

Agilent 4200 TapeStation System

System Manual



Agilent Technologies

Notices

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Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a **WARNING** notice until the indicated conditions are fully understood and met.

In this Guide...

This manual covers the 4200 TapeStation system.

1 Safety

This chapter provides information on safety symbols and safety messages.

2 Legal and Regulatory

This chapter provides additional information on legal and regulatory aspects.

3 Product Description

This chapter gives an introduction to the instrument and provides information on intended use and instrument specifications.

4 Installation

This chapter gives information about the installation of the system.

5 TapeStation Software

This chapter gives an overview on the three packages of software that come preinstalled with your 4200 TapeStation system.

6 Operating Instructions

This chapter explains the operation of the 4200 TapeStation system.

7 Good Measurement Practices

This chapter summarizes information on good measurement practices for ScreenTape assays.

8 Instrument Diagnosis

This chapter contains information on instrument diagnosis, available tests, and reporting.

In this Guide...

9 Maintenance

This chapter describes the maintenance of the 4200 TapeStation system.

10 Troubleshooting

This chapter gives an overview about the troubleshooting features of the 4200 TapeStation system. It includes information about application troubleshooting as well.

11 Appendix

This chapter contains parts information.

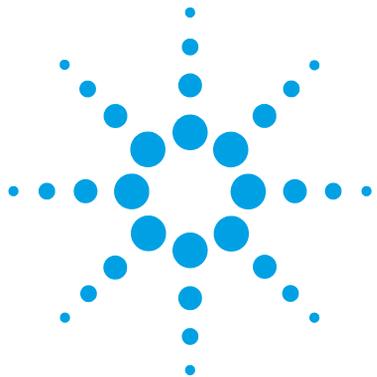
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Contents



1 Safety

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Safety Messages 12

This chapter provides information on safety symbols and safety messages.



Symbols

Table 1 Symbols

	The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect risk of harm to the operator and to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected ground terminal.
	Confirms that a manufactured product complies with all applicable European Community directives. The European Declaration of Conformity is available at: http://regulations.corporate.agilent.com/DoC/search.htm
	Manufacturing date.
	Power symbol indicates On/Off. The apparatus is not completely disconnected from the mains supply when the power switch is in the Off position
	Pacemaker Magnets could affect the functioning of pacemakers and implanted heart defibrillators. A pacemaker could switch into test mode and cause illness. A heart defibrillator may stop working. If you wear these devices keep at least 55 mm distance to magnets. Warn others who wear these devices from getting too close to magnets.
	Magnetic field Magnets produce a far-reaching, strong magnetic field. They could damage TVs and laptops, computer hard drives, credit and ATM cards, data storage media, mechanical watches, hearing aids and speakers. Keep magnets at least 25 mm away from devices and objects that could be damaged by strong magnetic fields.

Table 1 Symbols

	Indicates a pinching or crushing hazard
	Indicates a piercing or cutting hazard.

Safety Messages

WARNING

A WARNING

alerts you to situations that could cause physical injury or death.

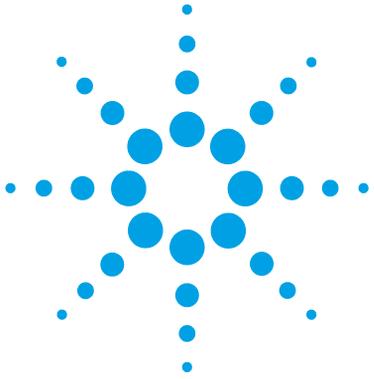
- Do not proceed beyond a warning until you have fully understood and met the indicated conditions.
-

CAUTION

A CAUTION

alerts you to situations that could cause loss of data, or damage of equipment.

- Do not proceed beyond a caution until you have fully understood and met the indicated conditions.
-



2 Legal and Regulatory

Sound Emission 14

Waste Electrical and Electronic Equipment (WEEE) Directive 15

This chapter provides additional information on legal and regulatory aspects.



Sound Emission

Manufacturer's Declaration

This statement is provided to comply with the requirements of the German Sound Emission Directive of 18 January 1991.

This product has a sound pressure emission (at the operator position) <70 dB.

- Sound Pressure L_p <70 dB (A)
- At Operator Position
- Normal Operation
- According to ISO 7779:1988/EN 27779/1991 (Type Test)

Waste Electrical and Electronic Equipment (WEEE) Directive

This product complies with the European WEEE Directive marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.



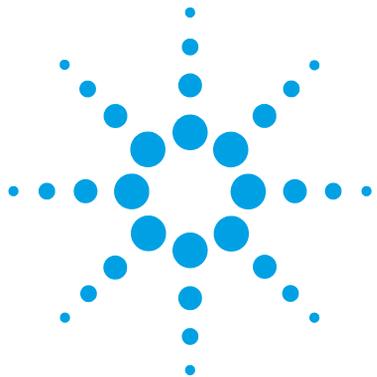
NOTE

Do not dispose of in domestic household waste

To return unwanted products, contact your local Agilent office, or see <http://www.agilent.com> for more information.

2 Legal and Regulatory

Waste Electrical and Electronic Equipment (WEEE) Directive



3 Product Description

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This chapter gives an introduction to the instrument and provides information on intended use and instrument specifications.



3 Product Description

Intended use of the 4200 TapeStation System

Intended use of the 4200 TapeStation System

The 4200 TapeStation system is intended to separate nucleic acids by means of electrophoresis. The system detects:

- Fluorescently stained double stranded DNA including genomic DNA
- Fluorescently stained total RNA (Eukaryotic and Prokaryotic)

The 4200 TapeStation system is designed for professional use to exclusively run Agilent ScreenTape kits and specified consumables.

For Research Use Only. Not for use in diagnostic procedures.

Do not use this product in any manner not specified by the manufacturer. The protective features of this product may be impaired if it is used in a manner not specified in the operation instructions.

Overview of the System and ScreenTape Architecture

Overview of the System

The Agilent 4200 TapeStation system offers automated sample processing of ScreenTape applications for DNA and RNA at scalable and high throughput. The system includes the 4200 TapeStation instrument and a laptop.



For the Agilent 4200 TapeStation system, a portfolio of ScreenTape assays is available. The credit card-sized, disposable ScreenTape device carries multiple separation lanes for analyzing DNA and RNA samples. Each ScreenTape device has 16 lanes so that each sample is analyzed in an individual lane, completely eliminating contamination and carryover.

A suite of intuitive software provides instrument control for creation of experimental data and streamlines data analysis and reporting. It also includes tools for system diagnosis.

3 Product Description

Overview of the System and ScreenTape Architecture

The TapeStation software comes preinstalled with the 4200 TapeStation system and consists of three packages.



TapeStation Controller software



TapeStation Analysis software



Agilent Information Center

The TapeStation software is simple and intuitive, streamlining instrument control as well as data analysis, reporting, and system diagnosis. You can display your results as an electropherogram, as a familiar gel image or in tabular format for effortless sample comparison. Depending on your application, the software automatically determines size, quantity, purity, RIN^e or ribosomal ratios, saving you time and reducing errors. Reports can easily be generated and saved in PDF format, or exported for editing in Microsoft Word or Excel.



Figure 1 ScreenTape devices for DNA and RNA assays

The TapeStation Controller software also offers advanced system diagnostics through a Diagnostics and Functional Verification context. It can be used for routine confirmation of the system functionality or for instrument troubleshooting. Using a dedicated TapeStation Test Tape device, you can run an instrument self-test and check the performance of electrical and optical components. Furthermore, the computer specification and installation status of all software components are evaluated. Test reports can easily be generated and saved in PDF format.



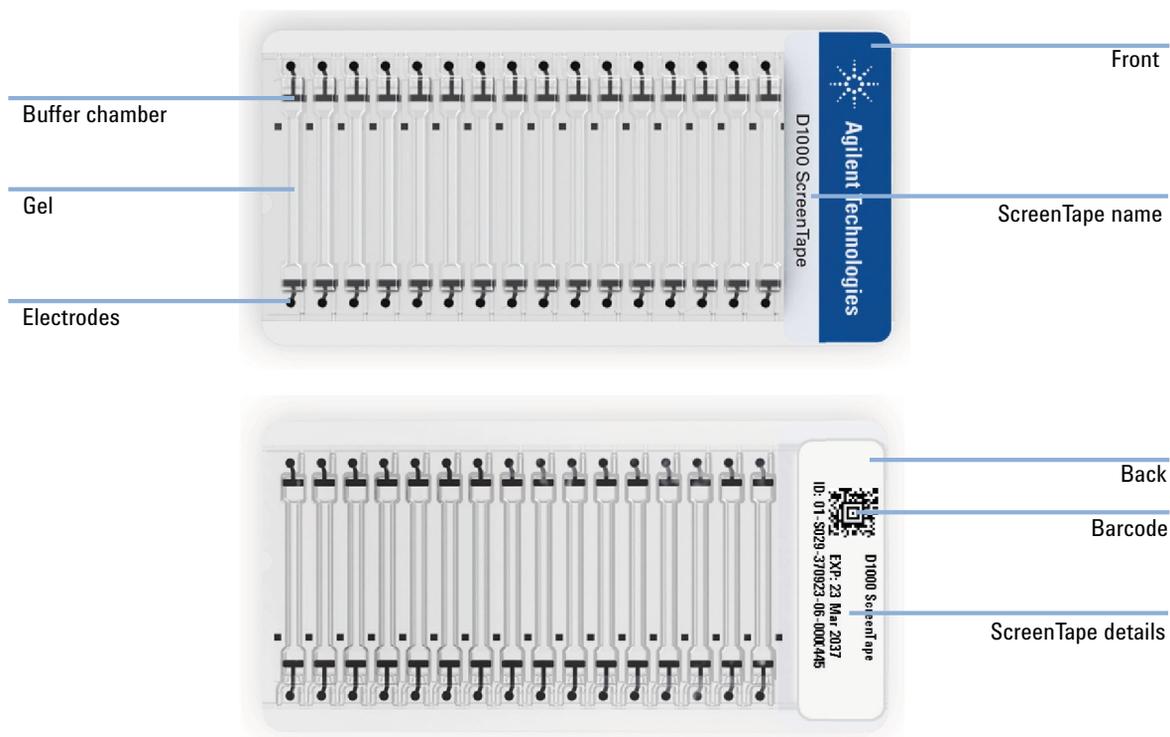
Figure 2 TapeStation Test Tape

3 Product Description

Overview of the System and ScreenTape Architecture

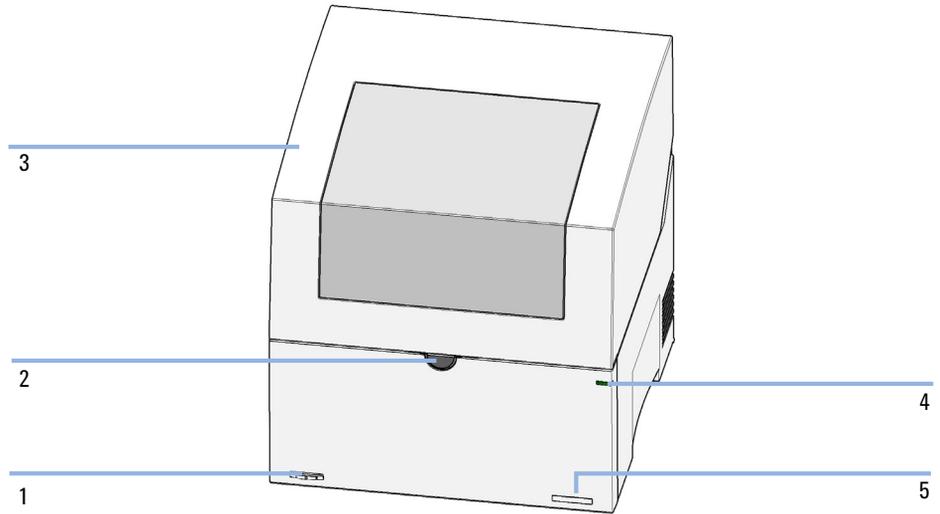
ScreenTape Architecture

- Barcode:** The unique barcode allows tracking of lane usage within each ScreenTape device and provides traceability of results. Information is stored locally on the bundled laptop.
- Buffer chamber:** The buffer chamber is located at the top of each channel and contains optimized buffers for the effective separation of nucleic acid fragments.
- Electrodes:** The integrated electrodes apply a current across the ScreenTape device and eliminate the need for any additional electrophoresis equipment.
- Gel:** The gel contained within the ScreenTape device has been developed specifically to resolve nucleic acids.
- ScreenTape device product details:** The information is unique to each ScreenTape device. This includes: ScreenTape assay type, product expiry date and a unique serial number.



Product Descriptions

1 Overview of the 4200 TapeStation instrument



1 Power button

2 Lid release button

3 Lid

4 Status LED

5 Serial number

WARNING

Heart pacemakers

Magnets could affect the functioning of pacemakers and implanted heart defibrillators.

A pacemaker could switch into test mode and cause illness.

A heart defibrillator may stop working.

→ Bearers of heart pacemakers or implanted defibrillators must stay off at least 55 mm from the magnets.

CAUTION

Magnetic fields

Magnets produce a far-reaching, strong magnetic field.

You can damage for example televisions, laptops, computer harddisks, credit cards, magnetic cards may be damaged as well.

→ Keep magnets at least 25 mm away from devices and objects that could be damaged by strong magnetic fields.

2 Lid operation

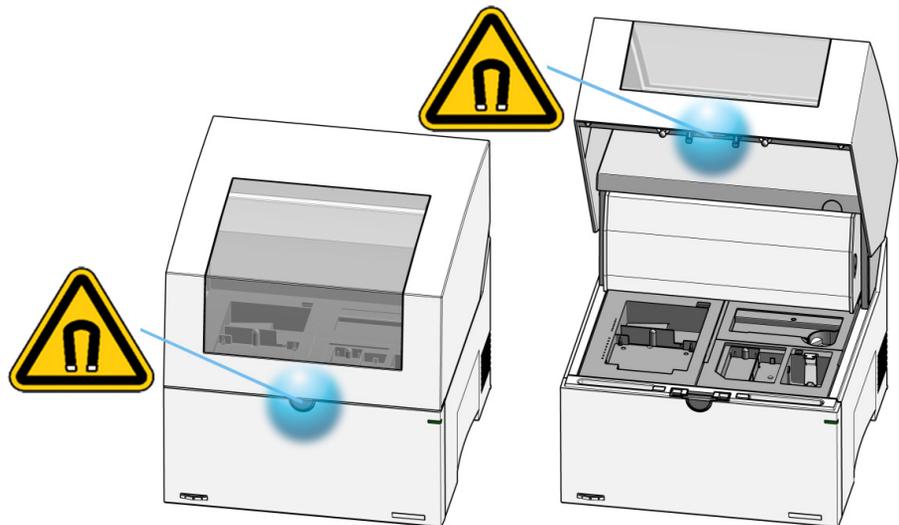


Figure 3 Position of magnet

The 4200 TapeStation instrument lid is opened by pressing the lid release button. Subsequently the lid swings open and allows access to the working area. Opening and closing of the lid is best assisted by two hands guiding the lid up or down. The lid should be closed each time the 4200 TapeStation instrument is initialized or when the TapeStation Controller software is started. Close the lid by moving it gently downwards until latching is complete.

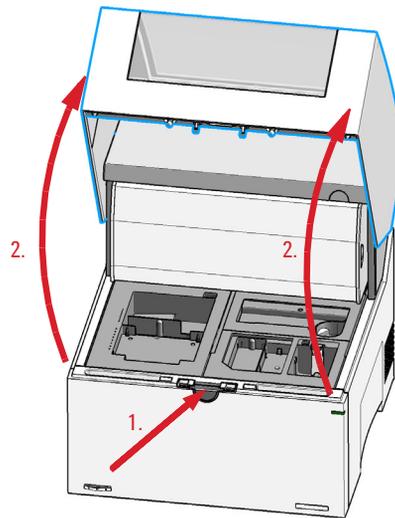
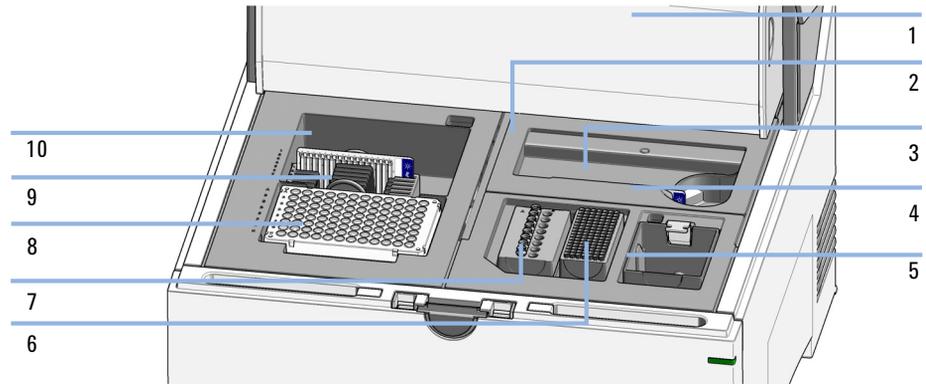


Figure 4 Lid operation

3 Product Description

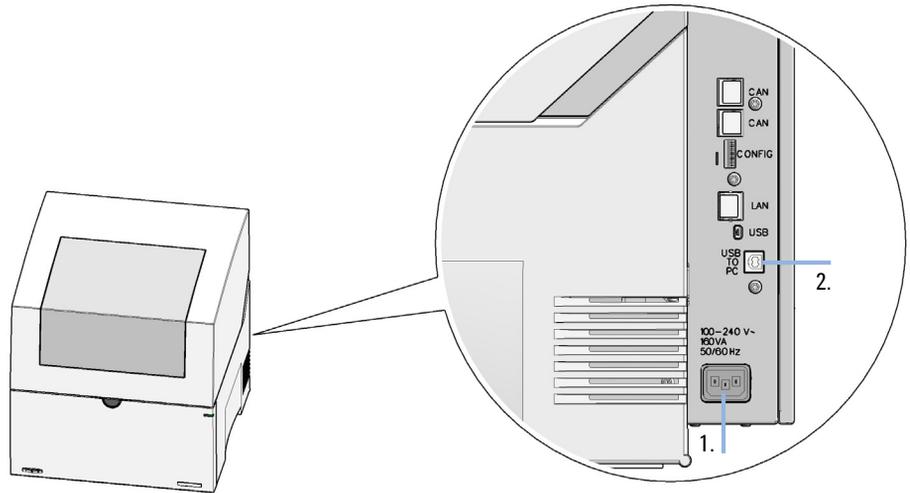
Product Descriptions

3 Overview of the instrument workspace



1	Gantry cover
2	Upper nest cover
3	Lower nest cover
4	ScreenTape nest
5	Tip waste
6	Tip rack
7	Tube strip holder
8	96-well sample plate
9	ScreenTape rack
10	ScreenTape waste location

4 Electrical connections



1. Power line in, 2. USB-B to PC

Remaining connectors are blocked by plugs and reserved for factory use.

