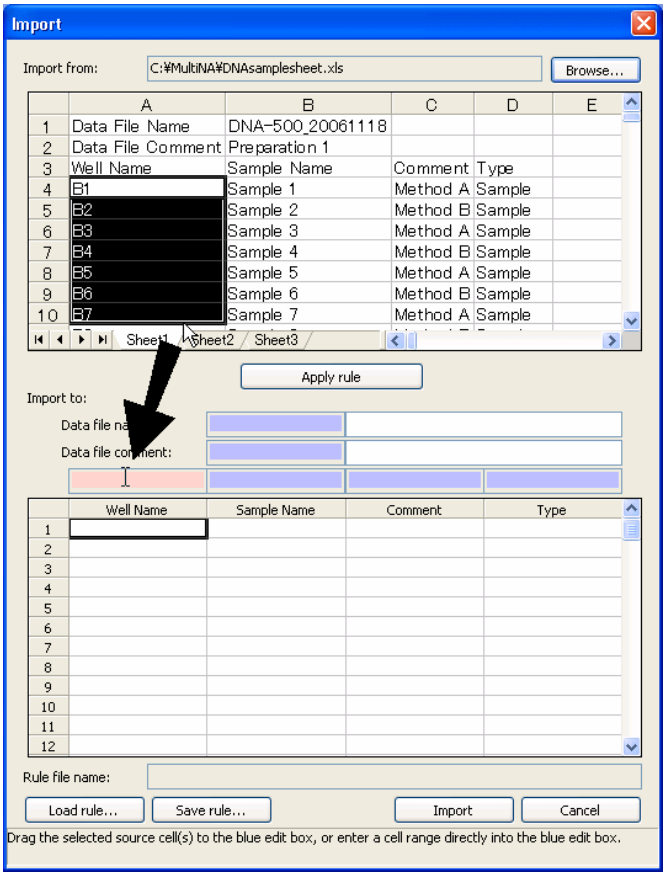
3 Create a rule for converting the [Import from] information to [Import to].

1 Drag the mouse to select the import source cell, and align the mouse cursor with the selected range.

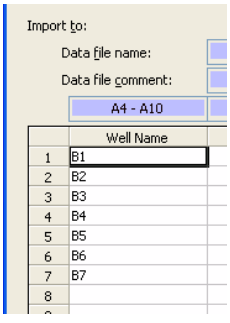
2 With the mouse cursor in arrow state, drop the selection into the blue box above the import destination column.

The box color changes to pink.

The figure below is an example of import source cells A4 to A9 selected, and then dropped into the box above the well name.

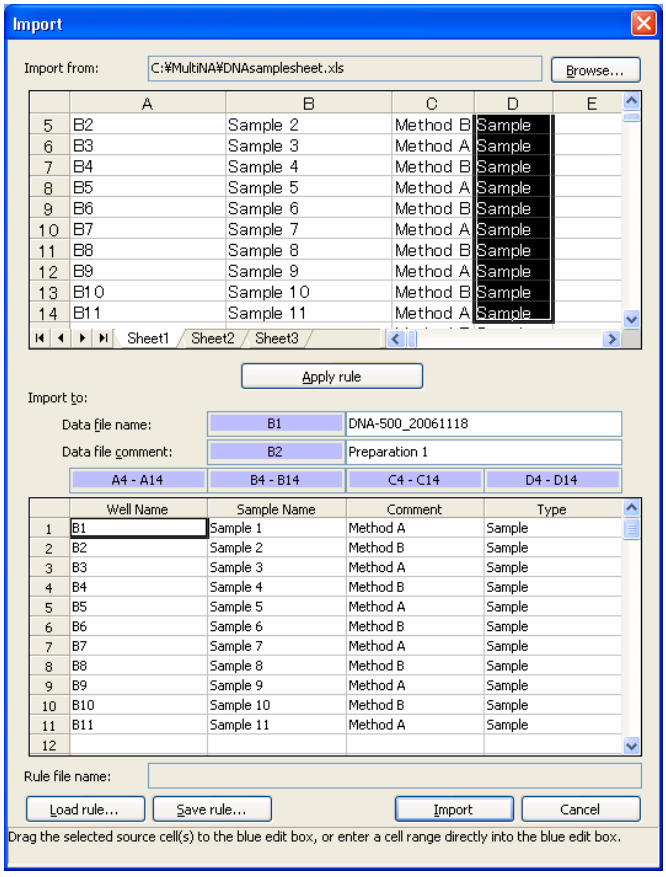


3 The selected import source information (cell range) is entered in the blue boxes above the import destination column. The import source information is copied under these boxes.



Or you can enter the desired import source cell information directly into the import destination blue boxes, and click the [Apply Rule] button in the center, to display the content at the import destination.

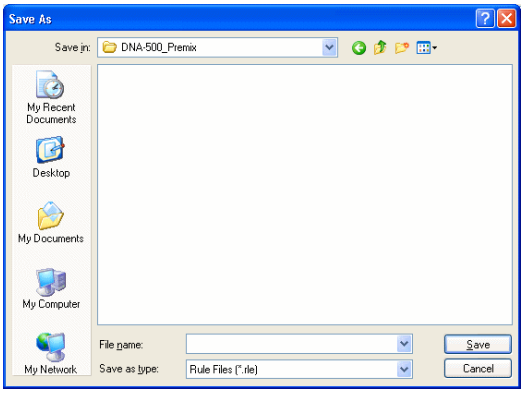
4 **Use the same operation as Step 3 to create a rule for importing another import content (sample name, comment, type, data file name, and data file comment).**



Saving the Rule

If saving the rule, you can easily convert the import source information to the import destination when importing the file with the same format next time.

1 Click [Save rule] to display the [Save As] window.

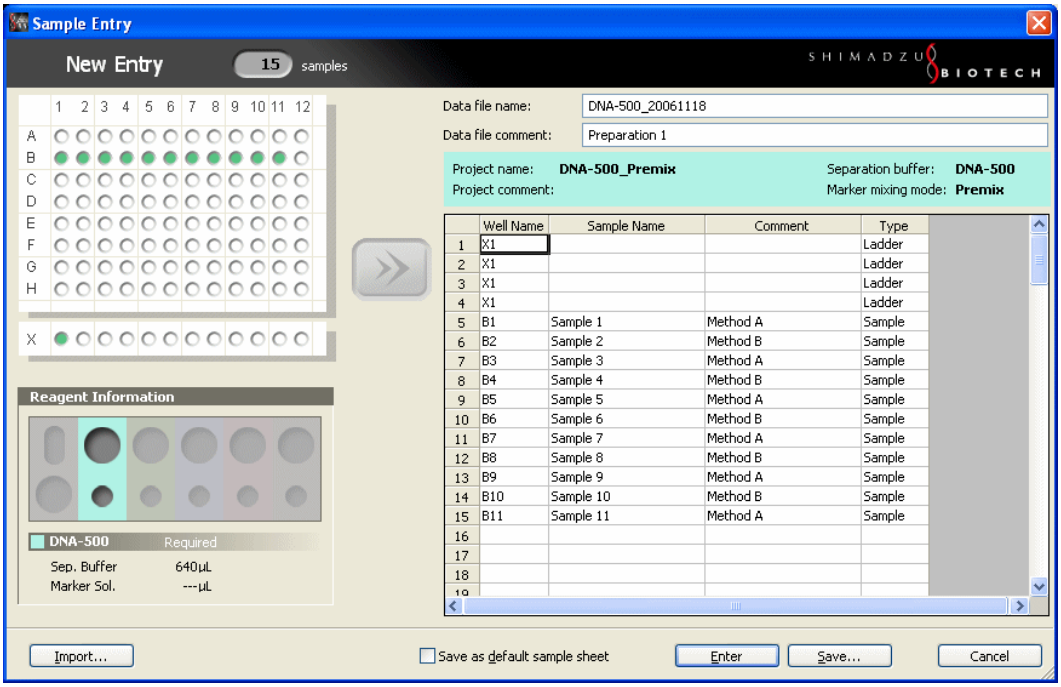


2 Enter a file name and click the [Save] button.
Cell information converted from the import source to import destination is saved as a rule file (extension: .rle).

3 When the next analysis schedule is created, click [Load Rule] in the [Import] window to open the saved rule file.

4 Click the [Browse] button to open the import source file, and the information is automatically input at the import destination.

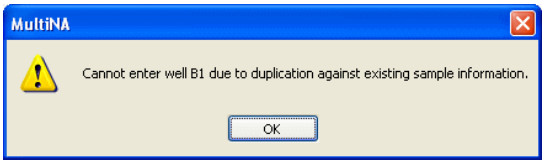
5 **Click the [Import] button to import the content of the import destination to the sample sheet.**



	Well Name	Sample Name	Comment	Type
1	X1			Ladder
2	X1			Ladder
3	X1			Ladder
4	X1			Ladder
5	B1	Sample 1	Method A	Sample
6	B2	Sample 2	Method B	Sample
7	B3	Sample 3	Method A	Sample
8	B4	Sample 4	Method B	Sample
9	B5	Sample 5	Method A	Sample
10	B6	Sample 6	Method B	Sample
11	B7	Sample 7	Method A	Sample
12	B8	Sample 8	Method B	Sample
13	B9	Sample 9	Method A	Sample
14	B10	Sample 10	Method B	Sample
15	B11	Sample 11	Method A	Sample
16				
17				
18				

NOTE

If a sample sheet has already been created before the import, and a well name is duplicated, the information is not imported and the following message is displayed.



2.2.2 Add

Multiple sample sheets can be individually added to an analysis schedule.

The [Add Entry] button is enabled on the analysis schedule toolbar after the first (new) sample sheet is entered in the analysis schedule.

NOTE

If a sample sheet is added to an analysis schedule using a project name that is already entered in the analysis schedule, all of the data will be stored in the same data file.

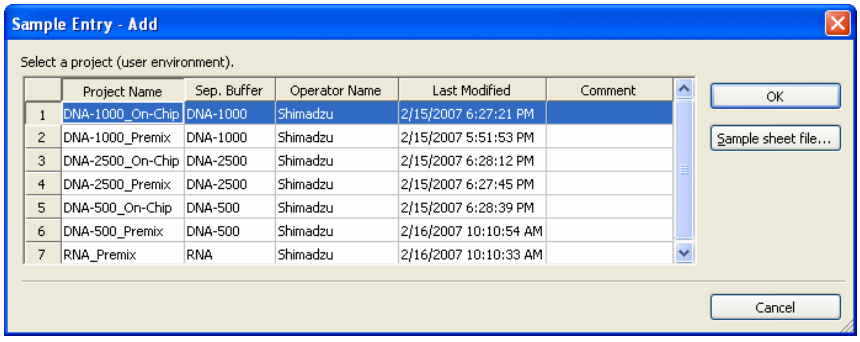
Typically, data from separate sample sheets is stored in separate data files. A separate data file is created even when a sequence-number format has been applied to the data file name.

Reference

"Fig.1-14 Work Flow from Project Creation to Data Acquisition" P.19

1 **Select [Add] on the [Sample Entry] pull-down menu.**

The [Sample Entry - Add] window is displayed.

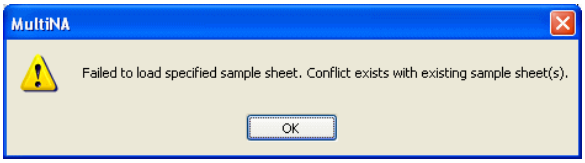


2 **Select a project in the window above, and click the [OK] button.**

Or click the [Sample sheet file] button to open an existing sample sheet.

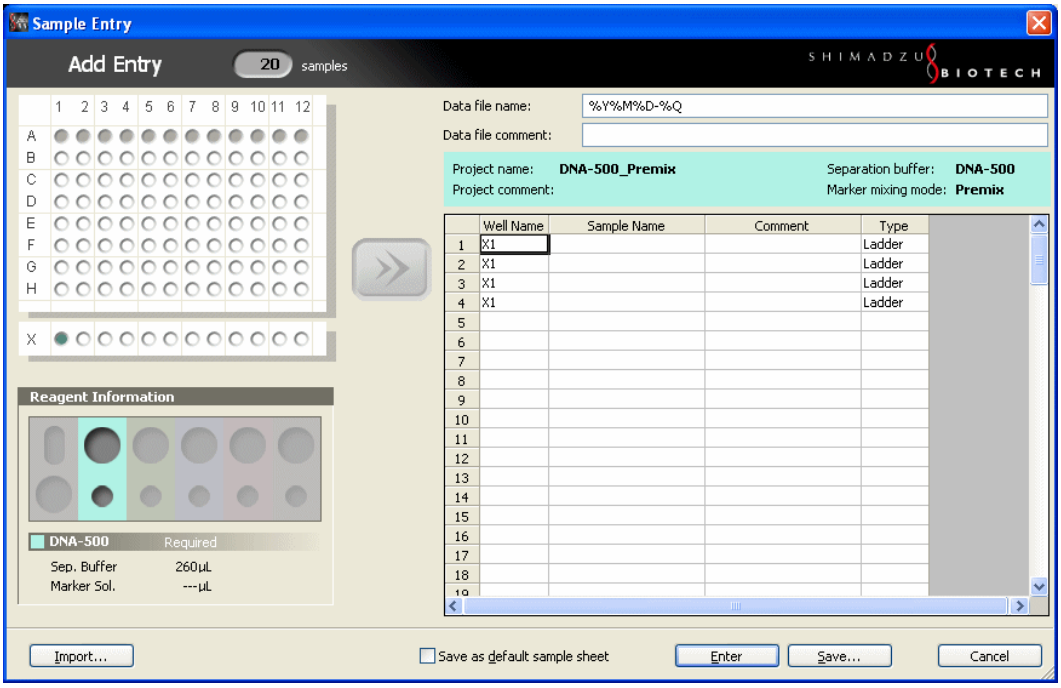
NOTE

If a well in the existing sample sheet is already being used by the samples in the analysis schedule, the sample sheet file fails to load and the following error message is displayed.



3 **The [Add Entry] window is displayed.**

Wells already registered in the analysis schedule are grayed out.



4 **Select any open well, and use the same procedure as new entries to insert the sample.**

The additional entry is inserted next to the existing analysis schedule.

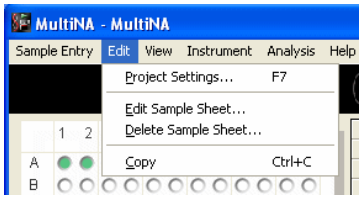
2.2.3 Exit

The MultiNA Control Software is closed.

NOTE

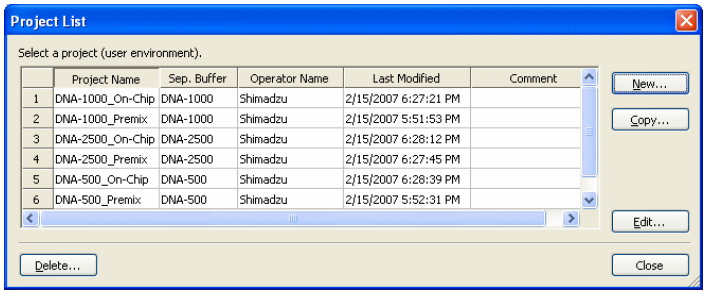
This menu item cannot be selected during analysis, cleaning, or other instrument operation.

2.3 Edit Menu



2.3.1 Project Settings

Create new projects or edit existing projects in the [Project List] window.



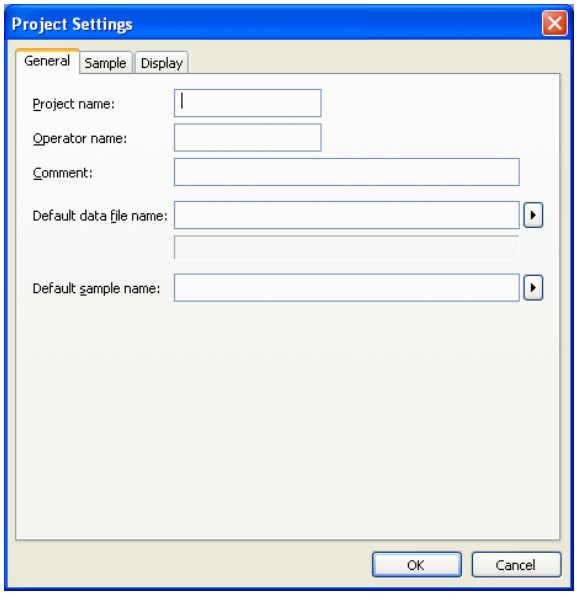
- [New] button: Creates a new project. *(P.103)*
- [Copy] button: Copy and edit an existing project. *(P.105)*
- [Edit] button: Edit an existing project. *(P.105)*
- [Delete] button: Delete an existing project. *(P.106)*

■ New

Create a new project. Enter and select the necessary items on the various tabs shown below, and then click [OK].

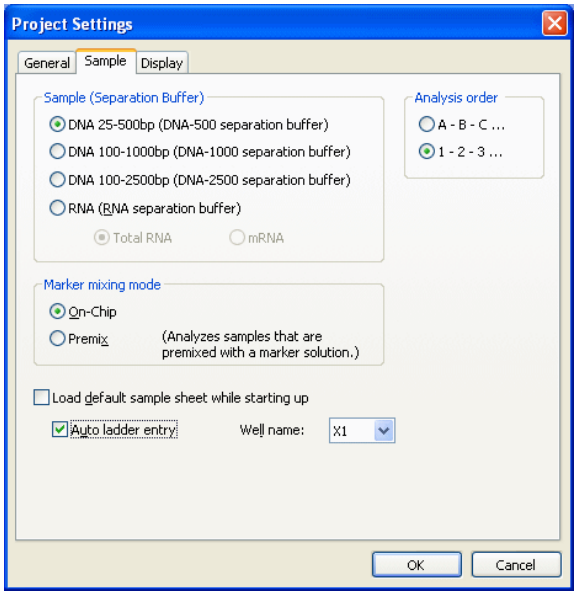
1 **[General] tab**

Enter the displayed items in [General] tab. Refer to *"1.5.7 Project Creation"*, step*"3" P.22* for more details.



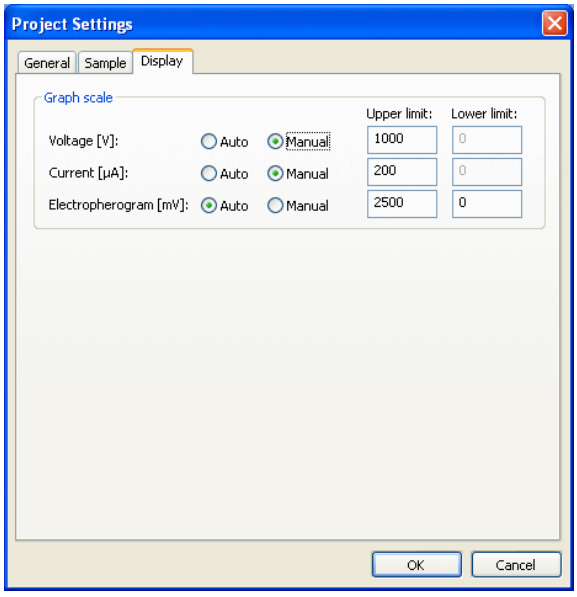
2 [Sample] tab

Select the displayed items in the [Sample] tab. Refer to *"1.5.7 Project Creation"*, step*"4" P.23* for more details.



3 [Display] tab

Select each item in the following window.



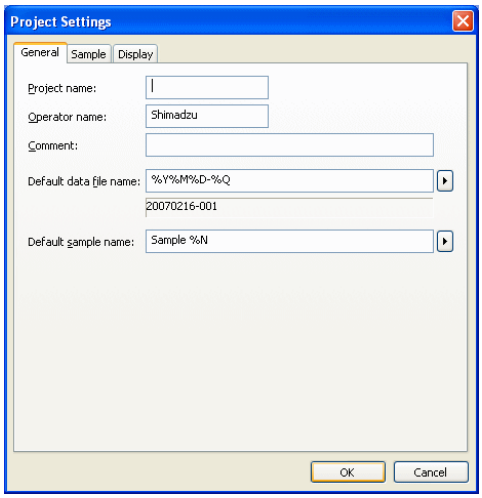
Item	Explanation
Graph scale	Configure the graph scales described in *"2.1.8 Chip Status Window" P.88*. The possible manual selection ranges are as follows. Voltage: 1 to 1500 (V) Current: 1 to 300 (µA) Electropherogram: 0 to 2500 (mV)

■ Copy

Copy and edit an existing project.

1 **Select an existing project in the [Project list] window *(P.103)*, and click the [Copy] button.**

A window is displayed with a blank project name and all of the other items copied from the existing project.



2 **Enter a new project name.**

3 **After completing entry and selection for the necessary items on each tab, click the [OK] button.**

The project is saved.

■ Edit

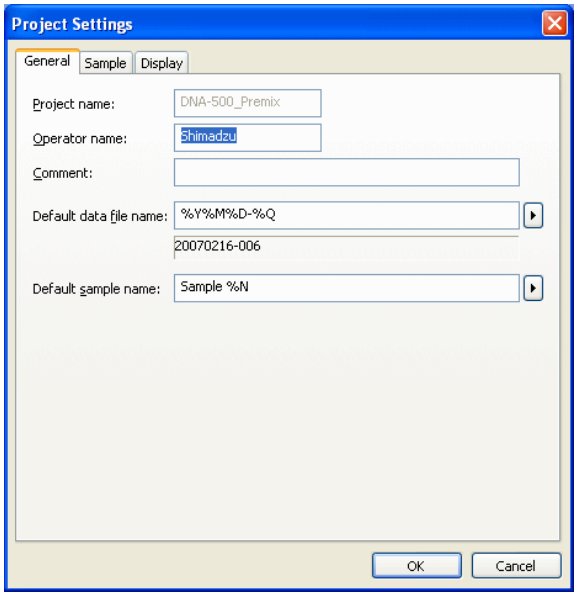
Edit the content of an existing project.

NOTE

The project name cannot be changed. To change the project name, [Copy] the project and save it with a different name.

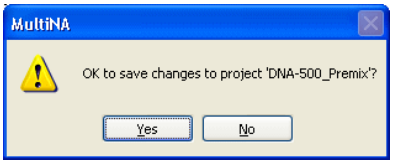
1 **Select an existing project in the [Project list] window *(P.103)*, and click the [Edit] button.**

2 **Change the necessary items in the [Project Settings] window.**



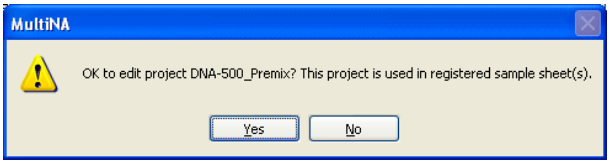
3 **Click the [OK] button to display the following message.**

Click [Yes] to save the changed content.



NOTE

The following message is displayed when an attempt is made to edit a project that is currently associated with samples entered in the analysis schedule. Select [Yes] to open the [Edit] window. Some items such as, type of separation buffer, will be disabled and cannot be changed.

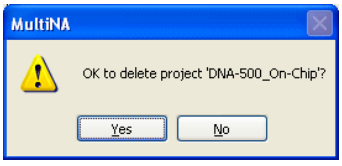


■ Delete

Delete an existing project.

1 **Select an existing project in the [Project list] window *(P.103)*, and click the [Delete] button.**

The following confirmation message is displayed.

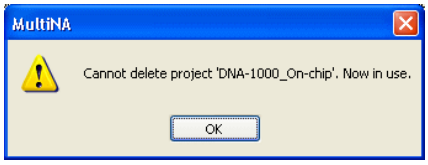


2 **Verify the project name, and click [Yes].**

The project is deleted.

NOTE

A project cannot be deleted if it is associated with a sample in the analysis schedule. The following message is displayed.

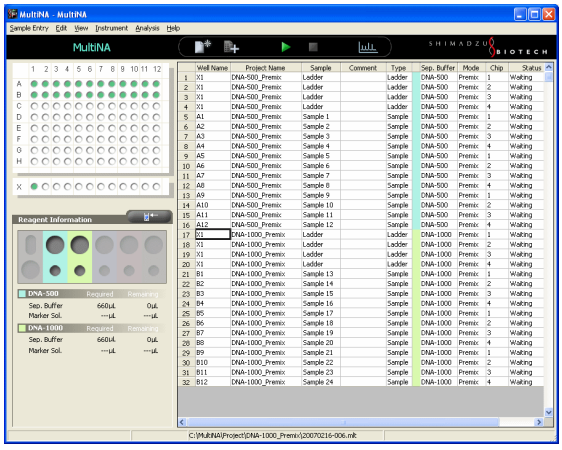


2.3.2 Edit Sample Sheet

Use this menu to edit sample sheet that is entered in analysis schedule.

1 **Click any one cell in the in the analysis schedule for the sample sheet to be edited.**

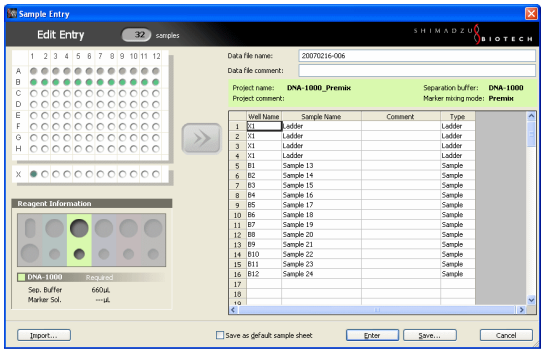
If only one sample sheet has been added to the analysis schedule, this procedure is not required.



2 **Select [Edit Sample Sheet] on the [Edit] pull-down menu.**

(You can also right-click on the sample sheet to bring up a menu, then select [Edit Sample Sheet].)
The [Sample Entry] window is displayed.

3 **Edit the sample sheet in the [Sample Entry] window.**



4 **After editing the sample sheet file, click the [Enter] button.**

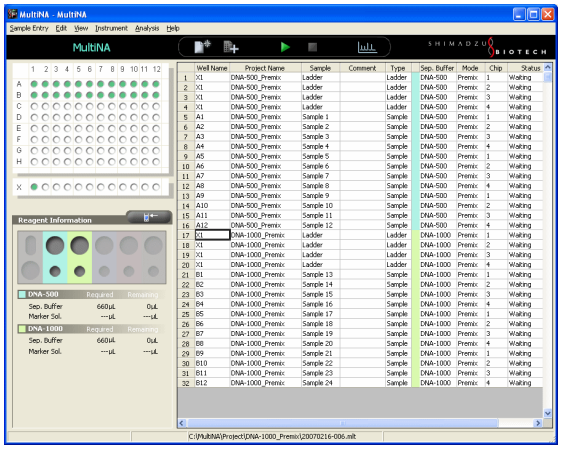
The sample sheet is re-entered into the analysis schedule.

2.3.3 Delete Sample Sheet

Use this menu to delete a sample sheet from the analysis schedule.

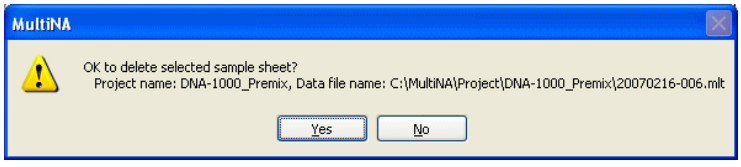
1 **Click any one cell in the in the analysis schedule for the sample sheet to be deleted.**

If only one sample sheet has been added to the analysis schedule, this procedure is not required.



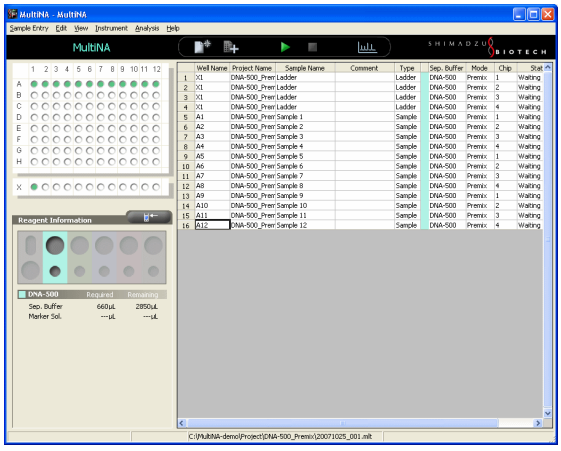
2 **Select [Delete Sample Sheet] on the [Edit] pull-down menu.**

Or right-click on the sample sheet and select [Delete Sample Sheet].
The following confirmation message is displayed.



3 **Check the project name and data file name, and click [Yes].**

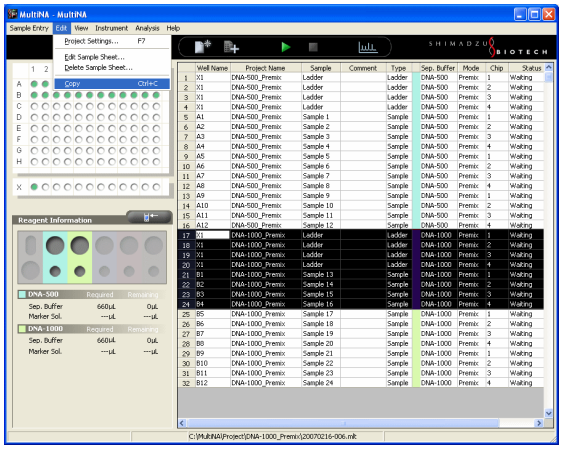
The sample sheet is deleted, and the analysis schedule in the main window is changed.



2.3.4 Copy

Use this menu to copy the content (text) selected in the analysis schedule to the clipboard.

1 **Drag the mouse over the range to be copied in the analysis schedule to select it.**

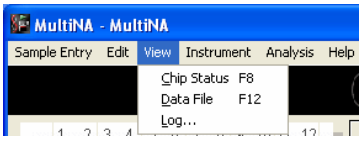


2 **Select [Copy] on the [Edit] pull-down menu.**

The selected range is copied to the clipboard with partition tabs.

3 **Paste the content into another application (Excel, etc.).**

2.4 View Menu



2.4.1 Chip Status

The [Chip Status] window displays the electropherogram and current/voltage graphs for each microchip.

Reference

"2.1.8 Chip Status Window" P.88

To return the display to [Reagent Information] *(P.83)*, either select [Chip Status] on the [View] menu, or press the [F8] key.

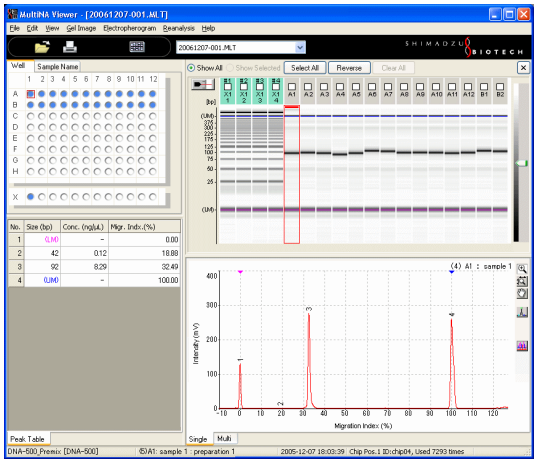
V1.05

The window will automatically switch to the [Chip Status] window when starting the analysis (when starting to fill the first sample).

2.4.2 Data File

Start the MultiNA Viewer to display the analyzed data.

(Or you can click the [View Data File] button on the right end of the toolbar.)



NOTE

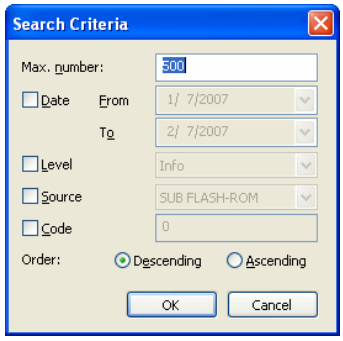
If the analysis schedule contains more than one sample sheet, only the data file for the selected sample sheet is displayed when the MultiNA Viewer is opened.

2.4.3 Log

Follow the procedures below to displays the instrument events recorded in the log.

1 **Select [Log] on the [View] pull-down menu.**

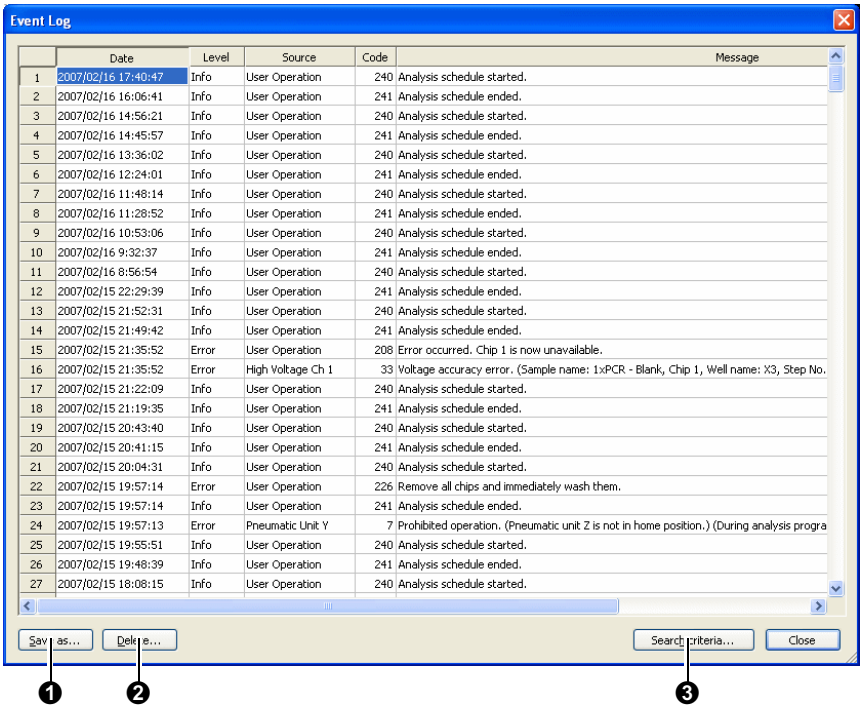
The [Search Criteria] window is displayed.



2 **To narrow the search criteria, enter or select the necessary conditions, and click [OK].**

Search criteria	Explanation
Max. number	Indicates the number of cases to be displayed. A maximum of 5000 cases can be entered.
Date (From/To)	Specifies the event generation range.
Level	Specifies the event level (Info, Warning, Error, Fatal).
Source	Specifies the event source.
Code	Specifies the event code.

An [Event Log] window is displayed.

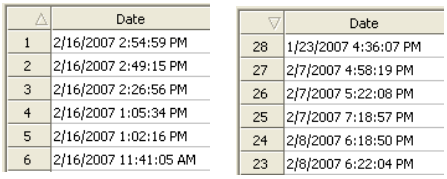


NOTE

Levels in the event log are classified as follows.

Level	Explanation
Info	Event (information) generated in the instrument (Analysis start or end, etc.).
Warning	Event that will not impede the continuous analysis but could have an effect on data. See *-Instrument and System- "Warning"*.
Error	Event that disables continuation of automatic analysis. See *-Instrument and System- "Error"*.
Fatal	Event that requires an instrument status check and could lead to a situation where continuation of operations in this condition is either impossible or dangerous. See *-Instrument and System- "Fatal"*.

- To rearrange the list displayed in the [Event Log] window, click the header of [Date] or [Level].

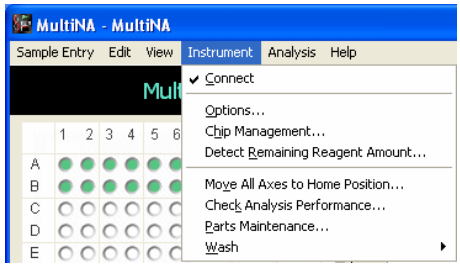


- Use the mouse to select the range, then right-click and select [Copy] to copy the selected range to the clipboard.In the displayed [Event Log] window, you can click the header of [Date] or [Level], etc., to rearrange the list.

[Event Log] Window Button

No	Button	Explanation
❶	Save as	Use this button to save the displayed content to a CSV file. Click the button, and then enter a name in the file save dialogue box.
❷	Delete	Use this button to delete the selected event log line. Click the button, and select [Yes] in the displayed confirmation dialogue box to delete the content. (The content is deleted not only from the display, but also from the file where the event log is saved.)
❸	Search criteria	The event log search window is re-displayed. Change the search criteria and perform search.

2.5 Instrument Menu



2.5.1 Connect

Communication between the MultiNA instrument and the PC is connected or disconnected with this menu.

- When connected, the check box of this menu is selected.
- During analysis, this menu is disabled and cannot be selected.
- The characters in the [Instrument name] display (“MultiNA” in the figure below) on the left end of the toolbar are light blue when connected and pale orange when disconnected.

NOTE

Select the [Connect at start up] check box to automatically establish communication between the PC and the instrument when the MultiNA software is opened. Refer to *"2.5.2 Options" P.114*.

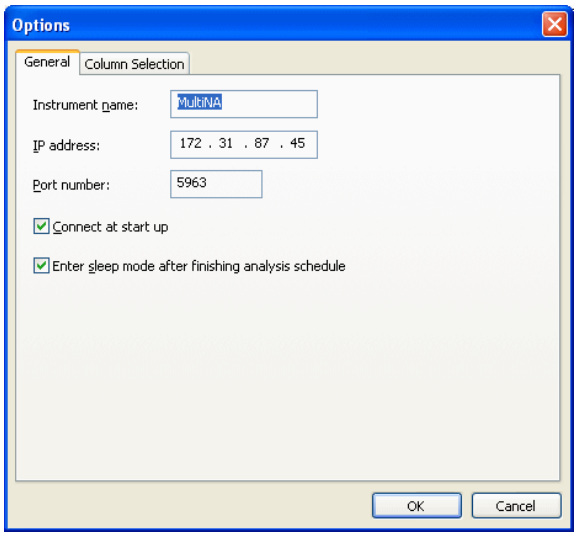
Automatic connection is not performed in the following cases, Verify that the instrument power is ON and that the LAN cable is properly connected, then use this menu to establish communication.

- Instrument was not turned ON when the software was started up
- [Connect at start up] check box is cleared
- LAN cable is not correctly connected
- Communication was canceled from the instrument for some reason

2.5.2 Options

The instrument options and display items common to all projects are set with this menu.

■ [General] tab

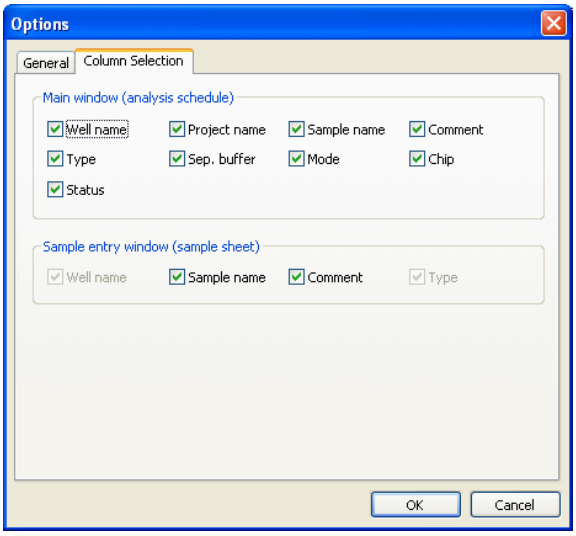


Item	Explanation
Instrument name	Instrument name displayed on left end of toolbar. (Up to 16 characters.)
IP address	Instrument IP address (entered during installation)
Port number	Port number used for socket communication (cannot be changed)
Connect at start up	Determines whether automatic connection to the instrument is established when the software is opened.
Enter sleep mode after finishing analysis schedule	Enter sleep mode after finishing analysis schedule - Determines whether the instrument enters the sleep mode after completion of the analysis schedule. If selected: • The instrument enters into the sleep mode when all instrument operations (analysis, washing, and all other operations) are complete, and there are no PC mouse or keyboard operations for a period of at least one hour. • In the sleep mode, all motor power, chip stage temperature adjustment, and fan operation stop, to reduce power consumption. • Operating the PC mouse or keyboard cancels the sleep mode. • It takes up to 5 minutes for the chip stage to reach the designated temperature after the sleep mode is canceled. (If analysis is started during this warm-up period, actual analysis will not begin until the chip stage reaches the designated temperature.)

NOTE

Do NOT change the IP address from the address set during the instrument installation. If any change is necessary, contact your Shimadzu service representative.

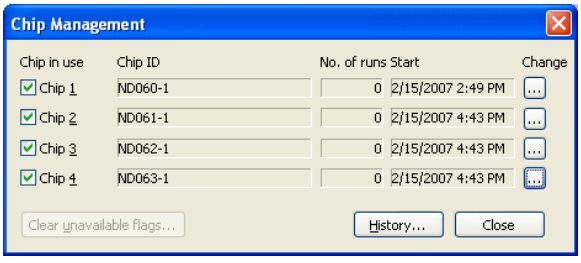
■ [Column Selection] tab



Item	Explanation
Main window (analysis schedule)	Determines the columns to be displayed in the analysis schedule.
Sample entry window (sample sheet)	Determines the columns to be displayed in the sample sheet. (The well names and types are essential for sample entry and cannot be changed.)

2.5.3 Chip Management

Follow the procedures below to control information for microchips installed in the instrument.



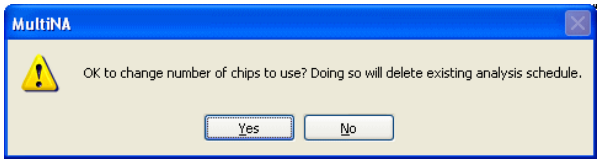
- The chip No. at [Chip in use] shows the chip position in the instrument. (Chip 1 is farthest back, followed in order by chip 2 and chip 3, with chip 4 in front.)
- Removing the [Chip in use] check box prevents use of that microchip for analysis.

NOTE

Changing the chip to be used after a sample has been entered will invalidate the analysis schedule. The following warning message is displayed.

To delete the analysis schedule and enter a new sample sheet, select [Yes].

To use the same analysis schedule, select [No], and use the procedure below to enter the sample into the analysis schedule.



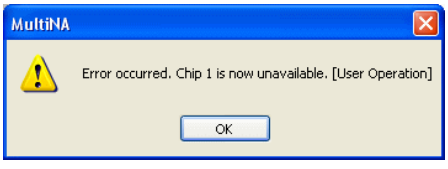
1. Click [No] in the message dialog box.
   The [Chip management] window is displayed.
2. Click the [Close] button, and close the [Chip management] window.
   Returns to the main window.
3. Select [Edit Sample Sheet] on the [Edit] pull-down menu.
   Or right-click on the analysis schedule list, and select [Edit Sample Sheet] on the displayed menu.
4. Click the [Save] button in the [Sample Entry] window.
   Name the sample sheet, and save it.
5. Click the [Cancel] button in the [Sample Entry] window to close the window.
6. Select [Chip Management] on the [Instrument] pull-down menu, to change the microchip to be used.
   If the warning message in the figure above is displayed, click [Yes].
7. After changing the microchip to be used, select [New] on the [Sample Entry] pull-down menu.
8. In the [Sample Entry - New] window, select a project, and click the [Sample sheet file] button.
9. Select the sample sheet given a name and saved in Step 4, and load it.
10. Click the [Enter] button in the [Sample Entry] window to enter the sample into the analysis schedule.

Reference

"Sample Sheet File" P.94

■ [Clear unavailable flag]

A microchip judged during analysis to be unavailable cannot be used any further and the following message is displayed. Manually clean the microchip, to eliminate clogging or other problems.
Refer to *-Instrument and System- "6.2.7 Microchip Reservoirs"*.



- If using a previously cleaned microchip, click the [Clear unavailable flag] button in the [Chip Management] window, to allow the chip to be used.

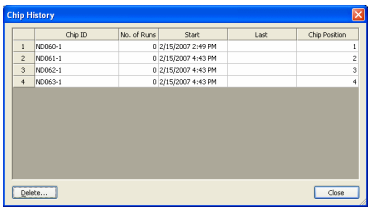
■ [History]

Click the [History] button to display the history of the microchip being used.

The following items are included in the history file.

- [Chip ID]
- [No. of runs]
- [Start] (date of initial use)
- [Last] (date of last use)
- [Chip position]

Click the [Delete] button to delete the chip history. Histories of microchips currently in use cannot be deleted.



■ Exchanging the microchip

Use the [Chip Management] window to control microchip exchange or removal.

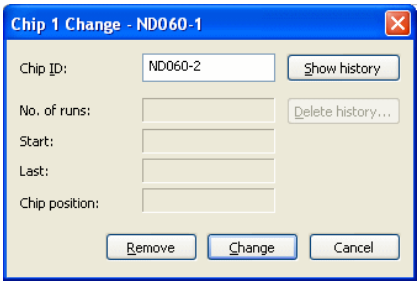
To exchange the microchip

1 **Click the [...] [Change] button next to the microchip to be exchanged.**

The [Chip x Change] window is displayed.

2 **Enter the microchip serial number (chip ID) in the [Chip x Change] window, and click the [Change] button.**

If the microchip was previously used, the history of its usage is displayed.

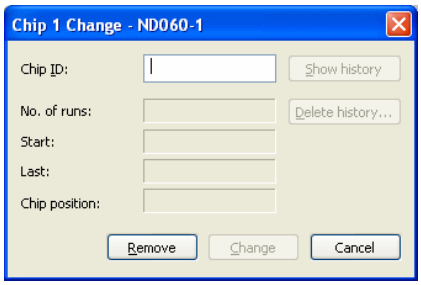


To remove the microchip

The following describes the procedure for removing a microchip, using chip position 1 for the example.

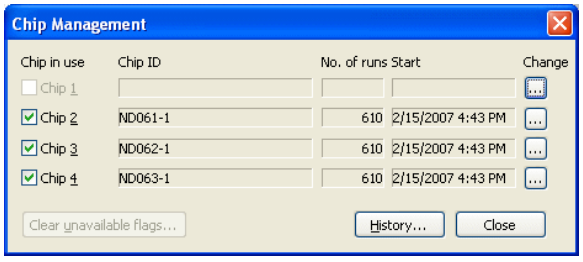
1 **Click the [...] [Change] button next to chip 1, in the [Chip Management] window.**

The [Chip 1 Change] window is displayed.



2 **Click the [Remove] button in the [Chip 1 Change] window.**

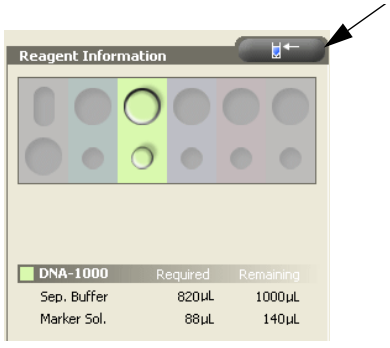
Chip 1 is grayed out, and the check box is cleared.



3 **Click the [Close] button.**

2.5.4 Detect Remaining Reagent Amount

Follow the procedures below to check the residual amount of separation buffer or marker solution. Or press the button on the top right corner of the [Reagent Information] window.



- The residual reagent amount is calculated by inserting a sample probe into the reagent bottle to detect the reagent liquid level.
- The residual reagent amount is automatically checked when an analysis sequence is started. This function can also be used to check the residual reagent amount without starting the analysis sequence.

1 **Place the reagents and samples in the appropriate positions and close the instrument cover.**

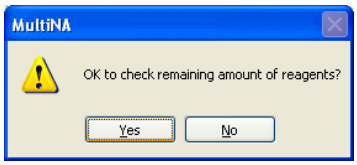
2 **Verify that the analysis schedule has been entered.**

3 **Select [Detect Remaining Reagent Amount] from the [Instrument] pull-down menu.**

Or click the button on the upper right corner of the [Reagent Information] window.

4 **If the following message is displayed, click [Yes].**

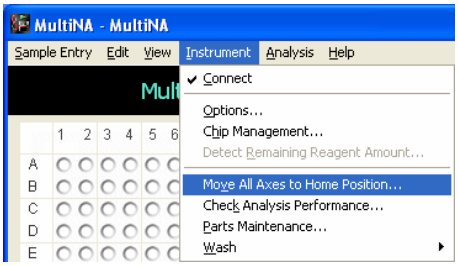
The check is started.



5 **The residual amount of reagent is displayed in the [Reagent Information] window.**

If the amount of reagent is insufficient, the location for the separation buffer or marker solution is displayed in red. Then add that reagent.

2.5.5 Move All Axes to Home Position



Use this menu to return the instrument drives (autosampler, pneumatic unit, and syringe pump) to the home position.

- The drives automatically return to the home position when analysis is completed normally. If an error occurs during operation the drives may stop without returning to the home position. Move all axes to the home position before performing other operations such as, micro chip removal, reagent replenishment, etc.
- This function is also used for parts replacement. Refer to Section *-Instrument and System- "6.4 Part Replacement and Inspection"*.

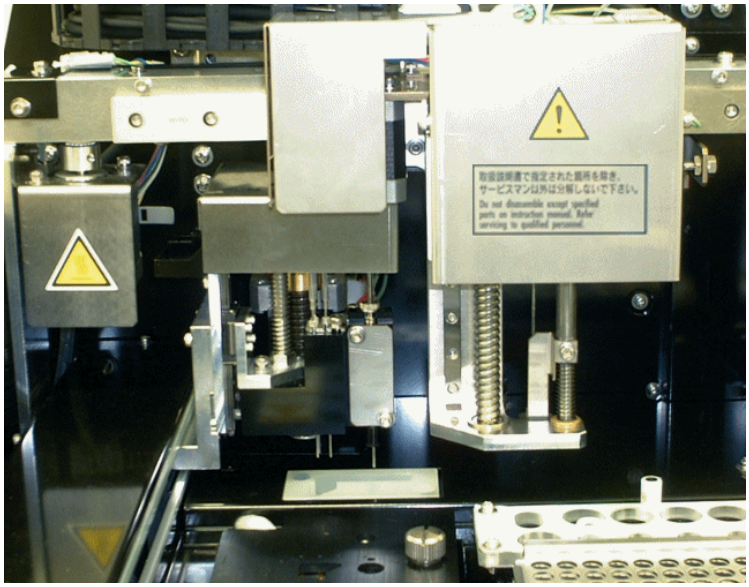


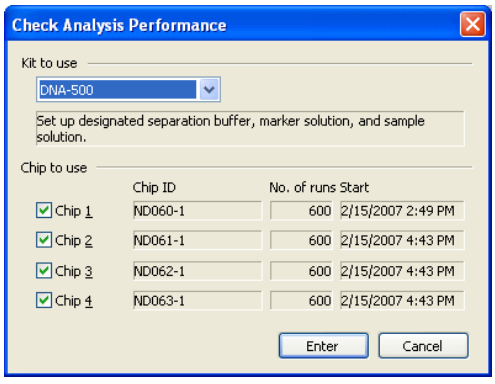
Fig.2-1 Home Positions for Autosampler and Pneumatic Unit

NOTE

This function will not operate if the chip cover or instrument cover are open. Close the chip cover and instrument cover before starting this operation.

2.5.6 Check Analysis Performance

Select [Check Analysis Performance] from the [Instrument] drop-down menu to create a schedule for checking the analysis performance.



- The [Check Analysis Performance] function checks for problems with the instrument, microchips, and reagent kit.

  Regular checks are recommended. Perform the evaluation as necessary if the sample analysis results are unfavorable.
- Use the TE buffer (10 mM Toris, 1 mM EDTA, pH 8.0) for the DNA kit sample and THE RNA Storage Solution for the RNA kit. (DNA, RNA, and ladders are not required.)

NOTE

The check is performed using the on-chip mix mode for the DNA kit (DNA-500, DNA-1000, or DNA-2500) and premix for the RNA kit.

- See *(P.123)* for a list of all of the items that are evaluated during [Check Analysis Performance]. “Passed” or “Failed” is displayed as the general result and for each specific item.
- The following files are created when the analysis performance check is executed.

Type	File name (base name)	Extension	Save folder
Raw data	Name showing date and time when check was performed (Ex.: Check was executed at 14:50' 20", September 13, 2006: 20060913_45020.MLT).	MLT	[Reagent kit name] folder in the Project folder located inside the data folder (Regularly, C:\MultiNA\).
Analyzed data	Same as raw data	MLA	Same folder as raw data
Analysis performance check results log	Same as raw data	LOG	Same folder as raw data

- If the check results raw data file (MLT file) is opened in MultiNA Viewer, the check results log file (LOG file) is also automatically opened. ()
- Since the LOG file is text data, it can also be opened with a [Memo] program or editor program.

1 **1Refer to the chapters in this manual listed below to prepare the analysis performance check for the appropriate reagent kit.**

(No need to prepare a ladder.)

- DNA-500: *"1.6.1 Requirements" P.32*
- DNA-1000: *"1.7.1 Requirements" P.40*
- DNA-2500: *"1.8.1 Requirements" P.48*
- RNA: *"1.9.1 Requirements" P.56*

2 **Prepare the same diluted dye solution as for the regular analysis.**

- DNA-500: *"1.6.3 Diluted Dye Solution Preparation" P.34*
- DNA-1000: *"1.7.3 Diluted Dye Solution Preparation" P.43*
- DNA-2500: *"1.8.3 Diluted Dye Solution Preparation" P.51*
- RNA: *"1.9.3 Diluted Dye Solution Preparation" P.59*

3 **Select the appropriate items in the [Check Analysis Performance] window.**

Select the kit to be used from among DNA-500, DNA-1000, DNA-2500, and RNA. Select all the microchips to be used.

NOTE

- Multiple performance checks can be entered into a single analysis schedule by repeatedly selecting [Check Analysis Performance] to add these checks to the analysis schedule. The results for each of the performance checks are saved in separate files.
- Performance checks can be performed on the same DNA kit up to 26 times and up to 8 times on the same RNA kit (upper limit for liquid in sample tube: 30 µL).
- Regular analysis samples cannot be added to an analysis schedule that contains performance checks.
- After the performance checks are added to the analysis schedule, [Delete Sample Sheet] can be performed, but [Edit Sample Sheet] cannot be performed.

4 Click the [Enter] button, prepare the necessary amounts of the reagent shown in [Reagent Information], and place them in the reagent holder.

DNA kit

1 Dispense the DNA kit separation buffer into the buffer bottle, and add the diluted dye solution.

To perform two checks on four microchips (8 analyses), add 5 µL of diluted dye solution to 495 µL of separation buffer. Refer to the following chapters for more details.

- DNA-500: *"1.6.5 Separation Buffer Preparation" P.36*
- DNA-1000: *"1.7.5 Separation Buffer Preparation" P.44*
- DNA-2500: *"1.8.5 Separation Buffer Preparation" P.52*

2 Cap the bottle, and agitate the solution with the vortex mixer for at least 10 seconds.

3 Remove the cap, and place the bottle in the reagent holder position that corresponds to the kit that was used.

4 Dispense the required amount of marker solution into a marker solution vial, and place it in the reagent holder position that corresponds to the kit that was used. Refer to the “On-chip mix” sections in the following chapters.

- DNA-500: *"1.6.6 Ladder, Sample and Marker Solution Usage" P.38*
- DNA-1000: *"1.7.6 Ladder, Sample, and Marker Solution Usage" P.46*
- DNA-2500: *"1.8.6 Ladder, Sample and Marker Solution Usage" P.54*

5 Use a TE buffer (10 mM Toris, 1 mM EDTA, pH 8.0) for the sample. Refer to the table below to dispense the required volume of sample into a sample tube, and place the tube into the specified well in the extra sample stand.

For example, to make one DNA-500 kit check on four microchips (4 analyses), place at least 11 µL of TE buffer in the X1 well.

Minimum amount of TE buffer required	5 + 2 x (Number of analyses - 1) µL
Container	Sample tube
Maximum amount of solution	30 µL

Reagent kit	Sample well
DNA-500	X1
DNA-1000	X2
DNA-2500	X3

NOTE

The sample wells cannot be changed, place the samples in the specified wells.

RNA kit

1 Dispense the RNA separation buffer into the buffer bottle, and add the diluted dye solution and formamide.

For example, to perform one check on four microchips (4 analyses), add 5 µL of diluted dye solution and 100 µL of formamide to 395 µL of separation buffer. Refer to the following chapters for more details.

- RNA: *"1.9.5 Separation Buffer Preparation" P.60*

2 Cap the bottle, and agitate the solution with the vortex mixer for at least 10 seconds.

3 Remove the cap, and place the bottle in the RNA position in the reagent holder.

4 Use THE RNA Storage Solution for the sample. Refer to the table below to dispense the required volume of THE RNA Storage Solution into a sample tube, add an equal amount of marker solution, mix the solution, and place the tube into the X4 well in the extra sample stand.

For example, to make one check on four microchips (4 analyses), add 7.5 µL of marker solution to 7.5 µL of THE RNA Storage Solution, mix them together, and place the sample tube in the X4 position of the extra sample stand.

Minimum amount of mixed solution required	3 x (Number of analyses + 1) µL
Amount of THE RNA Storage Solution	1.5 x (Number of analyses + 1) µL
Amount of marker solution	1.5 x (Number of analyses + 1) µL
Container	Sample tube
Maximum amount of solution	30 µL
Sample position	X4

NOTE

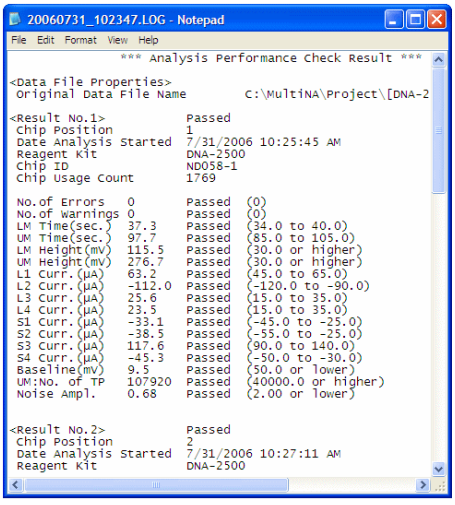
The sample position cannot be changed, place the sample in the specified well.

5 Start analysis as usual.

Either select [Start] on the [Analysis] pull-down menu, or click the [Start] button on the toolbar.

6 Use MultiNA Viewer to review the analysis performance check results.

Either select [Data File] on the [View] pull-down menu, or click the [View Data File] button on the toolbar. The check results are automatically displayed.



Check item	Explanation
No. of Errors	Number of “Errors” that occurred during sample analysis
No. of Warnings	Number of “Warnings” that occurred during sample analysis
LM Time (sec.)	Peak detection time for the lower marker
UM Time (sec.)*	Peak detection time for the upper marker
LM Height (mV)	Peak height for the lower marker
UM Height (mV)*	Peak height for the upper marker
L1 to L4 Curr. (µA)	Current value for sample injection in microchip reservoirs No.1 to No.4
S1 to S4 Curr. (µA)	Current value for sample separation in microchip reservoirs No.1 to No.4
Baseline (mV)	Baseline height in [Raw Data]
UM No. of TP*	Theoretical plate number for the upper marker peak
Noise Ampl.	Noise amplitude in electropherogram

- Items marked with asterisk (*) are not displayed for RNA checks (RNA uses lower marker only).
- “Passed” or “Failed” is displayed for each check item. The acceptable range is in the parentheses and varies depending on the reagent kit used.
- If any one of the check items has a “Failed” result, the general result (<Result No. #>) is also “Failed”.

7 **If the check result is “Failed”, the following causes can be considered.**

Check item	Symptom	Possible cause
No. of Error/ Warning	An error or warning occurred during analysis.	“Error” or “Warning” occurred in the instrument during sample analysis. Display the log (*"2.4.3 Log" P.111*) and check the content. For error messages, see *-Instrument and System- "9.2.1 MultiNA Control Software"*.
LM Time	Value under standard range (detection is too fast)	Low separation buffer concentration. Kit type used differs from the recorded kit.
	Value over standard range (detection is too slow)	Incorrect current running. High separation buffer concentration. Kit type used differs from the recorded kit.
UM Time	Value under standard range (detection is too fast)	Low dye concentration in separation buffer. Kit type used differs from the recorded kit.
	Value over standard range (detection is too slow)	Incorrect current running. High dye concentration in separation buffer. Kit type used differs from the recorded kit.
LM Height	Value under standard range (peak is too low)	Lower marker has deteriorated. Microchip surface is contaminated. Microchip not installed correctly on chip stage. (For RNA analysis) Formamide has deteriorated.
UM Height	Value under standard range (peak is too low)	Dye has deteriorated. Separation performance degraded due to microchip deterioration. Separation performance degraded due to separation buffer deterioration. Insufficient marker solution dispensed to microchip (degraded instrument dispensing precision). Microchip surface is contaminated. Microchip not installed correctly on chip stage. (For RNA analysis) Formamide has deteriorated.
L1 to L4 Curr.	Value under standard range (value is too low.)	Microchip flow channel is clogged, or partially clogged. Bubbles generated in microchip flow channel. Insufficient amount of separation buffer in microchip reservoir. Insufficient amount of sample dispensed to microchip. Kit type used differs from the recorded kit.
	Value over standard range (value is too high.)	Separation buffer concentration increase. Foreign material (salt) intruded into separation buffer. High salt concentration in sample. Kit type used differs from the recorded kit.

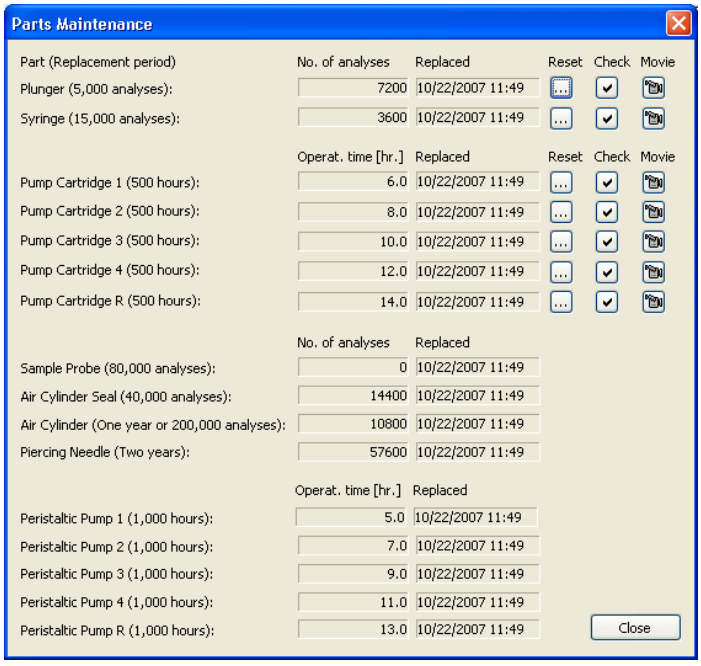
Check item	Symptom	Possible cause
S1 to S4 Curr.	Value under standard range (value is too low.)	Microchip flow channel is clogged, or partially clogged. Bubbles generated in microchip flow channel. Insufficient amount of sample dispensed to microchip. Kit type used differs from the recorded kit.
	Value over standard range (value is too high.)	Separation buffer concentration increase. Foreign material (salt) intruded into separation buffer. High salt concentration in sample. Kit type used differs from the recorded kit.
Baseline	Small value (baseline is too low.)	Low dye concentration in separation buffer.
	Large value (baseline is too high.)	High dye concentration in separation buffer. Microchip has deteriorated. Microchip surface is contaminated.
UM No. of TP	Small value (theoretical plate number is too small)	Microchip has deteriorated. Reagent kit has deteriorated, and salt concentration changing (increase).
Noise Ampl.	Large value (noise is large)	Microchip surface is contaminated.