3 Product Description

Product Descriptions

5 Status indicators (LED)

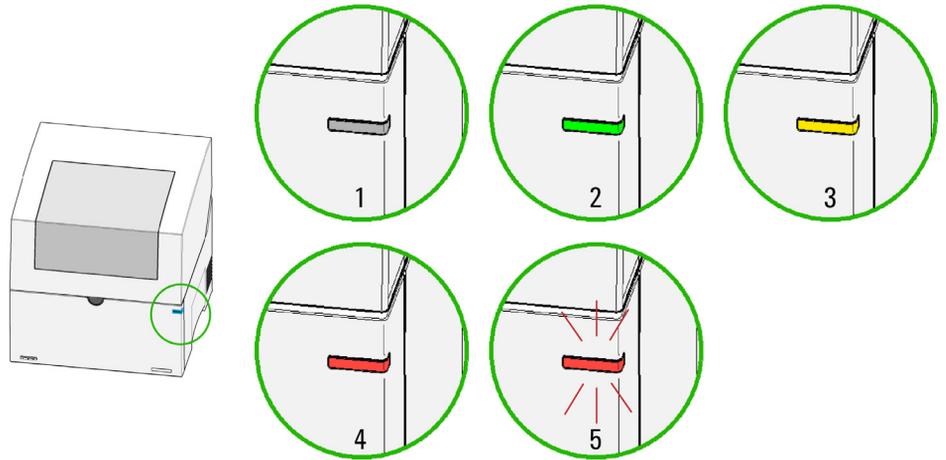


Figure 5 Status indicators

1	off	Idle
2	green	Run mode
3	yellow	Not-ready, waiting for a specific pre-run condition to be reached or completed
4	red	Error mode (restart required)
5	red blinking	Error mode (restart required) or ongoing firmware update

6 Status indicators (Lid)

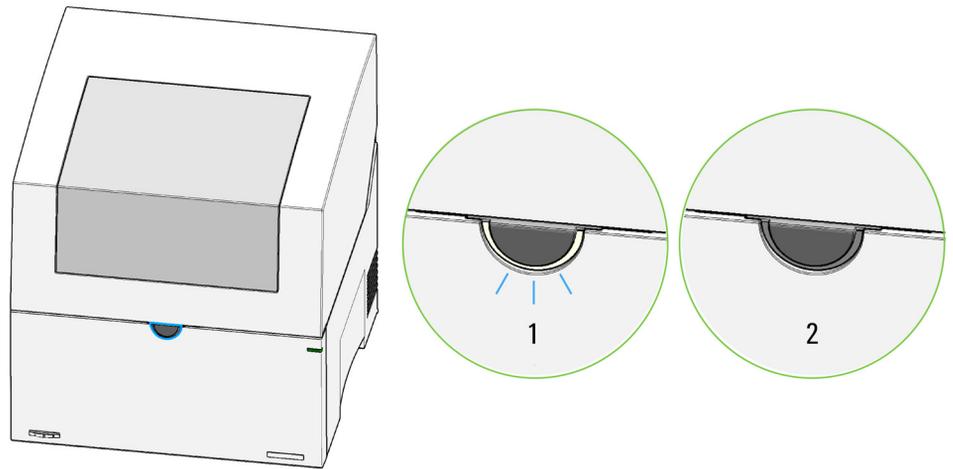


Figure 6 Lid release button status light

1. On; 2. Off.

Normal status is off. Blinking indicates an input request from the TapeStation Controller software to confirm a run stop request.

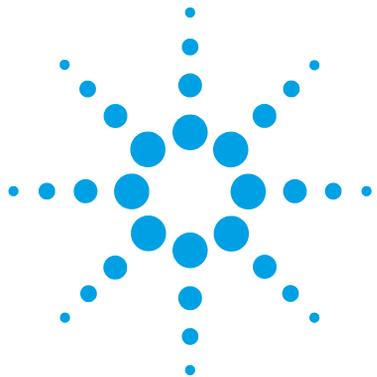
Physical Specification

Table 2 Physical Specifications

Type	Specification
Weight	25.5 kg (56.3 lbs)
Dimensions (w×d×h)	435 x 510 x 445 mm (17.1 x 20.0 x 17.5 inches)
Line voltage	100 – 240 VAC ± 10 %
Line frequency	50 or 60 Hz ± 5 %
Power consumption	50 VA / 50 W
Interface	USB2 (Instrument: USB-B; PC: USB-A)
Ambient operating temperature	10 – 40 °C (50 – 104 °F)
Operating humidity	15 – 80 %, non-condensing
Storage temperature	-40 to 70 °C (-40 to 158 °F)
Operating altitude	Up to 3000 m (9842 ft)
Safety standards	IEC, EN, CSA, UL, Installation category II, Pollution degree 2 For indoor use only.
ISM Classification	ISM Group 1 Class A According to CISPR 11
Sound pressure	<70 dB (A)

NOTE

This is an ISM Group 1 Class A product intended for use in industrial environment. In a domestic environment, this product may cause radio interference, in which case the user may be required to take adequate measures.



4 Installation

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Bench Space Required	34
Unpacking	36
Initial Setup of the System	39
Prepare the Working Area	44

This chapter gives information about the installation of the system.



Site Requirements

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

WARNING

Ensure the proper usage of the equipment.

The protection provided by the equipment may be impaired.

- The operator of this instrument is advised to use the equipment in a manner as specified in this manual.
-

WARNING

Wrong voltage range, frequency or cabling

Personal injury or damage to the instrument

- Verify that the voltage range and frequency of your power distribution matches to the power specification of the individual instrument.
 - Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.
 - Make all connections to the unit before applying power.
-

WARNING

Missing electrical ground

Electrical shock

- If your product is provided with a grounding type power plug, the instrument chassis and cover must be connected to an electrical ground to minimize shock hazard.
 - The ground pin must be firmly connected to an electrical ground (safety ground) terminal at the power outlet. Any interruption of the protective (grounding) conductor or disconnection of the protective earth terminal will cause a potential shock hazard that could result in personal injury.
-

WARNING

Instrument covers removed

Electrical shock

- Do Not Remove the Instrument Cover
 - Only Agilent authorized personnel are allowed to remove instrument covers. Always disconnect the power cables and any external circuits before removing the instrument cover.
-

WARNING

Damage to the instrument

Personal injury (for example electrical shock, intoxication)

- Instruments that appear damaged or defective should be made inoperative and secured against unintended operation until they can be repaired by qualified service personnel.
-

Bench Space Required

WARNING

Heavy weight

The instrument is heavy.

- Avoid back strain or injury by following all precautions for lifting heavy objects.
 - Ensure that the load is as close to your body as possible.
 - Ensure that you can cope with the weight of your load.
-

CAUTION

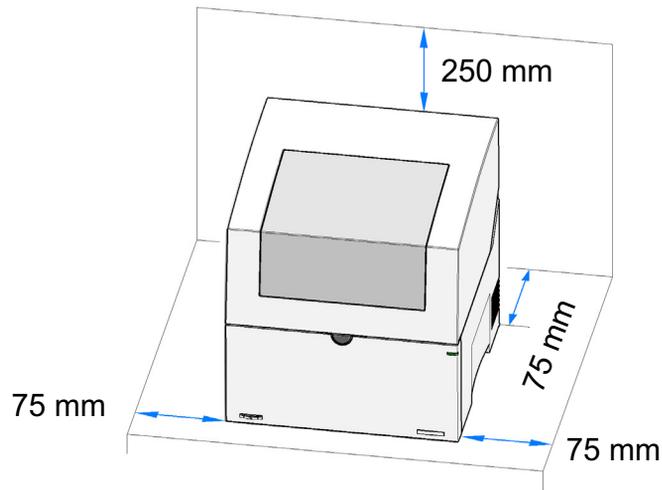
Condensation within the module

Condensation will damage the system electronics.

- Do not store, ship or use your module under conditions where temperature fluctuations could cause condensation within the module.
 - If your module was shipped in cold weather, leave it in its box and allow it to warm slowly to room temperature to avoid condensation.
-

Identify the laboratory bench space based on the specifications table (see “[Physical Specification](#)” on page 30).

Pay special attention to the total height and total weight requirements for all system components you have ordered. Also pay special attention to the total weight of the modules you have ordered to ensure your laboratory bench can support this weight. The work bench should be stable and vibration free.



Allow approximately 75 mm (3 inches) of clear space around the instrument for air circulation and electrical connections.

Performance can be affected by sources of heat and cold, for example direct sunlight, heating/cooling from air conditioning outlets, drafts and/or vibrations.

A computer system is supplied with your instrument. Account for respective electrical outlets. Two plug sockets must be available near the allocated bench space for the instrument itself and a laptop.

NOTE

Additional space of approximately 25 cm is required on top of the listed height in order to open the TapeStation lid. Please avoid locations with low overhanging shelves.

Unpacking

For unpacking the instrument follow the instructions listed below.

Do not remove the instrument from its original shipping box immediately. Upon delivery, store in a temperature stable, dry environment suitable for operation, see “[Physical Specification](#)” on page 30. If actual shipment conditions are unknown or were outside operating conditions, wait for at least 5 h before unpacking to allow the instrument to adjust to the ambient temperature and humidity of the operating environment.

WARNING

Be careful when lifting the instrument.

→ Because the instrument is heavy, lifting it will require the assistance of a second person to prevent injury.

CAUTION

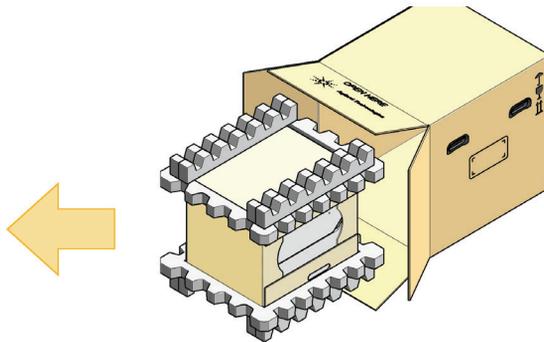
Condensation within the module

Condensation can damage the system electronics.

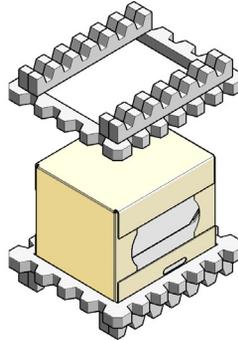
→ Do not store, ship or use your module under conditions where temperature fluctuations could cause condensation within the module.

→ If your module was shipped in cold weather, leave it in its box and allow it to warm slowly to room temperature to avoid condensation.

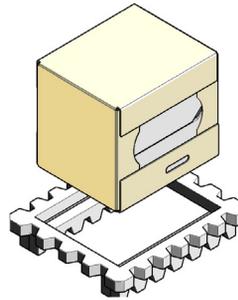
1 Pull the instrument out of the outer cardboard box.



- 2 Remove the top foam of the inner cardboard box.



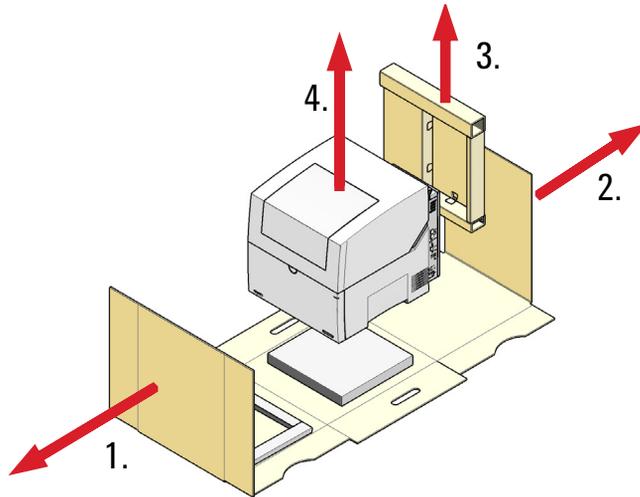
- 3 Lift the instrument to remove the bottom foam packaging.



4 Installation

Unpacking

- 4 Open the inner cardboard box and move the instrument to its bench.



Initial Setup of the System

This procedure explains the initial setup of the system after unpacking.

WARNING

Hazard of electrical shock or damage of your instrumentation can result, if the devices are connected to a line voltage higher than specified.

→ Connect your instrument to the specified line voltage only.

WARNING

Electrical shock hazard

The module is partially energized when switched off, as long as the power cord is plugged in.

The cover protects users from personal injuries, for example electrical shock.

→ Do not open the cover.

→ Do not operate the instrument and disconnect the power cable in case the cover has any signs of damage.

→ Contact Agilent for support and request an instrument repair service.

WARNING

Inaccessible power plug.

In case of emergency it must be possible to disconnect the instrument from the power line at any time.

→ Make sure the power connector of the instrument can be easily reached and unplugged.

→ Provide sufficient space behind the power socket of the instrument to unplug the cable.

4 Installation

Initial Setup of the System

WARNING

Unintended use of power cords

Using power cords for unintended purposes can lead to personal injury or damage of electronic equipment.

- Never use a power cord other than the one that Agilent shipped with this instrument.
 - Never use the power cords that Agilent Technologies supplies with this instrument for any other equipment.
 - Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.
-

WARNING

Absence of ground connection

The absence of ground connection can lead to electric shock or short circuit.

- Never operate your instrumentation from a power outlet that has no ground connection.
-

NOTE

The fixation foam holds covers in place and is only required during shipments. Keep the foam in a safe place for further use.

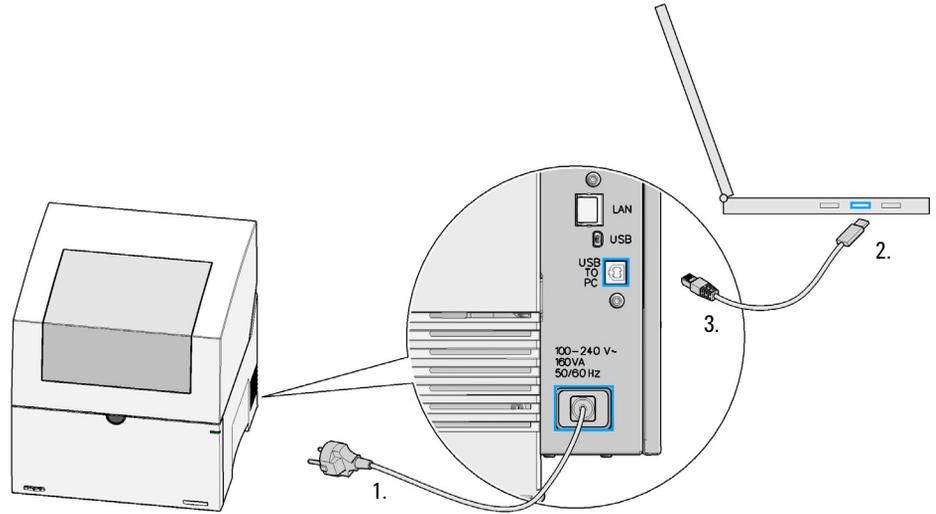
NOTE

The transportation lock holds robotic parts in a safe position during shipment. If needed, the transportation lock can only be engaged by the TapeStation Controller software again.

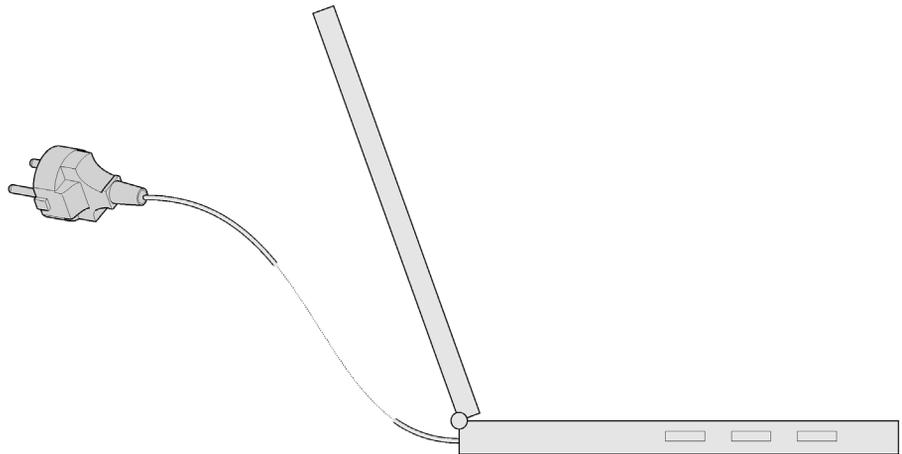
NOTE

An instrument with engaged transportation lock cannot perform analysis. The TapeStation Controller software will prompt you for powercycling releasing the transportation lock in case of an analysis start.

- 1 Connect the instrument in the following order: 1. Power plug to power socket, 2. USB-A to laptop, 3. USB-B to TapeStation instrument.



- 2 Connect laptop to power according to its accompanying documentation.



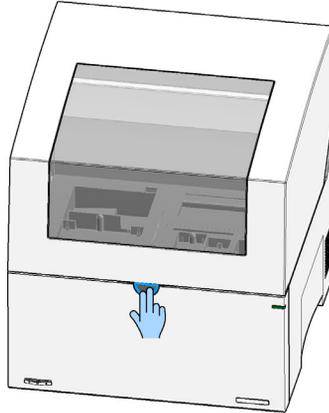
NOTE

Refer to “Preparing the Instrument” on page 61 for the required order when turning the instrument on.