- Corresponding to the presumed cause, clean the microchip surface, or replace the reagent kit, dye, microchip, and formamide (RNA analysis only) with new items, and repeat the analysis performance check again.
- If this does not solve the problem, contact your Shimadzu service representative.

2.5.7 Parts Maintenance

This window displays the previous number of analyses, their operation times, and the previous replacement dates and times.

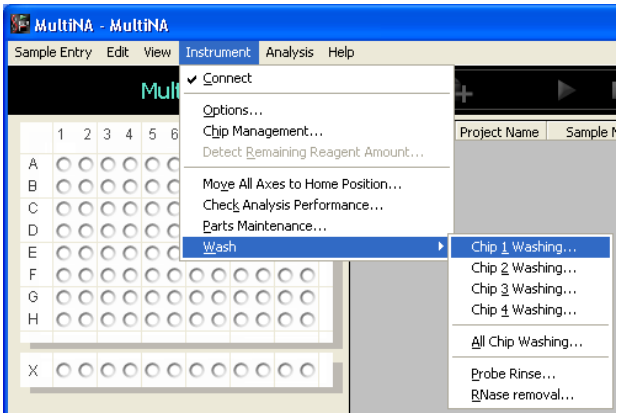
Access this window regularly to determine if any of the parts need to be replaced.



Reference

-Instrument and System- "6.3.1 Parts Maintenance", "6.4 Part Replacement and Inspection"

2.5.8 Wash

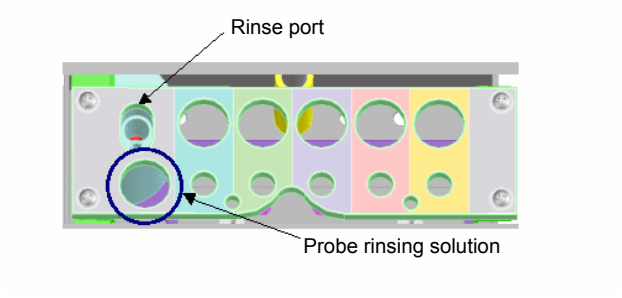


Select the following items from the pull-down menu, and perform each rinsing operation.

Menu item	Explanation
Chip 1 to Chip 4 Washing	Rinse each microchip with water.
All Chip Washing	Rinse all the microchips installed with water. Even chips that are not selected in the [Chip Management] window are washed.
Probe Rinse	Rinse the sample probe with water.
RNase Removal	Rinse the sample probe with probe rinsing solution and water.

Before rinsing, verify that the glass rinse water bottle for rising contains at least 100 mL of Milli-Q ultrapure water or distilled water.

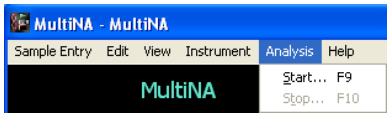
Before RNase removal, insert 1 mL of probe rinsing solution (RNaseZAP® Applied Biosystems (formerly Ambion)) into the buffer bottle, and place it in front of the rinse port in the reagent holder.



2.5.9 Periodic maintenance

Operate the peristalic pump, so that the operation prevents the pump from performance decrement when the instrument is out of use for an extended period (for 1 month or more). See *-Instrument and System- "7.3.3 Peristalic Pump Storage"*.

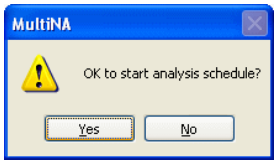
2.6 Analysis Menu



2.6.1 Start

After the analysis schedule is entered, [Start] on the [Analysis] pull-down menu (and the [Start] button on the toolbar) is enabled.

Select either on to display the following message. Click [yes] to start the analysis operation.



NOTE

- The instrument automatically checks the following items before starting an analysis sequence. If an error occurs during this evaluation, perform appropriate measures. See *-Instrument and System- "9.2.1 MultiNA Control Software"* for more details.
- Do NOT leave the instrument unattended until the check is complete and the analysis starts.

Check item	Explanation
Control power supply unit	Check the voltage.
Top cover/chip cover open/close	The instrument will not operate while the covers are open. Close the covers and then start analysis.
Sample holder conditions	Verifies that the sample holder is correctly installed. Uses a sample probe to perform contact detection. Analysis will not start if the sample holder is not correctly installed. *"1.10.1 Sample Holder Installation" P.64*
Microchip in position	Verifies that a microchip is installed in the specified position. Uses a sample probe to perform contact detection. Analysis will not start if the microchip is not installed in the specified position. Install the microchip in the specified position, and restart the analysis. *"2.5.3 Chip Management" P.115*
Amount of remaining reagent	Determines the residual reagent amount, Uses a sample probe to perform liquid level detection. Analysis will not start if the residual reagent amount is insufficient. Refer to the [Reagent Information] window and dispense the required amount of reagent, and then restart the analysis.
Chip temperature	Verifies that the microchip temperature, is at least 37°C. If the temperature is low it is warmed to the set temperature. Analysis starts when the temperature is reached.

- After analysis starts, check the analysis status in the main window. See *"2.1 Explanation of Windows" P.83*.
- Select [Chip Status] on the [View] pull-down menu to switch between the chip status display and reagent information display. See *"2.1.8 Chip Status Window" P.88*.

2.6.2 Stop

After analysis, the instrument performs microchip rinsing and other post-processing, and then automatically stops.

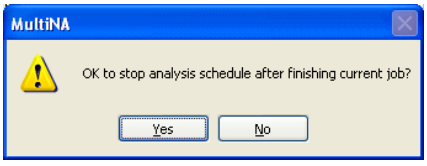
Use this menu to interrupt analysis.

1 Either select [Stop] on the [Analysis] pull-down menu, or click the [Stop] button on the toolbar.

Depending on the instrument status, one of the two messages below is displayed.

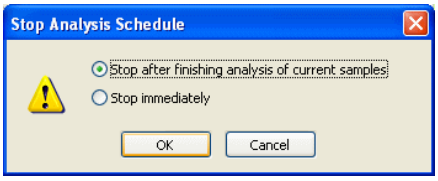
1 Before starting sample analysis

The following message is displayed. Click [yes] to stop analysis after completing the processes in progress.



2 During sample analysis

The following message is displayed. Select either [Stop after finishing analysis of current sample] or [Stop immediately], and click [OK].

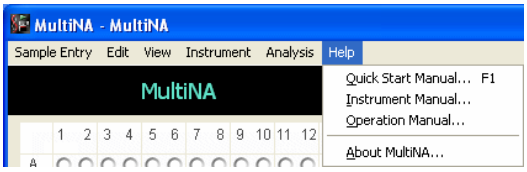


- [Stop after finishing analysis of current sample]
  Selecting this check box continues analysis of sample currently being analyzed or currently being loaded. After the analysis is completed, rinsing of the microchip and sample probe is automatically executed, and the operation is ended.
- [Stop immediately]
  Selecting this check box immediately stops analysis even if a sample is currently being analyzed. Rinsing of the microchip and sample probe is then automatically executed, and operation ends.

  Samples currently in loading or analysis have a data file prepared for partway through the analysis.

2 Check the instrument indicators, etc., to confirm that operation has stopped, and open the top cover.

2.7 Help Menu



2.7.1 Manual

Select the menu below to display the PDF files for the corresponding instruction manuals.

[Quick Start Manual]: Analysis operations flow (Operating Procedure P.2 to P.3)

[Instrument Manual]: Instrument and System

[Operation manual]: Operating Procedure

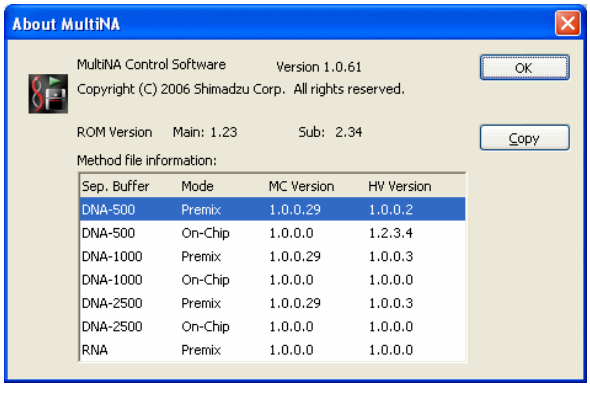
NOTE

Adobe® Reader® distributed by Adobe Systems Co. or other PDF file viewing software (provided free) is required to view the PDF file. Adobe® Reader® (Ver. 4 or later) can be downloaded from the following website:

http://www.adobe.com/products/acrobat/readstep2.html

2.7.2 About MultiNA

Use this menu to display the software version information. The MultiNA Control Software version information is shown as well as version information for the current method.



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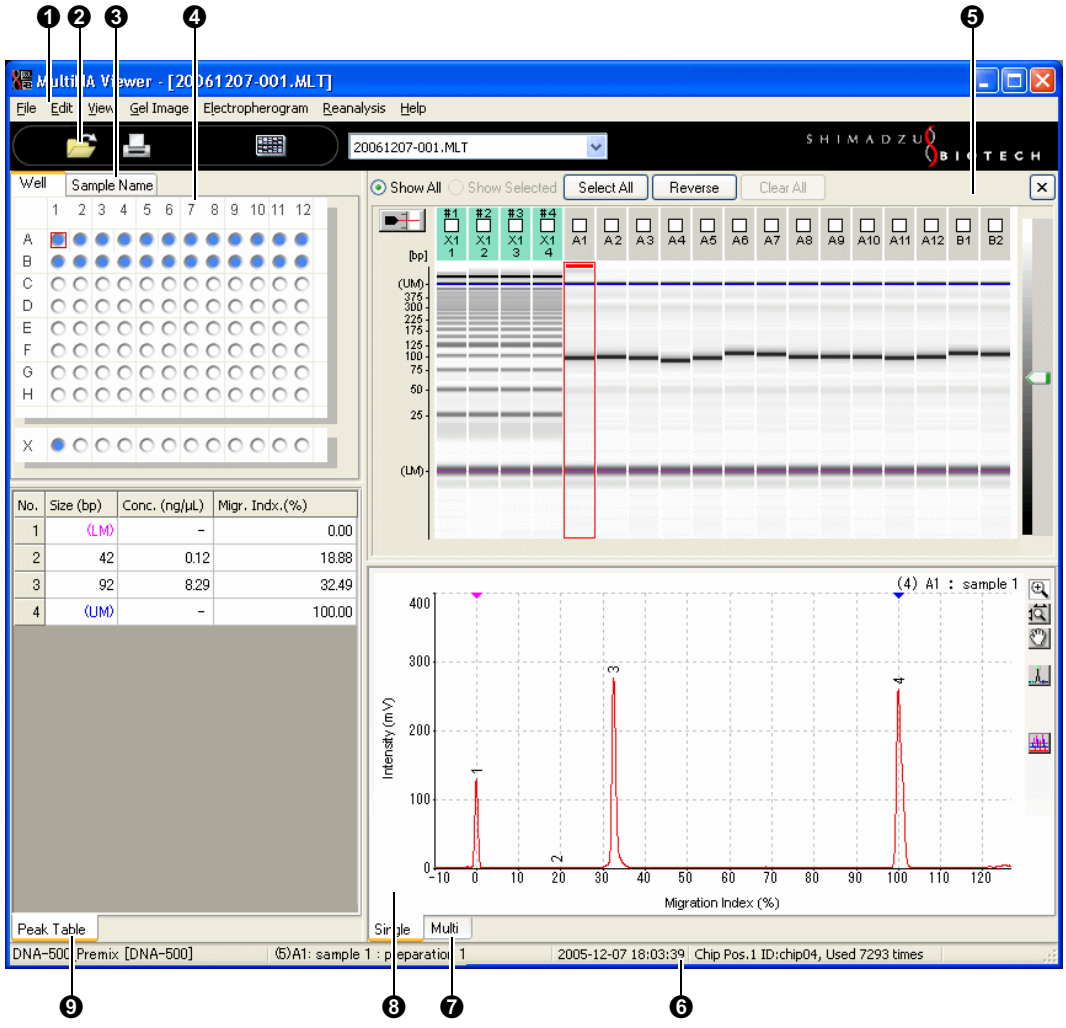
3 MultiNA Viewer Functions

3.1 Explanation of the Window



No.	Name	Reference
❶	Pull-down menu	*"3.1.1 Pull-down Menu List" P.132*
❷	Toolbar	*"3.1.2 Toolbar" P.134*
❸	Sample Name Tree	*"3.1.5 Sample Name Tree" P.135*
❹	Well Display	*"3.1.3 Focused data and selected data" P.134*
❺	Gel Image	*"3.1.6 Gel Image" P.135*
❻	Status Bar	*"3.4.3 Status Bar" P.165*
❼	Electropherogram (Multi)	*"3.1.10 Electropherogram (Multi)" P.142*
❽	Electropherogram (Single)	*"3.1.9 Electropherogram (Single)" P.140*
❾	Peak Table	*"3.1.7 Peak Table" P.139*

3.1.1 Pull-down Menu List

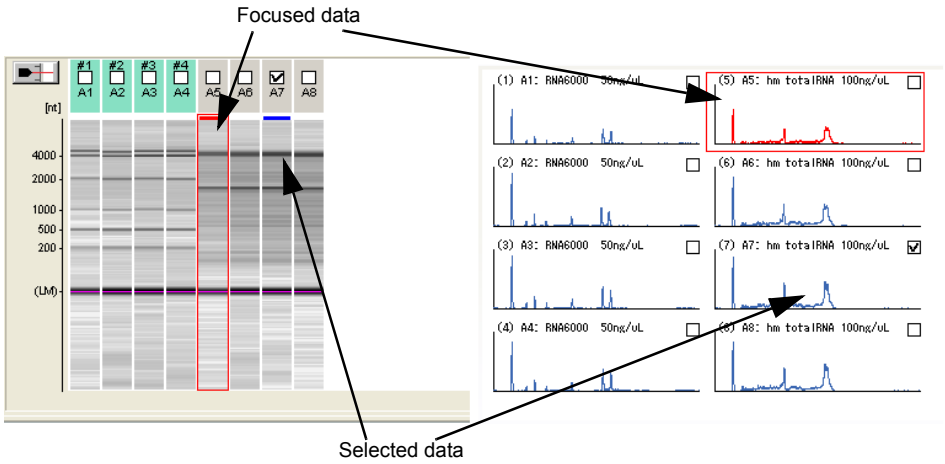
Menu Item		Outline of Function	Shortcut Key	Reference Page
File	Open	Open a data file.	Ctrl + O	P.143
	Close	Close a data file being viewed.		P.145
	Save	Rewrite and save a data file.	Ctrl + S	P.145
	Save As	Save a data file with a different name.		P.146
	V1.05 Save Selected Data	Save only the selected data in a separate file.		P.147
	Search	Search data files.		P.149
	Export	Export data in CSV format.		P.150
	Print	Print out data.	Ctrl + P	P.152
	Print Setup	Set up a printer.		P.159
	Data File Properties	Display conditions where a data file is acquired.		P.160
	Sample Properties	Display conditions where each sample data item in a data file is acquired.		P.160
	(File Name)	Reopen a file that has already been opened.		
	Exit	Quit the data analysis software MultiNA Viewer.		P.160
Edit	Copy	Copy information displayed on [Gel Image], [Electropherogram], [Peak Table], or [RNA Report] to the clipboard.		P.162
	V1.05 Save Image As	Save what is displayed on [Gel image] and [Electropherogram] as an image file.		P.163
View	Refresh	Reopen a data file.		P.164
	Marker	Switch between hide and display marker peak sign.		P.164
	Status Bar	Switch between hide and display Status Bar.		P.165
	V1.05 Title	Tooltip displayed in the upper right area of [Electropherogram (Single)] and at the top of [Gel Image].		P.165
	Analyzed Data / Raw Data	Switch between analyzed data and raw data.		P.166
	Normal Sensitivity Data / Low Sensitivity Data	Switch between normal sensitivity data and low sensitivity data.		P.166
	Comparison	Display multiple data files for comparison.		P.167
	Size Calibration Curve	Display the size calibration curve used for data analysis.		P.171
	Analysis Performance Check Result	Display the results of analysis performance check.		P.171
	Select Ladder Used for Analysis	Select and display the ladder used to analyze the focused sample.		P.172
	Select All Samples Analyzed Using This Ladder	Select and display the samples analyzed using the focused ladder.		P.173
	Options	Set options for items to be displayed or scale.		P.174

Menu Item		Outline of Function	Shortcut Key	Reference Page
Gel Image	Invert Black and White	Switch black and white highlighting.		P.176
	V1.05 Vertical Axis	Select the image to be displayed on the vertical axis from among [Distance Image], [Size Image], or [Time Image].		P.177
	Gel Image List	Display the [Gel Image List] window.		P.178
	Undo Zoom	Undo the last zooming operation on [Gel Image].	Ctrl + Z	P.180
	Undo Zoom All	Undo all zooming operations on [Gel Image].	Ctrl + R	P.180
Electro-phero-gram	Peak Top	Select the item displayed at the top of the peak.		P.181
	V1.05 Font Size	Select size of text displayed at the top of the peak.		P.181
	Show Baseline	Switch between show and hide baseline.		P.181
	V1.05 Horizontal Axis	Switch the item to be displayed on the vertical axis from among [Migration Index], [Size], or [Migration Time].		P.182
	V1.05 Overlay	Switches between [Ascending Order] and [Descending Order] for displaying overlays.		P.182
	Undo Zoom	Undo the last zooming, enlargement, and pan operations on [Electropherogram].		P.182
	Undo Zoom All	Undo all zooming, enlargement, and pan operations on [Electropherogram].		P.182
Reanal-ysis	Automatic	Reanalyze all data by automatic operation.		P.183
	Manual Edit Mode	Switch to the mode where addition or deletion of peaks or attribution of peaks are manually edited.		P.184
	Change Ladder and Analyze	Reanalyze with a different ladder data.		P.189
Help	Quick Start Manual	Display the Analysis Operation Flow (Operating Procedure P.2 to P.3).	F1	P.194
	Instrument Manual	Display the Instruction Manual (Instrument and System)		
	Operation Manual	Display the Instruction Manual (Operating Procedure).		
	About MultiNA Viewer	Display the version information of the program.		P.194

3.1.2 Toolbar

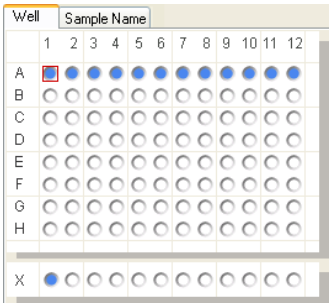
No.	Explanation
❶	[Open existing data file] button ([File] - [Open]): Open an existing data file (*.MLT).
❷	[Print active data file] button ([File] - [Print]): Display the [Print] dialog box and start printing.
❸	[View Gel Image List] button: Display the gel image list. There are three types of layouts available to display the gel image list, 12-well unit, 8-well unit, or sort by chip position. The list is displayed in a layout previously displayed by selecting the [Gel Image List] on the [Gel Image] menu.
❹	[Data file name] list box: Display the name of the data file currently open. When multiple files are loaded, click the downward arrow on the right to select a data file to be displayed.
❺	[Open active data file] button ([File] - [Close]): Close the currently displayed data file.

3.1.3 Focused data and selected data



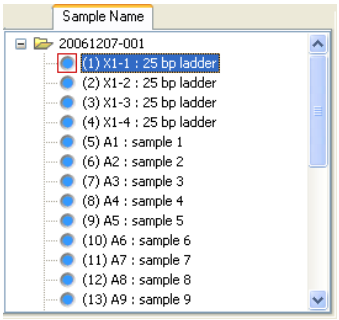
- “To focus” means to click specific data displayed in [Well Display], [Sample Name Tree], [Gel Image], or [Electropherogram (Multi)] and outline it with red lines. The outlined data is called “focused data.”
- “To select” means to select a check box of data displayed on [Gel Image] or [Electropherogram (Multi)]. Two or more data items can be selected. The data with a check mark is called “selected data.”

3.1.4 Well Display



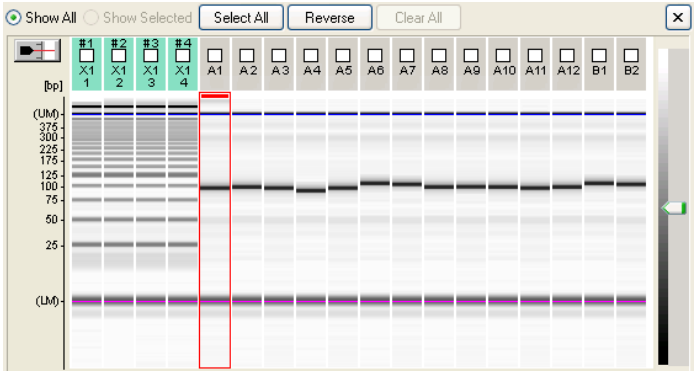
- Samples analyses that completed normally are displayed in blue. If a “Warning” occurred during the analysis, the sample is displayed in yellow. If an “Error” or “Fatal” error occurred during the analysis, the sample is displayed in red.
- Click a colored well to focus on that sample. The data on the [Gel Image] and [Electropherogram (Multi)] is outlined in red. The applicable data is displayed for [Electropherogram (Single)].

3.1.5 Sample Name Tree

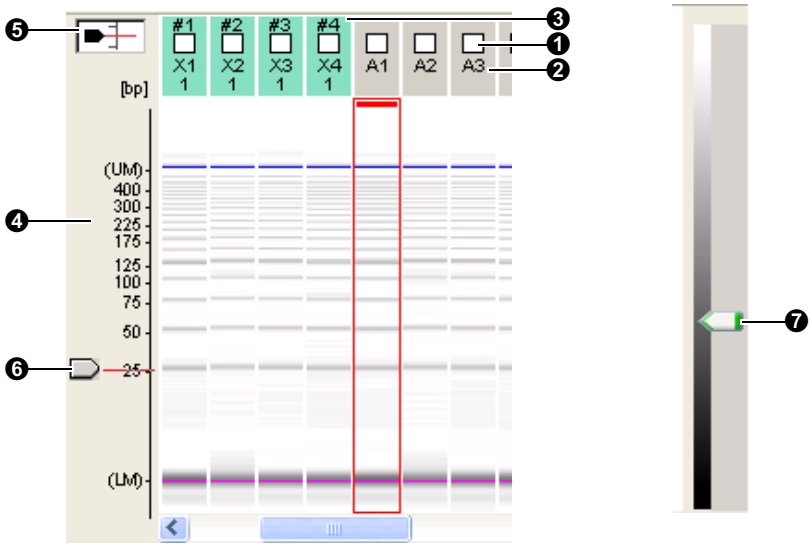


- Click the [Sample Name] tab to display the [Sample Name Tree].
- The original file name (one used to save the sample sheet) is displayed at the top of the tree. The name remains the same even if the file is saved with a different name.
- Analysis order (number), well, and sample name are displayed under the original file name.
- The well and sample name were entered on the sample sheet.
- Click a sample name to focus on that sample. The data on the [Gel Image] and [Electropherogram (Multi)] is outlined in red. The applicable data is displayed for [Electropherogram (Single)].

3.1.6 Gel Image

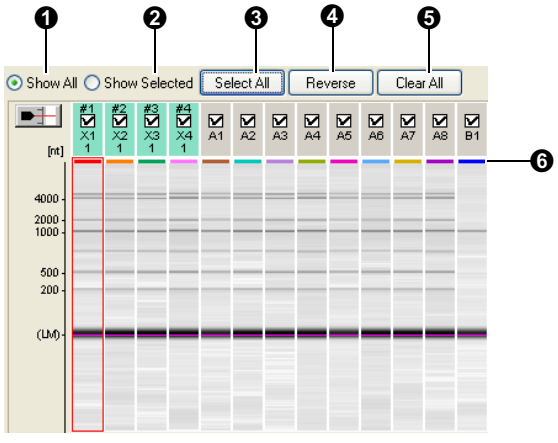


- When a regular data file is open, [Analyzed Data] ([Distance Image]) is displayed in gel image.
- Click a desired piece of [Gel Image] to focus on that sample. The corresponding [Electropherogram] is displayed below.
- The upper tab of the [Gel Image] is gray for sample data and pale green for ladder data.
- For DNA analysis, [Gel Image] ([Analyzed Data]) is displayed so that the position of the lower marker (LM) and upper marker (UM) align. For RNA analysis, it is displayed so that the position of the lower marker (LM) and the end of electrophoretic data align.



No.	Explanation
❶	Click the check box. The respective data becomes “selected data.”
❷	Indicates a well name. More than one analysis can be performed from the extra sample stand and analysis order is displayed below the well name.
❸	The “#” mark and chip position (1 to 4) is displayed above the ladder data check box.
❹	Size axis. (It is based on the position of fragment peaks in the ladder data.)
❺	Click the button to display the knob (6) and a red horizontal on [Gel Image].
❻	Drag the knob up or down to move the red line.
❼	Drag the knob up or down to adjust color contrast of [Gel Image]. While dragging the knob, the color contrast adjustment is applied only to the focused data. After adjustment, the color contrast is applied to all of the data displayed.

An example of selected check boxes on the [Gel Image] is displayed below.



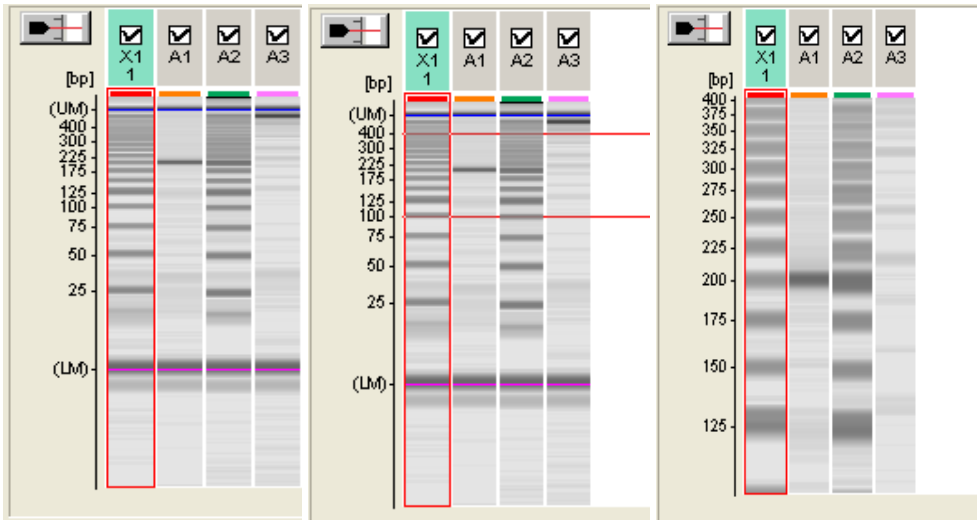
No.	Explanation
❶	[Show All] is selected in the initial display. All data (with and without a check mark) is displayed.
❷	Select [Show Selected] to display only the data that is selected with a check mark. This button is enabled when at least one data item is selected with a check mark.
❸	Click [Select All] to select all of the data in a data file with check marks.
❹	Click [Reverse] to switch between the selected and non selected data.
❺	Click [Clear All] to remove the check marks from all of the samples.
❻	A small color bar is displayed at the top of the gel image for the selected data. The color of the bar corresponds to the color in the [Electropherogram] overlay (see *"3.1.9 Electropherogram (Single)" P.140*).

NOTE

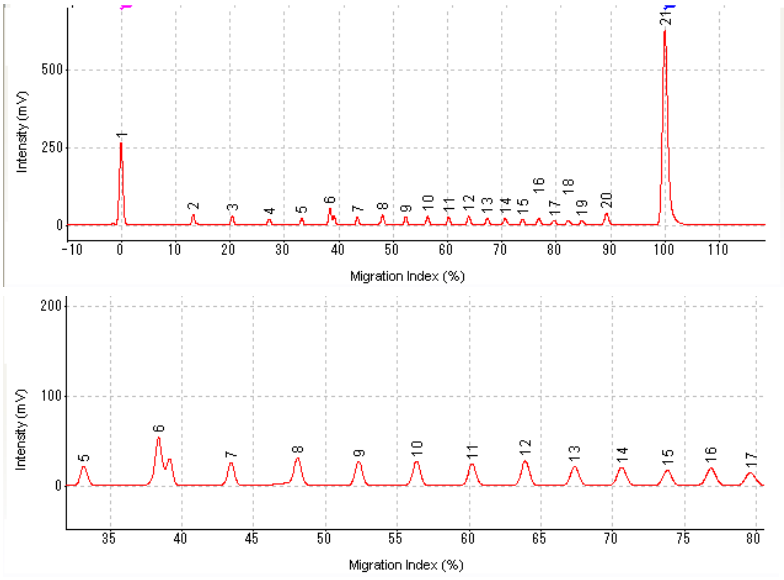
- If the check mark is cleared from a selected data when [Show Selected] is selected, the respective data disappears from [Gel Image] because the data is no longer selected.
- If the check mark is cleared from all of the displayed data and [Show Selected] is selected, the display state automatically changes to [Show All].

Zooming of [Gel Image]

- Select an area by dragging the mouse and then releasing it. While dragging the mouse up or down on [Gel Image], red lines are displayed at the starting and ending points of the area to be enlarged.
- To return to the original display, select [Undo Zoom]/[Undo Zoom All] on the [Gel Image] menu or use the right click pop-up menu.
- When the display range on [Gel Image] is changed, the display range of the horizontal axis of [Electropherogram (Single)] is scaled to the same. (To return to the original display in [Electropherogram (Single)], select [Undo Zoom]/[Undo Zoom All] on the [Electropherogram] menu or right click pop-up menu on [Electropherogram (single)].)



Example of [Gel Image] Zooming in
(Left: Before Zooming in, Center: While Specifying the Range, Right: After Zooming in)

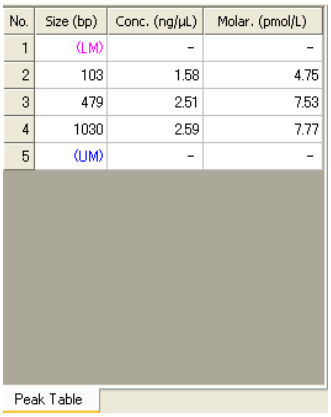


[Electropherogram] Zoomed in
(Top: Before Zooming in, Bottom: After Zooming in)

■ [Gel Image] Right Click Pop-up Menu

Menu	Explanation	Reference Page
Undo Zoom	Undo the last zooming operation on [Gel Image].	*P.180*
Undo Zoom All	Undo all zooming operations on [Gel Image] to recover the initial display state.	*P.180*
Copy	Copy the image data displayed on [Gel Image] to the clipboard. (Or select [Copy] on the [Edit] pull-down menu and point to [Gel Image].)	*P.162*
V1.05 Save Image As	Save the image data displayed on [Gel Image] to a file. (Or select [Save Image As] on the [Edit] pull-down menu and point to [Gel Image].)	*P.163*
Add Focused Data to Comparison	Add focused data to the [Comparison] view.	*P.167*
Add All Selected Data to Comparison	Add all selected data (ones with a check mark) to the [Comparison] view.	
Delete Focused Data from Comparison	Delete focused data from the [Comparison] view.	
Delete All Selected Data from Comparison	Delete all selected data (ones with a check mark) from the [Comparison] view.	
Select Ladder Used for Analysis	Select and display ladder data used for analyzing the focused sample and the sample data.	*P.172*
Select All Samples Analyzed Using This Ladder	Select and display sample data analyzed using the focused ladder and the ladder	*P.173*
Sample Properties	Display properties for acquiring data of each sample.	*P.160*

3.1.7 Peak Table

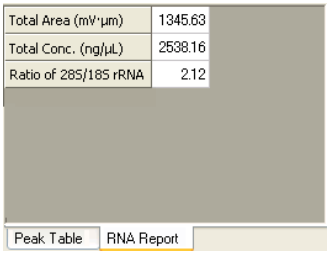


- The peak table of the focused sample is displayed.
- (LM) represents the lower marker peak and (UM) represents the upper marker peak.
- To customize Items displayed on the columns, select [Options] on the [View] pull-down menu (see *"3.4.12 Options" P.174*).
- Double-click a cell in the [Peak Table] or a peak to apply a "*" mark next to the peak no. and cause the corresponding peak top information on [Electropherogram] to be displayed in red.
- Double-click the peak with the "*" mark again to remove the mark and recover the original display.