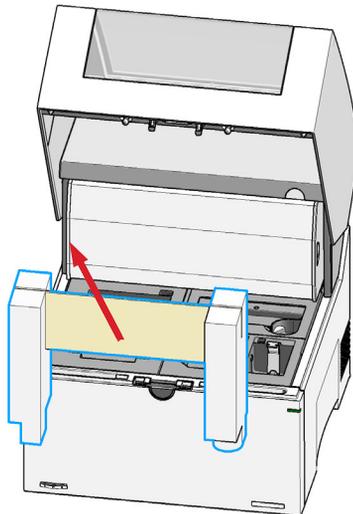
4 Installation

Initial Setup of the System

- 3 Open the lid by pressing the lid release button.



- 4 Move the fixation foam carefully upwards.

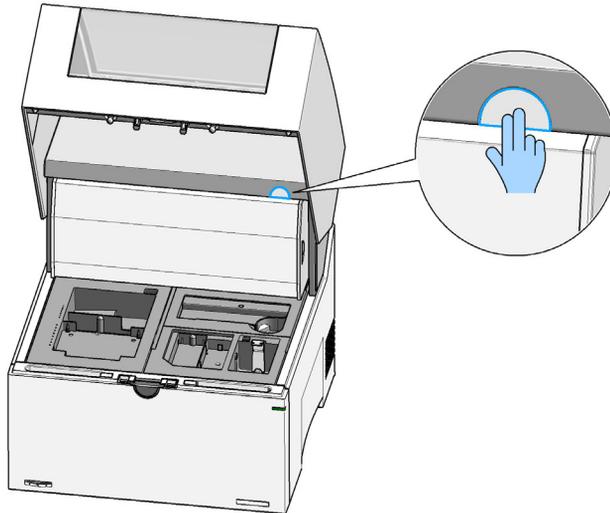


WARNING

A piercing needle is located behind the gantry cover.

- Do not reach under the cover.
- Do not remove the cover.

5 Release the transportation lock.



Pressing the release button of the transportation lock frees parts of the robotics from internal clamping. This mechanical unlocking activity enables the robotics to move and the instrument to operate. See details on transportation lock ([“Transportation Lock”](#) on page 137).

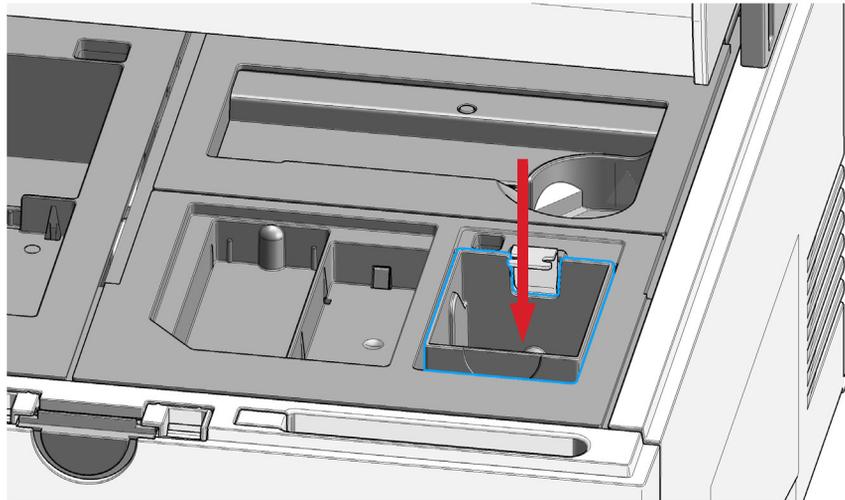
NOTE

The release of the transportation lock should always be done prior to powering on the instrument. The turn-on process requires the robotics to be unlocked for proper initialization.

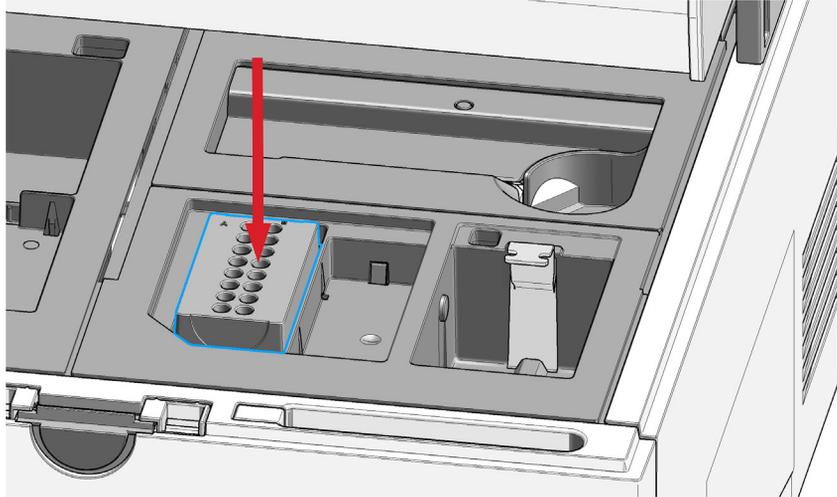
Prepare the Working Area

The steps below outline how to prepare the instrument working area with material provided as accessories. A shipment list of this material can be found in “[Accessories Shipment List](#)” on page 204.

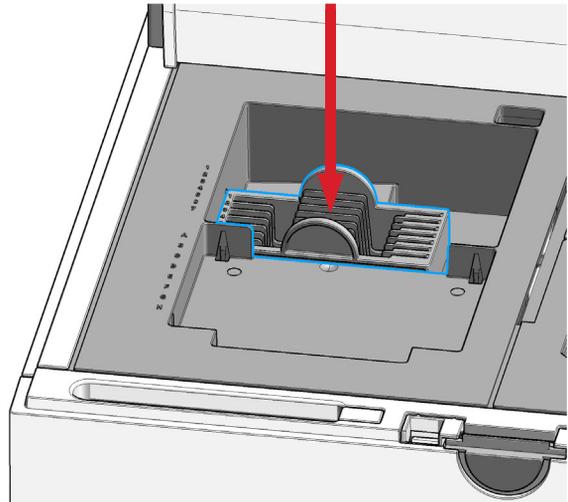
- 1 Install the tip waste bucket.



- 2 Install the sample tube strip holder.



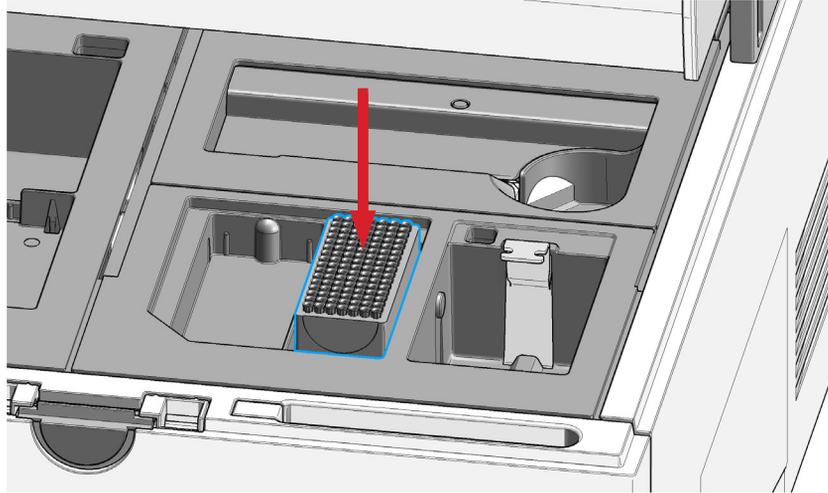
- 3 Install the ScreenTape rack.



4 Installation

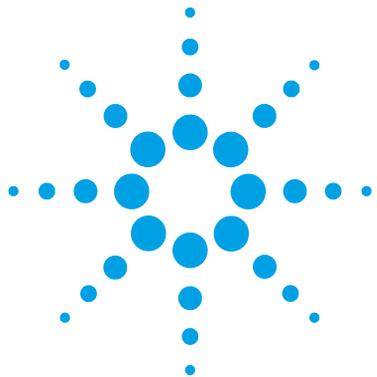
Prepare the Working Area

- 4 Install the tip rack and remove the lid. Ensure sufficient tips are present.



NOTE

The sample tube holder and ScreenTape rack have recesses for orientation and only fit in one way. The tip rack is symmetrical and can be placed either way.



5 TapeStation Software

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TapeStation Controller Software User Interface	49
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This chapter gives an overview on the three packages of software that come preinstalled with your 4200 TapeStation system.



TapeStation Controller Software

The TapeStation Controller software is designed to control the instrument during the analysis. It allows the automatic connection through USB and controls the robotics and vision system. It runs the desired assay based on the inserted ScreenTape device barcode. Data files are saved by this software and automatically opened in the TapeStation Analysis software for further processing. The TapeStation Controller software allows you to select sample location and information, to configure the file save settings and preselect assay parameters. This software reports the instrument status and allows the review of diagnostic counter.

Refer to “[Operating Instructions](#)” on page 59 for a guideline for starting an analysis.

The TapeStation Controller software also provides access to the hardware diagnostics and functional verification context, see “[Instrument Diagnosis](#)” on page 113.

NOTE

In order to keep software and firmware up to date visit www.agilent.com regularly and review the respective *readme.pdf* of any successor revision.

NOTE

The connection of only one single TapeStation system at a time to a computer running the TapeStation Controller software is allowed. Multiple instruments or fast switching between them will cause communication errors.

TapeStation Controller Software User Interface

The TapeStation Controller software user interface consists of following items.

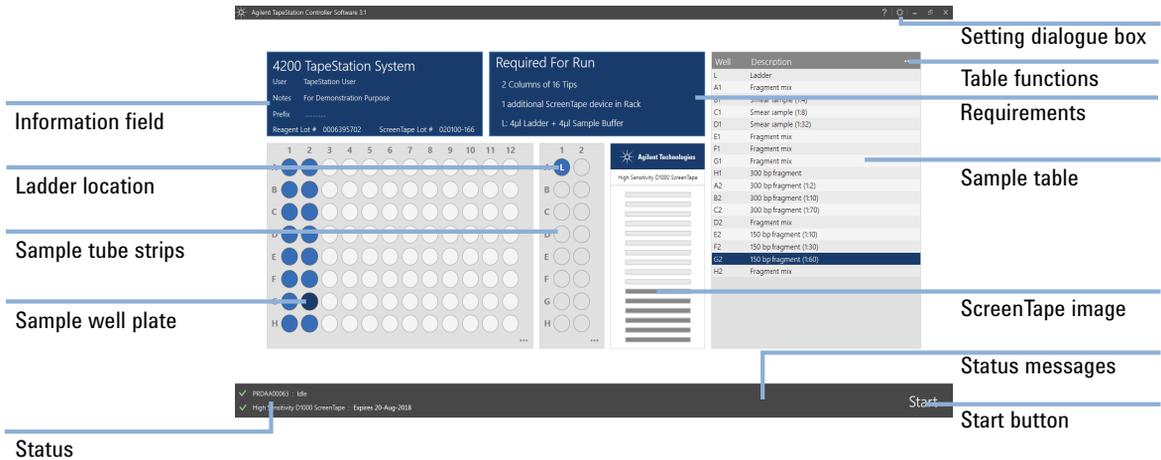


Figure 7 TapeStation Controller software user interface for 4200 TapeStation instrument

- The central ScreenTape image displays the assay selected based on the ScreenTape barcode. For partially used ScreenTape devices, lanes used in earlier runs are dark gray whereas unused lanes are light gray.
- The information field reports the name of the user logged on to the system. It allows you to enter study-related notes and change the file name prefix. For general file save settings, see the setting dialogue (see [“TapeStation Controller Software Settings”](#) on page 51)
- 96-well sample plate and Sample tube strips Sample locations can only be selected once a ScreenTape device is inserted. Samples can then be selected by clicking and dragging across either the tube strip or 96-well sample plate locations in either the tube strip or 96-well sample plate. When samples are selected, the sample table populates the corresponding lines to allow you to enter sample descriptions.

- 96-well sample plate and tube strip functions, accessible by clicking onto '...', will allow you to select or clear all position and to decide if a run ladder or an electronic ladder is used. Table functions allow you to import or export sample descriptions or to clear the table.

The ladder is always loaded from location A1 on the strip tube position. Some assays allow the use of an electronic ladder, although running a ladder with the samples is recommended. The electronic ladder can be selected by right-clicking on the ladder location (L).

Once the samples are selected the graphical user interface is updated to show the exact consumables required for the number of samples selected, including the number of ScreenTape devices, the number of tips, and the volumes required to prepare the ladder.

NOTE

Typically, one lane of ladder is run for every ScreenTape device used. Higher volumes of ladder are required for runs with multiple ScreenTape devices.

The required tips are given in full columns of 16 tips for any run from 96 well plates. For runs from tube strips the exact number of required tips is given. The ScreenTape devices required in the ScreenTape rack are in addition to the ScreenTape device inserted in the nest. If samples run from tube strips and the number of free lanes is not sufficient the TapeStation Controller software will prompt for insertion of the second ScreenTape during the run. A time window of 15 min for this applies. A brief description of the ladder volume required is given. Please refer to the assay-specific Quick Guide for more details, see [Table 3](#) on page 60.

The current statuses of the instrument and ScreenTape device are listed at the bottom of the screen. Status messages show important information relating to the instrument, the ScreenTape device, or the planned run. Any status which prevents the run initiating is described, for example if the instrument lid is still open, or the ScreenTape device is fully used or expired. In the status message area, upcoming maintenance and errors are also displayed.

TapeStation Controller Software Settings

TapeStation Controller Software Settings

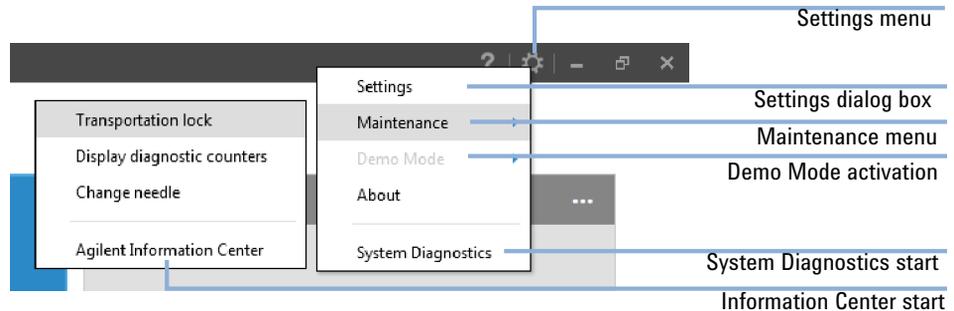


Figure 8 TapeStation Controller software settings menu for 4200 TapeStation instrument

Clicking on the icon for TapeStation Controller software settings menu allows you to

- Access the **Settings** dialog box.
- **Maintenance** menu: Activate the **Transportation lock** (see “[Engaging the Transportation Lock](#)” on page 138).
- Read diagnostic values from the instrument, see “[Needle Pierce Counter](#)” on page 126.
- Perform a needle exchange, see “[Exchange the Piercing Needle](#)” on page 129.
- Run in instrument **Demo Mode** with virtual ScreenTape assays.
- Start Hardware Diagnostics and Functional Verification context.
- Start the **Agilent Information Center**.
- See information about the software.

The **Settings** dialog box consists of following items.

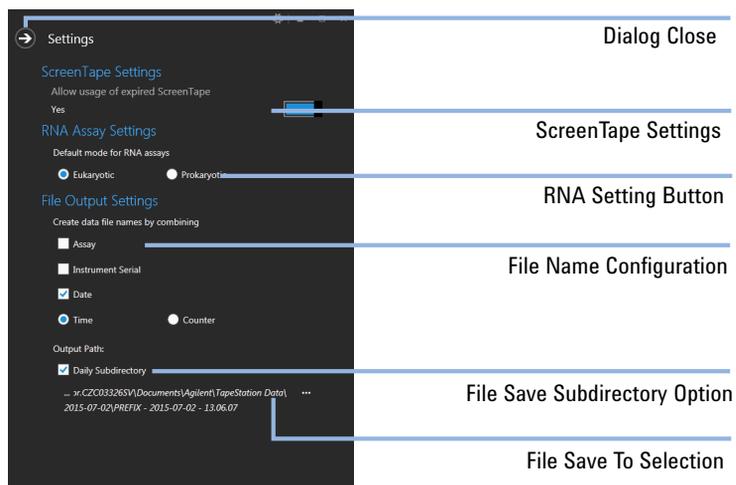


Figure 9 TapeStation Controller software settings dialog box

NOTE

This exception for expired ScreenTape devices is not recommended. Usage of expired ScreenTape devices will be recorded in the data file.

Clicking on the **ScreenTape Settings** allows you to:

- Use expired ScreenTape devices beyond the general expiry date that is printed on the ScreenTape device, or
- Use ScreenTape devices beyond the period of two weeks, which is the time allowed for using up a partially used ScreenTape device

The **RNA Assay Settings** button preselects the analysis mode for RNA assays (default setting Eukaryotic). The selected value can also be changed after the run, in the TapeStation Analysis software.

The **File Name Configuration** allows you to customize the data file name. Assay name, instrument serial number and date can be selected. The file will have either time or a number from a continuous counter for identification.

You can create daily subdirectories within the location chosen as output path. The **File Save To Selection** allows you to change to a different location on your hard disk drive.

System Diagnostics

The **Hardware Diagnostics and Functional Verification** context within the TapeStation Controller software offers you advanced instrument diagnosis for routine confirmation of the system functionality and troubleshooting purposes.

Software Installation Verification evaluates computer specifications and verifies that all components have been installed correctly. Using a dedicated TapeStation Test Tape device (see “[TapeStation Test Tape](#)” on page 118), you can check the performance of electrical and optical components using a **System Verification** (see “[System Verification](#)” on page 116) or more extended **Hardware Troubleshooting** tests (see “[Hardware Troubleshooting](#)” on page 117). The **Software Installation Verification** is included in the **System Verification**, which is itself included in the **Hardware Troubleshooting** tests.

Test results can be stored, reviewed and used for the generation of PDF reports on the tests (see “[Reviewing Test Results](#)” on page 124). By default result files from these test suites are collected into exported log files for remote support purposes (see “[Collecting Troubleshooting Information](#)” on page 202).