■ [Peak Table] Right Click Pop-up Menu

Menu	Explanation	Reference Page
Copy Selected Cells	Copy the data selected in [Peak Table] to the clipboard.	*P.162*
Set to Lower Marker	Set the peak right-clicked in [Peak Table] as the lower marker when [Manual Edit Mode] is selected on the [Reanalysis] pull-down menu.	*P.184*
Set to Upper Marker	Set the peak right-clicked in [Peak Table] as the upper marker when [Manual Edit Mode] is selected on the [Reanalysis] pull-down menu.	
Set to 18S rRNA	Set the peak right-clicked in [Peak Table] as 18S rRNA in the case of RNA sample (total RNA) when [Manual Edit Mode] is selected on the [Reanalysis] pull-down menu.	
Set to 28S rRNA	Set the peak right-clicked in [Peak Table] as 28S rRNA in the case of RNA sample (total RNA) when [Manual Edit Mode] is selected on the [Reanalysis] pull-down menu.	
Delete Peak	Delete the peak right-clicked in [Peak Table] when [Manual Edit Mode] is selected on the [Reanalysis] pull-down menu].	

3.1.8 RNA Report



- For RNA analysis data, the [RNA Report] tab is displayed. The following items are displayed.

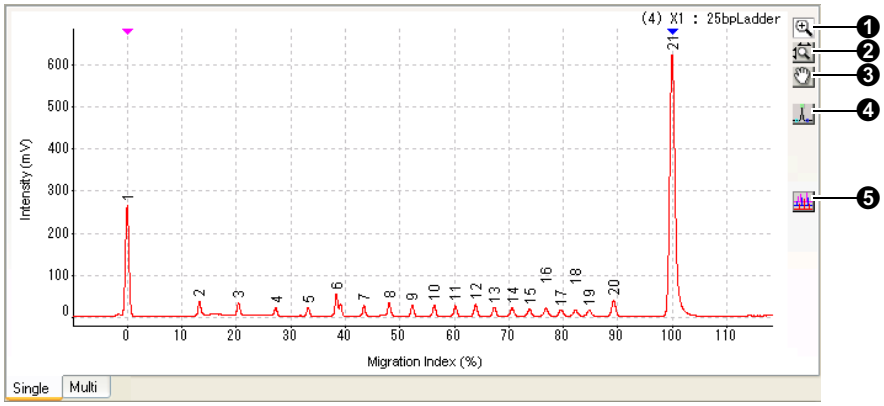
  Total RNA: [Total Area], [Total Conc.], [Ratio of 28S/18S rRNA]

  mRNA: [Total Area], [Total Conc.]
- Although peaks of only 18S rRNA and 28S rRNA are displayed on [Electropherogram], the area and concentration of all RNA peaks are calculated.

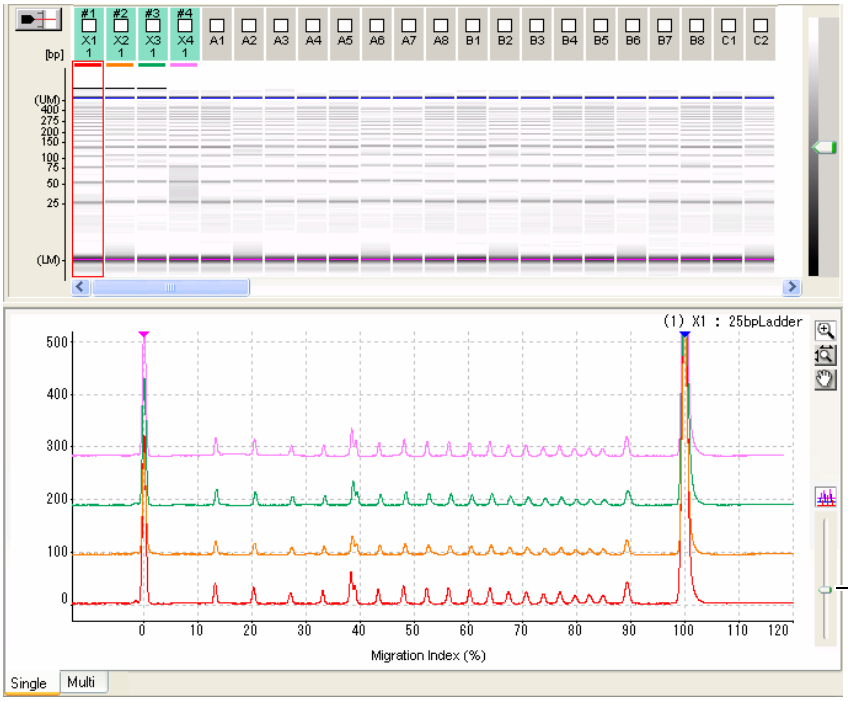
■ Right Click Pop-up Menu in [RNA Report]

Menu	Explanation	Reference Page
Copy Selected Cells	Copy the data selected in [Peak Table] to the clipboard.	*P.162*

3.1.9 Electropherogram (Single)



No.	Explanation
❶	[Rectangle Zoom] button: [Rectangle Zoom] where this button is pressed mode appears in the default setting. Drag the rectangular box in the graph to zoom in on the area surrounded by the box. To return the display to its original state, select [Undo Zoom] or [Undo Zoom All] on the [Electropherogram] menu or right-click pop-up menu.
❷	[Horizontal/Vertical Zoom] button: Click this button to start the [Horizontal/Vertical Zoom] mode. Drag the mouse vertically or horizontally. The selected area is zoomed in or out in the respective direction according to the dragged distance. To return the display to its original state, select [Undo Zoom] or [Undo Zoom All] on the [Electropherogram] menu or right-click pop-up menu.
❸	[Pan] button: Click this button to start the [Pan] mode. Drag the mouse in a desired direction in the graph to move the displayed range in that direction. To return the display to its original state, select [Undo Zoom] or [Undo Zoom All] on the [Electropherogram] menu or right-click pop-up menu.
❹	[Manual Edit Mode] button: Click this button to switch ON/OFF of manual editing of analysis results. (This operation is also available by selecting [Manual Edit Mode] on the [Reanalysis] menu.) (See *"3.7.2 Manual Edit Mode" P.184*.)
❺	[Overlay] button: Clicking this button starts the [Overlay] mode, and selected data (data with a check mark) and focused data (data framed in red) on [Gel Image] are overlaid. *V1.05* [Ascending Order] / [Descending Order] display setting is specified by selecting the [Electropherogram] menu and pointing to [Overlay].
❻ Next figure	[Slider]: (Displayed only when data is overlaid.) Drag the slider to adjust the width to display overlaid data that is off vertically. Focused data is displayed on the bottom.



- The horizontal axis of analyzed data indicates time index while the vertical axis indicates signal strength (with background being removed).
- Raw data can be displayed by selecting [Raw data] on the [View] menu. In this case, the horizontal axis indicates migration time and the vertical axis indicates signal strength.
- The information displayed at the top of the peak can be selected from [Peak Top] on the [Electropherogram] menu.
- Point to [Electropherogram] and select [Show Baseline] to display the peak baseline and vertical parting line.
- Select [Show Baseline] from the [Electropherogram] menu to display the baseline and vertical peak parting line.
- Double-click a cell in the [Peak Table] or a peak to apply a “*” mark next to the peak no. and cause the corresponding peak top information on [Electropherogram] to be displayed in red.
- Double-click the peak with the “*” mark again to remove the mark and recover the original display.

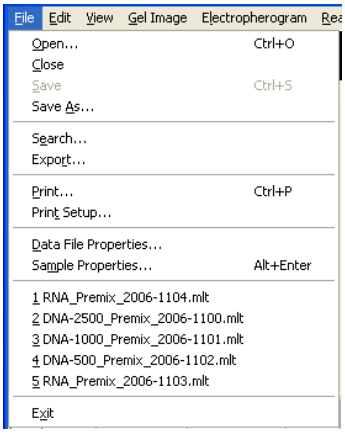
■ [Electropherogram] (Single) Right-click Pop-up Menu

Menu	Explanation	Reference Page
Undo Zoom	Undo the previous zooming or pan operation on [Electropherogram].	P.182
Undo Zoom All	Undo all zooming or pan operations on [Electropherogram] to recover the initial display state.	P.182
Copy	Copy image data of [Electropherogram] to the clipboard. (Or on the [Edit] pull-down menu, select [Copy] and point to [Electropherogram].)	P.162
V1.05 Save Image As	Saves the image data displayed on [Electropherogram] to a file. (Or select [Save Image As] on the [Edit] pull-down menu and point to [Electropherogram])	P.163
Set to Lower Marker	When there is a peak near the cursor where the mouse is right-clicked in the Manual Edit mode, the peak is set as the lower marker. When there is no peak near the cursor, a peak for the lower marker is added.	P.184
Set to Upper Marker	When there is a peak near the cursor where the mouse is right-clicked in the Manual Edit mode, the peak is set as the upper marker. When there is no peak near the cursor, a peak for the upper marker is added.	
Set to 18S rRNA	When there is a peak near the cursor where the mouse is right-clicked in the Manual Edit mode, the peak is set as 18S rRNA. When there is no peak near the cursor, a peak for 18S rRNA is added.	
Set to 28S rRNA	When there is a peak near the cursor where the mouse is right-clicked in the Manual Edit mode, the peak is set as 28S rRNA. When there is no peak near the cursor, a peak for 28S rRNA is added.	
Add Peak	In the Manual Edit mode, the peak closest to the cursor where the mouse is right-clicked is detected and added.	
Delete Peak	In the Manual Edit mode, peaks near the cursor are deleted when the mouse is right-clicked.	

3.1.10 Electropherogram (Multi)

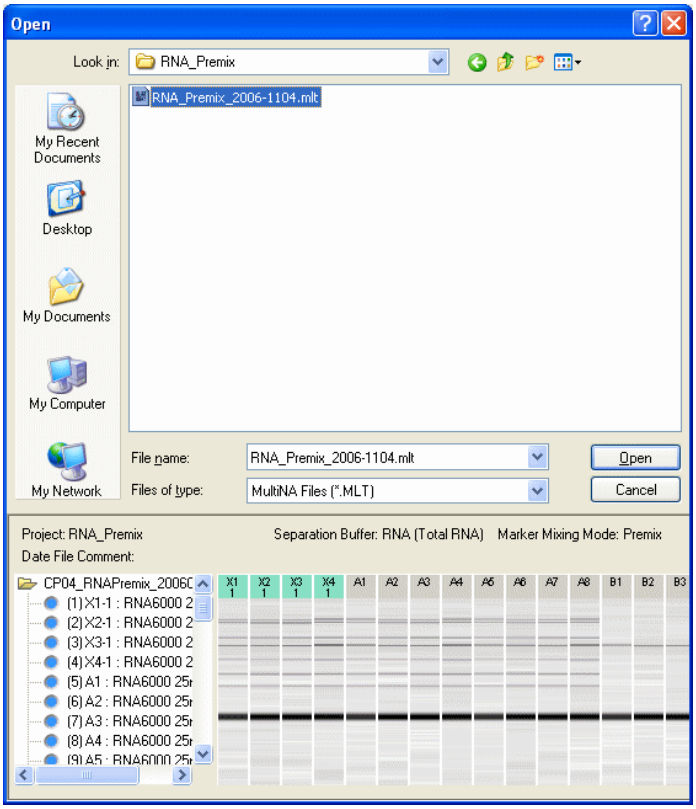
- Click the [Multi] tab to display multiple electropherograms.
- If the check box on the upper right of an electropherogram is selected, the data becomes “selected data.” (Or select the check box on [Gel Image].)

3.2 File Menu



3.2.1 Open

1 **Select [Open] on the [File] pull-down menu or click the [Open existing data file] button at the left end of the toolbar.**



- Data files are saved in a folder named with the project name used for analysis.
- When a data file (extension: .MLT) is selected, the content of the data file is displayed as preview (project name, separation buffer used, marker mix mode, data file comment, sample name tree, and gel image).

NOTE

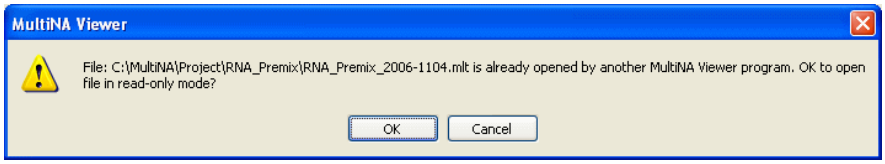
A data folder contains two files: a raw data file (extension: .MLT) and analyzed data file (extension: .MLA). When [Open] is selected on the [File] menu, only a raw data file (extension: .MLT) is displayed. When this file is opened, the analyzed data file is also loaded and automatically displayed.

2 To open a data file, select a data file and click [Open] or double-click the file.

- Two or more files can be loaded by repeatedly selecting [Open] on the [File] menu. Switch the file to be displayed on the File Name list box on the toolbar (see *"3.1.2 Toolbar" P.134*).
- Data files can be opened by double-clicking them on “Windows Explorer” (single-clicking in some settings on Windows). They can also be opened by dragging and dropping to the [MultiNA Viewer] window from Explorer.

NOTE

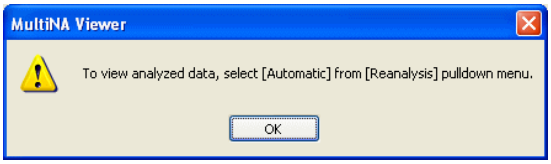
If multiple iterations of the MultiNA Viewer are open, the following message is displayed when an attempt is made to open a data file that is already open in one of the other iterations. Click [OK] to open the file in read-only mode or click [Cancel] to stop opening the file.



- [OK]: Opens the file in read-only mode. Reanalysis (automatic, manual edit or ladder change) is disabled.
- [Cancel]: Stops the open file process.

NOTE

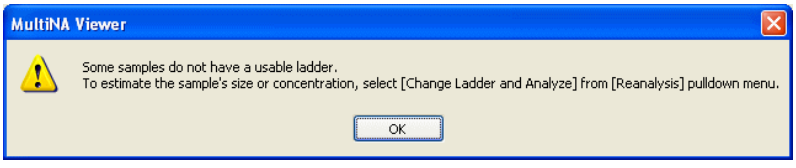
If only raw data (no analyzed data) exists in the folder, the following message is displayed and only raw data is opened. This occurs when the analyzed data file has been deleted or moved to a different folder.



Select [Automatic] from the [Reanalysis] menu, and then select either [Fine], [Standard] or [Coarse] to re-analyze the data. An analyzed data file is created and displayed. [Fine], [Standard] and [Coarse] indicate the level of peak detection precision (see *"3.7.1 Automatic" P.183*).

NOTE

The following message is displayed when there is no ladder data selected for analysis.



In this case, select [Change Ladder and Analyze] on the [Reanalysis] menu and add ladder data (see *"3.7.3 Change Ladder and Analyze" P.189*).

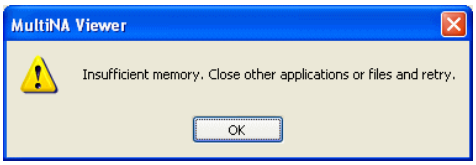
NOTE

In the following cases, data files are opened in the read-only mode and “(Read only)” is displayed at the end of the file name on the window title and the file selection drop-down list box.

- When the specified file is currently being acquired in the MultiNA Control Software
- When [Comparison View] is displayed

NOTE

The following message is displayed when a file, being analyzed by the instrument, is opened or updated by a PC with insufficient memory.



Click [OK] and close other applications or close the other files open in the MultiNA Viewer then repeat the operation.

If this memory error appears frequently, a PC memory upgrade is recommended.

3.2.2 Close

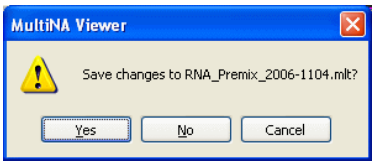
Select [Close] to close the file currently being displayed.

(Or click the [×] [Close] button under the toolbar.)

NOTE

The following message is displayed when the results were not saved after editing using [Manual Edit Mode] on the [Reanalysis] menu or [Change Ladder and Analyze] on the [Reanalysis] menu.

Click [Yes] to save the results and close the file, [No] to close the file without saving the results, or [Cancel] to cancel the closing operation.



3.2.3 Save

Select [Save] to overwrite an analyzed data file that has been changed.

Analyzed data can be changed and saved in the following cases.

- Manually edit the file using [Manual Edit Mode] on the [Reanalysis] menu.
- Reanalyze the file using [Change Ladder and Analyze] on the [Reanalysis] menu.

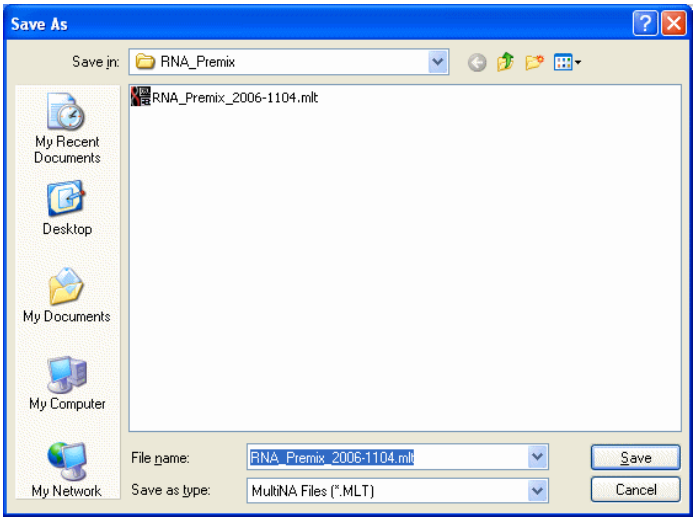
3.2.4 Save As

Select [Save As] to save a data file with a new name.

Raw and analyzed data are saved with different names at the same time.

1 **Select [Save As] on the [File] pull-down menu.**

The [Save As] dialog box is displayed.

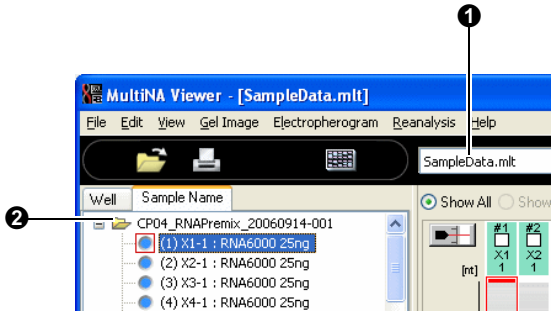


2 **Enter a new file name in the [File name] box and click [Save].**

The data file (raw and analyzed data) is saved with the new name.
(/\<>:"|?* cannot be used for a file name.)

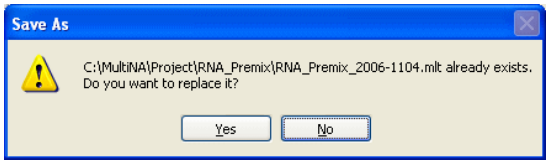
NOTE

Although the data file is saved with the new name, the data file name given when the sample sheet was created is kept in the data file as “Original data file name.” “Original data file name” is displayed on the top ❷ of [Sample Name Tree]. (❶ is displayed by the new name.)



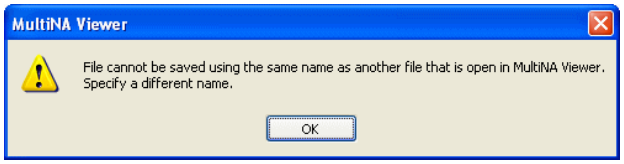
NOTE

The following message is displayed if an attempt is made to save the file using a previously used file name.



Click [Yes] to overwrite the file and [No] to cancel the saving operation.

When [Yes] is clicked and the applicable file is open on MultiNA Viewer, the following message is displayed. In this case, the file cannot be saved with the specified name. Click [OK] and enter a different file name to save.



3.2.5 Saving Selected Data

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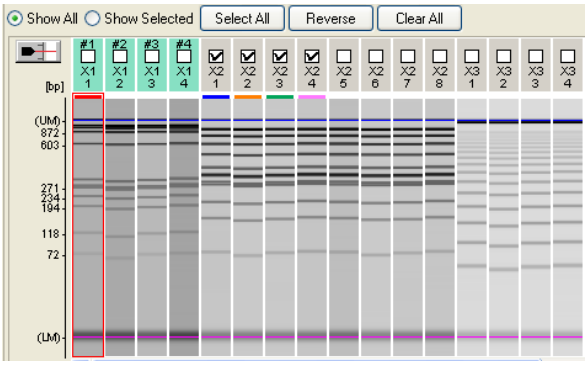
Save only data indicated with a selected checkbox to a separate file.

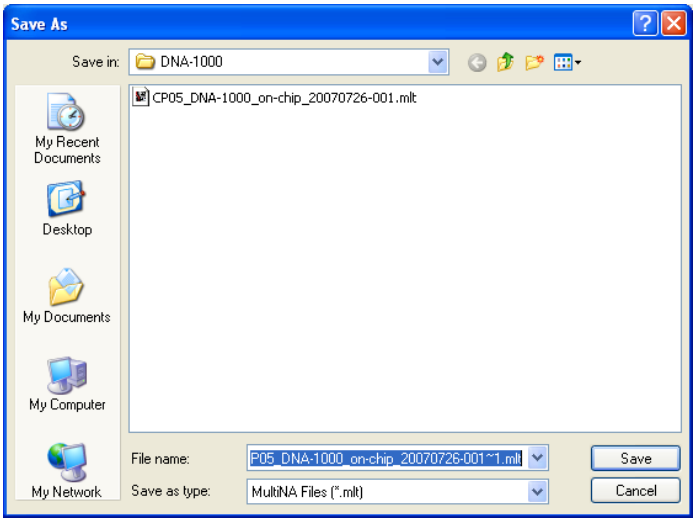
1 **Select data (sample) to be saved in a different file with a check mark on a gel image (or an electropherogram (multi)).**

Ladders used for sample analysis are automatically saved. Therefore, they do not need to be selected with a check mark.



2 **Select [Save Selected Data] on the [File] pull-down menu.**

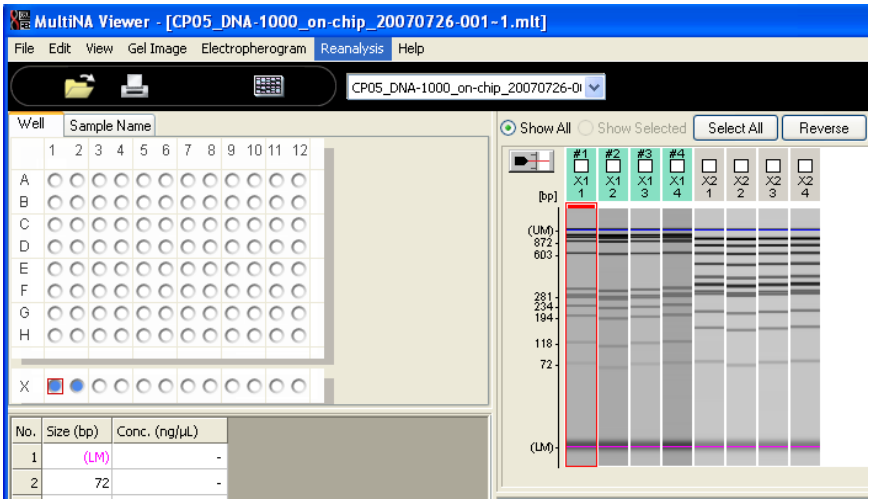
The [Save As] dialog box is displayed.



3 **Enter a new file name, which is different from the original data file, in the [File name] box and click [Save].**

By default, the new file name has "~" and numbers at the end of the original data file name.

4 **The saved file is automatically loaded and displayed.**

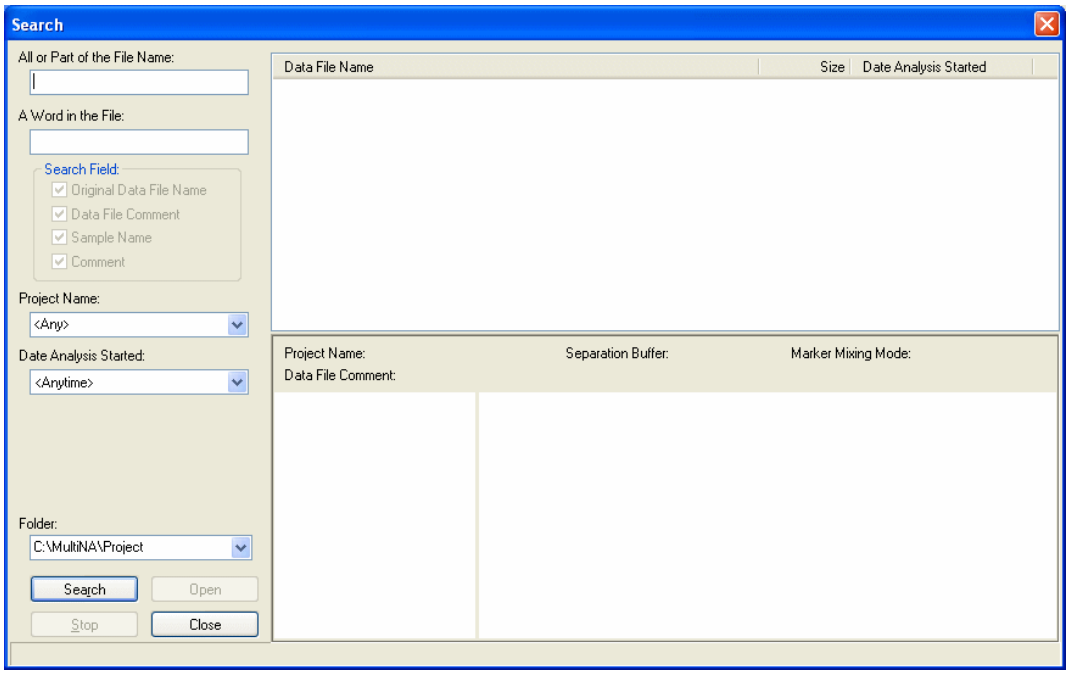


3.2.6 Search

Select [Search] to search data files in a specified folder with specific screening conditions such as data file names, keywords, or date analysis started.

1 Select [Search] on the [File] pull-down menu.

The [Search] dialog box is displayed.



2 Enter or select search conditions.

[Data file name]: Enter all or part of a data file name.
[A Word in the File]: Enter a keyword for searching in selected search fields.
[Project Name]: Select or enter a project name.
[Date Analysis Started]: Select the date when analysis started.
[Folder]: Select or enter a target folder.

- “AND” is applied to each condition entered in search fields.
- Only one target keyword can be used for searching. Select at least one search field from [Original data file], [Data file comment], [Sample name], and [Comment].
- To search by specifying a project name, select a project name from the combo box below [Project Name] or enter a project name (or part of it). Select <Any> when not specifying any name.
- To specify [Date Analysis Started], select the range of date analysis started from the list in the combo box ([Today], [Within the last week], [Within the last 30 days], [Within the last 90 days]) or select [Custom] and then specify the start and end of the time range.
- To search by specifying a folder, select the folder from the combo box or enter the folder name. Click <Browse…> from the list in the combo box. The [Select Folder] dialog box is displayed and a folder can be selected.

NOTE

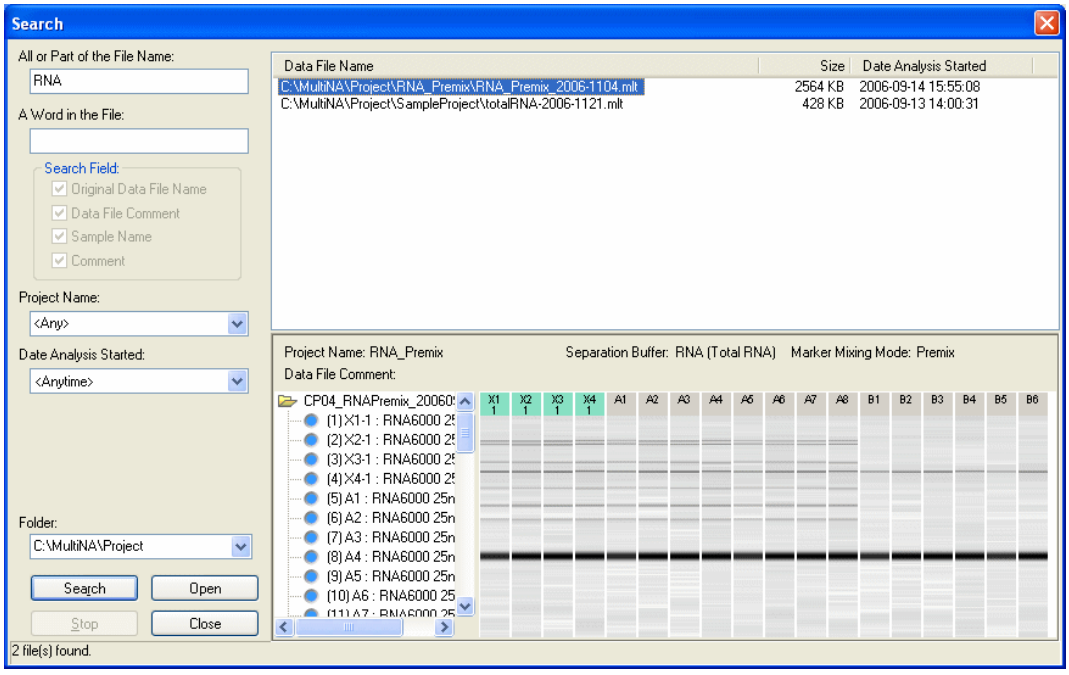
[Date Analysis Started] represents date and time where analysis of each data saved in MLT files started (see *"3.4.3 Status Bar" P.165*). It may be different from the time when the file was updated.

3 **Click [Search] to start searching.**

Click [Stop] to stop searching.

4 **Extracted data files are displayed on the list to the right with information such as data file name and date analysis started.**

Click a file name to display the preview of gel image and sample names.



5 **Select a file and click [Open] or double-click the file name to load the data.**

NOTE

Only one data file can be selected or loaded at a time.

3.2.7 Export

Select [Export] to export sample sheet or peak table as a CSV format file.