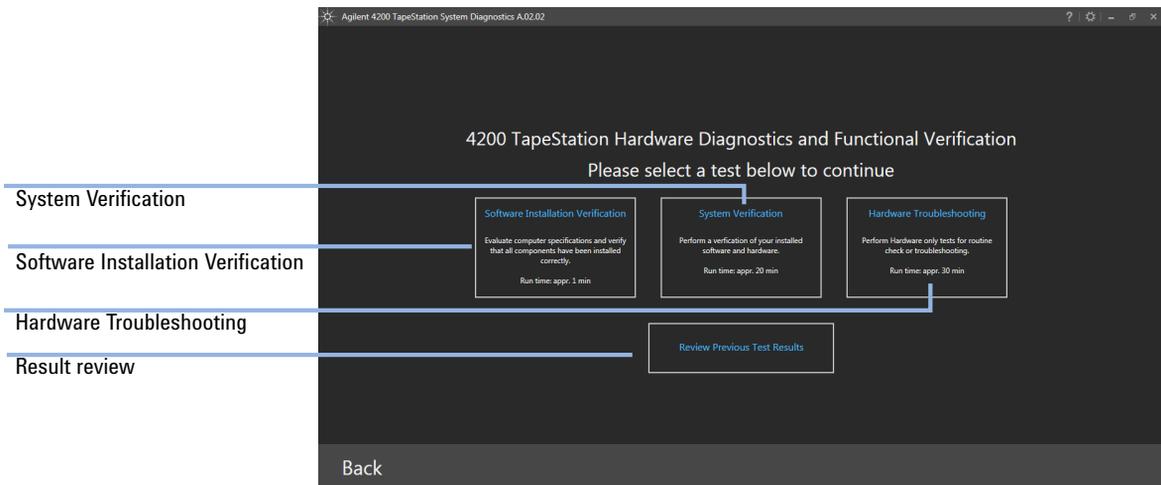
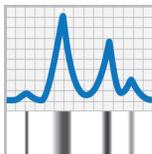


Figure 10 TapeStation Controller software: **System Diagnostics** context

TapeStation Analysis Software



The TapeStation Analysis software is a simple and intuitive software for data analysis and reporting. You can display your results as an electropherogram, as familiar gel image or in tabular format for effortless sample comparison. Depending on your application, the software automatically determines size, quantity, molarity, purity, RIN^e, DIN, %cfDNA or ribosomal ratios. Reports can easily be generated and saved in PDF format, or exported for editing in Microsoft Word. For context-specific help within the software press F1 on your keyboard.

Within the Agilent Information Center (see “[Agilent Information Center](#)” on page 56) you will find a detailed description for the TapeStation Analysis software user interface. Under *Software Operations* you can find How To videos for most common tasks.

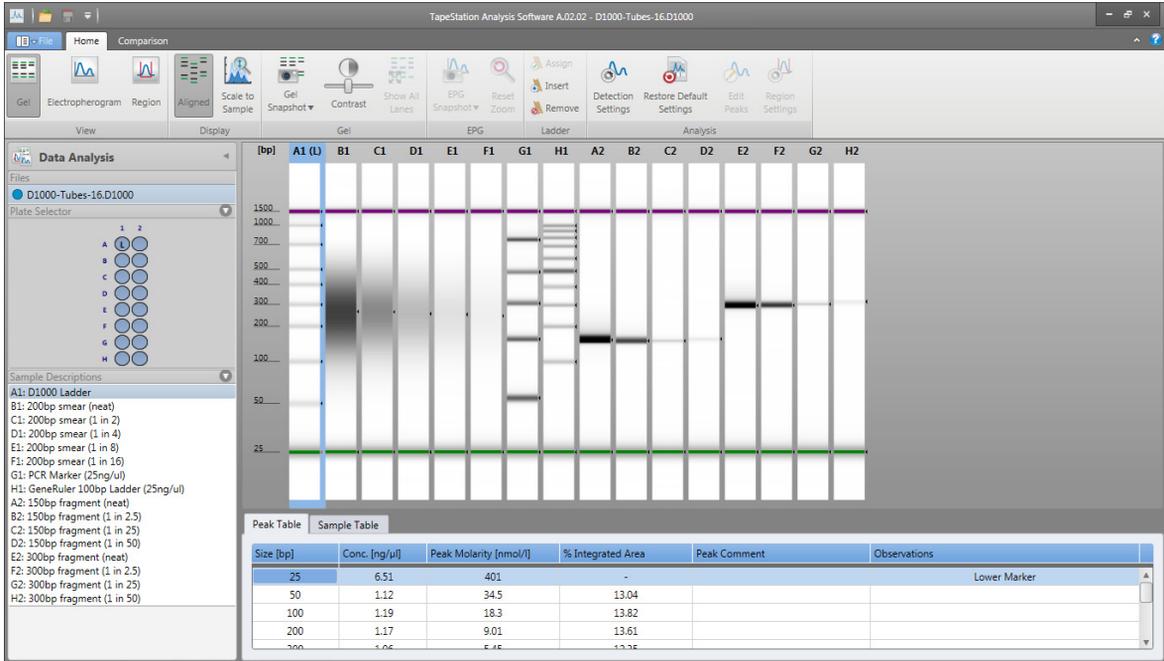


Figure 11 TapeStation Analysis software graphical interface

Agilent Information Center



The Agilent Information Center is a repository for all TapeStation user information. It offers documentation on operating instructions of the TapeStation instrument as machine in English and instructions translated into multiple European and international languages.

The Agilent Information Center has four TapeStation system-related chapters:

- The TapeStation instrument operation, explained using animated workflows (multiple languages).
- The TapeStation software, with reference help and How To videos for the most common tasks.
- The ScreenTape assays, with PDF Quick Guides and good measurement practices recommendations as well as troubleshooting.
- System Diagnostics of the TapeStation instrument, with animated workflows on its operations.

The Agilent Information Center gives you access to the PDF Quick Guides for all assays, and also access to the System Manual. The Agilent Information Center also includes a copy of the context-sensitive help on the TapeStation software.

NOTE

The Agilent Information Center should be tailored to your needs with two simple steps:
Select your language and select your instrument type.

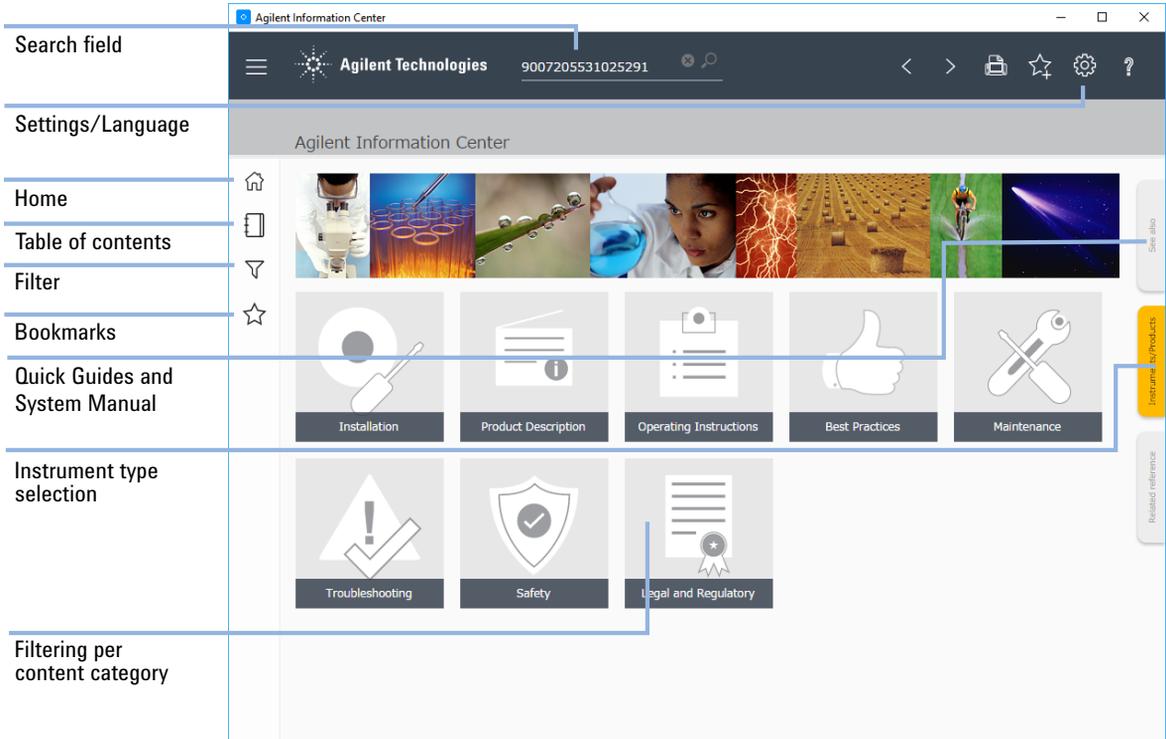
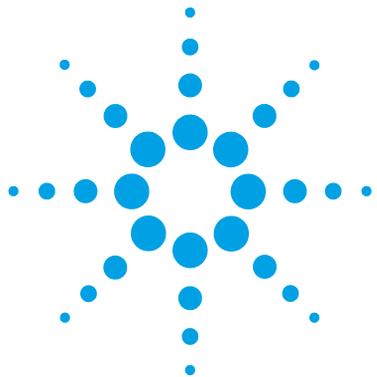


Figure 12 Agilent Information Center for the TapeStation system



6 Operating Instructions

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This chapter explains the operation of the 4200 TapeStation system.



Sample Preparation

Prepare the samples according to the assay-specific Quick Guide. Printed Quick Guides are part of system shipment (“[Accessories Shipment List](#)” on page 204). Quick Guides in electronic format are part of the Agilent Information Center which is installed along with your TapeStation software. They provide analytical and physical specifications such as sizing range, resolution, sensitivity, maximum sample buffer strength, quantitative range, analysis time, and the assay operating procedure.

NOTE

Please notice additional documentation on assays in the chapter ScreenTape Assays in the Agilent Information Center.

Table 3 ScreenTape Assay portfolio

ScreenTape Assay	Quick Guide Number
Agilent D1000 ScreenTape Assay	G2991-90031
Agilent High Sensitivity D1000 ScreenTape Assay	G2991-90131
Agilent RNA ScreenTape Assay	G2991-90021
Agilent High Sensitivity RNA ScreenTape Assay	G2991-90121
Agilent Genomic DNA ScreenTape Assay	G2991-90041
Agilent D5000 ScreenTape Assay	G2991-90051
Agilent High Sensitivity D5000 ScreenTape Assay	G2991-90151
Agilent Cell-free DNA ScreenTape Assay	G2991-90060

Please notice a documentation on “[Good Measurement Practices](#)” on page 81.

Find information on “[Troubleshooting DNA Applications](#)” on page 160 and “[Troubleshooting RNA Applications](#)” on page 181.

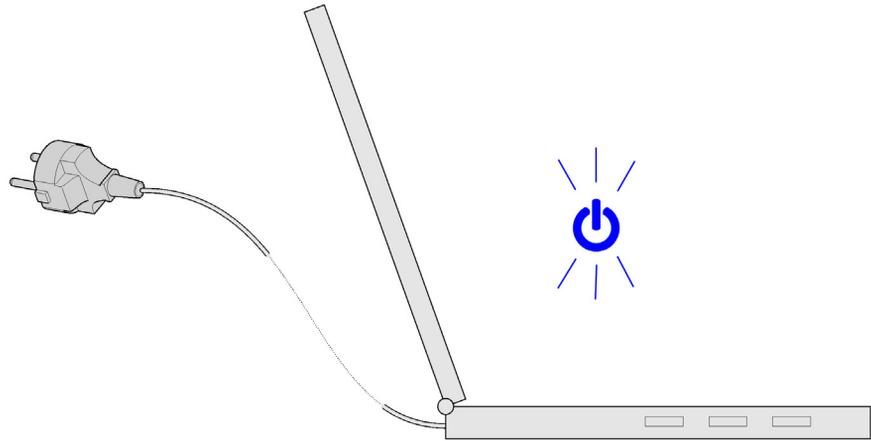
NOTE

The assay-specific Quick Guides contain important information on the ladder, see also “[Ladder Considerations](#)” on page 91. Please read this section carefully.

Preparing the Instrument

Preparing the 4200 TapeStation Instrument for an analysis is outlined below.

- 1 Power on the laptop, wait until it is booted.



- 2 Turn On

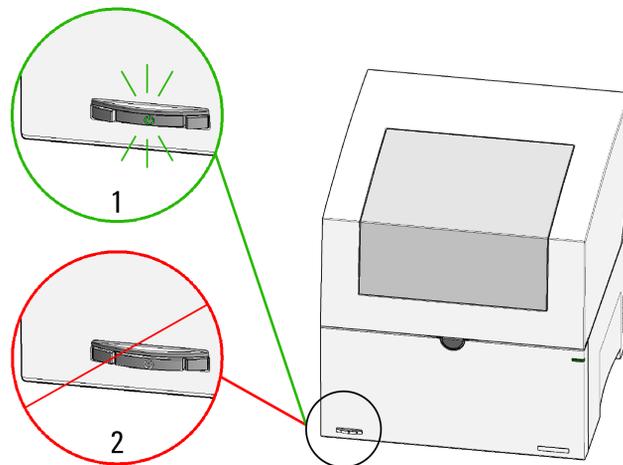
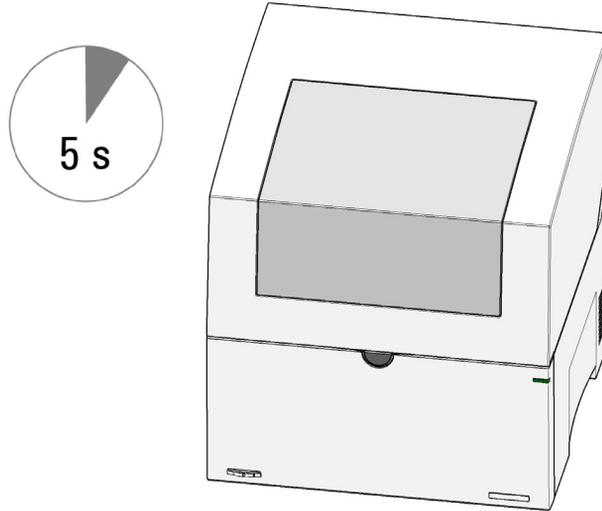


Figure 13 Power switch. 1: On; 2: Off

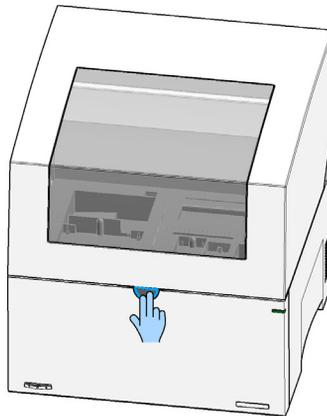
6 Operating Instructions

Preparing the Instrument

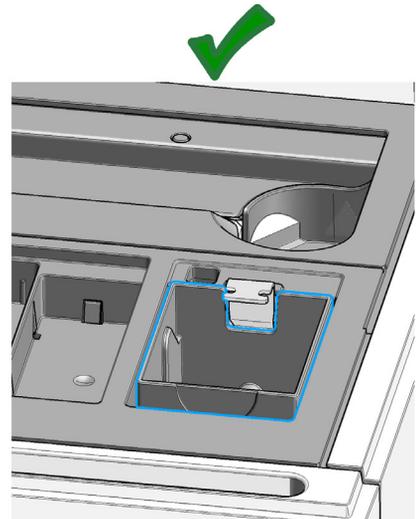
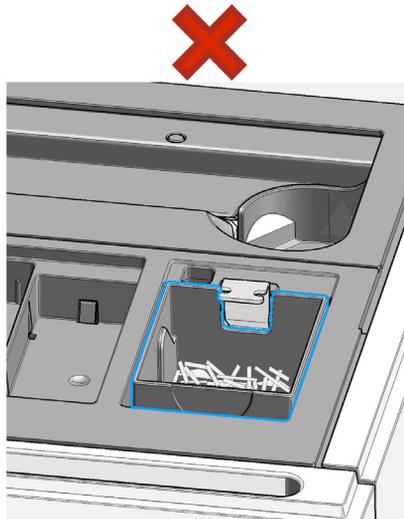
- 3 Allow the instrument to initialize.



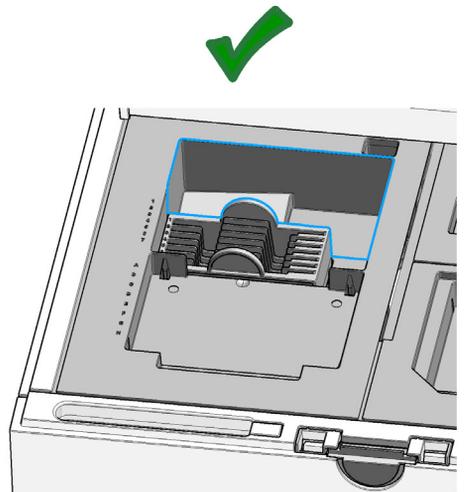
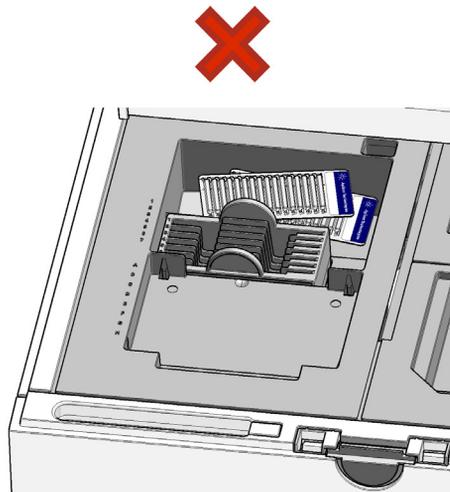
- 4 Start the TapeStation Controller software. In case the software doesn't start properly, see [“Instrument and Laptop”](#) on page 193.
- 5 Open the lid by pressing the lid release button.



6 Empty the tip waste.



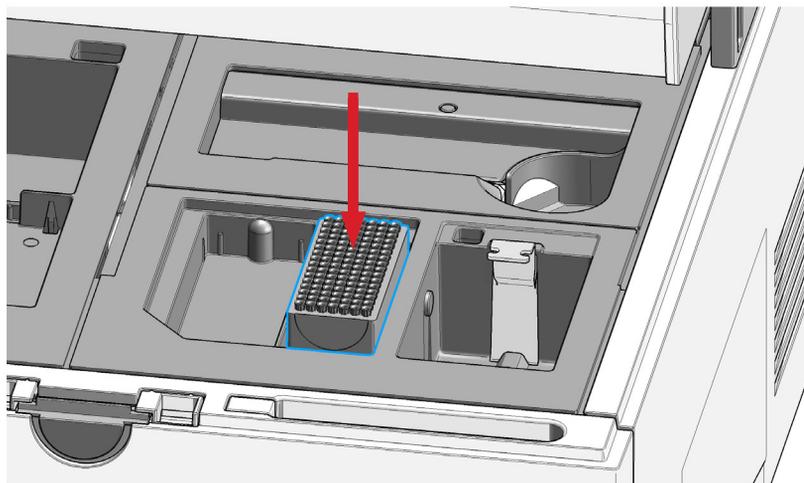
7 Discard the used up ScreenTape devices present in the waste location.



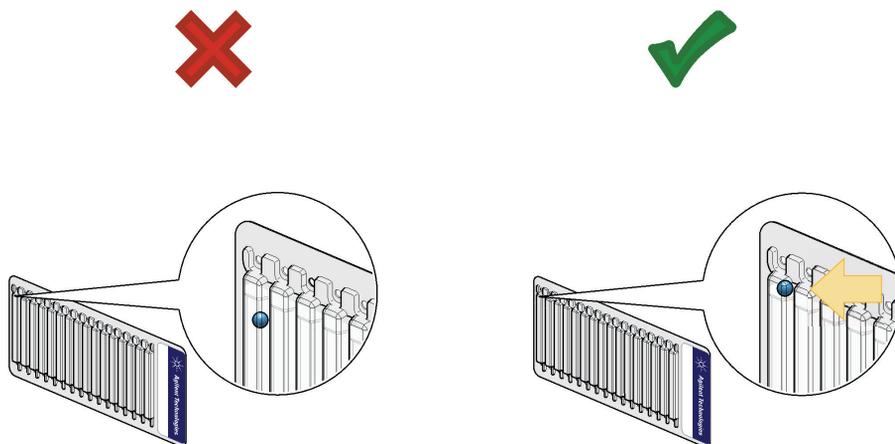
6 Operating Instructions

Preparing the Instrument

- 8 Install the tip rack and remove the lid. Ensure sufficient tips are present.



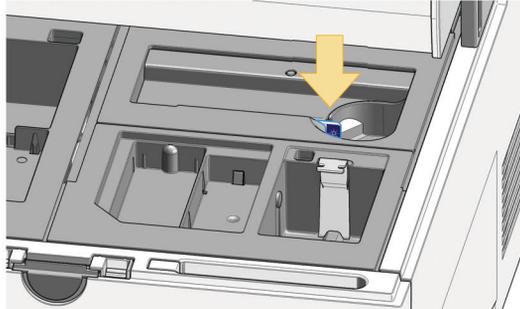
- 9 Flick the ScreenTape device. Trapped air bubbles will move up in the buffer chamber. For details, see “ScreenTape Devices” on page 86



NOTE

The presence of small bubbles within the buffer chamber of the ScreenTape devices is normal and acceptable. These bubbles often occur at the gel/buffer interface and need to be displaced prior to running. Failure to remove bubbles from the gel/buffer interface is detrimental to the performance of the ScreenTape assay.

- 10 Place the first ScreenTape device into the ScreenTape nest. Make sure it is horizontal within the nest and that the label faces toward the front.



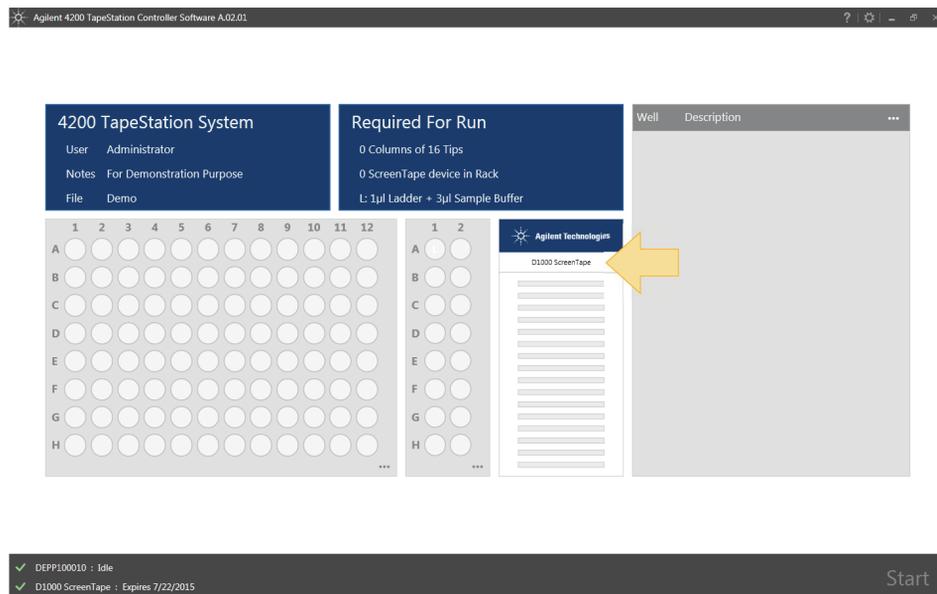
NOTE

This first ScreenTape device might be new or partially used with remaining lanes from previous analysis on the same system. For details, see [“Partially Used ScreenTape Devices”](#) on page 89.

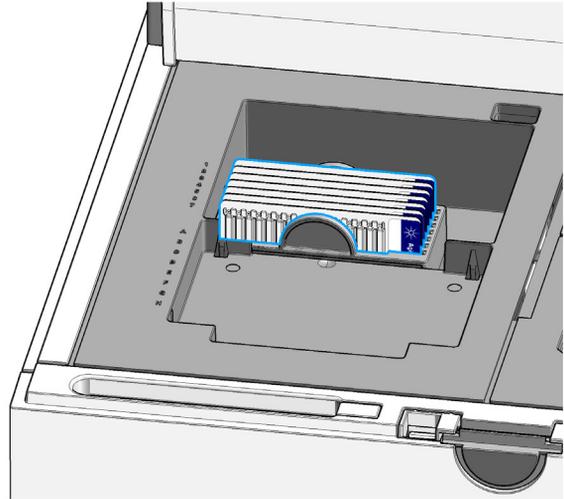
6 Operating Instructions

Preparing the Instrument

- 11 The TapeStation Controller software interface will change and displays the ScreenTape assay recognized. For details, see “[TapeStation Controller Software](#)” on page 48



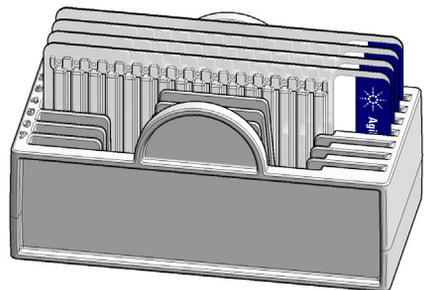
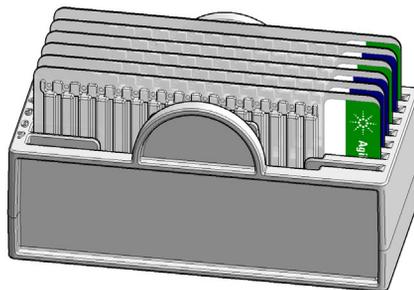
12 Insert flicked ScreenTape devices into the rack.



NOTE

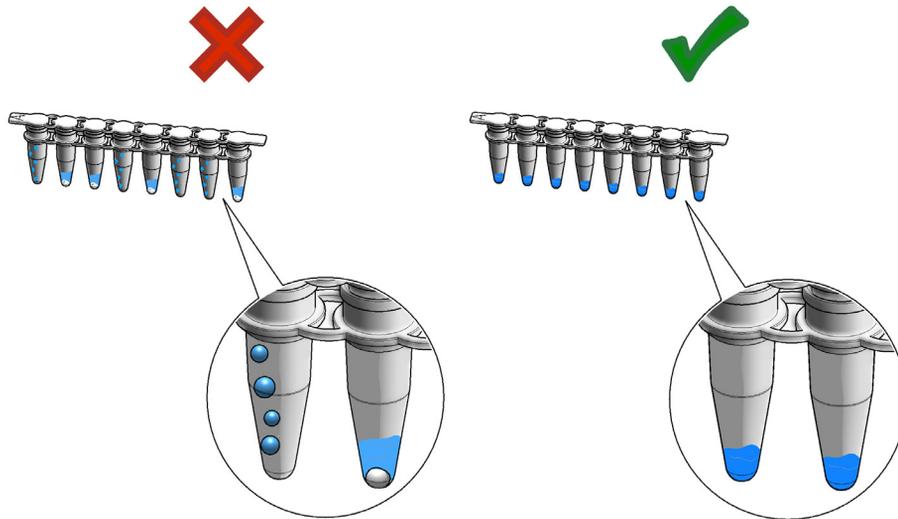
Any partially used ScreenTape devices must be used first, and should be placed into the ScreenTape nest. Fill ScreenTape rack only with fresh and unused ScreenTape devices. Flick all ScreenTape devices to remove bubbles prior to loading them into the rack.

13 Only use ScreenTape devices of identical assays.



6 Operating Instructions
Preparing the Instrument

14 Centrifuge samples to remove spills and air bubbles. For details, see “[Centrifugation Recommendations](#)” on page 96.

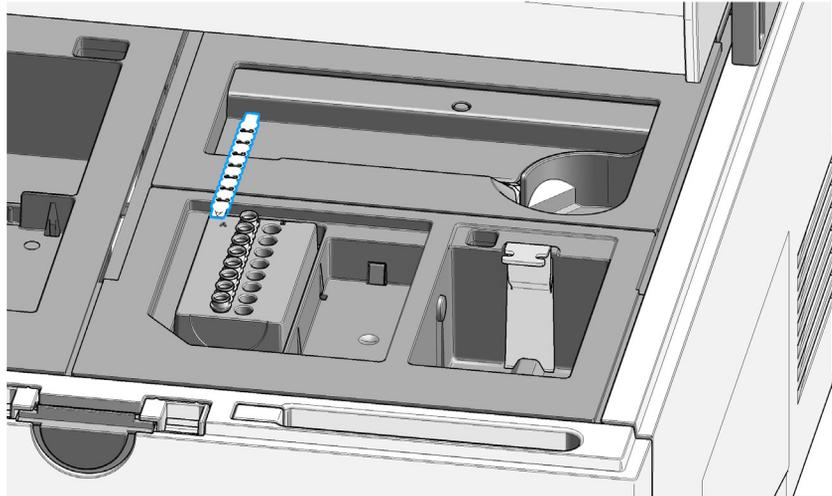


CAUTION

Blockage of the robotic system

- Remove the lid from the sample tube strips to prevent blockage of the robotics system during pipetting steps.

- 15 Insert sample tube strips. Remove the tube lids carefully ensuring not to create bubbles in the samples.

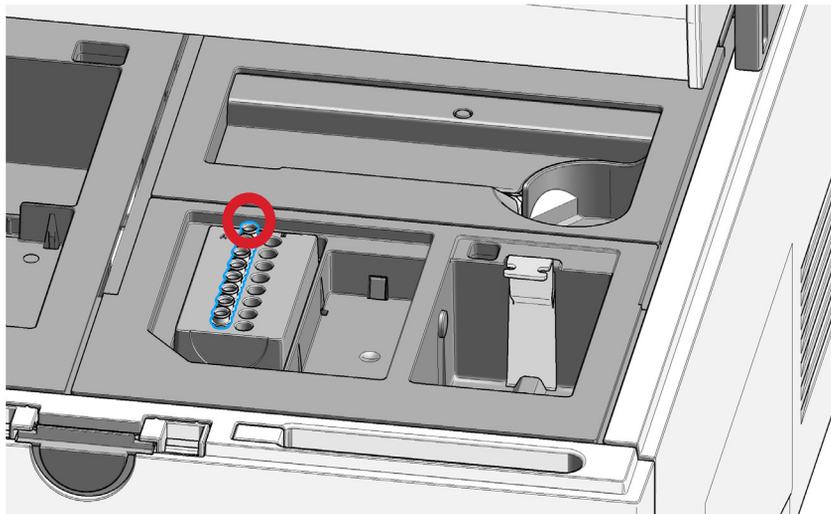


- 16 Ladder is exclusively provided from location A1 on the sample tube strip holder. For further details on handling the ladder within assays see the

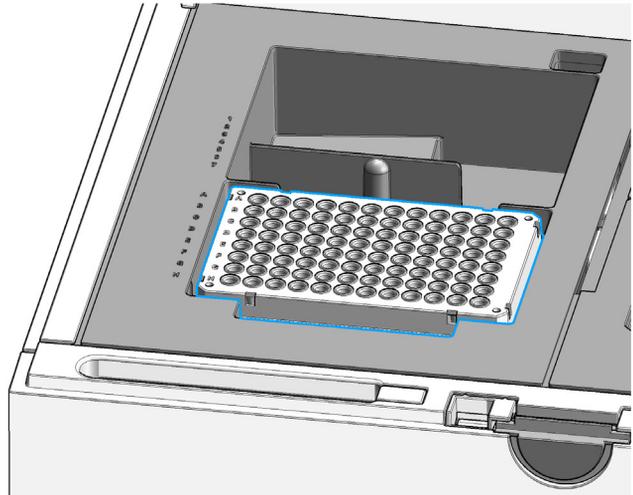
6 Operating Instructions

Preparing the Instrument

assay-specific Quick Guides or the section “[Ladder Considerations](#)” on page 91.



- 17 Alternatively, samples can be run from a sealed 96-well sample plate by inserting a sealed 96-well sample plate in correct orientation (The letters A to H are embossed for reference on the left hand side of the recess for the 96-well sample plate). Vortex and centrifuge 96-well sample plates before insertion.



NOTE

Use 96-well plate foil seals to protect samples from evaporation during analysis.
Use only Agilent approved 96-well plate foil seals and plates to avoid damaging the instrument.

6 Operating Instructions

Preparing the Instrument

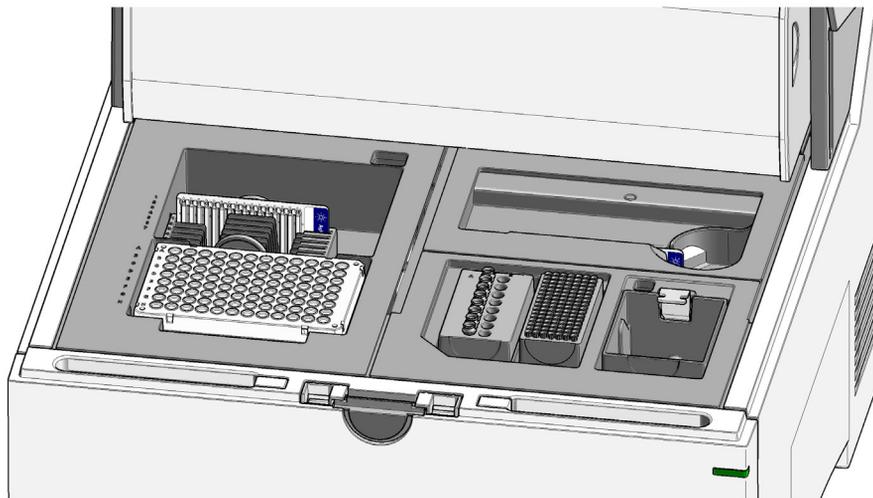
CAUTION

Failures and errors during the run

Any spare material left in the instrument will cause failures and errors during the run. The instrument might get damaged by this.

→ Ensure the working area is cleared before starting the run.

18 Keep the working area clean and tidy.



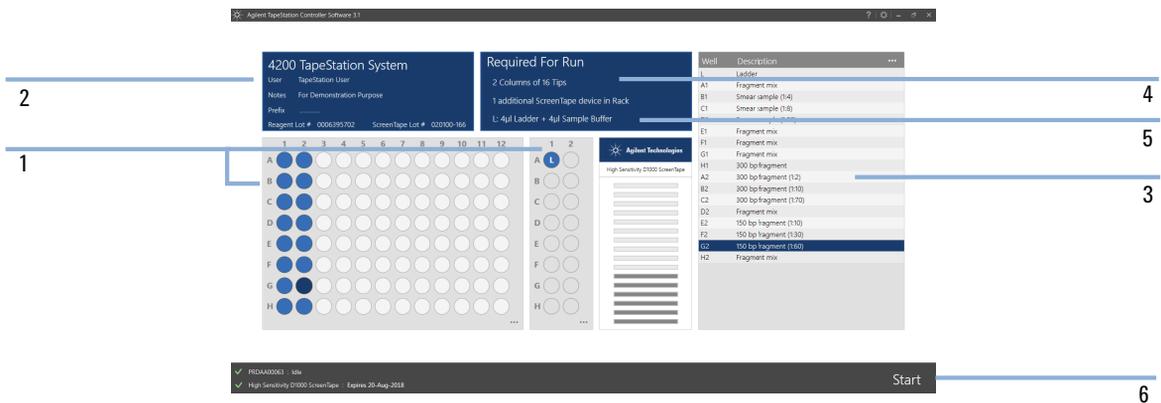
NOTE

Keep in mind the robotics movements during an analysis. Let material snap in place where appropriate. Do not leave lids, pipettes or packaging material in the working area.

19 Close the lid.

Starting the Analysis

The following steps describe the essential steps for starting a 4200 TapeStation analysis. Please refer to section “[TapeStation Analysis Software](#)” on page 54 for further details. Press **F1** for context-sensitive help on TapeStation Controller software questions or consult the Agilent Information Center.



Numbers in Figure refer to steps below.

- 1 Select samples for the analysis either on the 96-well sample plate or on the tube strip holder.

Always load the ladder in location A1 on the tube strip holder.