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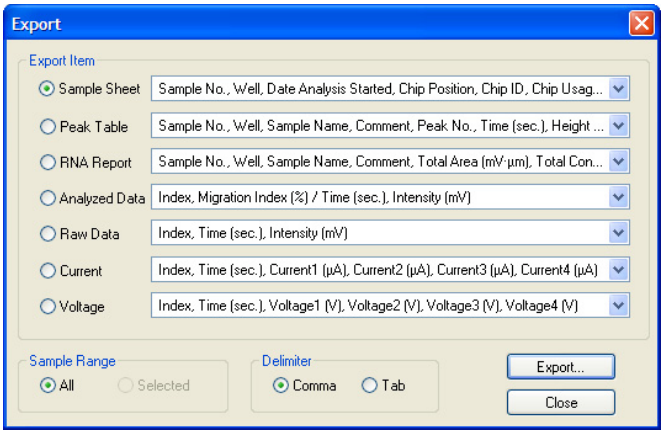
This allows selecting all detailed items to be exported.

1 **Select data to be exported by selecting the respective check box on [Gel Image] (or [Electropherogram (Multi)]).**

When exporting all data, data selection is unnecessary.

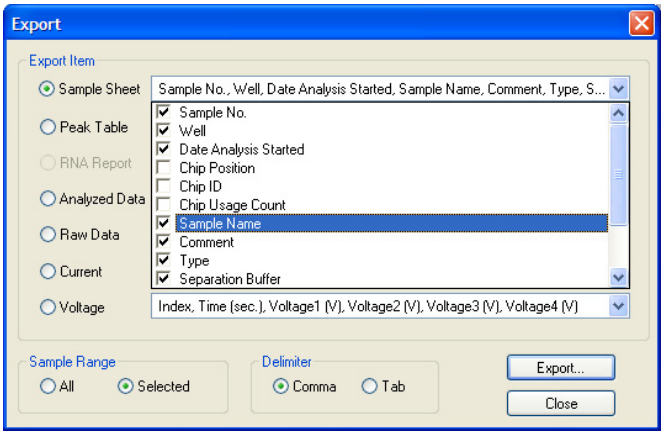
2 Select [Export] on the [File] pull-down menu.

The [Export] dialog box is displayed.



3 Specify items, range, and delimination mark to be exported in the dialog box above.

Click the arrow facing downward on the right side of the items to display a drop-down list. Select necessary detailed items with a check mark.



Item	Explanation
[Sample Sheet]	Well name, sample name, comment, chip ID, date analysis started, etc.
[Peak Table]	Peak table of sample data (size, concentration, height, time, area, etc.)
[RNA Report]	Total area, total concentration, and 28S rRNA/18S rRNA ratio for RNA sample (total RNA analysis only)
[Analyzed Data]	Migration index/time and signal strength at each data point in analyzed data
[Raw Data]	Time and signal strength at each data point in raw data
[Current]	Time and current on four electrodes of a microchip at each data point
[Voltage]	Time and voltage on four electrodes of a microchip at each data point

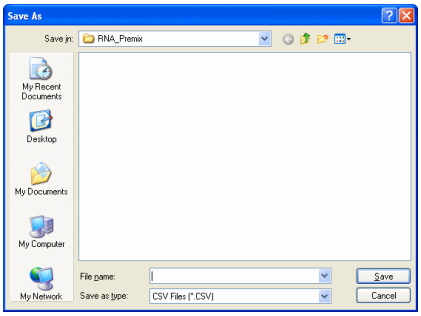
NOTE

If the horizontal axis of the analyzed data on [Electropherogram] represents migration index or size, the migration index data is exported. If the horizontal axis represents time, then time data is exported.

Range	Explanation
[All]	All data in the data file
[Selected]	Selected data with a check mark on [Gel Image] (or [Electropherogram (Multi)])

Delimination mark	Explanation
[Comma]	Data items are separated by commas.
[Tab]	Data items are separated by tabs and enclosed by double quotation marks.

4 **Click [Export] to display the [Save As] dialog box.**



5 **Enter a file name and click [Save].**

6 **The display returns to the dialog box shown in step 2.**

To continuously export another item, select the item and export it in the same procedure. Click [Close] to finish the exporting procedure.

3.2.8 Print

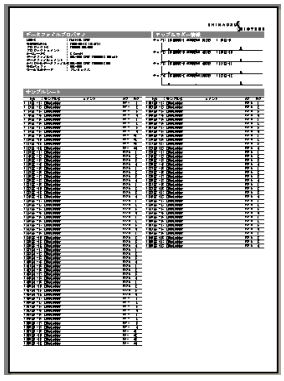
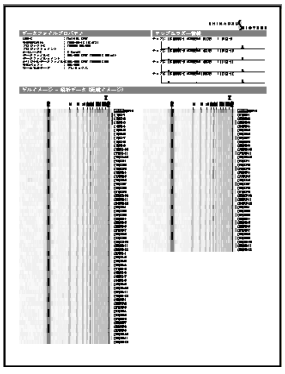
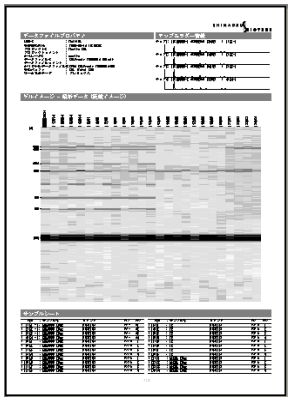
Select [Print] from the [File] menu to print data.

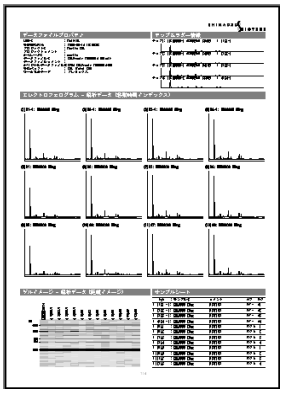
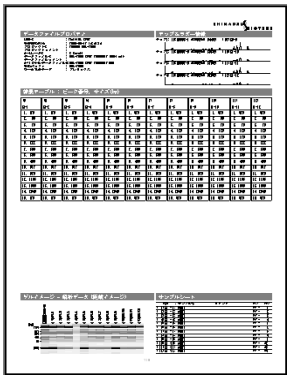
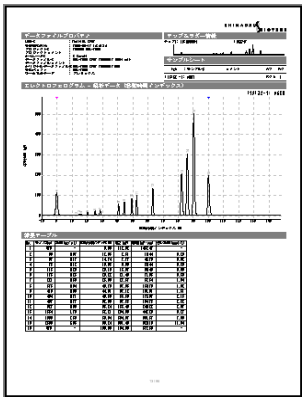
Check the following menu parameters to select the printed material.

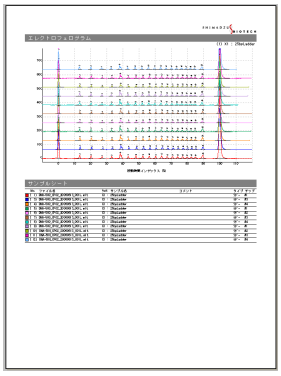
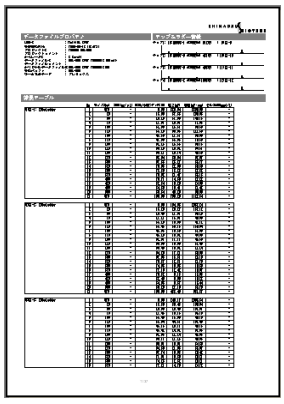
Matters reflected in printing	Settings in windows displayed	Reference
• Both Gel Image and Electropherogram		
Selection between [analyzed data]/[raw data]	[View] - [Analyzed Data] / [Raw Data]	*P.166*
Scale	[View] - [Options] - [Scale of Default View]	*P.174*
• Gel Image		
Selection of inversion of black and white	[Gel Image] - [Invert Black and White]	*P.176*
Brightness adjustment	Slider at the right of [Gel Image]	*P.135*
Selection between time image/distance image	[Gel Image] - [Time Image] / [Distance Image]	*P.178*
• Electropherogram (whole)		
Show or hide baseline	[Electropherogram] - [Show Baseline]	*P.181*
• Electropherogram (single)		
Show or hide marker signs	[View] - [Marker]	*P.164*
Peak top item	[Electropherogram] - [Peak Top]	*P.181*
• Analysis result (excluding 12 samples/page layout)		
Selection of items on the peak table	[View] - [Options] - [Peak Table]	*P.139*
• Overlaying selected data		
Width to make overlaid data vertically off	Slider during overlaying on [Electropherogram (Single)]	*P.140*

Available print layouts are shown in the table below. Layout diagrams are shown assuming that printing is carried out vertically. The layout is changed when printing horizontally,

Refer to *"Printing Procedure" P.158*.

Layout Category	Printed Subjects	Explanation
[120 Samples/Page]	Sample sheets (Data file properties, chip and ladder information)	A sample sheet containing up to 120 samples is printed on a page. 
	Gel Image (Data file properties, chip and ladder information)	Gel images for up to 120 samples are printed on a page. 
[24 Samples/Page]	Sample sheet, gel image (Data file properties, chip and ladder information)	A sample sheet and gel images for up to 24 samples are printed on a page. Ladder data is added to the left end of the gel image. (Among the ladders displayed, the ladder used for the first analysis is printed.) 

Layout Category	Printed Subjects	Explanation
[12 Samples/Page]	Electropherogram, gel image (Data file properties, chip and ladder information, sample sheet)	Electropherograms, gel images, and sample sheet for up to 12 samples are printed on a page. 
	Analysis result, gel image (Data file properties, chip and ladder information, sample sheet)	Analysis results, gel images, and sample sheet for up to 12 samples are printed on a page. Concerning analysis results, only the size of each peak is printed for DNA analysis. Only RNA report contents and concentration of each peak are printed for RNA analysis. (For one analysis, up to 60 lines of data can be printed.) 
[1 Sample/Page]	Electropherogram, analysis result (Data file properties, chip and ladder information, sample sheet)	An electropherogram and analysis result for one sample are printed on a single page. More than one page may be used if the analysis result display will not fit on a single page. 

Layout Category	Printed Subjects	Explanation
[Overlay Selected Data]	Electropherogram (sample sheet)	Electropherograms of selected data and focused data are printed overlaid. 
Other	All analysis results (Data file properties, chip and ladder information)	Analysis results for all data to be printed are printed. 

NOTE

Imported ladder data is not printed except for “Chip and Ladder Information” (see *"3.7.3 Change Ladder and Analyze" P.189*).

NOTE

For printing [Comparison View], “1 sample/page” layout cannot be used. Some displayed contents are different from ones of ordinary data files (see *"3.4.7 Comparison" P.167*).

■ Contents printed

Data File Properties

Properties such as data acquisition date and conditions set for the instrument are printed.

Data File Properties

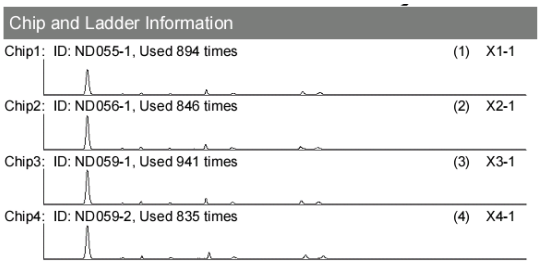
Instrument Name	: MultiNA
Date Analysis Started	: 2006-09-14 15:55:08
Project Name	: RNA_Premix
Project Comment	: Total RNA
Operator	: shimadzu
Data File Name	: RNA_Premix_2006-1104.mlt
Data File Comment	: Preparation 1
Original Data File Name	: RNAPremix_2006-0914-0000001
Separation Buffer	: RNA (Total RNA)
Marker Mixing Mode	: Premix

NOTE

If a large number of characteristics are selected for each item, all of the information may not be printed. (Printable range differs according to the printing conditions such as paper size and margin.)

Chip and Ladder Information

Microchip ID, number of uses (analysis schedule starting point), no., well, and electropherogram for the ladder used is printed for each chip position.



Sample Sheet

The sample sheet is printed (no., well, sample name, comment, type, and microchip position).

A “#” mark is added on the left of the chip position number to indicate the ladder used for analysis.

NOTE

If a large number of characteristics are selected for each item, all of the information may not be printed. (Printable range differs according to the printing conditions such as paper size and margin.)

Sample Sheet

	Well	: Sample Name	Comment	Type	Chip
(1)	X1 -1	: RNA6000 25ng		Ladder	#1
(2)	X2 -1	: RNA6000 25ng		Ladder	#2
(3)	X3 -1	: RNA6000 25ng		Ladder	#3
(4)	X4 -1	: RNA6000 25ng		Ladder	#4
(5)	A1	: RNA		Sample	1
(6)	A2	: RNA		Sample	2
(7)	A3	: RNA		Sample	3
(8)	A4	: RNA		Sample	4
(9)	A5	: RNA		Sample	1
(10)	A6	: RNA		Sample	2
(11)	A7	: RNA		Sample	3
(12)	A8	: RNA		Sample	4

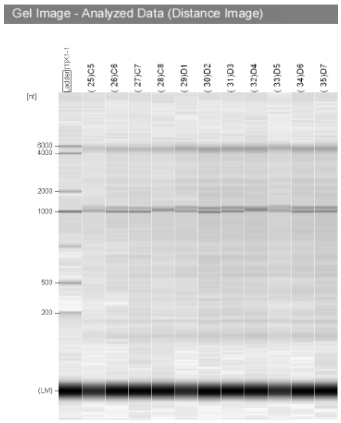
Gel Image

[Ladder] (the ladder used for the first analysis among ladder data displayed in Gel Image) is added to the left end of each gel Image. A size scale can be marked according to the band of this ladder.

Contrast is adjusted by the slider on the right end in the [Gel Image] window (see *"3.1.6 Gel Image" P.135*).

There are three types of gel images; for analyzed data (distance image), for analyzed data (time image), and for raw data.

The following is an example of analyzed data (distance image).

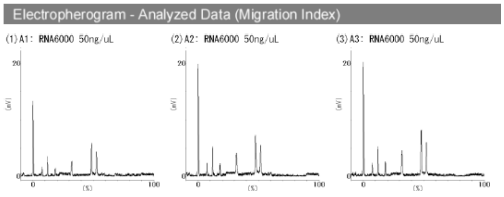


Electropherogram

Each electropherogram is printed with (number), well, and sample name. The vertical scale, set in [Options] on the [View] menu of the [Electropherogram] window is applied.

There are three types of electropherograms; analyzed data (migration index), analyzed data (migration time), and raw data.

The following is an example of analyzed data (migration index).



Results Table: DNA analysis

In the [12 samples/page] layout, (number), well, and predicted size of each peak are printed.

In the [1 sample/page and all results table] layout, (number), well, and items selected in [Options] on the [View] menu are printed.

Results Table: RNA analysis

In the [12 samples/page] layout, (number), well, concentration of each peak, and contents of RNA report (total area, total concentration, and the 28S rRNA/18S rRNA ratio for Total RNA) are printed.

In the [1 sample/page and all results table] layout, (number), well, and items selected on [Options] on the [View] menu are printed.

Overlay Selected Data

On sample sheet, data file names, (numbers), wells, sample names, comments, types, and chip positions of overlaid data.

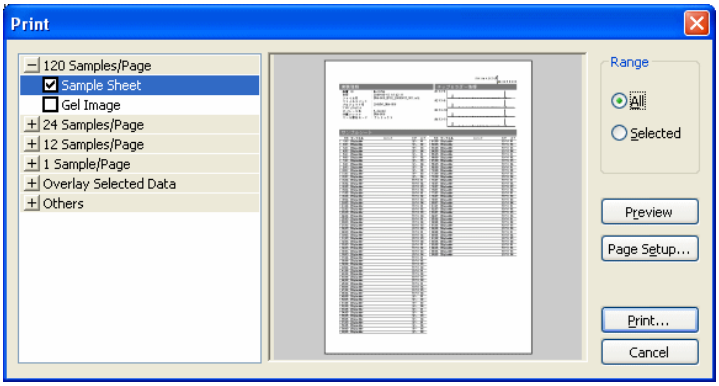
■ Printing Procedure

1 **Select the data to be printed.**

This process is unnecessary when printing all data.

2 **Select [Print] on the [File] pull-down menu or click the [Print active data file] button on the toolbar.**

The [Print] dialog box is displayed.

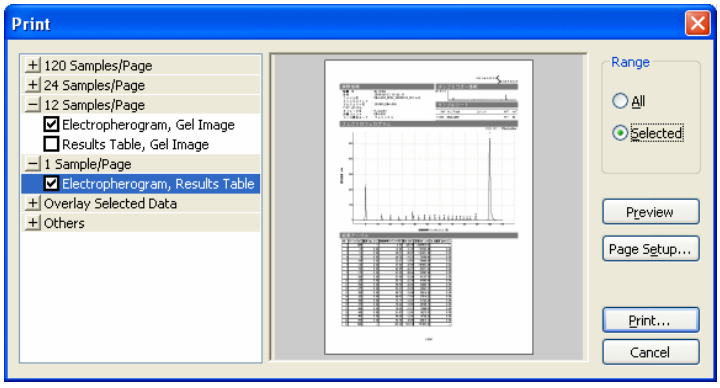


3 **Select a layout for printing.**

- Click a “+” mark displayed with printing layouts at the left to display items to be printed such as sample sheet and gel image under each printing layout category.
- Select an item to be printed and the image of the respective layout is displayed in the right field of the dialog box.
- Click [Page Setup] to set paper size, orientation, page margins, and other items.

4 **Select the items to be printed.**

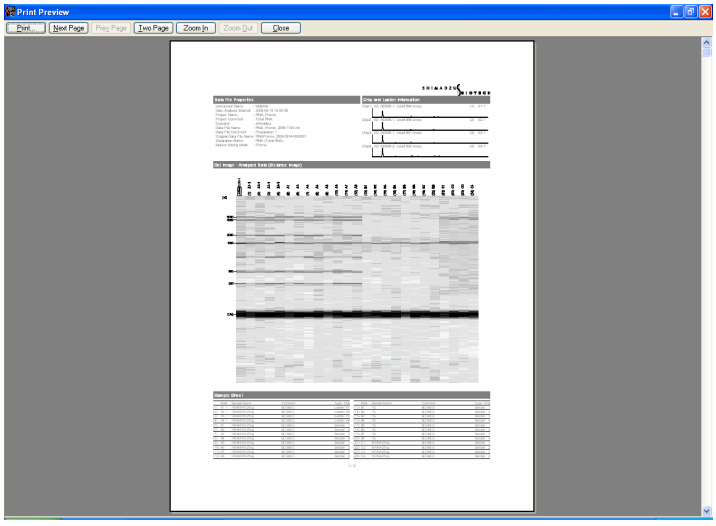
The [Preview] and [Print] buttons are enabled.



5 **Select the data range to be printed.**

Click [All] to print all of the data in the file or click [Selected] to print only the selected data.

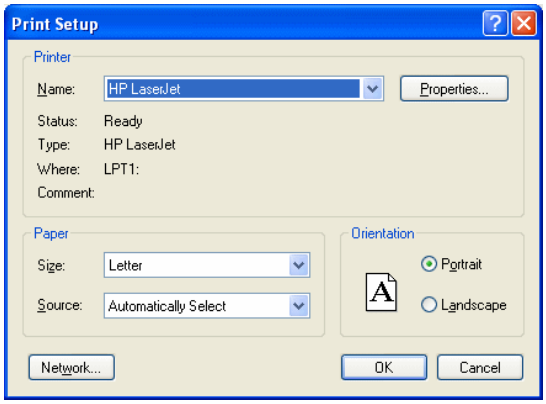
6 **Click [Preview] to review the print image and determine the number of pages.**



7 **Click [Print] to start printing.**

3.2.9 Print Setup

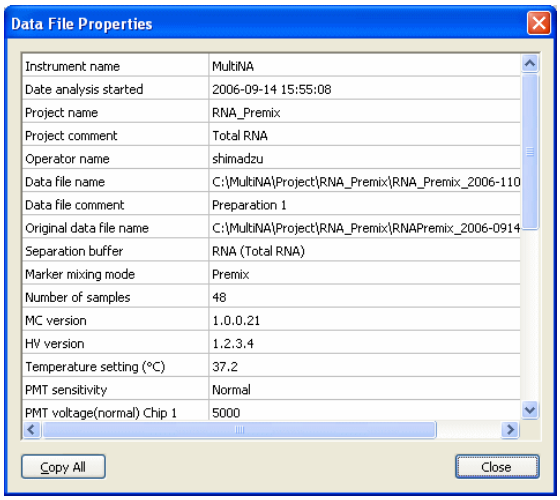
The [Print Setup] dialog box is displayed. Select a printer and set the printer properties such as paper size and orientation.



3.2.10 Data File Properties

Select [Data File Properties] to display the instrument conditions when a data file was acquired.

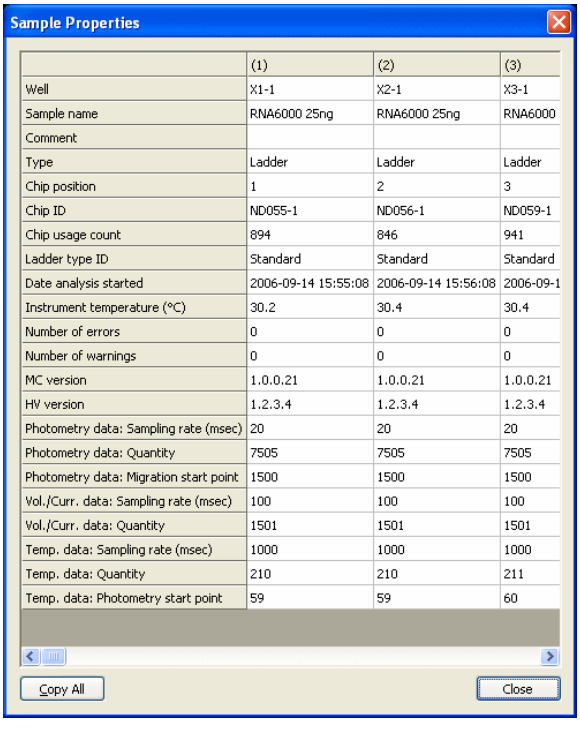
- Click [Copy All] to copy all of the displayed contents to the clipboard.
- Select cells, lines, or columns and then right-click and select [Copy Selected Cells] to copy the selected cells to the clipboard.



3.2.11 Sample Properties

Select [Sample Properties] to display all of the information in the file such as the conditions when the data was acquired.

- Click [Copy All] to copy all of the displayed contents to the clipboard.
- Select cells, lines, or columns and then right-click and select [Copy Selected Cells] to copy the selected cells to the clipboard.



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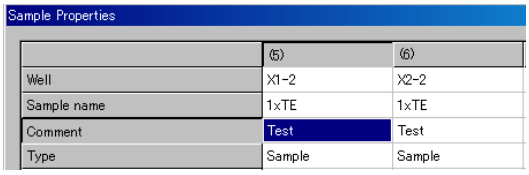
Sample names and comments can be edited using the following procedure.

1 **On the [File] pull-down menu, click [Sample Properties]. Or click [Sample Properties] on the right click pop-up menu displayed on the gel image.**

The [Sample Properties] window is displayed.

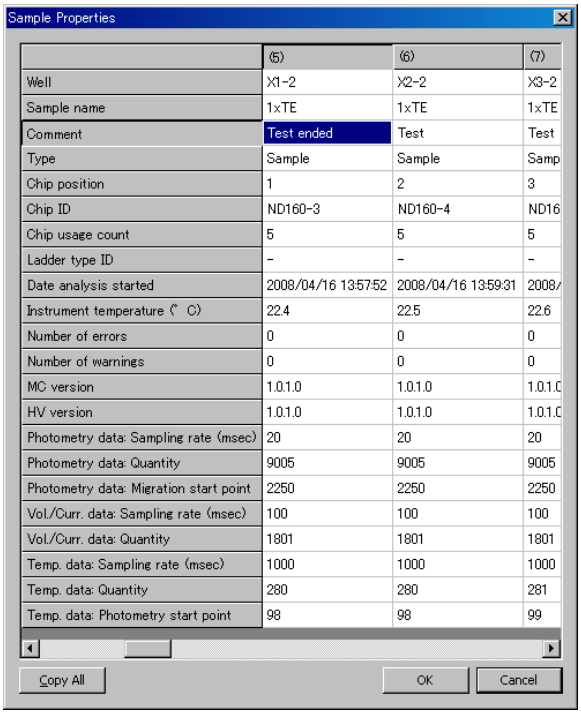
2 **Double-click the sample name or comment box to be edited.**

When the cursor appears, the content can be edited.



3 **When finished editing, click [OK] to close the window.**

Clicking [Cancel] aborts any editing changes made.

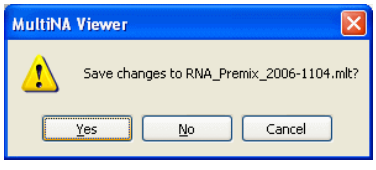


4 **On the [File] pull-down menu, select [Save] or [Save As] to save the file.**

NOTE

The message below is displayed when an attempt is made to close a data file without saving the changes.

- Click [Yes] to save the changes in the initial data file.
- Click [No] to cancel the changes.
- Click [Cancel] to stop the closing process.

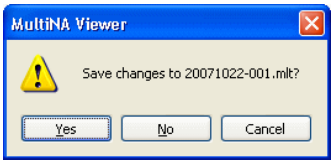


3.2.12 Exit

Click [Exit] to quit MultiNA Viewer.

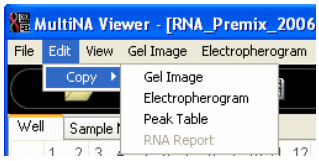
NOTE

The following message is displayed when the results were not saved after editing using [Manual Edit Mode] on the [Reanalysis] menu or [Change Ladder and Analyze] on the [Reanalysis] menu. Click [Yes] to save the changes and close the MultiNA Viewer, [No] to close the MultiNA Viewer without saving the changes, or [Cancel] to cancel the exit operation.



3.3 Edit Menu

3.3.1 Copy



Select either [Gel Image], [Electropherogram], [Peak Table], or [RNA Report] (RNA analysis only) from [Copy] on the [Edit] menu. The corresponding image and data is copied to the clipboard.

- [Gel image] / [Electropherogram]

  Image data (except for the red focus outline) is copied.

  The same result can be achieved by selecting [Copy] on the right click pop-up menu on [Gel Image] or [Electropherogram].

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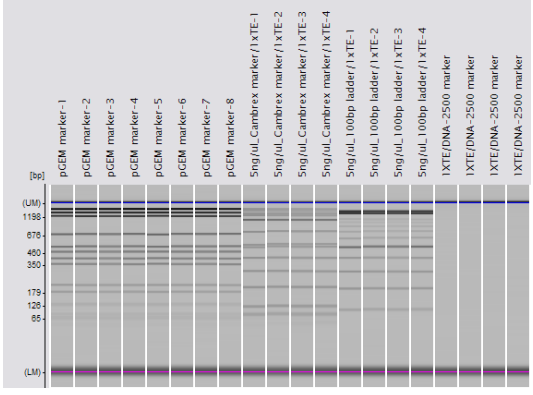
  If [Gel Image] is selected, each data number, well, sample name, and comment can be copied to the top of the gel image. Select the items to copy by clicking [View] and pointing to [Title] on the pull-down menu.



- [Peak table], [RNA report]

  When [Copy] is selected on the [Edit] menu:

  All cells including item names and line numbers are copied.

  When “Copy Selected Cells” is selected on the right click pop-up menu:

  Only selected cells are copied. Item names and line numbers are not copied.

NOTE

The [Copy] function of [Gel Image] and [Electropherogram] is used for copying the window currently being displayed. Note that if a different window is displayed over the MultiNA Viewer window immediately after selecting the menu (within approx. one second), the content of the different window is copied.

3.3.2 Saving Images to File

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On the [Edit] pull-down menu, clicking [Save Image As] and pointing to [Gel Image] or [Electropherogram] saves the gel image or electropherogram currently displayed as an image file.

The same result can be achieved by selecting [Save Image As] on the right click pop-up menu on [Gel Image] or [Electropherogram].

If [Gel Image] is selected, items selected by clicking [Title] on the [View] pull-down menu (number, well, sample name, and comments) are inserted at the top of the gel image.

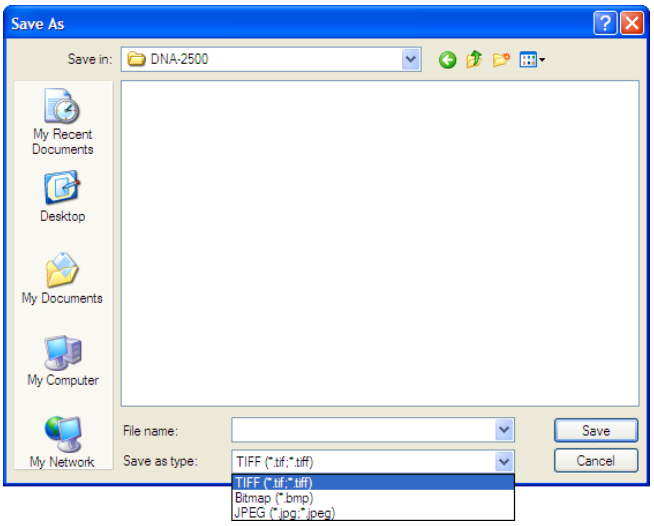
NOTE

The [Save Image As] function of [Gel Image] and [Electropherogram] is used for copying the window currently being displayed. Note that if a different window is displayed over the MultiNA Viewer window immediately after selecting the menu (within approx. one second), the content of the different window is saved.

1 **On the [Edit] pull-down menu, select [Save Image As], then click [Gel Image] or [Electropherogram].**

Or click [Save Image As] on the right click pop-up menu displayed on [Gel Image] or [Electropherogram].

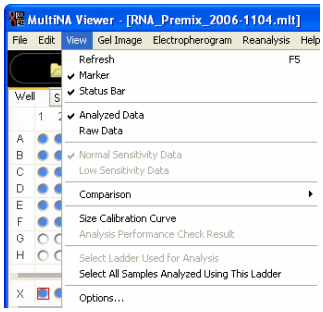
2 **When the [Save As] window appears, select [TIFF], [Bitmap], or [JPEG] for the file type.**



3 **Select [Save in], enter the file name, and click the [Save] button.**

File extensions are automatically added to file names.

3.4 View Menu

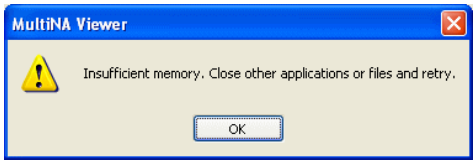


3.4.1 Refresh

Select [Refresh] to reload a data file and update it. Use this function to update data being analyzed.

NOTE

The following message is displayed when a file, being analyzed by the instrument, is opened or updated by a PC with insufficient memory.

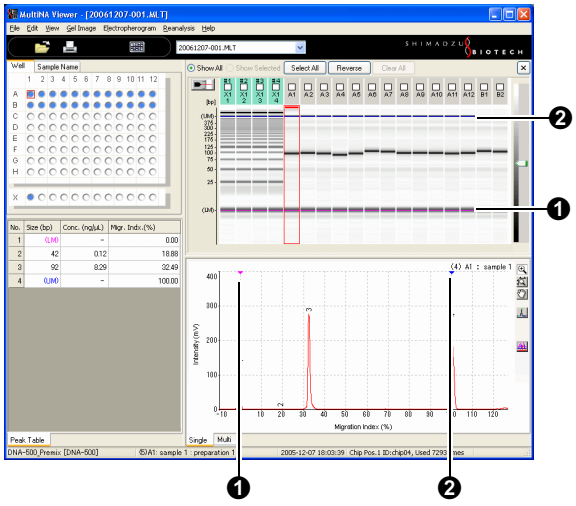


Click [OK] and close other applications or close the other files open in the MultiNA Viewer then repeat the operation.

If this memory error appears frequently, a PC memory upgrade is recommended.