NOTE

Running samples from the 96-well sample plate and from sample tube strips at the same time is not supported. Combine all samples either to the 96-well sample plate or to the tube strips.

- 2 Modify user name, notes, lot information and filename prefix as required.
- 3 Enter sample descriptions.

- 4 Make sure sufficient tips and ScreenTape devices are present as directed to in the **Required for Run** section.

The graphical user interface shows the required number of columns of tips and ScreenTape devices for the selected number of samples. The requested number of ScreenTape devices in the rack is based on calculation with full ScreenTape devices in the rack and the number of available lanes on the ScreenTape device in the nest. For reasons of simplification, required tips for analysis from 96-well sample plates are expressed in columns (16 tips) and therefore possibly rounded up.

NOTE

- 96-well sample plates: samples from 96-well sample plates can be run on different ScreenTape devices which are provided in the tape rack.
- Tube strips: samples from tube strips can be run on up to two ScreenTape device. The second ScreenTape device is provided manually during the run.

Make sure that you have as many unused lanes on a partially used ScreenTape device as samples in the tube strips. If you want to use multiple ScreenTape devices in one analysis or want to avoid the need to insert a second ScreenTape device, transfer samples from tube strips to 96-well sample plates.

-
- 5 Make sure sufficient ladder is present in L respectively position A1 on the sample tube strip holder.

The volume of ladder and sample buffer to be mixed is displayed. These volumes differ from assay to assay. They also differ for analysis with one ScreenTape device or multiple ScreenTape devices. For further details, see the assay-specific Quick Guide of the respective ScreenTape assay or the section “[Ladder Considerations](#)” on page 91.

6 Start the run.

After starting the analysis, a dialogue box will request that you confirm tip and ScreenTape waste are empty, and that tube strip lids have been removed. During the initialization phase of an analysis, the presence of components such as tube strip lids, tips, and ScreenTape devices are checked.

NOTE

Instrument initialization and sample analysis take place after starting an analysis. If desired, an analysis may be paused or aborted from the graphical user interface.

NOTE

Pressing the lid release button in order to open the lid while an analysis takes place does not open the lid immediately. The area around the button starts blinking. The TapeStation Controller software prompts you for confirmation.

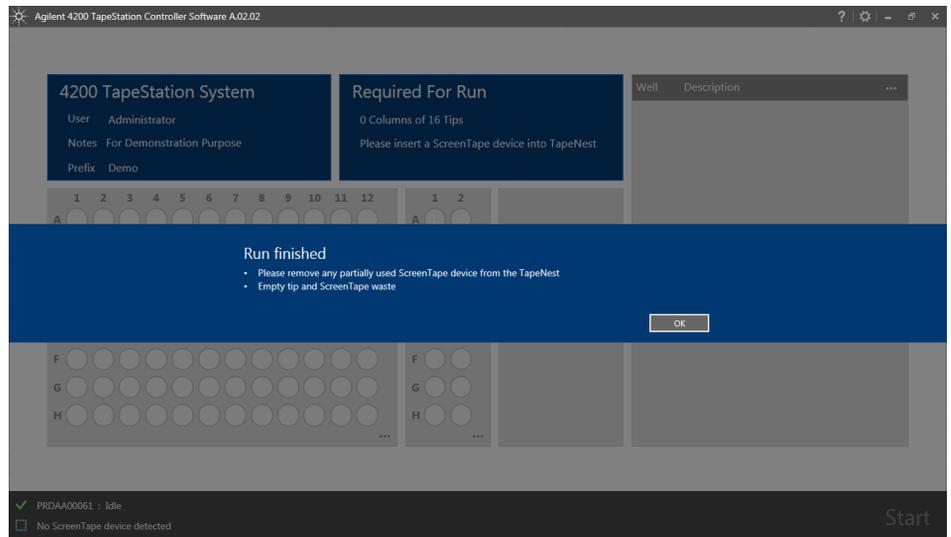
NOTE

A check for properly inserted nest covers and correct movement of the lower nest cover is done during the initialization phase prior to a run. In case of stopped runs, see [“Assembling Nest Covers”](#) on page 143, [“Insertion of the Lower Nest Cover”](#) on page 145 and [“Software Messages”](#) on page 153.

Analysis end

Once the entire analysis is complete the instrument is reset and the lid unlocked. After acquisition of data from 16 lanes or when all samples are run, the TapeStation Analysis software starts automatically. The TapeStation Controller software is kept active; therefore its graphical interface may remain in the background. Please notice any remaining dialogue.

1 Analysis end.



2 Data acquired is displayed in the TapeStation Analysis Software.

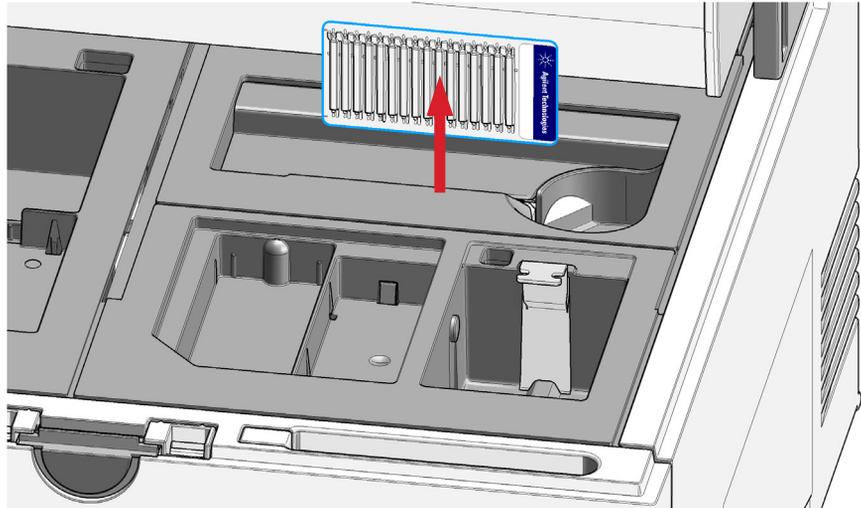
NOTE

For analysis runs which involve multiple ScreenTape devices, the TapeStation Analysis Software will launch in read-only mode after data from the first ScreenTape device is available. Data from each additional ScreenTape device will be added to this read-only file, until the last ScreenTape device has been run, and the file will be finalized.

Follow up

After the analysis end follow the post run steps listed below.

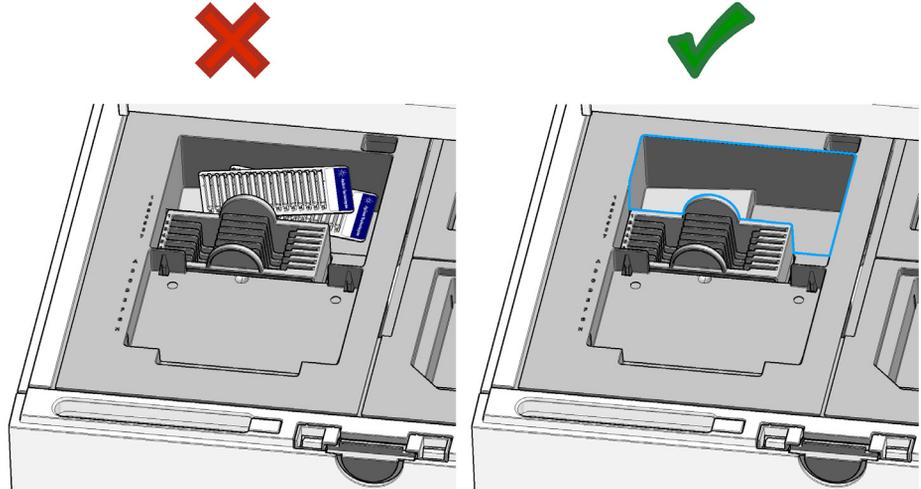
- 1 Gather any partially used ScreenTape device from the nest and store it according to the Quick Guide instructions.



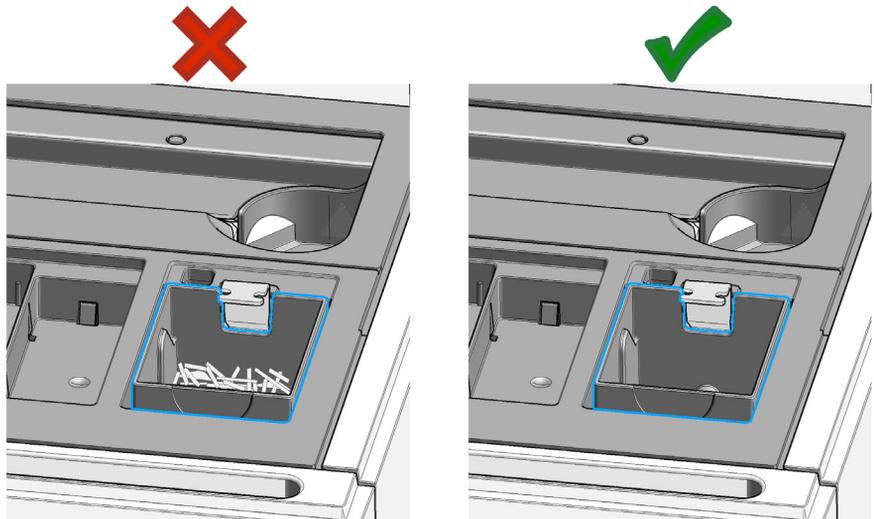
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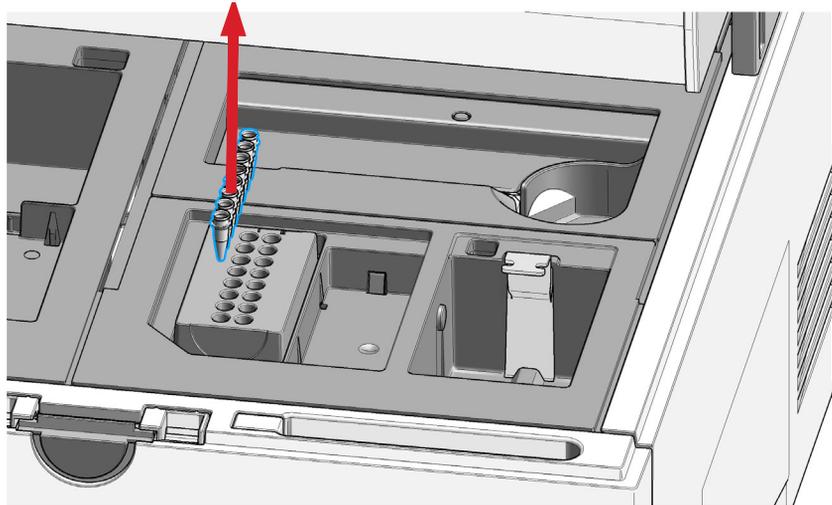
- 2 Discard the used up ScreenTape devices present in the waste location.



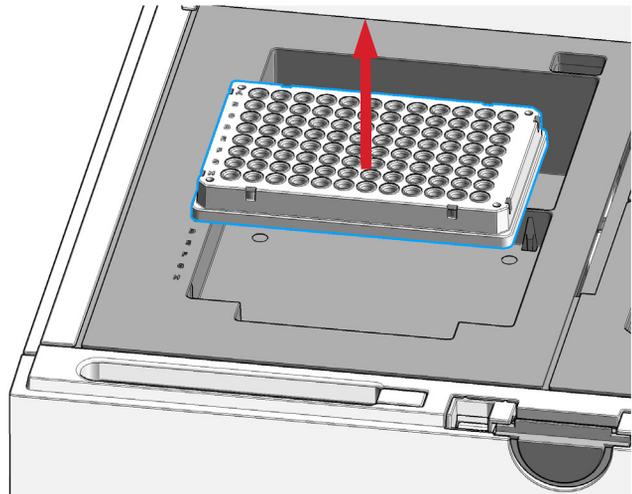
- 3 Empty the tip waste.



- 4** Remove the sample tube strip.



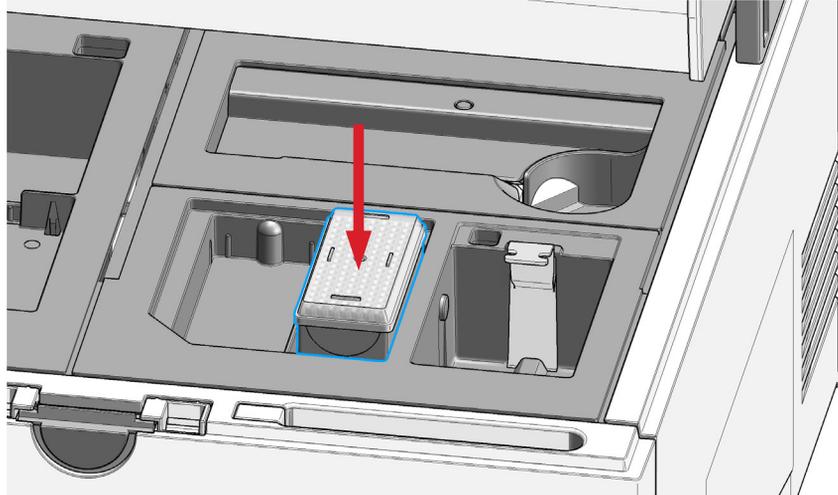
- 5** Remove the 96-well sample plate.

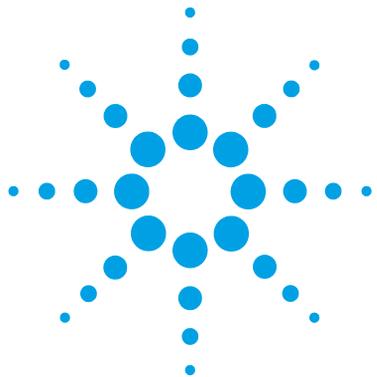


6 Operating Instructions

Follow up

- 6 Replace the tip rack cap until next use.





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This chapter summarizes information on good measurement practices for ScreenTape assays.



7 Good Measurement Practices

Follow up

For accurate results using the TapeStation systems, instructions regarding reagent preparation and instrument maintenance must be strictly followed. Important technical details are described in the Quick Guides.

This section describes techniques for ensuring reliable quantification and sizing results using all assays on the TapeStation systems.

Latest Technical Notes as well as all other user information can be found on the Agilent website:

<https://www.agilent.com/en/product/tapestation-automated-electrophoresis>.

Use of Correct Tools

- Use calibrated pipettes which are suitable for the volume required.
- Ensure correct pipetting technique, so that volumes are precise, and that the concentrations can be calculated correctly.
- Use a vortexer that is designed for mixing 8-way tube strips, or 96-well sample plates respectively.
- For more details, please see [“Mixing Recommendations”](#) on page 93.
- Use only the correct, Agilent supplied consumables including:
 - Loading tips (5067-5598; 5067-5599)
 - Tube strips (401428)
 - Tube strip caps (401425)
 - 96-well sample plates (5042-8502)
- Robotics are calibrated to dimensions of the validated consumables. Using other consumables may lead to either blockage errors or insufficient volume being drawn by the instrument pump.
- To run samples from a 96-well sample plate, also use the Agilent seal foil (5067-5154) to prevent the sample from leaving the plate during vortexing.
- Tips used are designed to be able to pierce the validated Agilent seal foil (5067-5154). Using third-party seal foils may lead to either blockage errors or insufficient volume being drawn by the instrument pump.
- After vortexing, use an appropriate centrifuge for either 96-well sample plates, or tube strips to ensure that all of the samples are at the bottom of the tube before placing in the TapeStation instrument.

Tools and Handling

- Always follow the GLP-rules established in the laboratory.
- Always wear gloves to prevent contamination.
- When pipetting samples, use loading tips that are of adequate size. Loading tips that are too large lead to poor quantitation accuracy.
- Change loading tips between steps to avoid cross contamination.

Good Pipetting Practice

A consistent pipetting technique is essential to achieve reliable and reproducible results. Always follow the recommended good pipetting practice guides from the respective manufacturer.

- Pipettes possess different pressure points and volume controls depending on the manufacturer. Familiarize yourself with the pipettes used.
- Quality pipettes possess a tolerance range which can be checked in the respective user documentation.
- Always ensure that pipettes that are used are calibrated.
- Pipette with a slow, smooth action.
- Hold the pipette vertically when drawing liquid.
- Immerse the tip only as deep as necessary when drawing liquid, otherwise the outside of the tip will be coated with liquid as well.
- Always pipette reagents against the side of the 96-well sample plate or sample tube.
- When pipetting small volumes ensure that no liquid remains within the tip or on the outside of the tip.
- Use a pipetting technique suitable for heterogenous samples.
- Care must be taken due to viscosity of sample buffer. Highly viscous solutions may require drawing liquid more slowly to ensure that the proper volume is filled.

Reagents and Reagent Mixes

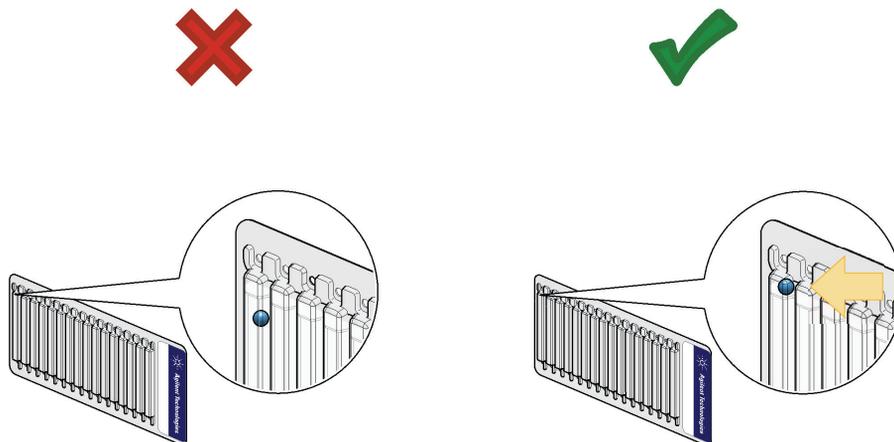
- Handle and store all reagents according to the instructions given in the appropriate Quick Guide ([Table 3](#) on page 60).
- Keep all reagents at the directed temperature when not in use. Reagents that are left at room temperature for a long period of time may decompose, leading to poor results.
- Allow all reagents to equilibrate to room temperature for 30 min. Mix and spin down prior to use.
- When pipetting sample buffer, ensure that excess buffer droplets are removed from the tip before transfer to the sample tubes. Care must be taken due to viscosity of sample buffers.
- When adding sample buffer to samples, please ensure that they are mixed correctly. See “[Mixing Recommendations](#)” on page 93, “[Good Pipetting Practice](#)” on page 84, and the appropriate Quick Guide ([Table 3](#) on page 60).
- When pipetting small volumes ensure that no sample remains within the tip. Special care must be taken with high sensitivity assays which use small volumes of sample buffer.