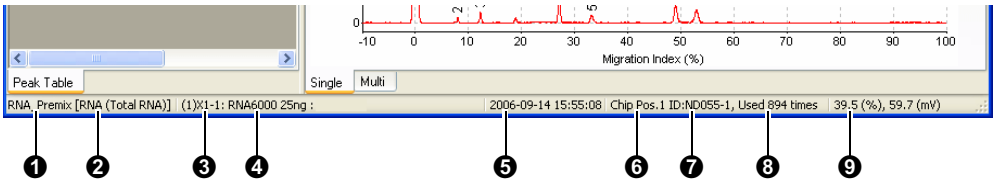
3.4.2 Marker

Select [Marker] to switch between display/hide the marker on [Gel Image] or [Electropherogram (Single)]. When the [Marker] menu is selected with a check mark (default), the lower marker (magenta line and triangle 1) and the upper marker (blue line and triangle 2) are displayed as shown in the figure below.



3.4.3 Status Bar

Select [Status Bar] on the [View] menu to display or hide the status bar at the bottom of the window. The information displayed on the status bar pertains to the focused data and is described below.



No.	Explanation
❶	Project name
❷	Separation buffer (TotalRNA or mRNA distinction for RNA analysis)
❸	(Analysis No.)Well name:
❹	Sample name: Comment
❺	Date analysis started
❻	Chip position
❼	Chip ID
❽	Chip use count
❾	Information from MultiNA Viewer (cursor position); values on vertical and horizontal axes on single electropherogram

3.4.4 Title

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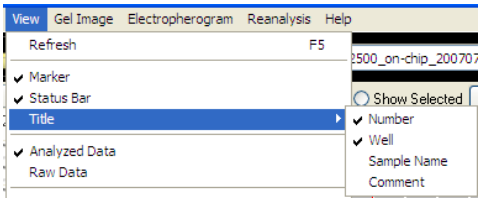


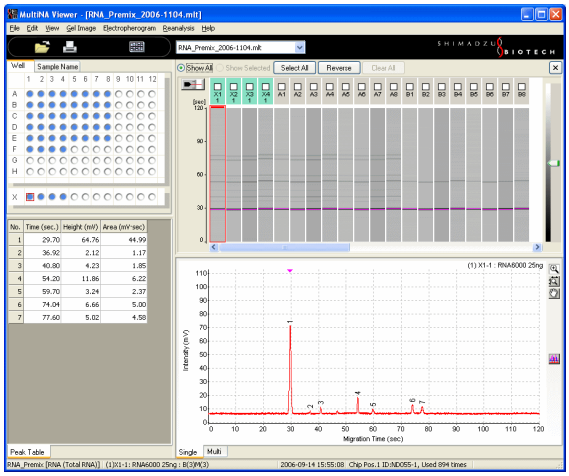
Items selected with checkmarks on this menu will be reflected in the following displayed or printed information.

- Title displayed in upper right area on [Electropherogram (Single)]
- Tooltip displayed when the cursor rolls over the top (light green or gray) area of gel images
- Information displayed in lower right area of respective overlaid electropherograms
- Information inserted at top of gel images when they are copied or saved by clicking [Copy] or [Save Image As]
- Title printed in upper right area of electropherograms by clicking [Sample] or [Page Print]
- Information printed in lower right area of each image overlaid when printing overlays of selected data

3.4.5 Analyzed Data/Raw Data

Analyzed data is displayed when a data file is opened. To display raw data, select [Raw Data] on the [View] pull-down menu.

To display analyzed data again, select [Analyzed Data] on the [View] pull-down menu.

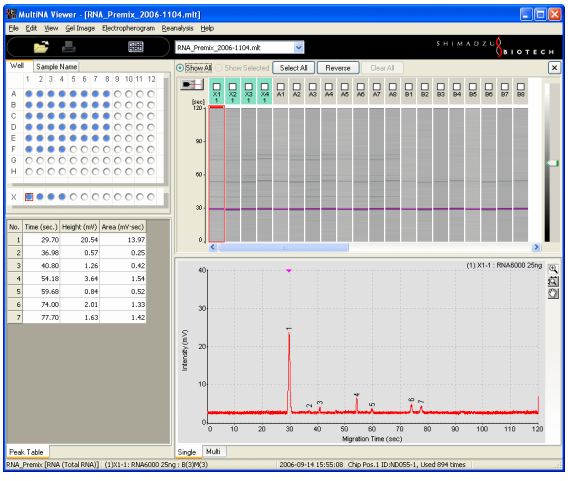


The following contents are displayed in the raw data view.

Window	Contents Displayed
[Gel Image]	Vertical axis: Migration time (sec)
[Electropherogram]	Horizontal axis: Migration time (sec), Vertical axis: Signal strength before the removal of baseline (mV)
[Peak Table]	Time (sec), height (mV), area (mV•sec) (Items to be displayed cannot be changed.)

3.4.6 Normal Sensitivity Data/Low Sensitivity Data

Select [Low Sensitivity Data] from the [View] menu to display low sensitivity raw data.

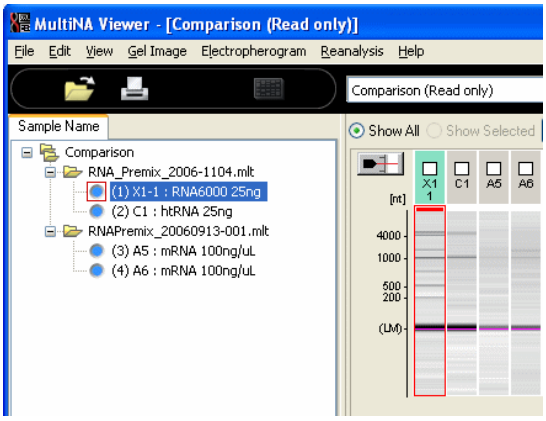


- Signal intensity of low sensitivity data is approximately half as compared to normal sensitivity data.
- If signal saturation occurs on normal sensitivity data due to high sample concentration (50 ng/µL min of DNA concentration), the sample may need to be diluted. Refer to the peak height obtained in low sensitivity data to decide how much the sample should be diluted.
- Reanalysis, print, or export is not available with low sensitivity data because it contains raw data only.
- To return the display to analyzed data, select [Normal Sensitivity Data] on the [View] menu, and select [Analyzed Data] on the [View] menu.

3.4.7 Comparison

Select [Comparison] to display data in different data files in one window. Only data obtained with the same type of separation buffer can be displayed.

- Well numbers and sample names of the samples that have been added to the [Comparison] view are displayed under the original data file names in the comparison sample name tree. A sequential number (displayed in parentheses) is added to each sample name on the view.
- Data displayed in the [Comparison] view is “read only.” The [Save], [Save As], and [Reanalysis] functions are not enabled.

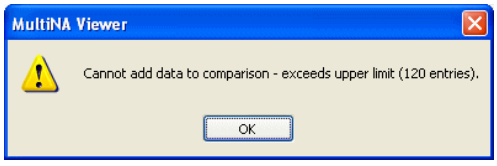


NOTE

Only data obtained with the same type of separation buffer can be compared. The following message is displayed if an attempt is made to add data obtained with a different type of separation buffer.

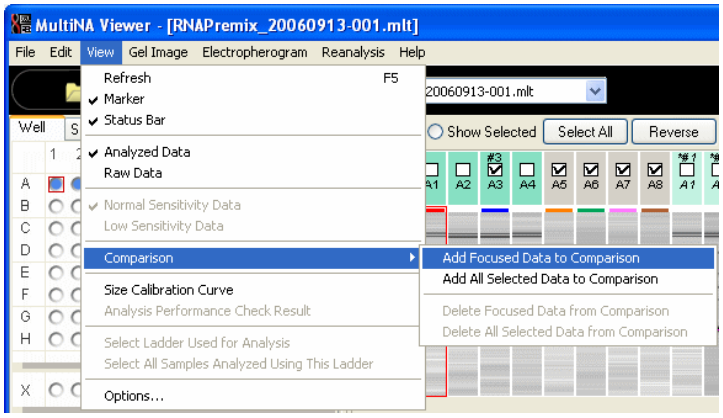
NOTE

Up to 120 data can be added to the [Comparison] view. The following message is displayed if an attempt is made to add more than 120 data to the [Comparison] view.



■ Add Data to Comparison

This section describes the addition of focused or selected data to the comparison view.



Add Focused Data to Comparison

1 **Focus (Click) the data to be added to Comparison on Gel Image.**

2 **On the [View] pull-down menu, select [Comparison] and point to [Add Focused Data to Comparison].**

Or select [Add Focused Data to Comparison] on the right click pop-up menu on Gel Image.

3 **The data is added to the Comparison view.**

Add All Selected Data to Comparison

1 **Select the check box of the data to be added to Comparison on Gel Image.**

2 **On the [View] pull-down menu, select [Comparison] and point to [Add All Selected Data to Comparison].**

Or select [Add All Selected Data to Comparison] on the right click pop-up menu on Gel Image.

3 **The data is added to comparison view.**

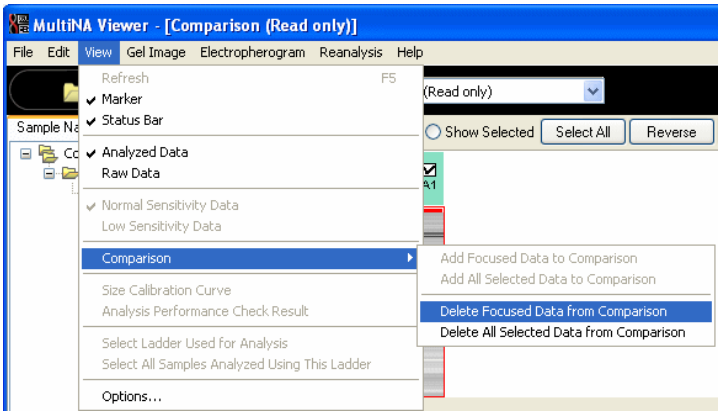
NOTE

When the first data is added to the [Comparison] view, the window automatically changes to [Comparison]. However, the [Comparison] window is not automatically displayed when the next or following data is added.

Add all data to be compared then select [Comparison] in the file name list box on the toolbar.

■ Delete Data From Comparison

This section describes procedures to delete focused or selected data from the [Comparison] view.



Delete Focused Data from Comparison

1 **Focus (click) the data to be deleted on [Gel Image] at the [Comparison] view.**

2 **On the [View] pull-down menu, select [Comparison] and point to [Delete Focused Data from Comparison].**

Or select [Delete Focused Data from Comparison] on the right click pop-up menu on Gel Image.

3 **The focused data is deleted from the Comparison view and the view is updated.**

Delete All Selected Data from Comparison

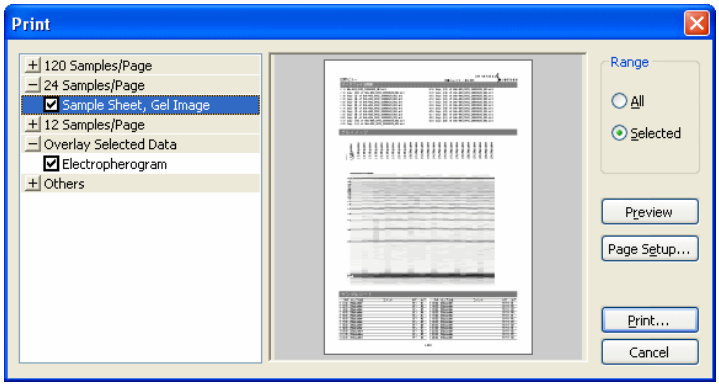
1 **Select the check box of the data to be deleted on Gel Image at the Comparison view.**

2 **On the [View] pull-down menu, select [Comparison] and point to [Delete All Selected Data from Comparison].**

Or select [Delete All Selected Data from Comparison] on the right click pop-up menu on Gel Image.

3 **The selected data is deleted from the [Comparison] view and the view is updated.**

■ Print



Data displayed on the Comparison view can be printed by selecting the [Print] menu. Refer to *"3.2.8 Print" P.152*. Some differences from printing ordinary data files are described below.

- The [1 Sample/Page] layout is not available.
- <Data file numbers> and the original data file names are printed in the [Data File Information] section.
- (Sequential data numbers), wells in the original data file, and <data file numbers> are printed in the [Sample Sheet] section. Sample names, comments, types, and positions of the chip used are also printed to the right.

Comparison

Data File Information

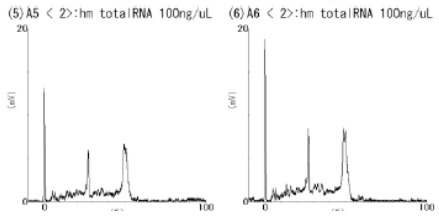
<1> RNA_Premix_2006-0913-001.mlt
<2> RNA_Premix_2006-1104.mlt

Sample Sheet

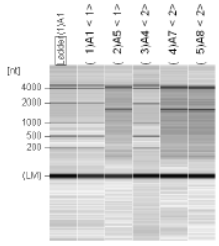
	Well		: Sample Name	Comment	Type	Chip
(1)	A3	<1>	: RNA6000 50ng/uL	B(2) M(2)	Ladder	3
(2)	C1	<2>	: htRNA 25ng	B(3)M(3)	Sample	1
(3)	C2	<2>	: htRNA 25ng	B(3)M(3)	Sample	2
(4)	C3	<2>	: htRNA 25ng	B(3)M(3)	Sample	3
(5)	C4	<2>	: htRNA 25ng	B(3)M(3)	Sample	4

- The (sequential data number), well, and <data file number> are printed for each data in the [Electropherogram] and [Gel Image] sections. The left end ladder on Gel Image is the first ladder displayed on the comparison view. The ladder is automatically added to the Gel Image.

Electropherogram - Analyzed Data (Migration Index)



Gel Image - Analyzed Data (Distance Image)



- The (sequential data number), well, <data file number>, and sample name of each sample are printed in the left cell of the [Results Table].

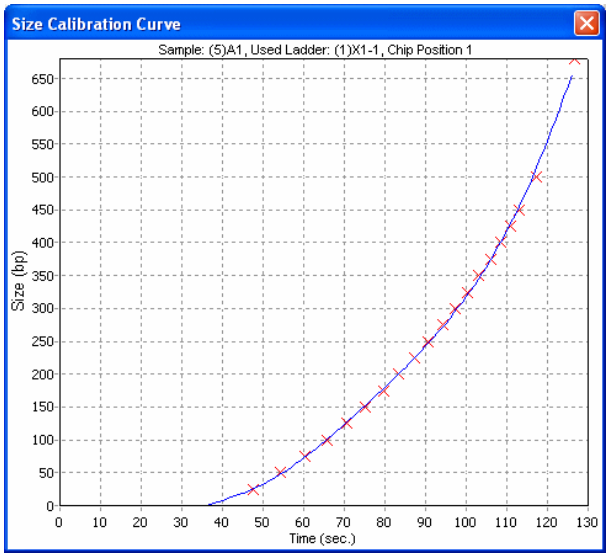
Results Table

	No.	Attrib.	Conc. (ng/µL)
(1)A3 <1>:RNA6000 50ng/uL	1	(LM)	-
	2	200 nt	-
	3	500 nt	-
	4	1000 nt	-
	5	2000 nt	-
	6	4000 nt	-
	7	6000 nt	-

3.4.8 Size Calibration Curve

Select [Size Calibration Curve] to display a graph of the size calibration curve used in analysis of focused data (sample and ladder).

- Size calibration curve is created for each chip position based on the ladder that was analyzed on that chip. For the ladder used for analysis, see *"1.11.2 Data Examination and Reanalysis" P.73*.
- When Raw Data is displayed, size calibration curve is not displayed.
- Click the ☒ button on the upper right corner to close the window.



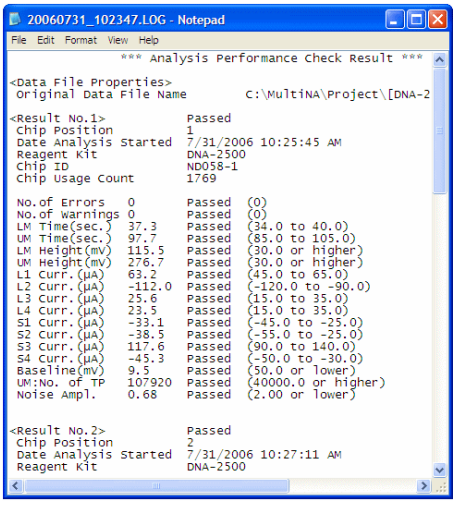
3.4.9 Analysis Performance Check Result

Select [Analysis Performance Check Result] to display the result of analysis performance check.

- Normally, when an analysis performance check data file is loaded, the result is automatically displayed as well. Select this menu to display the result window again after closing it.
- The result (LOG file) is saved in a [reagent kit name] folder in the project folder as plain text and can be opened on “Notepad” or an editor program. (The data is easier to view when it is displayed in single spaced font.)

☞ Reference

"2.5.6 Check Analysis Performance" P.120, check item: *P.123*

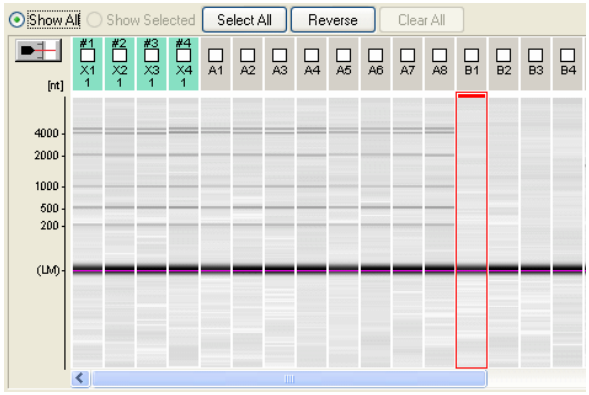


3.4.10 Select Ladder Used for Analysis

Select the [Select Ladder Used for Analysis] menu item from the [View] menu to display the ladder data used to analyze the focused sample data.

1 **Focus (click) the sample data on Gel Image.**

The upper area of the Gel Image is gray for sample data.

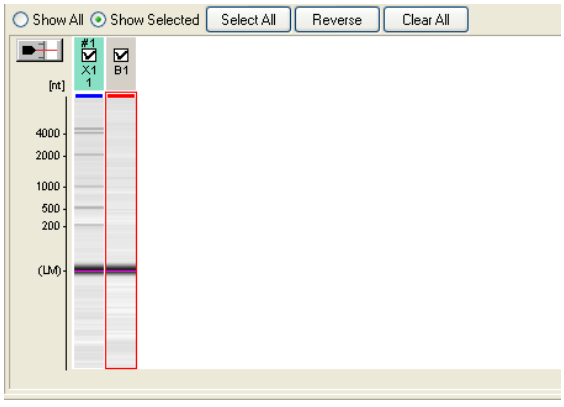


2 **Select [Select Ladder Used for Analysis] on the [View] pull-down menu.**

Or select [Select Ladder Used for Analysis] on the Gel Image right click pop-up menu.

3 **The check box of the focused sample and the corresponding ladder data is selected and only the "selected data" is displayed ([Show Selected]) on Gel Image.**

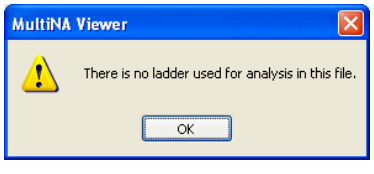
To return to the original display, select [Show All].



Refer to *"3.4.11 Select All Samples Analyzed Using This Ladder" P.173* to display all of the data that was analyzed using the selected ladder.

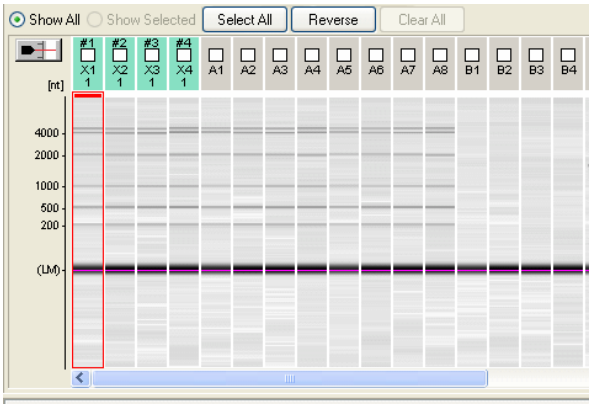
NOTE

The following message is displayed if there is no ladder data associated with the focused sample data.



3.4.11 Select All Samples Analyzed Using This Ladder

Select the [Select All Samples Analyzed Using This Ladder] menu item from the [View] menu to display all of the sample data that was analyzed using the focused ladder.



1 **Focus (click) the ladder data on the Gel Image.**

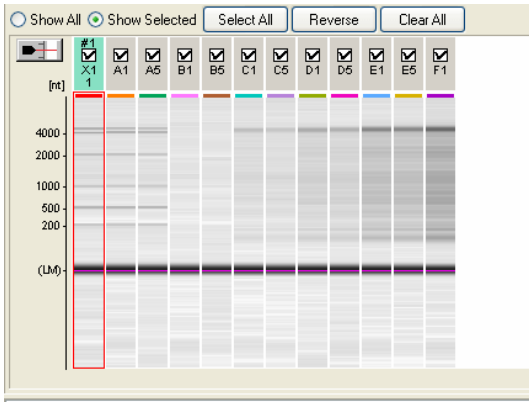
For ladders used for analysis, numbers #1 to #4 (numbers indicate chip positions) are displayed above the check box.

2 **Select [Select All Samples Analyzed Using This Ladder] on the [View] pull-down menu.**

Or select [Select All Samples Analyzed Using This Ladder] on the Gel Image right click pop-up menu.

3 **Check boxes of the ladder and all the corresponding sample data are selected and only the "selected data" is displayed ([Show Selected]) on the Gel Image.**

To return to the original display, select [Show All].

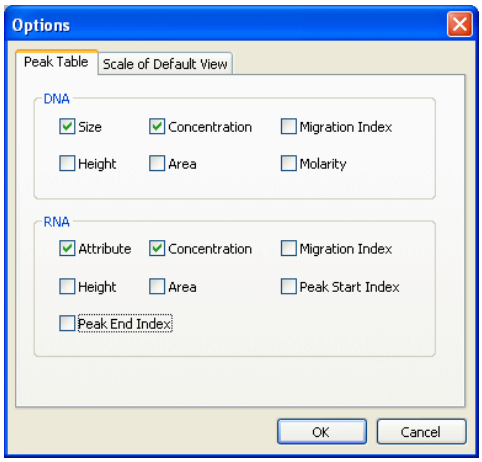


3.4.12 Options

Select [Options] from the [View] menu to change the following items.

■ [Peak Table] Tab

Select the items to be displayed on the peak table.

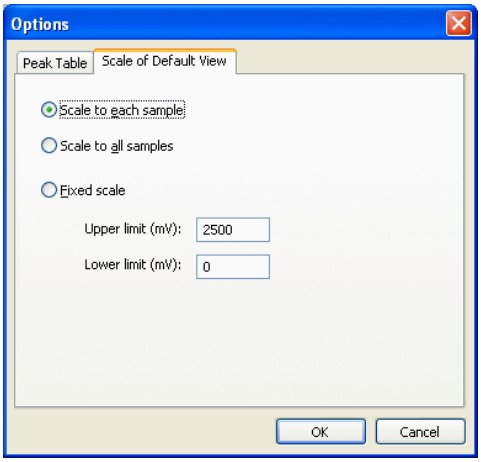


Item		Explanation
DNA (Display items for DNA data)	Size (bp)	Specified size for ladder data. Size calculated using the size calibration curve for sample data
	Concentration (ng/µL)	Concentration calculated using the fragment and marker peak areas.
	Migration Index (%)	Migration index based on marker detection time (Lower marker: 0%, Upper marker: 100%).
	Height (mV)	Peak height measured from baseline
	Area (mV•µm)	Peak area calculated by converting the migration time into the migration distance
	Molarity (pmol/L)	Mol concentration calculated from the concentration assuming that the average molecular mass is 333/1 mer
RNA (Display items for RNA data)	Attribute	Specified size for lower marker (LM), rRNA (18S, 28S), and ladder data V1.05 Size for samples (reference value only.)
	Concentration (ng/µL)	Concentration calculated using the fragment and marker peak area
	Migration Index (%)	Migration index based on marker detection time (Lower marker: 0%, End point of data: 100%)
	Height (mV)	Peak height measured from baseline
	Area (mV•µm)	Peak area calculated by converting the migration time into the migration distance
	Peak start index (%)	Migration index at the peak start point
	Peak end index (%)	Migration index at the peak end point

NOTE

- For [Raw data] only time (sec), height (mV), and area (mV•sec) are displayed and these settings cannot be changed.
- For RNA analysis, there is only one marker, making accurate size prediction impossible. Size values for RNA analysis are not included in the instrument specifications.

■ [Scale of Default View] Tab



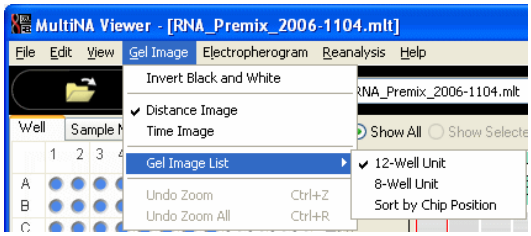
Item	Explanation
Scale to each sample	The maximum concentration displayed on Gel Image and the maximum vertical axis value on Electropherogram are displayed according to the maximum value of each data.
Scale to all samples	The maximum concentration displayed on Gel Image and the maximum vertical axis value on Electropherogram are displayed according to the maximum value of all data (except for import ladder) included in the data file.
Fixed scale	The maximum and minimum values both for contrast displayed on Gel Image and the vertical axis on Electropherogram are displayed according to the entered values.

NOTE

Scale changes are not immediately reflected when the [Electropherogram] is zoomed in. [Scale of Default View] changes are applied when the default display is recovered by selecting [Undo Zoom All].

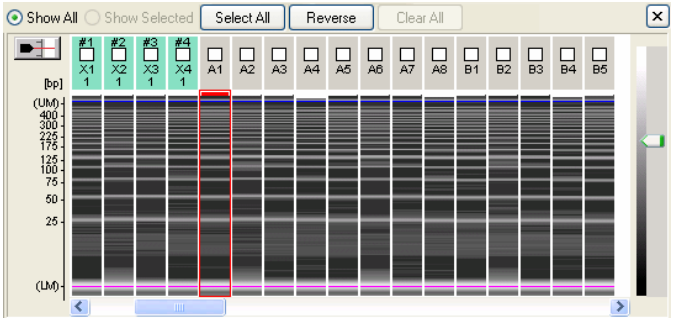
The graph may be displayed over the display range even after [Undo Zoom All] has been selected if [Fixed scale] has been selected.

3.5 Gel Image Menu



3.5.1 Invert Black and White

Select [Invert Black and White] from the [Gel Image] menu to change the background of the Gel Image display from white to black and back again.



- Select [Invert Black and White] on the [Gel Image] pull-down menu to invert black and white on Gel Image.
- Brightness can be adjusted by dragging the slider at the right end up or down.
- To revert the black and white display, select [Invert Black and White] again.

3.5.2 Vertical Axis

V1.05

Select the display on the vertical axis of the analyzed data from among [Distance Image], [Size Image], or [Time Image].

- [Distance Image] is displayed by default when a data file is opened.
- When [Distance Image] is selected, the value calculated by converting the migration time into the migration distance is displayed on the vertical axis. This gel image is similar to one obtained by Agarose gel electrophoresis.
- When [Size Image] is selected, the image displayed is obtained by correcting the distance image based on one reference ladder size. Same-size peaks are displayed in the same-position band. The reference ladder is the ladder used for the first analysis.

NOTE

For raw data or data without a ladder, or if there is data from a failed analysis, size cannot be calculated. Consequently, [Size Image] cannot be selected. If this occurs, reanalyze or manually edit the data, or click [Show Selected] on [Gel Image] to display only data that has been successfully analyzed.

- When [Time Image] is selected, the vertical axis is displayed as migration index for analyzed data and migration time for raw data.
- For raw data, [Time Image] is always displayed and this menu is not selectable (see *"3.4.5 Analyzed Data/Raw Data" P.166*).

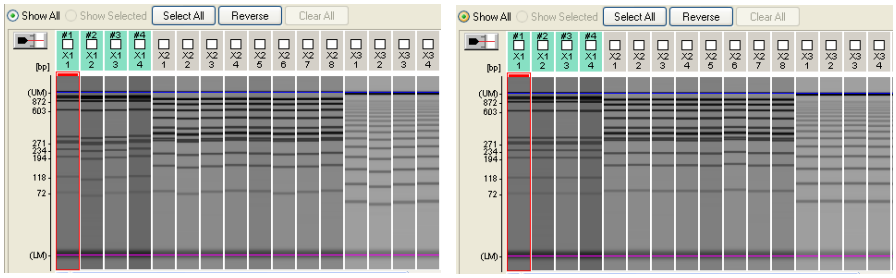


Fig.3-1 Distance Image (Left) and Size Image (Right)

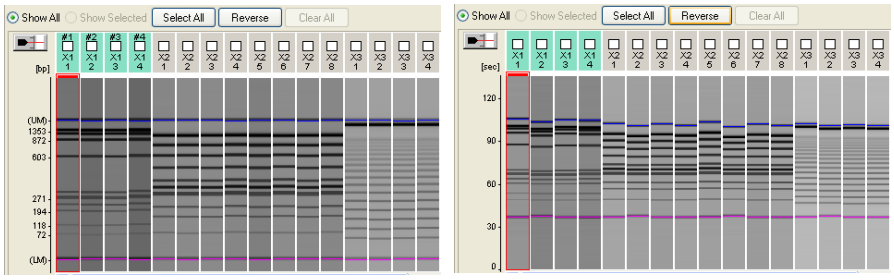


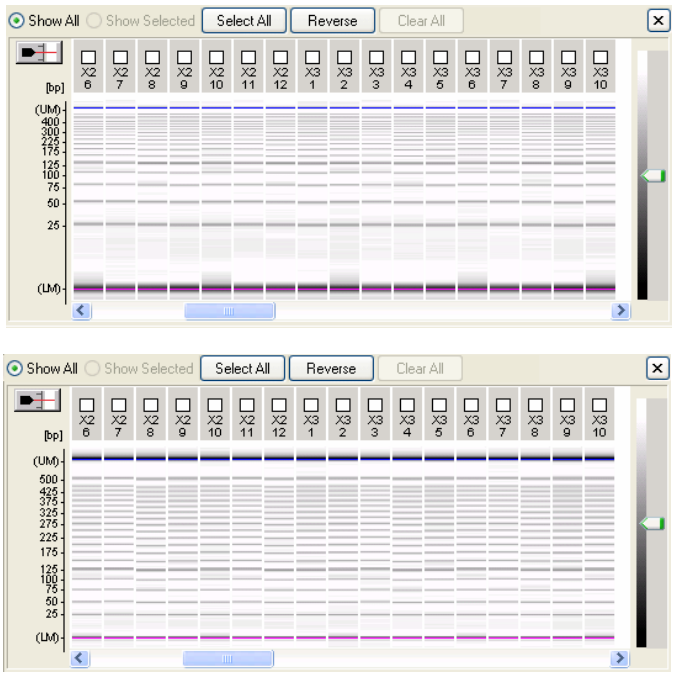
Fig.3-2 Analyzed Data (Left) and Raw Data (Right) of Time Image

3.5.3 Distance Image/Time Image

Select the [Distance Image] or [Time Image] item from the [Gel Image] menu to change vertical axis displayed on the Gel Image.