ScreenTape Devices

- Protect the gel lanes of the ScreenTape device from excessive force - do not bend or flex the ScreenTape device. Bent ScreenTape devices can potentially lead to blockage errors.
- Store the ScreenTape devices in the provided packaging between 2 – 8 °C. ScreenTape devices that are stored in the back of a fridge or in contact with the wall may also be subject to freezing.
- ScreenTape devices can be used straight from the fridge with no equilibration time.
- Handle ScreenTape devices carefully to avoid fingerprints or fibers, which can affect imaging.

Pay special attention to not damage the backside of the ScreenTape device.

- Ensure that the ScreenTape device is flicked before inserting it into the instrument. If there are any small bubbles present at the gel/buffer interface, flicking the ScreenTape device will move them to the top of the chamber.



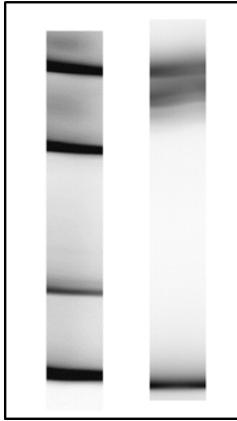


Figure 14 Effect of bubbles at the gel interface on TapeStation Analysis software results - Gel view/unaligned

Air bubbles present at the top of the buffer chamber are normal and acceptable.

NOTE

The presence of small bubbles within the buffer chamber of the ScreenTape devices is normal and acceptable. These bubbles often occur at the gel/buffer interface and need to be displaced prior to running. Failure to remove bubbles from the gel/buffer interface is detrimental to the performance of the ScreenTape assay.

NOTE

ScreenTape devices have an expiration date. If a ScreenTape device beyond the expiration date is used, a message will show up in the TapeStation Controller software interface. Starting a sample analysis with an expired ScreenTape device is not allowed by the software. Assay performance specifications do not apply beyond the expiration date.

Changing TapeStation Controller software settings will permit the instrument running expired ScreenTape devices, see [“TapeStation Controller Software Settings”](#) on page 51.

Resulting data are marked accordingly.

7 Good Measurement Practices

ScreenTape Devices

- Inspect any ScreenTape device before every run to ensure that no bubbles are located within the gel lane.

NOTE

Bubbles within the gel lane significantly affect results.

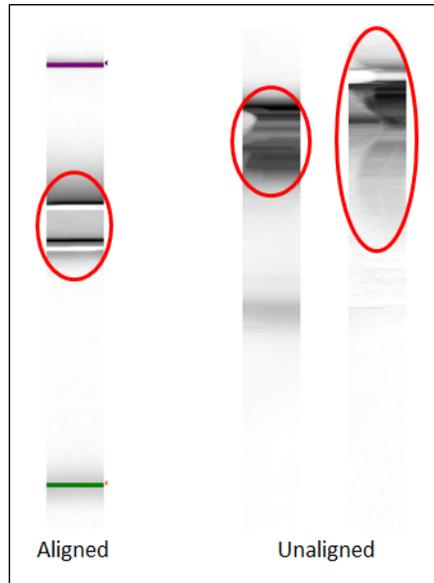


Figure 15 Different effects of bubbles in the gel matrix on TapeStation Analysis software results - Gel view

NOTE

Failure to remove air bubbles at the gel/buffer interface might significantly affect results.

Partially Used ScreenTape Devices

Depending on the number of samples run, a ScreenTape device may not be used up completely and can be stored for later use. The TapeStation Controller software keeps track of each ScreenTape device.

- The next time the ScreenTape device is inserted to the ScreenTape nest, the number of available lanes is displayed.
- Please insert partially used ScreenTape devices only to the ScreenTape nest of the instrument, never into the ScreenTape rack.

NOTE

Partially used ScreenTape devices should only be placed in the ScreenTape nest of the instrument. The ScreenTape rack should only be filled with unused ScreenTape devices. This ensures the proper prediction of required ScreenTape devices during analysis with multiple ScreenTape devices.

Partially used ScreenTape devices (those that contain lanes that were run on previous occasions) should be stored upright between 2 – 8 °C for a maximum of 2 weeks.

NOTE

The laptop that is utilized for performing any previous use(s) of the ScreenTape device must be utilized for all further re-use. ScreenTape run data is stored locally on the instrument laptop. Changing or updating the laptop can cause this information to be lost, resulting in partially used ScreenTape lanes being reused.

NOTE

Partially used ScreenTape devices have a 2 weeks use period after first use. Discard partially used ScreenTape devices beyond this time. A related message will show up in the TapeStation Controller software interface.

Sample Preparation

- Refer to the assay-specific Quick Guide for the recommended sizing and concentration ranges. Using samples outside the stated ranges can affect performance (Table 3 on page 60).
- For salt tolerance guidelines, please refer to the assay-specific Quick Guide. Please ensure that sample buffers are below the maximum recommendation (Table 3 on page 60).
- If frozen, allow all samples to become liquid. Mix and spin down liquid samples before use.
- Always pipette reagents against the side of the sample tube.
- Ensure that no residual material is left on the outside of the tip.
- When adding sample buffer to sample, please ensure that they are mixed correctly by following the instructions in the Quick Guide and “Good Pipetting Practice” on page 84. Improper mixing can lead to quantification errors, see “Mixing Recommendations” on page 93 (Table 3 on page 60).
- Once mixed, briefly centrifuge to collect the contents at the base of tubes. Visually confirm that all liquid is collected at the bottom of the vials.
- If running samples using tube strips, remove the strip caps carefully and ensure that all liquid is still at the bottom after removal. If droplets are formed after removing the strip caps, shortly centrifuge the vials before placing them inside the instrument.
- Dispose of used sample tube strips and tips in accordance to local safety regulations.

NOTE

For successful loading, the sample solution must be placed at the bottom of the tube or well without any air-bubbles. The TapeStation systems load a sample from a minimum of 3 μ L onto the ScreenTape device.

CAUTION

Lids on sample tubes

Failure to remove sample tube lids can cause damage to the TapeStation instrument and impact the performance.

→ Ensure that lids are removed from the sample tube strips before starting the run.

NOTE

For successful sample analysis, 96-well sample plates must be covered by 96-well plate foil seal (96 -well Plate Foil Seal, Pack of 100 foils (5067-5154)) to prevent evaporation. The TapeStation system is capable of piercing through the foil, providing only Agilent approved foils are used.

Ladder Considerations

The ScreenTape assays allow determination of the size of unknown sample fragments or smear regions. This size determination is based on a ladder which is a sample with specific sizing standards. The required ladder for an assay is provided with the ScreenTape Reagents or can be purchased standalone.

For best sizing precision and accuracy, a ladder per ScreenTape device is recommended. Alternatively, an electronic ladder is also available for the D1000 and High Sensitivity D1000, as well as the Cell-free DNA Screentape assay. The electronic ladder can be selected in the TapeStation Controller software and is added to the data automatically.

For the TapeStation systems, the ladder is exclusively loaded from location A1 on the tube strip holder. A ladder is typically prepared like a sample. For an analysis that consists of runs with multiple ScreenTape devices a distinct higher ladder volume is prepared. Please refer to the assay-specific Quick Guide of the assay you plan to run for details on the preparation of this higher ladder volume (Table 3 on page 60).

Some ScreenTape assays such as the standard D5000 and High Sensitivity D5000 ScreenTape assay, as well as the Genomic DNA ScreenTape assay require a ladder run per ScreenTape device. These assays do not offer usage of electronic ladders. Running a ladder per ScreenTape device is a must in analyses which involve multiple ScreenTape devices of these assays.

RNA ScreenTape assays require only one ladder per complete analysis even if this analysis is involving multiple ScreenTape devices. For RNA assays an electronic ladder can be selected in the TapeStation Controller software and would then be added to the data automatically.

NOTE

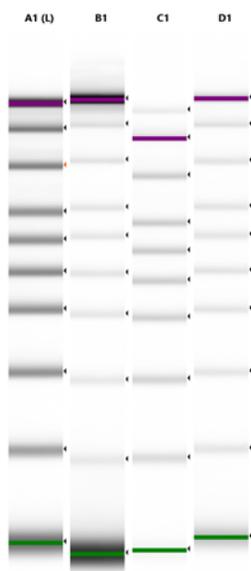
To select usage of an electronic ladder instead of a real ladder run on the ScreenTape device, refer to inserting electronic ladder in [“TapeStation Controller Software User Interface”](#) on page 49.

NOTE

Typically, one lane of ladder is run for every ScreenTape device used. Higher volumes of ladder are required for runs with multiple ScreenTape devices.

Use the Correct Protocol

- Each ScreenTape type is designed for use with its corresponding Reagent kit. Reagent kits are not interchangeable between standard and high sensitivity assays due to different dye and marker concentrations.
- Ensure that sample and sample buffer volumes from the correct protocol are followed. The assays require a specific sample to buffer ratio. If not followed correctly, sizing may be impaired due to altered run distance as well as quantification may be affected due to altered dye and marker concentration.
- Ensure that the reagents are used with the corresponding ScreenTape type.
- It is important to choose the correct assay based on the concentration of the sample. Using sample concentrations outside the specified quantitative ranges will lead to inaccurate quantification.
- For correct volumes and quantitative range, please refer to the appropriate Quick Guide (“[Sample Preparation](#)” on page 60).



A1 – 3 μ l D1000 Sample Buffer + 1 μ l D1000 Ladder ✓
B1 – 3 μ l D1000 Sample Buffer + 1 μ l High Sensitivity D1000 Ladder ✗
C1 – 2 μ l High Sensitivity D1000 Sample Buffer + 2 μ l D1000 Ladder ✗
D1 – 2 μ l High Sensitivity D1000 Sample Buffer + 2 μ l High Sensitivity D1000 Ladder ✗

Figure 16 Effect of correct (A1) and incorrect consumables/protocol (B1 – D1) on the D1000 Ladder peak intensity and Marker assignment in the D1000 ScreenTape assay.

Mixing Recommendations

Use a vortexer which is designed for mixing tube strips, or 96-well sample plates respectively. The IKA MS3 vortexer is recommended for all ScreenTape assays. TapeStation systems are supplied with an optional IKA MS3 vortexer which includes a 96-well sample plate adaptor suitable for both 96-well PCR plates and tube strips.

- Close tube strips with tube strip caps and seal 96-well sample plates with 96-well plate foil seal.
- Set the IKA MS3 vortexer to 2000 rpm.
- Vortex for 1 min by pressing the start button.



Figure 17 IKA MS3 vortexer

If no IKA MS3 vortexer is available, please ensure thorough manual vortex mixing – 1 min on maximum speed.

- Sample and sample buffer must be vortex mixed according to protocol, followed by centrifugation to remove any bubbles.
- Insufficient mixing can cause discrepancies in quantification.
- Poor mixing dramatically affects the reported sample concentration. Following the recommended procedure is the best way to attain accurate quantification results.

The effects of insufficient mixing were investigated using the D1000 ScreenTape assay, and results are presented in the figures and table below.

7 Good Measurement Practices Mixing Recommendations

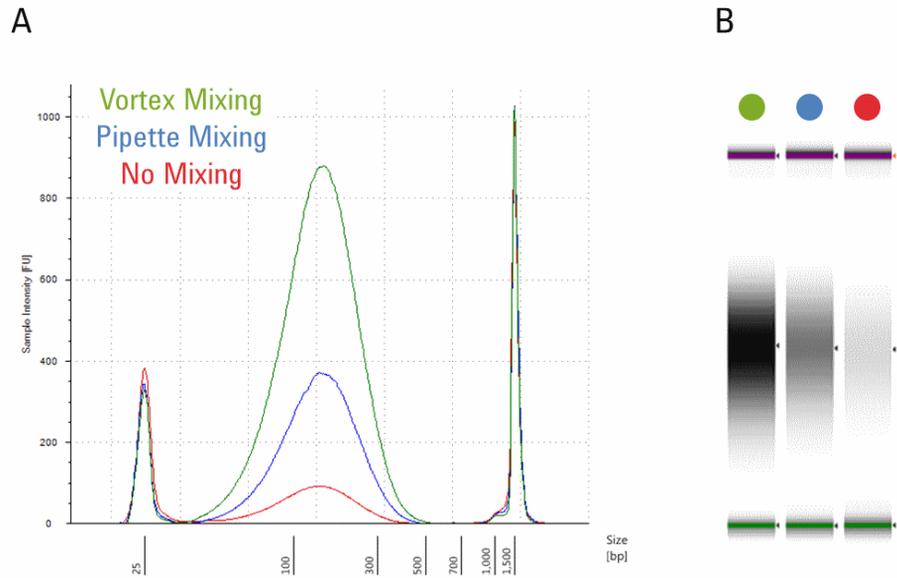


Figure 18 The electropherogram (A) and the gel image (B) of the ScreenTape reagent mixing tests.

The green trace shows the recommended protocol of vortex mixing using the IKA vortexer and adaptor at 2000 rpm for 1 minute followed by brief centrifugation. Blue shows results for pipette mixing only. Red shows the effect of no mixing. Images were taken from the TapeStation Analysis software.

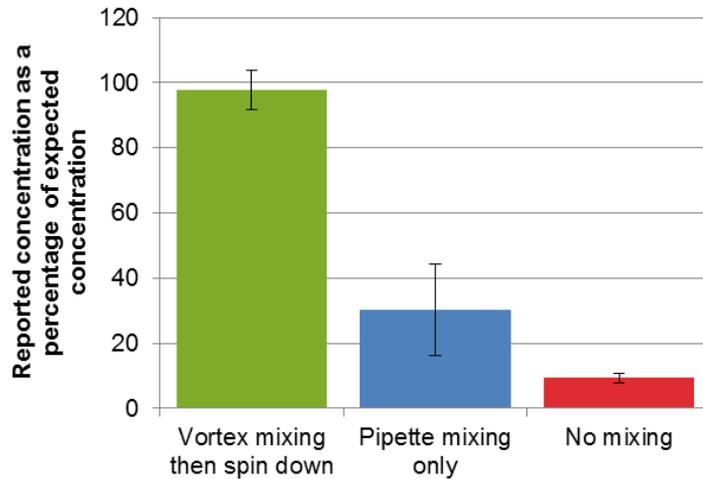


Figure 19 Chart of reported concentrations for the ScreenTape reagent mixing tests, using the D1000 ScreenTape assay.

The concentrations are expressed as a percentage of the nominal concentration for the three mixing methods. Again, the green bar represents the recommended protocol of vortex mixing using the IKA vortexer and adaptor at 2000 rpm for 1 minute followed by brief centrifugation; blue - pipette mixing only; red - no mixing.

Table 4 ScreenTape reagent mixing tests

	Measured concentration (ng/μL)	Theoretical concentration (ng/μL)
Vortex mixing then centrifugation	68.5	70
Pipette mixing only	21.2	70
No mixing	6.5	70

The table lists the quantification values that are obtained from the TapeStation Analysis software and the Agilent D1000 ScreenTape assay when using the correct protocol (vortex mix followed by brief centrifugation) and two incorrect mixing protocols (pipette mixing only and no mixing) as illustrated in the figures above.

Centrifugation Recommendations

Spinning down samples during sample preparation is required to collect all liquid in one droplet for effective mixing, to collect liquids at the bottom of the vial so that they can be picked up by the pipetting robotics of the TapeStation system, and to remove trapped air bubbles at the bottom of the vial.

A centrifuge for tube strips and 96-well sample plates is required. This centrifuge is not supplied with the system. A centrifuge with a 8-tube strip rotor and a centrifuge with a rotor for 96-well sample plates can be used, as well as devices with interchangeable rotors. A suitable centrifuge should allow spinning at a centrifugal force of approximately 500 G and, for example, for the duration of 1 min. The effectiveness of this step should be confirmed by a visual check.

CAUTION

Improper or skipped centrifugation

Failure to spin down samples can cause empty or smeared lanes on the ScreenTape device as no or insufficient sample volume is transferred. Subsequently, no data or data of low quality are created.

→ Ensure that the sample liquid persists bubble free at the bottom of the sample tube or well.

NOTE

For successful sample analysis in 96-well sample plates as well as from sample tube strips, both must be centrifuged. A centrifuge with a 8-tube strip rotor and a centrifuge with a rotor for 96-well sample plates can be used as well as devices with interchangeable rotors.

Centrifuge samples to remove spills and air bubbles.