- By default, [Distance Image] is displayed when a data file is opened.
- When [Distance Image] is selected, the value calculated by converting the migration time into the migration distance is displayed on the vertical axis. This gel image is similar to one obtained by Agarose gel electrophoresis.
- When [Time Image] is selected, the vertical axis is displayed as migration index for analyzed data and migration time for raw data.
- [Time Image] is always displayed for raw data, and selection between [Distance Image] and [Time Image] is not available (see *"3.4.5 Analyzed Data/Raw Data" P.166*).

[Distance Image] is selected in the upper example and [Time Image] in the lower example.



3.5.4 Gel Image List

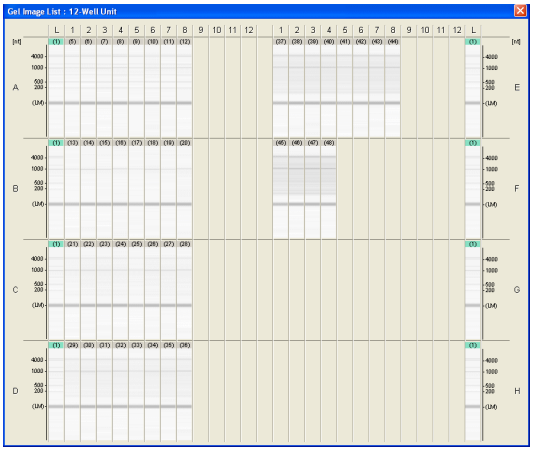
On to the [Gel Image] pull-down menu, point to [Gel Image List], and select [12-well Unit], [8-well Unit], or [Sort by Chip Position]. The selected [Gel Image List] layout is displayed.

- Or click the [View Gel Image List] button on the toolbar to display the gel image list. When the [Gel Image List] is open from the toolbar icon, it is displayed the last selected layout ([12-well Unit], [8-well Unit], and [Sort by Chip Position]).
- The other Gel Image display (black and white inversion, selection between distance image and time image) are applied as they were last set.
- Click the button in the upper right corner to close the [Gel Image List] window. Or double-click on a data to close the [Gel Image List] window and focus the corresponding data on the Gel Image.
- To copy an image data to the clipboard, select [Copy] on the [Gel Image List] right click pop-up menu.
- To print an image data, select [Print] on the [Gel Image List] right click pop-up menu. (The paper margins previously specified in the [Page Setup] window are applied.)

NOTE

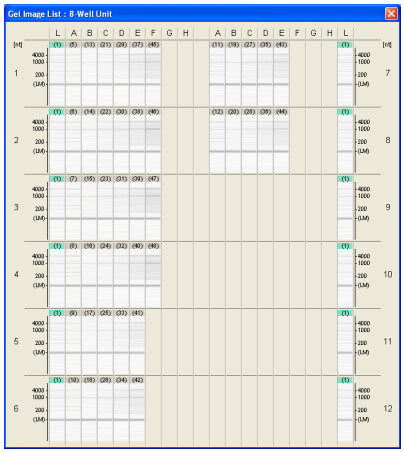
[Copy] and [Print] are functions are for the currently displayed window. If a different window is displayed over that window immediately (approx. one second) after selecting [Copy] or [Print], the content of the new window is copied or printed.

12-well Unit



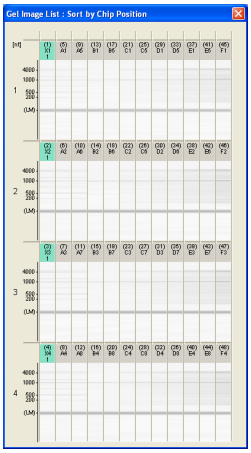
- Gel image list is displayed by a horizontal line on the sample stand (12 wells). The list is displayed in two separated portions; the left side (A to D) and the right side (E to H).
- “L” displays the ladder used for the first analysis in the data file.
- The number above each data represents (sample number) (see *"3.1.5 Sample Name Tree" P.135*).

8-well Unit



- Gel image list is displayed by a vertical column on the sample stand (8 wells). The list is displayed in two separate portions; the left side (1 to 6) and the right side (7 to 12).
- “L” displays the ladder used for the first analysis in the data file.
- The number above each data represents (sample number) (see *"3.1.5 Sample Name Tree" P.135*).

Sort by Chip Position



- Gel image list is displayed in sideways (1 to 4) for each chip position.
- The numbers above each data represent (sample number) (see *"3.1.5 Sample Name Tree" P.135*) and well name.

3.5.5 Undo Zoom

Select [Undo Zoom] to cancel the previous zooming operation on the Gel Image. The same operation is available using the Gel Image right click pop-up menu.

NOTE

When the display range in Gel Image is changed, the display range of the horizontal axis of Electropherogram (Single) is rescaled to match. To return to the original display in Electropherogram (Single), select [Undo Zoom]/[Undo Zoom All] on the [Electropherogram] menu or Electropherogram (Single) right click pop-up menu.

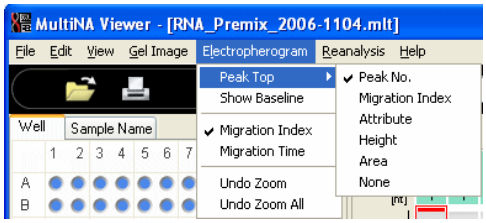
3.5.6 Undo Zoom All

Select [Undo Zoom All] to cancel all previous zooming operations on the Gel Image. The same operation is available on the Gel Image right click pop-up menu.

NOTE

When the display range in Gel Image is changed, the display range of the horizontal axis of Electropherogram (Single) is rescaled to match. To return to the original display in Electropherogram (Single), select [Undo Zoom]/[Undo Zoom All] on the [Electropherogram] menu or Electropherogram (Single) right click pop-up menu.

3.6 Electropherogram Menu



3.6.1 Peak Top

Select [Peak Top] to choose the information that is displayed at the top of the Electropherogram (Single) peak.

Item	Explanation
Peak No.	Peak number on the Peak Table
Migration Index / Migration Time	Value on the horizontal axis of Electropherogram
Size	Predicted size (DNA analysis only) When size prediction is not completed, (---) is displayed.
Attribute	Peak attribute of LM (lower marker), UM (upper marker), or 18S rRNA/28S rRNA (RNA analysis only) *V1.05* Reference values for size
Height	Peak height measured from baseline to the top of the peak
Area	Peak area
None	None

3.6.2 Font Size

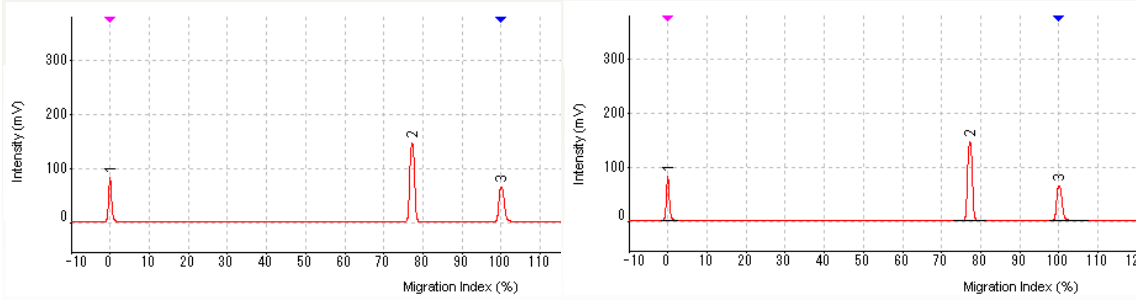
V1.05

Select [Large], [Medium], or [Small] as the font size for peak-top text and the title (displayed on the upper right) on [Electropherogram].

3.6.3 Show Baseline

Select this menu item to show peak baseline and auxiliary vertical line partitioning on the Electropherogram (Single).

Select the menu again and eliminate the check mark and hide baseline and auxiliary line (left graph below).



3.6.4 Horizontal Axis

V1.05

Select the display on the horizontal axis of the analyzed data from among [Migration Index], [Size], or [Migration Time].

- When a normal data file is opened, [Migration Index] is displayed on the horizontal axis.
- When [Migration Index] is selected, data in terms of relative index values based on marker detection times is displayed.

  DNA Analysis: Detection time for the lower marker and upper marker are set to 0% and 100%, respectively.

  RNA Analysis: Detection time for the lower marker and end point for the electropherogram are set to 0% and 100%, respectively.
- When [Size] is selected, the data displayed is obtained by correcting the migration index based on the reference ladder size. The reference ladder is the ladder used for the first analysis.

  NOTE

  For raw data or data without a ladder, or if there is data from a failed analysis, size cannot be calculated. Consequently, [Size] cannot be selected. If this occurs, reanalyze or manually edit the data, or click [Show Selected] to display only data that has been successfully analyzed.
- When [Manual Edit Mode] is selected on the [Reanalysis] pull-down menu, the horizontal axis is automatically set to [Migration Time].
- When raw data is displayed (see *"3.4.5 Analyzed Data/Raw Data" P.166*), only migration time can be displayed on the horizontal axis and this menu disabled.

3.6.5 Overlaying Images

V1.05

When displaying overlaid data, select either [Ascending Order] or [Descending Order].

Reference

"3.1.9 Electropherogram (Single)" P.140

3.6.6 Undo Zoom

Select [Undo Zoom] to cancel the previous zoom or pan operation on the Electropherogram.

The same operation is available on the Electropherogram right click pop-up menu.

NOTE

Display range on Gel Image does not change in conjunction with the Electropherogram display range.

3.6.7 Undo Zoom All

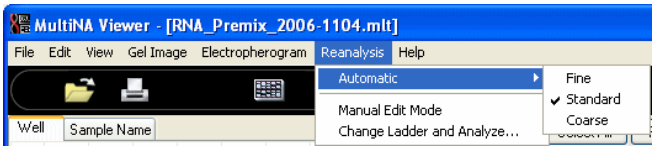
Select [Undo Zoom All] to cancel all of the previous zoom or pan operations on the Electropherogram.

The same operation is available on the Electropherogram right click pop-up menu.)

NOTE

Display range on Gel Image does not change in conjunction with the Electropherogram display range.

3.7 Reanalysis Menu



NOTE

Reanalysis is disabled in the following “read only” modes.

- Data currently being analyzed by the instrument is loaded and opened.
- The same file is open in another MultiNA Viewer iteration.
- Comparison view (see *"3.4.7 Comparison" P.167*) is displayed on MultiNA Viewer.

Imported ladders (see *"3.7.3 Change Ladder and Analyze" P.189*) cannot be manually edited.

3.7.1 Automatic

On the [Reanalysis] pull-down menu, point to [Automatic] and select [Fine], [Standard], or [Coarse] to automatically reanalyze all of the data in the data file.

[Fine], [Standard], and [Coarse] represent standards for peak detection.

- [Standard]: Regular detection for automatic analysis
- [Fine]: Select this detection level to detect smaller peaks that are not detected in [Standard] detection level.
- [Coarse]: Select this detection level to detect only the necessary peaks if excessive peaks are detected using the [Standard] detection level.

The difference in peak detection levels is shown below.

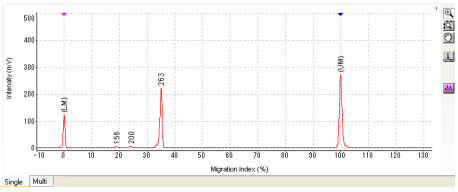


Fig.3-3 [Coarse]

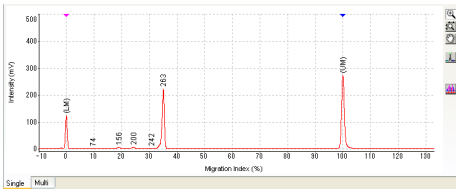


Fig.3-4 [Standard]

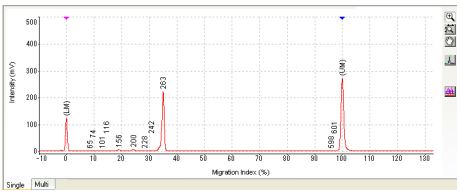
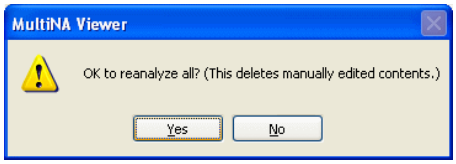


Fig.3-5 [Fine]

NOTE

Selecting [Automatic] on the [Reanalysis] menu deletes all previous editing results from the procedures described in *"3.7.2 Manual Edit Mode" P.184*. (The following message is displayed.)

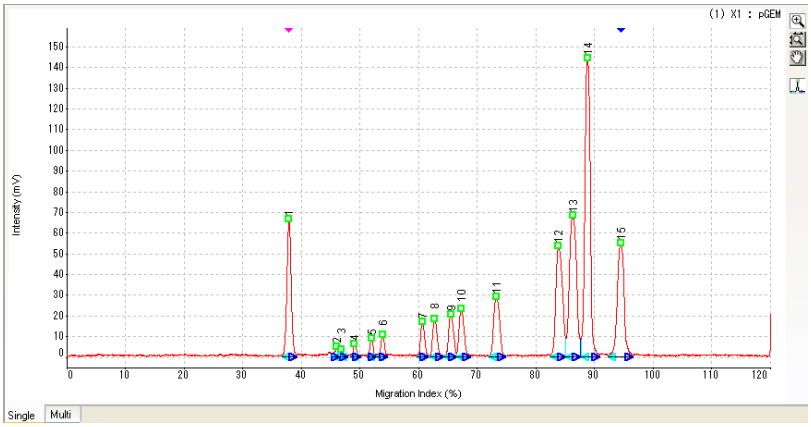


3.7.2 Manual Edit Mode

Select [Manual Edit Mode] to add or delete peaks, change the markers, or change the peak detection points.

1 Select [Manual Edit Mode] on the [Reanalysis] pull-down menu.

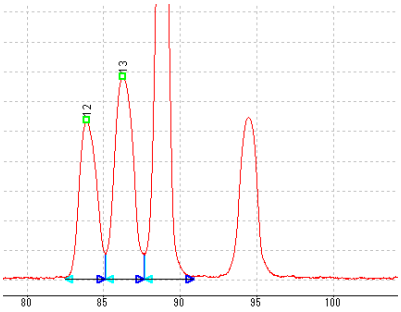
(Or click the [Manual Edit Mode] button on the upper right side of Electropherogram (Single).)
The peak detection points are displayed as shown below.



◁	Peak start (light blue triangle)
□	Peak top (yellow green rectangle)
▷	Peak end (blue triangle)

2 Edit peaks manually.

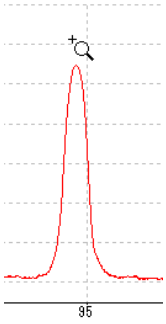
Enlarge a target peak by zooming or panning on the Electropherogram.



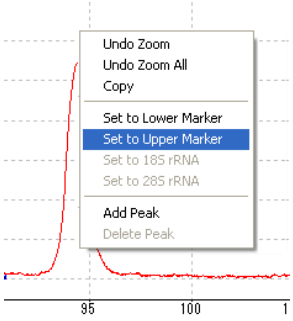
[Set to Lower Marker] / [Set to Upper Marker]

Select [Set to Lower Marker] or [Set to Upper Marker] to set the target peak to the lower maker or upper marker.

1 Place the cursor near the correct marker peak on the Electropherogram.
(It does not have to be a detected peak.)



2 Select [Set to Lower Marker] or [Set to Upper Marker] on the right click pop-up menu.



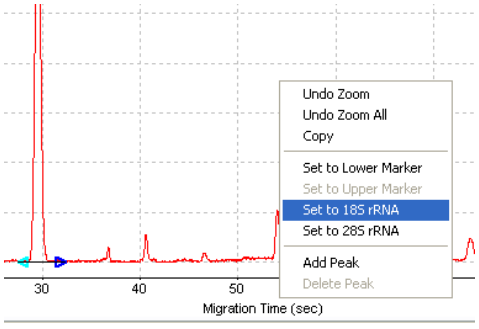
3 The marker is set to the new peak and the Peak Table is automatically updated.

[Set to 18S rRNA] / [Set to 28S rRNA]

Select [Set to 18S rRNA] or [Set to 29S rRNA] to change the 18S rRNA or 28S rRNA peaks in total RNA analyzed data.

1 Place the cursor near the peak of the correct rRNA peak on the Electropherogram.
(It does not have to be a detected peak.)

2 Select [Set to 18S rRNA] or [Set to 28S rRNA] on the right click pop-up menu.

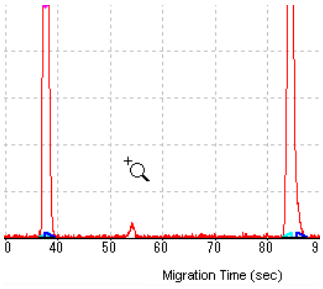


3 18S rRNA or 28S rRNA is set to the new peak position and recalculation is automatically performed. As a result, Peak Table and RNA Report are updated as well.

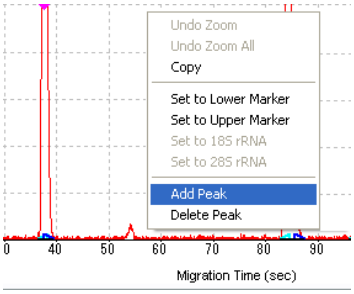
[Add Peak]

Select [Add Peak] to add a peak that has not been detected.

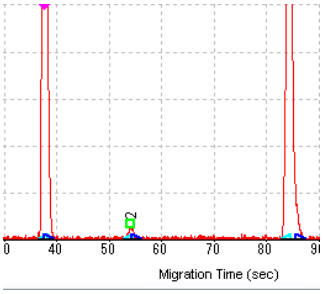
1 Place the cursor near the peak to be added on the Electropherogram.



2 Select [Add Peak] on the right click pop-up menu.



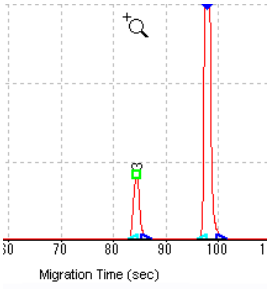
3 The peak is added and the [Peak Table] is automatically updated.



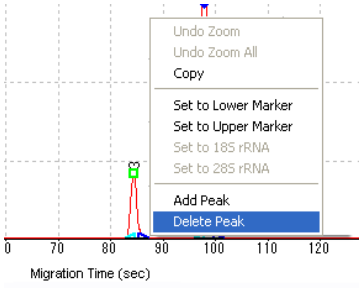
[Delete Peak]

Select [Delete Peak] to delete an unnecessary peak.

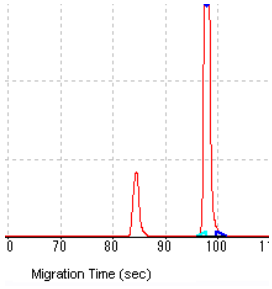
1 Place the cursor near the peak to be deleted on the Electropherogram.



2 Select [Delete Peak] on the right click pop-up menu.



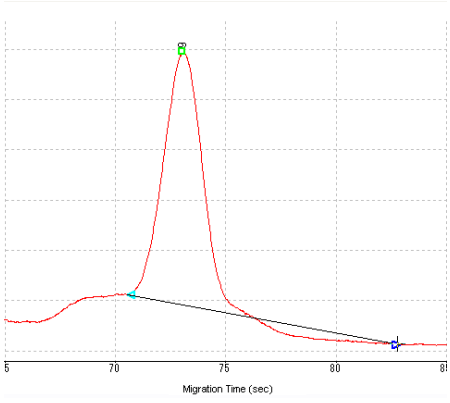
3 The peak is deleted and the [Peak Table] is automatically updated.



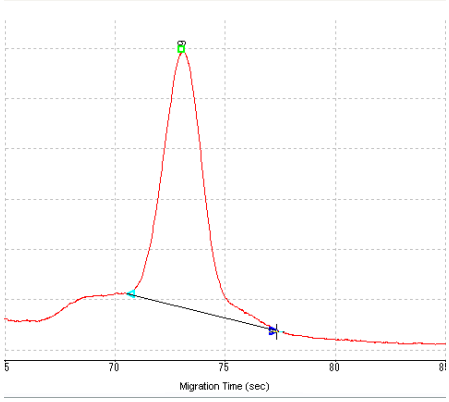
Changing the Peak Detection Position

The position of the peak start, top, and end can be changed.

1 Place the cursor at the peak mark (peak start, top, or end). The cursor becomes a “+.”
2 Drag the cursor to a desired peak position.



3 Release the mouse button. The peak detection point is changed and recalculation is automatically performed and the Peak Table is updated.



NOTE

In vertical partitioning, the end mark of a peak goes over the start mark of the next peak. The start mark and end mark can be separated or overlapped again by dragging the peak detection point mark.

3 Select [Manual Edit Mode] on the [Reanalysis] pull-down menu again.

(Or click the [Manual Edit Mode] button on the right side of Electropherogram (Single).)
The following message is displayed. Click [Yes] to end "Manual Edit mode and register the changes.

- [Yes]: Register the changes.
- [No]: Cancel the changes.
- [Cancel]: Return to the [Manual Edit Mode].

NOTE

Even after clicking [Yes] and registering the changes, they are not saved in the data file yet. Perform the procedure described in step 4 below to save the changes.

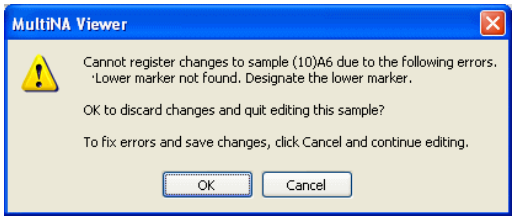
NOTE

The message shown above is displayed again as other data in the same data file is focused upon and edited in the [Manual Edit Mode].

NOTE

The message shown below is displayed when an error is detected in the changes and the changes cannot be registered. The message shows the content of the error and the procedure to solve the problem. To solve the problem, click [Cancel] and continue editing.

To cancel the changes, click [OK].

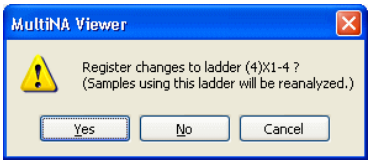


NOTE

The message shown below is displayed after editing ladder data. Click [Yes] to reanalyze all of the samples analyzed using this ladder.

Click [No] to discard the changes and make no changes to the sample data.

Click [Cancel] to return to the [Manual Edit Mode].



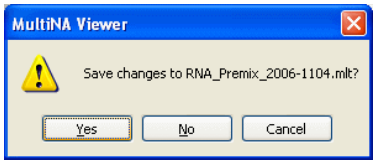
4 Select [Save] or [Save As] from the [File] pull-down menu.

Save the changes in the data file to the initial data file or to one with another name.

NOTE

The message below is displayed when an attempt is made to close a data file without saving the changes.

- Click [Yes] to save the changes in the initial data file.
- Click [No] to cancel the changes.
- Click [Cancel] to stop the closing process.



3.7.3 Change Ladder and Analyze

Select [Change Ladder and Analyze] to reanalyze sample data using a different ladder.

When a ladder is inappropriate or does not exist in the data file, ladder data from a different file can be loaded and the samples reanalyzed.

- The new ladder data is called an “imported ladder.”
- To display a ladder and samples analyzed using the ladder, see *"3.4.10 Select Ladder Used for Analysis"* or *"3.4.11 Select All Samples Analyzed Using This Ladder"*.

NOTE

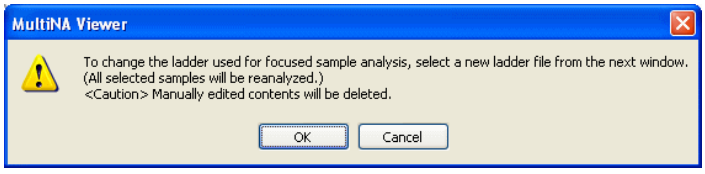
The accuracy of the results may not meet the specification when analysis is performed with an imported ladder.

NOTE

The imported ladder must be obtained using the same separation buffer and microchip (same ID) as the sample data.

NOTE

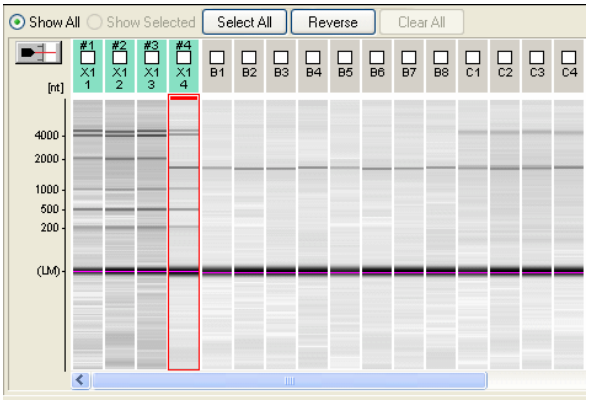
When [Change Ladder and Analyze] is selected for saved or registered data that has been changed through manual editing, the manual editing changes are deleted. The message below is displayed when [Change Ladder and Analyze] is selected for manually edited data. Click [OK] to delete the changes and [Cancel] to stop the ladder change process.



■ When the Ladder Used for Analysis is Inappropriate

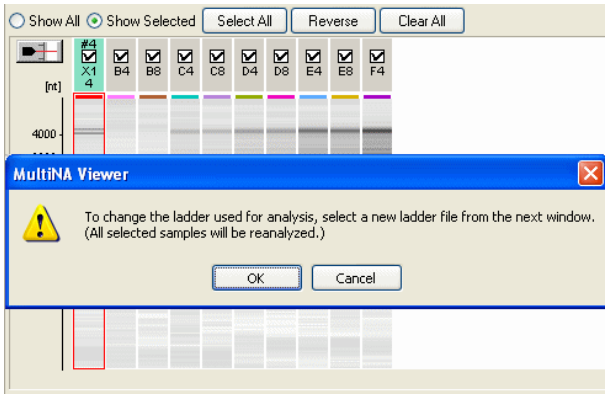
When the ladder is inappropriate in automatically analyzed data (ex., the ladder's electropherogram does not show fragment peak), change the ladder as described below and perform reanalysis.

1 Focus (click) the target ladder on Gel Image.



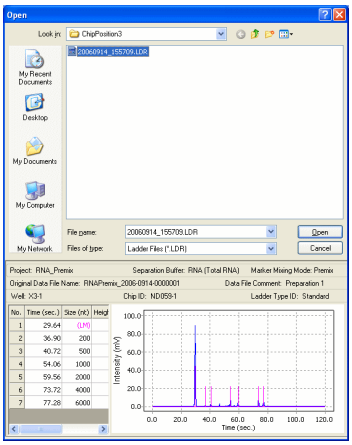
2 Select [Change Ladder and Analyze] on the [Reanalysis] pull-down menu.

The message below is displayed. The ladder and samples analyzed using the ladder are selected with a check mark.



3 **Review the selected Gel Image and click [OK].**

A folder is opened. This folder contains ladder data that was obtained under the same conditions as the ladder being replaced. The “Chip ID,” “Separation buffer,” “Marker mixing mode,” and “Chip position” match that of the ladder being replaced.



NOTE

The hierarchic structure of ladder data folders is shown below.

Folders are created according to the conditions where ladders are obtained as shown below and ladders are sorted and saved respectively.

[Data folder]

-[Ladder]

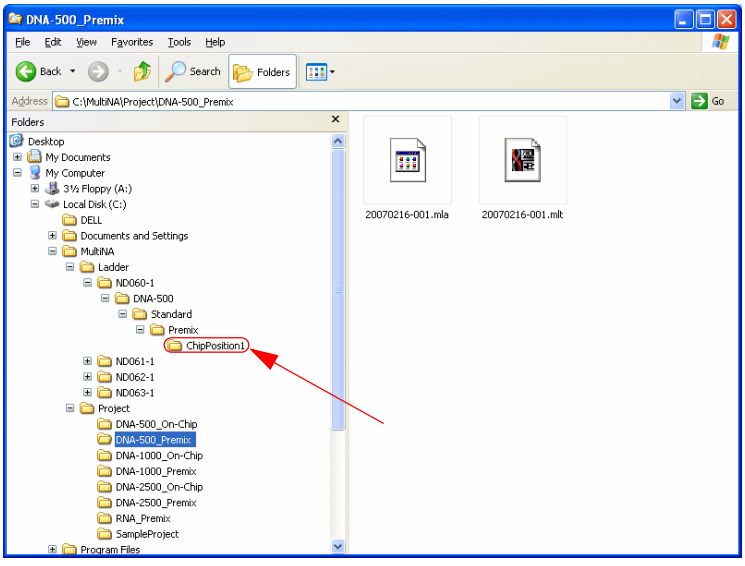
-[Chip ID] (ex., ND058-2)

-[Separation buffer] (DNA-500/DNA-1000/DNA-2500/RNA)

-[Ladder type ID] (regularly, “Standard” only)

-[Marker mixing mode] (Premix/On-chip)

-[Chip position] (ChipPosition1/ChipPosition2/ChipPosition3/ChipPosition4)



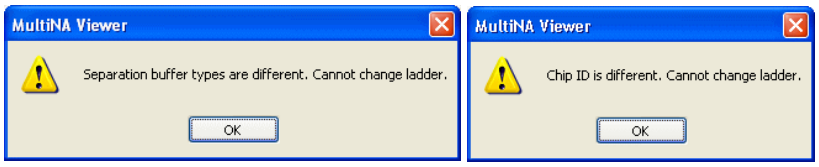
- Ladder data names reflect the time the ladder data analysis was started. They include year, date, hour, minute and second and end with “.ldr”. For example, ladder data that started analysis at 18:53:00 on June 7, 2006 is named “20060607_185300.ldr”.

  Example) C:\MultiNA\Ladder\ND058-2\DNA-2500\Standard\Premix\ChipPosition4\20060607_185300.ldr

- The lower windows of the ladder data folder displays a preview of the Electropherogram and Peak Table of the selected ladder data.
- When no appropriate ladder exists in the open folder, select ladder data from a ladder data folder where at least the “Chip ID” and “Separation buffer” match. Ladder data can be selected even if the “Marker mixing mode” and “Chip position” differ from ladder data being replaced.

NOTE

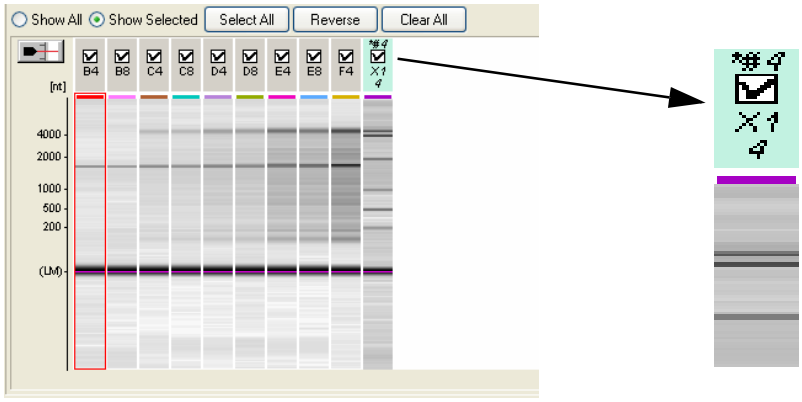
The error messages shown below may be displayed if an attempt is made to select ladder data in which the “Separation buffer” or “Chip ID” differ from that of the target ladder data.



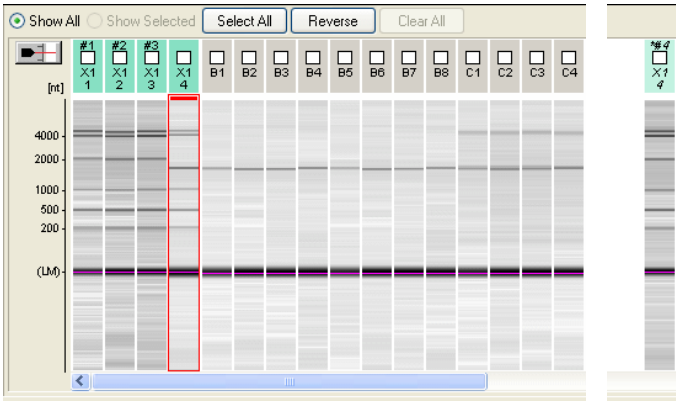
4 Select an appropriate ladder data file and click [Open].

Data is reanalyzed and the results are displayed.

- “Imported ladder” is displayed on the right end of [Gel Image]. An asterisk mark (*) is put above the check box of the imported ladder. The asterisk mark is followed by # and the “chip position.”
- The well name of the ladder initially used for analysis is displayed under the check box. All of the indications for the imported ladder are in italics.



- Clicking [Show All] displays all of the data in the data folder. The ladder used for analysis before ladder change (“X1-4” in this example) does not have the “#” mark, showing that it was not used for analysis.



5 **Go back to step 1 to change other ladder data.**

To change the imported ladder to a different ladder data, focus (click) on the imported ladder.

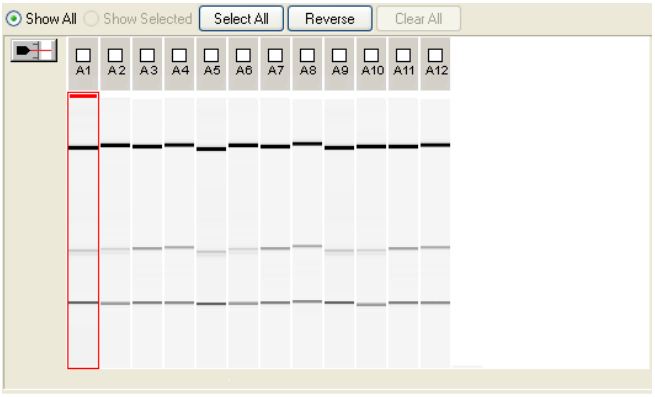
6 **Save the analysis results in the data file.**

Select [Save] on the [File] pull-down menu to overwrite the data file, and select [Save As] to save the results in a data file with a different name.

■ When No Ladder Data Exists in the Data File

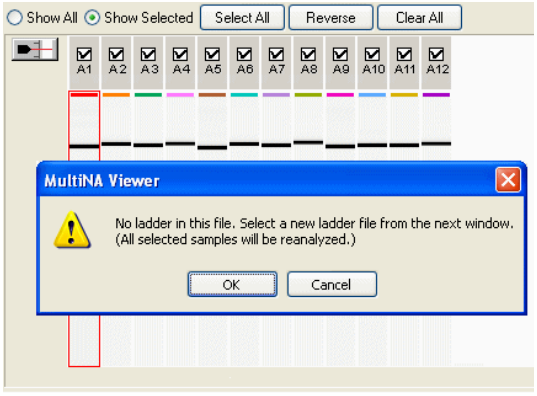
When no ladder data exists in the data file because no ladder was analyzed or for other reasons, perform reanalysis using the procedures described below.

1 **Focus (click) the Gel Image of the sample to be analyzed.**



2 **Select [Change Ladder and Analyze] on the [Reanalysis] pull-down menu.**

selected with a check mark.



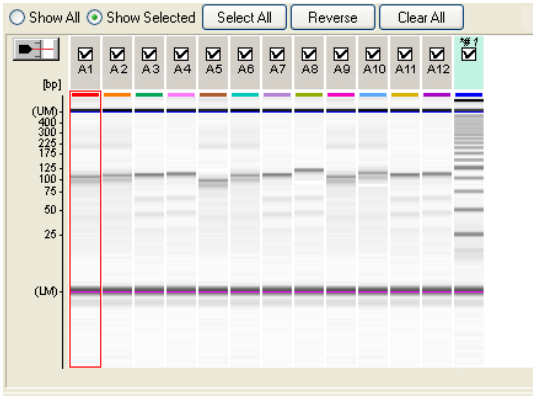
3 **Review the selected Gel Image and click [OK].**

The [Select Ladder Data] window is displayed. (Procedures to select ladder data are the same as the procedures described in “When the Ladder Used for Analysis Is Inappropriate” *(P.190)*.)

4 **Select an appropriate ladder data and click [Open].**

Data is reanalyzed and the results are displayed.

- "Imported ladder" is displayed on the right end of [Gel Image]. An asterisk mark (*) is put above the check box of the imported ladder. The asterisk mark is followed by # and "chip position".
- The area below the check box is blank. All of the indications for the imported ladder are in italics.



5 **Go back to step 1 to analyze another sample.**

Focus (click) the imported ladder to change the imported ladder to different ladder data.

6 **Save the analysis results in the data file.**

Select [Save] on the [File] pull-down menu to overwrite the data file and select [Save As] to save the results in a data file with a different name.

3.8 Help Menu

3.8.1 Manual

Select a menu listed below to display a PDF file for the respective manual.

- [Quick Start Manual]: Analysis operation flow (Operating Procedure P.2 to P.3)
- [Instrument Manual]: Instrument and System
- [Operation Manual]: Operating Procedure

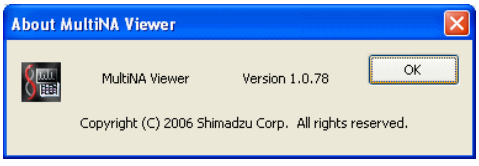
NOTE

Adobe® Reader® distributed by Adobe Systems Co. or other PDF file viewing software (provided free) is required to view the PDF file. Adobe® Reader® (Ver. 4 or later) can be downloaded from the following website:

http://www.adobe.com/products/acrobat/readstep2.html

3.8.2 About MultiNA Viewer

Select [About MultiNA Viewer] to display the version of the MultiNA Viewer data analysis software.



4 Appendix

Sections 4.1 to 4.7 briefly explain the preparation of each reagent kit. For preparation details, see the sections of this instruction manual listed below.

"1.6 Preparation for Analysis with the DNA-500 Kit" P.32

"1.7 Preparation for Analysis with the DNA-1000 Kit" P.40

"1.8 Preparation for Analysis with the DNA-2500 Kit" P.48

"1.9 Preparation for Analysis with the RNA Kit" P.56

4.1 Preparation of the DNA-500 Kit (Premix)

■ Requirements

Reagent	DNA-500 separation buffer (stored at 4°C to 8°C)
	DNA-500 marker solution (stored at -20°C)
	SYBR® Gold storage solution (diluted to 1/100 with 1xTE buffer)
	DNA ladder (Size marker: 25 bp DNA ladder diluted to 1/50, Concentration 20 ng/µL)
Containers and utensils	Sample containers (200-µL tube, 96-well plate + aluminum seal)
	Micro pipette (10 µL, 100 µL, 1000 µL)

NOTE

Confirm the caution item on *"1.6.1 Requirements" P.33*.

V1.05

When using an optional ladder, see *"4.10 Using Optional Ladders" P.205*.

■ Protocol (Necessary time: 5 to 10 min.)

❶ Add SYBR® Gold storage solution to DNA-500 separation buffer and mix them with a vortex mixer.

- Add the separation buffer into the buffer bottle so that SYBR® Gold storage solution is diluted to 1/100. (Final concentration is 1/10,000.)
  * For 24 analyses: 1,000 µL of buffer
  * For 96 analyses: 3,000 µL of buffer

⇩

❷ Mix DNA ladder and DNA-500 marker solution in the 200-µL sample tube to a 1:2 volume ratio, and set the sample tube on the extra sample stand.

- When four chips are used for analysis, the DNA ladder is analyzed four times, once per chip.
  * Mix 5 µL of DNA ladder and 10 µL of DNA-500 marker.
- Multiple analyses can be performed on the extra sample stand.

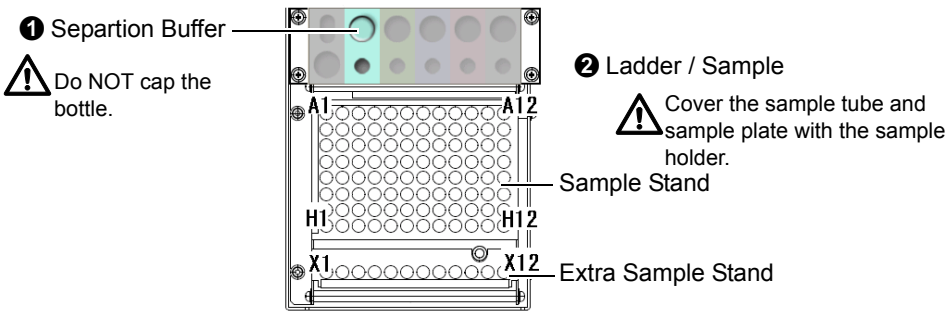
⇩

❸ Mix the sample and DNA-500 marker solution in the 96-well plate in a 1:2 volume ratio, seal with the aluminum seal, and place the plate on the sample stand.

- Mix 2 µL of the sample and 4 µL of DNA-500 marker for each well.
- On the sample stand, only one analysis is performed per well.
- For up to 12 samples, mix 3 µL of the sample and 6 µL of DNA-500 marker. Aluminum seal is unnecessary.

⇩

❹ Uncap the buffer bottle, place it in the blue reagent holder position and install the sample holder on the sample stand.



4.2 Preparation of the DNA-500 Kit (On-Chip mix)

■ Requirements

Reagent	DNA-500 separation buffer (stored at 4°C to 8°C)
	DNA-500 marker solution (stored at -20°C)
	SYBR® Gold storage solution (diluted to 1/100 with 1xTE buffer)
	DNA ladder (Size marker: 25 bp DNA ladder diluted to 1/50, Concentration 20 ng/µL)
Containers and utensils	Sample containers (200-µL tube, 96-well plate + aluminum seal)
	Micro pipette (10 µL, 100 µL, 1000 µL)

NOTE

Confirm the caution item on *"1.6.1 Requirements" P.33*.

V1.05

When using an optional ladder, see *"4.10 Using Optional Ladders" P.205*.

■ Protocol (Necessary time: 5 to 10 min.)

❶ Add SYBR® Gold storage solution to DNA-500 separation buffer and mix them with a vortex mixer.

- Add the separation buffer into the buffer bottle so that SYBR® Gold storage solution is diluted to 1/100. (Final concentration is 1/10,000.)
  * For 24 analyses: 1,000 µL of buffer
  * For 96 analyses: 3,000 µL of buffer

⇩

❷ Pour the DNA ladder into a sample tube and set it on the extra sample stand.

- When four chips are used for analysis, the DNA ladder is analyzed four times, once per chip.
  * For 4 analyses: 11 µL of DNA ladder
- Multiple analyses can be performed on the extra sample stand.

⇩

❸ Pour the DNA-500 marker solution into a vial and place it in the blue reagent holder position.

- Necessary amount: 2 x (the number of analyses) + 40 µL

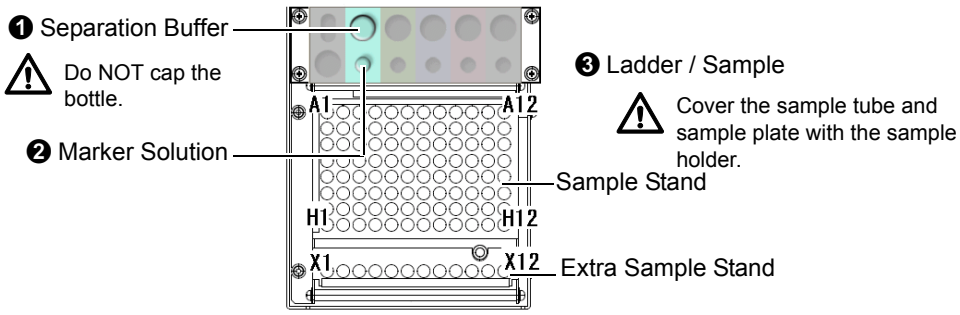
⇩

❹ Put the sample into a 96-well plate, seal with the aluminum seal, and place it on the sample stand.

- On the sample stand, only one analysis is performed per well.
- For up to 12 samples, put 9 µL of the sample in the tube. Aluminum seal is unnecessary.

⇩

❺ Uncap the buffer bottle, place it in the blue reagent holder position and install the sample holder on the sample stand.



4.3 Preparation of the DNA-1000 Kit (Premix)

■ Requirements

Reagent	DNA-1000 separation buffer (stored at 4°C to 8°C)
	DNA-1000 marker solution (stored at -20°C)
	SYBR® Gold storage solution (diluted to 1/100 with 1xTE buffer)
	DNA ladder (Size marker: ϕX174 DNA/Hae III Markers diluted to 1/100, Concentration: 10 ng/μL)
Containers and utensils	Sample containers (200-μL tube, 96-well plate + aluminum seal)
	Micro pipette (10 μL, 100 μL, 1000 μL)

NOTE

Confirm the caution item on *"1.7.1 Requirements" P.41*.

V1.05

When using an optional ladder, see *"4.10 Using Optional Ladders" P.205*.

■ Protocol (Necessary time: 5 to 10 min.)

❶ Add SYBR® Gold storage solution to DNA-1000 separation buffer and mix them with a vortex mixer.

- Add the separation buffer into the buffer bottle so that SYBR® Gold storage solution is diluted to 1/100. (Final concentration is 1/10,000.)
  * For 24 analyses: 1,000 μL of buffer
  * For 96 analyses: 3,000 μL of buffer

↓

❷ Mix DNA ladder and DNA-1000 marker solution in the 200-μL sample tube to a 1:2 volume ratio, and set the sample tube on the extra sample stand.

- When four chips are used for analysis, the DNA ladder is analyzed four times, once per chip.
  * Mix 5 μL of DNA ladder and 10 μL of DNA-1000 marker.
- Multiple analyses can be performed on the extra sample stand.

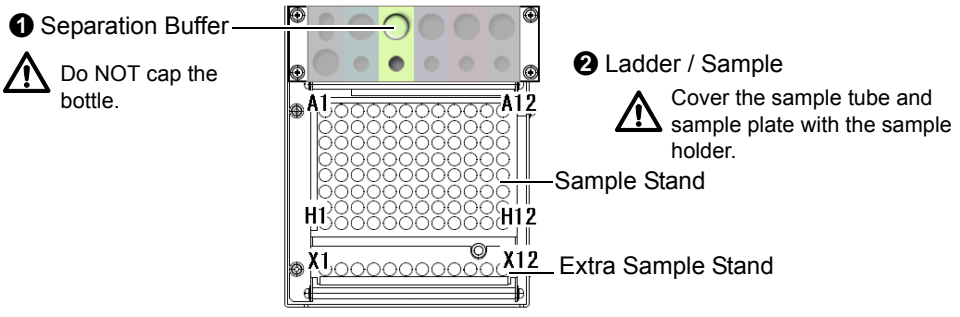
↓

❸ Mix the sample and DNA-1000 marker solution in the 96-well plate to a 1:2 volume ratio, seal with the aluminum seal, and place the plate on the sample stand.

- Mix 2 μL of the sample and 4 μL of DNA-1000 marker for each well.
- On the sample stand, only one analysis is performed per well.
- For up to 12 samples, mix 3 μL of the sample and 6 μL of DNA-1000 marker. Aluminum seal is unnecessary.

↓

❹ Uncap the buffer bottle, place it in the green reagent holder position, and install the sample holder on the sample stand.



4.4 Preparation of the DNA-1000 Kit (On-Chip mix)

■ Requirements

Reagent	DNA-1000 separation buffer (stored at 4°C to 8°C)
	DNA-1000 marker solution (stored at -20°C)
	SYBR® Gold storage solution (diluted to 1/100 with 1xTE buffer)
	DNA ladder (Size marker: ϕX174 DNA/Hae III Markers diluted to 1/100, Concentration: 10 ng/μL)
Containers and utensils	Sample containers (200-μL tube, 96-well plate + aluminum seal)
	Micro pipette (10 μL, 100 μL, 1000 μL)

NOTE

Confirm the caution item on *"1.7.1 Requirements" P.41*.

V1.05

When using an optional ladder, see *"4.10 Using Optional Ladders" P.205*.

■ Protocol (Necessary time: 5 to 10 min.)

❶ Add SYBR® Gold storage solution to DNA-1000 separation buffer and mix them with a vortex mixer.

- Add the separation buffer into the buffer bottle so that SYBR® Gold storage solution is diluted to 1/100. (Final concentration is 1/10,000.)
  * For 24 analyses: 1,000 μL of buffer
  * For 96 analyses: 3,000 μL of buffer

⬇

❷ Pour the DNA ladder into a sample tube and set it on the extra sample stand.

- When four chips are used for analysis, the DNA ladder is analyzed four times, once per chip.
  * For 4 analyses: 11 μL of DNA ladder
- Multiple analyses can be performed on the extra sample stand.

⬇

❸ Pour the DNA-1000 marker solution into a vial and place it in the green reagent holder position.

- Necessary amount: 2 x (the number of analyses) + 40 μL

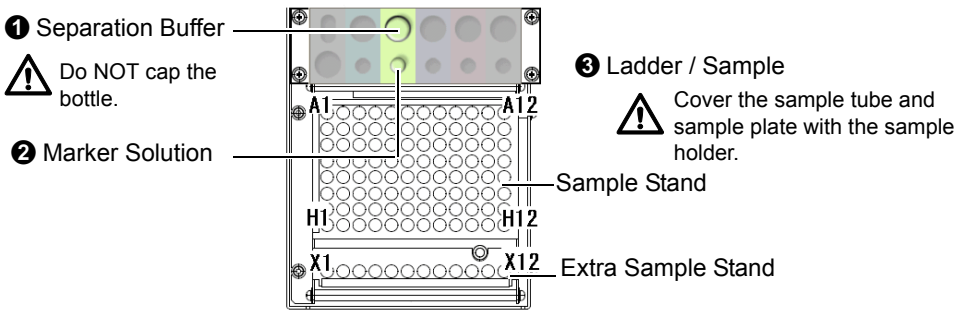
⬇

❹ Put the sample into a 96-well plate, seal with the aluminum seal, and place it on the sample stand.

- On the sample stand, only one analysis is performed per well.
- For up to 12 samples, put 9 μL of the sample in the tube. Aluminum seal is unnecessary.

⬇

❺ Uncap the buffer bottle, place it in the green reagent holder position, and install the sample holder on the sample stand.



4.5 Preparation of the DNA-2500 Kit (Premix)

■ Requirements

Reagent	DNA-2500 separation buffer (stored at 4°C to 8°C)
	DNA-2500 marker solution (stored at -20°C)
	SYBR® Gold storage solution (diluted to 1/100 with 1xTE buffer)
	DNA ladder (Size marker: pGEM® DNA Markers diluted to 1/100, Concentration: 10 ng/μL)
Containers and utensils	Sample containers (200-μL tube, 96-well plate + aluminum seal)
	Micro pipette (10 μL, 100 μL, 1000 μL)

NOTE

Confirm the caution item on *"1.8.1 Requirements" P.49*.

■ Protocol (Necessary time: 5 to 10 min.)

❶ Add SYBR® Gold storage solution to DNA-2500 separation buffer and mix them with a vortex mixer.

- Add the separation buffer into the buffer bottle so that SYBR® Gold storage solution is diluted to 1/100. (Final concentration is 1/10,000.)
  * For 24 analyses: 1,000 μL of buffer
  * For 96 analyses: 3,000 μL of buffer

⇩

❷ Mix DNA ladder and DNA-2500 marker solution in the 200-μL sample tube to a 1:2 volume ratio, and set the sample tube on the extra sample stand.

- When four chips are used for analysis, the DNA ladder is analyzed four times, once per chip.
  * Mix 5 μL of DNA ladder and 10 μL of DNA-2500 marker.
- Multiple analyses can be performed on the extra sample stand.

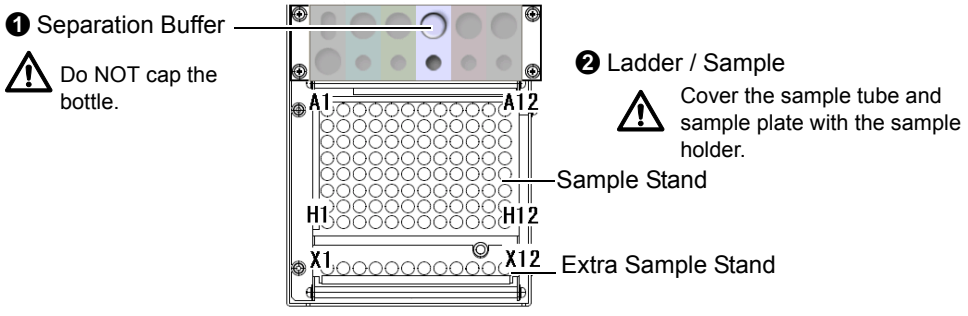
⇩

❸ Mix the sample and DNA-2500 marker solution in the 96-well plate to a 1:2 volume ratio, seal with the aluminum seal, and place the plate on the sample stand.

- Mix 2 μL of the sample and 4 μL of DNA-2500 marker for each well.
- On the sample stand, only one analysis is performed per well.
- For up to 12 samples, mix 3 μL of the sample and 6 μL of DNA-2500 marker. Aluminum seal is unnecessary.

⇩

❹ Uncap the buffer bottle, place it in the purple reagent holder position, and install the sample holder on the sample stand.



4.6 Preparation of the DNA-2500 Kit (On-Chip mix)

■ Requirements

Reagent	DNA-2500 separation buffer (stored at 4°C to 8°C)
	DNA-2500 marker solution (stored at -20°C)
	SYBR® Gold storage solution (diluted to 1/100 with 1xTE buffer)
	DNA ladder (Size marker: pGEM® DNA Markers diluted to 1/100, Concentration: 10 ng/μL)
Containers and utensils	Sample containers (200-μL tube, 96-well plate + aluminum seal)
	Micro pipette (10 μL, 100 μL, 1000 μL)

NOTE

Confirm the caution item on *"1.8.1 Requirements" P.49*.

■ Protocol (Necessary time: 5 to 10 min.)

❶ Add SYBR® Gold storage solution to DNA-2500 separation buffer and mix them with a vortex mixer.

- Add the separation buffer into the buffer bottle so that SYBR® Gold storage solution is diluted to 1/100. (Final concentration is 1/10,000.)
  * For 24 analyses: 1,000 μL of buffer
  * For 96 analyses: 3,000 μL of buffer

⇩

❷ Pour the DNA ladder into a sample tube and set it on the extra sample stand.

- When four chips are used for analysis, the DNA ladder is analyzed four times, once per chip.
  * For 4 analyses: 11 μL of DNA ladder
- Multiple analyses can be performed on the extra sample stand.

⇩

❸ Pour the DNA-2500 marker solution into a vial and place it in the purple reagent holder position.

- Necessary amount: 2 x (the number of analyses) + 40 μL

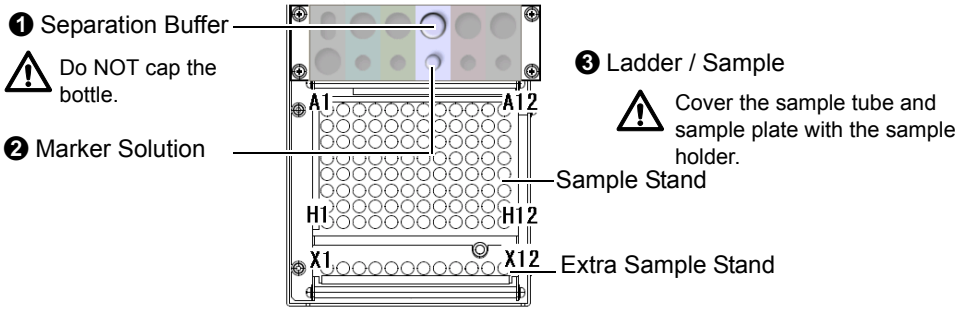
⇩

❹ Put the sample into a 96-well plate, seal with the aluminum seal, and place it on the sample stand.

- On the sample stand, only one analysis is performed per well.
- For up to 12 samples, put 9 μL of the sample in the tube. Aluminum seal is unnecessary.

⇩

❺ Uncap the buffer bottle, place it in the purple reagent holder position, and install the sample holder on the sample stand.



4.7 Preparation of the RNA Kit

■ Requirements

Reagent	RNA separation buffer (stored at 4°C to 8°C)
	RNA marker solution (stored at -20°C)
	SYBR® Green II storage solution (diluted to 1/100 with 1xTE buffer)
	RNA ladder (RNA6000 ladder diluted to 1/6, concentration: 25 ng/µL)
	Formamide
	THE RNA Storage Solution (Applied Biosystems)
Containers and utensils	Sample containers (200-µL tube, 96-well plate + aluminum seal)
	Micro pipette (10 µL, 100 µL, 1000 µL)

NOTE

Confirm the caution item on *"1.9.1 Requirements" P.57*.

■ Protocol (Necessary time: 5 to 10 min.)

❶ Add SYBR® Green II storage solution and formamide to RNA separation buffer and mix them with a vortex mixer.

- For 22 analyses, put 790 µL of separation buffer, 10 µL of SYBR® Green II storage solution, and 200 µL of formamide in the buffer bottle.

⇩

❷ Mix the RNA ladder and RNA marker solution in the 200-µL sample tube to a 1:1 volume ratio.

- When four chips are used for analysis, the RNA ladder is analyzed four times, once per chip.
  * Mix 7.5 µL of RNA ladder and 7.5 µL of RNA marker.
- Multiple analyses can be performed on the extra sample stand.

⇩

❸ Mix the sample and RNA marker solution them in the 96-well plate to a 1:1 volume ratio, and seal with the aluminum seal.

- Blank (THE RNA Storage Solution) is analyzed first (before the ladder) per microchip.
- Mix 3 µL of the sample and 3 µL of RNA marker for each well.
- On the sample stand, only one analysis is performed per well.

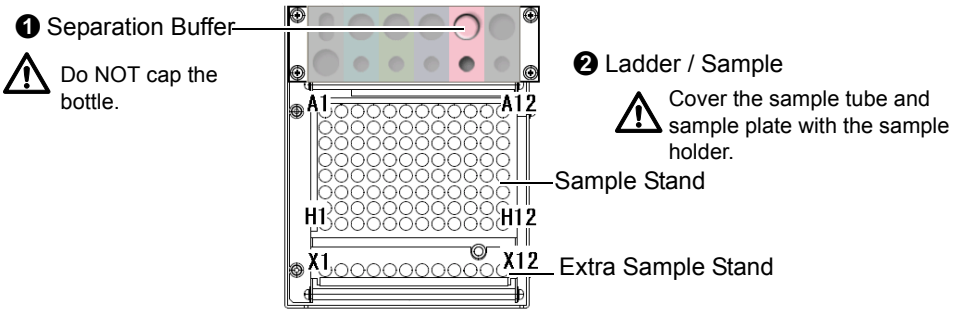
⇩

❹ Heat-denature the RNA ladder and sample.

- Cap the sample tube.
- Temperature condition: 65°C for 5 min. -> 4°C for 5 min.

⇩

❺ Uncap the buffer bottle and place it in the pink reagent holder position. Do NOT cap the sample tube. Install the sample holder on the sample stand.



4.8 Using a Chip for RNA Analysis after Using It for DNA Analysis

- Since different dyes are used for DNA analysis and RNA analysis, performing RNA analysis immediately after performing DNA analysis using the same microchip may negatively influence analysis results.
- Rinse the microchip using the procedure below before using it for a different type of analysis.

1 **Install the microchip in the instrument.**

☞ **Reference**

"1.5.4 Microchip Registration" P.13
"1.5.5 Microchip Installation" P.16

2 **From the MultiNA Instrument Control [Instrument] pull-down menu, point to [Wash], and select the target chip.**

☞ **Reference**

"2.5.8 Wash" P.126

3 **Prepare the RNA separation buffer (for three analyses per chip).**

Do not add diluted dye solution. Add formaldehyde only.

☞ **Reference**

"1.9.5 Separation Buffer Preparation" P.60

4 **Prepare a sample by mixing the same amounts of THE RNA storage solution and marker solution (for three analyses per chip).**

☞ **Reference**

"1.9.6 Ladder, Sample and Marker Solution Usage" P.62

5 **Perform analysis three times on the target chip.**

☞ **Reference**

"1.10 Analysis" P.64

4.9 Using a Chip for DNA Analysis after Using It for RNA Analysis

- Since different dyes are used for DNA analysis and RNA analysis, performing DNA analysis immediately after performing RNA analysis using the same microchip may negatively influence analysis results.
- Rinse the microchip using the procedure below before using it for a different type of analysis.
- The following procedure describes rinsing of the microchips in the premix mode using DNA separation buffer. Rinsing can also be performed in the on-chip mixing mode.

1 **Install the microchip in the instrument.**

☞ **Reference**

"1.5.4 Microchip Registration" P.13
"1.5.5 Microchip Installation" P.16

2 **From the MultiNA Instrument Control [Instrument] pull-down menu, point to [Wash], and select the target chip.**

☞ **Reference**

"2.5.8 Wash" P.126

3 **Prepare the DNA separation buffer (for three analyses per chip).**

Separation buffer can be selected from among DNA-500, DNA-1000, and DNA-2500. Add a diluted dye solution and mix.

☞ **Reference**

DNA-500: *"1.6.5 Separation Buffer Preparation" P.36*
DNA-1000: *"1.7.5 Separation Buffer Preparation" P.44*
DNA-2500: *"1.8.5 Separation Buffer Preparation" P.52*

4 **To prepare a sample, add the marker solution and TE buffer (1:2 volume ration) and mix (for three analyses per chip).**

☞ **Reference**

DNA-500: *"1.6.6 Ladder, Sample and Marker Solution Usage" P.38*
DNA-1000: *"1.7.6 Ladder, Sample, and Marker Solution Usage" P.46*
DNA-2500: *"1.8.6 Ladder, Sample and Marker Solution Usage" P.54*

5 **Perform analysis three times on the target chip in the premix mode.**

☞ **Reference**

"1.10 Analysis" P.64

4.10 Using Optional Ladders

Optional ladders are available for use with DNA-500, and DNA-1000 kits. The optional ladders and dilution conditions that can be used with respective kits are indicated below.

Type and Dilution Conditions for Optional Ladders

Applicable Kit	Ladder Type ID (abbreviated name)	Supplier	Name	Catalog No.	Dilution
DNA-500	Ladder1 (LD1)	Applied Biosystems	pUC19Hpa II UDigest	AM7770	1/50 dilution
DNA-1000	Ladder1 (LD1)	Takara Bio	100bp DNA Ladder	3407A	1/10 dilution

The Ladder Type ID is used by the software to differentiate between ladder types. The ID for standard ladders is "Standard (STD)".

NOTE

If an optional ladder is used, analytical performance (size accuracy, etc.) may be different than what is indicated in the MCE-202 MultiNA Analysis Performance Specifications (using a standard ladder, see *-Instrument and System- "8 Specifications"*).

The following describes how to prepare optional ladder solutions.

■ Requirements

- Optional ladder to be used (see the "Type and Dilution Conditions for Optional Ladders" table above)
- TE buffer
- Microtube (volumes from 200 µL to 1.5 mL)
- Micro pipettes
- Vortex Mixer

■ Procedures

1 **Use a micropipette to dispense TE buffer in the microtube.**

For 1/100 dilutions, dispense 99 µL in the tube, for 1/50 dilutions, dispense 49 µL, and for 1/20 dilutions, dispense 19 µL. (Confirm dilution conditions in the "Type and Dilution Conditions for Optional Ladders" table above.)

2 **Add 1 µL of optional ladder to the microtube and agitate the solution with a vortex mixer for at least 10 seconds.**

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