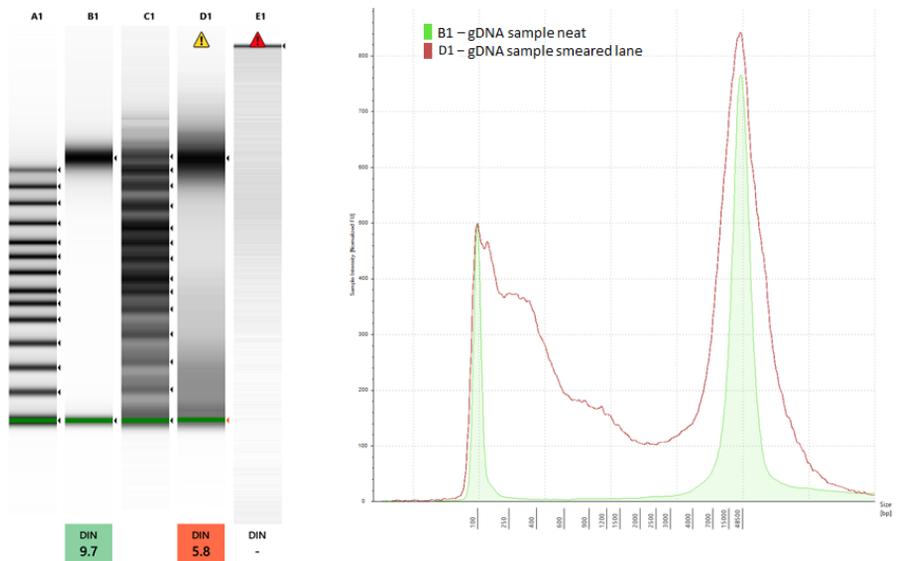
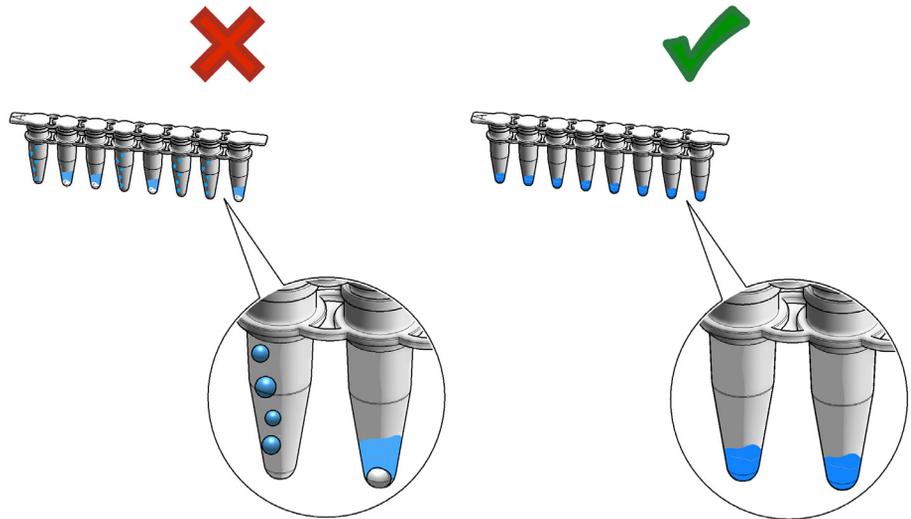


Figure 20 Example of a well separated Genomic DNA ladder and sample lane (A1 and B1) in comparison to a smeared Genomic DNA ladder and sample lane (C1 and D1) as well as a blank lane in the **Gel View** (Left) and **Electropherogram View** (Right) of the TapeStation Analysis software.

RNA Assays

Heat Denaturation

The two RNA assays below include a heat denaturation step in the workflow.

- RNA ScreenTape assay
- High Sensitivity RNA ScreenTape assay

For heat denaturation, an accurately calibrated block heater, or a PCR instrument fitting to 200 μ L vials is required. This equipment is not supplied with the TapeStation system. Before the heating step, close the tube strips or 96-well sample plates with lids or foil seal.

Heat denaturation typically consists of:

- Collecting sample and sample buffer at the bottom by centrifugation
- Incubation at 72 °C for 3 min
- Incubation on ice for 2 min
- Centrifuge to collect samples at the bottom of the vial

NOTE

Ensure the sample well on a 96-well sample plate is kept air tight with 96-well plate foil seal (PN 5067-5154) to prevent evaporation during the heating step. Use, for example, flat compression mats or screwed down PCR machine lids to achieve this.

NOTE

After each heating step, spinning down samples is required to collect condensed material from the lid or foil seal.

NOTE

Many heat blocks and PCR machines display a temperature that can be incorrect by up to 10 °C. Ensure that your heat block is appropriately calibrated before use.

See also [“Tools and Handling”](#) on page 84 and [“Troubleshooting RNA Applications”](#) on page 181.

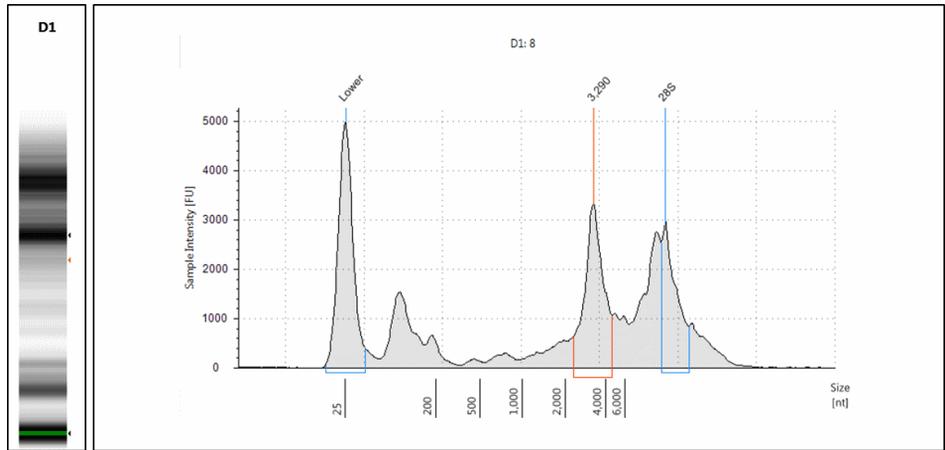


Figure 21 Example of extra peaks being detected at the 28S position due to insufficient denaturation in the **Gel View** (Left) and **Electropherogram View** (Right) of the TapeStation Analysis software.

General Recommendations When Working with RNA

When working with RNA samples, make sure to consider following recommendations:

- Always follow the GLP-rules established in the laboratory
- Wear gloves all the times to prevent contamination with RNAses
- Thaw RNA samples and ladder on ice and keep them on ice during sample preparation
- Ensure all working areas and plastic ware are RNase free

NOTE

Not following these general guidelines might result in RNA degradation and false data.

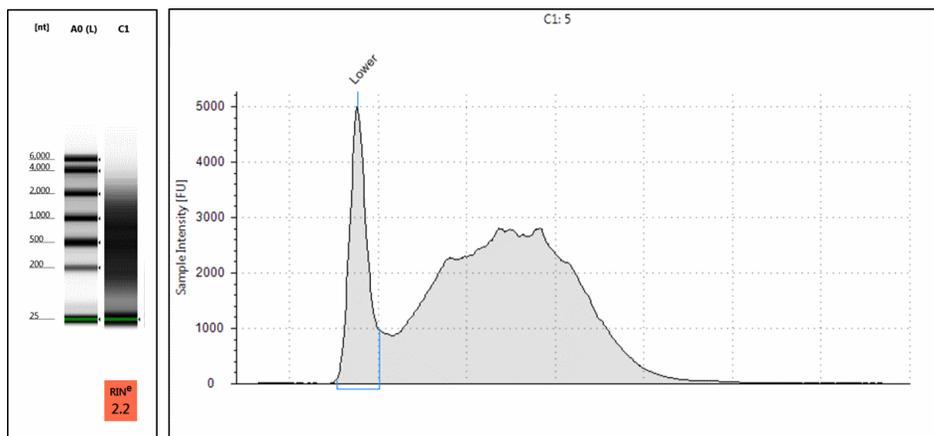


Figure 22 Example of a degraded RNA sample in the High Sensitivity RNA assay resulting in missing peak identification in the **Gel View** (Left) and **Electropherogram View** (Right) of the TapeStation Analysis software.

Peak Integration

In DNA assays, concentration values are calculated using the area of the sample peak compared to the known concentration of the upper marker.

The user must ensure that both marker and sample peaks are properly integrated, by manually adjusting the peak when necessary. An example of correct upper marker peak integration is shown below.

NOTE

In assays with no upper marker (RNA, High Sensitivity RNA, Genomic DNA, and Cell-free DNA assay) quantification is calculated using the lower marker.

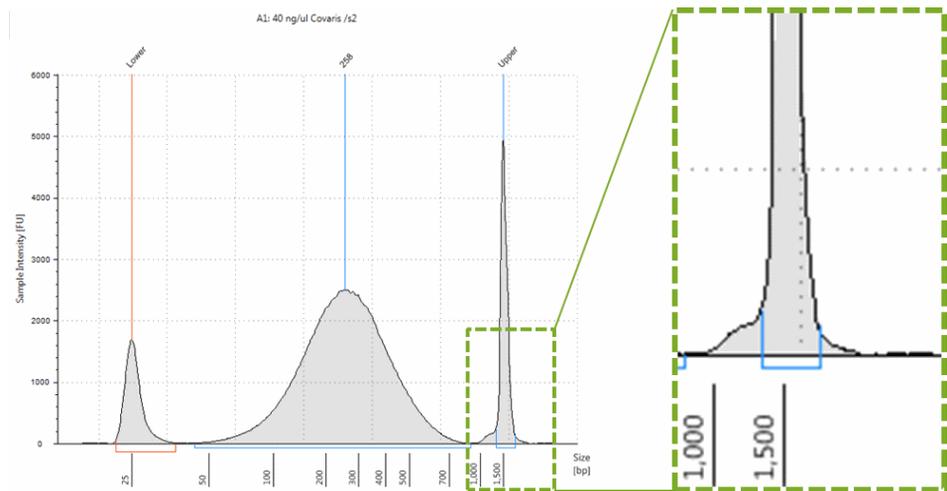


Figure 23 Correct upper marker integration for DNA ScreenTape assays. Figure shows D1000 ScreenTape assay.

7 Good Measurement Practices

Peak Integration

The second figure demonstrates the effect of peak integration on the quantitative results. Incorrect peak integration can bias the determined DNA sample concentration significantly:

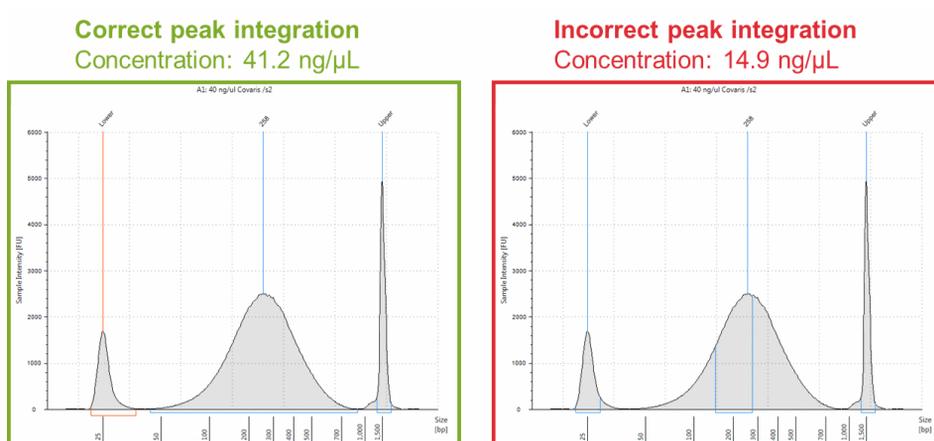


Figure 24 Example of correct (A) and incorrect (B) sample peak integration, and their effect on reported sample concentration.

NOTE

The displayed examples are chosen to show the effect of incorrect peak assignment to full extent. Samples with broad peaks as shown here should be analyzed in the Region view. See the Agilent Information Center for more information.

The user must ensure that all marker peaks are properly assigned. Use the **Aligned** button to detect the correct markers. Compare to aligned/unaligned data of good, for example the ladder, and questionable samples, then assign correct markers by right-clicking.

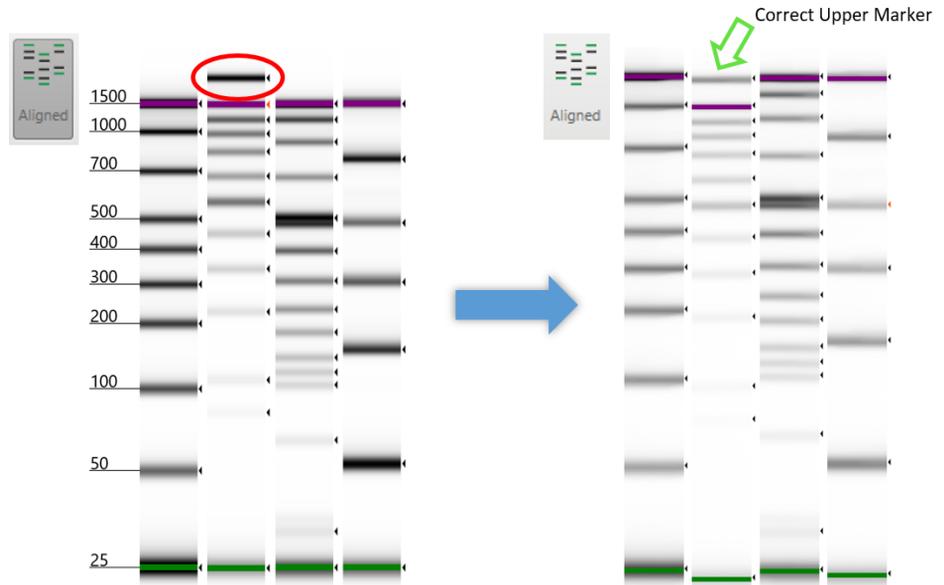


Figure 25 Example of an incorrect (Left) assigned Upper Marker and how to identify the correct Upper Marker (Right) in the D1000 ScreenTape assay in the TapeStation Analysis software.

Peak Maxima Versus Average Molecular Weight Sizing and Molarity

Within the TapeStation Analysis software, results can be accessed in both electropherogram and region mode/view. The sizing information presented in these modes is different.

Electropherogram view is designed for use with discrete peaks, and the default size reported is that of the highest point of the peak.

Region view calculates data over a whole smear or region (for example NGS libraries), and reports size as that of the center of the regions' mass. This gives the user an idea of the distribution of sizes within that sample.

The same holds true for quantification results, especially molarity as this is determined from both size and quantity.

NOTE

Errors in sizing and quantification result in erroneous molarity calculations.

Peak Maxima Versus Average Molecular Weight Sizing and Molarity

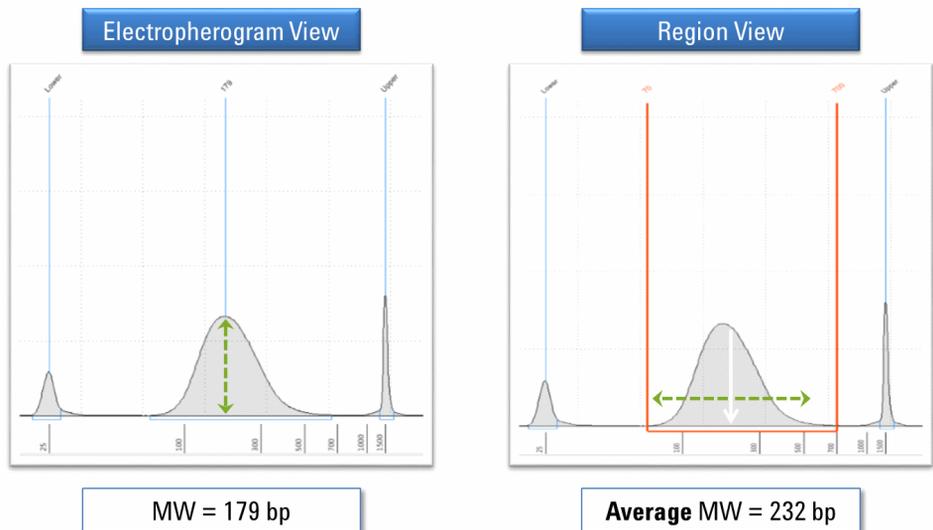


Figure 26 The sizing data obtained in Electropherogram and Region views of the TapeStation Analysis software.

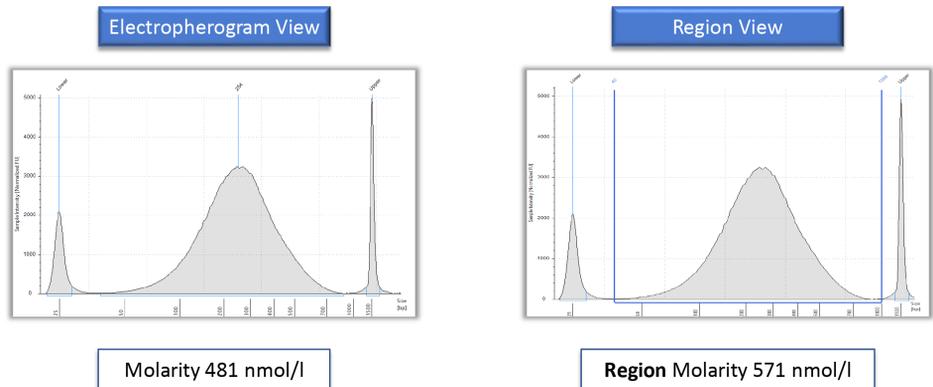


Figure 27 The concentration data obtained in Electropherogram and Region views of the TapeStation Analysis software.

Sizing Range

In order to achieve accurate results the samples used must be within the sizing range as stated in the respective assay Quick Guide. Signal of sample merging with either Upper and/or Lower Marker will not be taken into consideration in concentration calculation. Additional sample overlaying with the marker will alter the known concentration of the marker that is used for concentration calculation. This will influence the overall quantification performance of this lane (Table 3 on page 60).

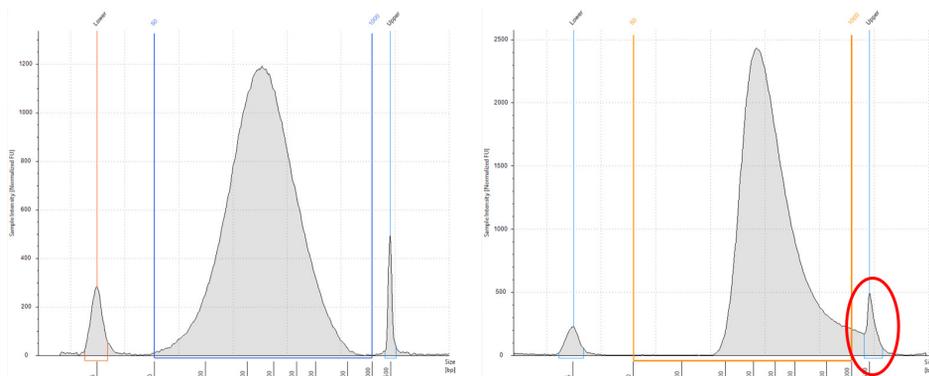


Figure 28 Example of a sample within the sizing range of the High Sensitivity D1000 assay (Left) and a sample merging with the Upper Marker (Right) in the **Region** view of the TapeStation Analysis software.

If applicable use an assay with a broader sizing range.

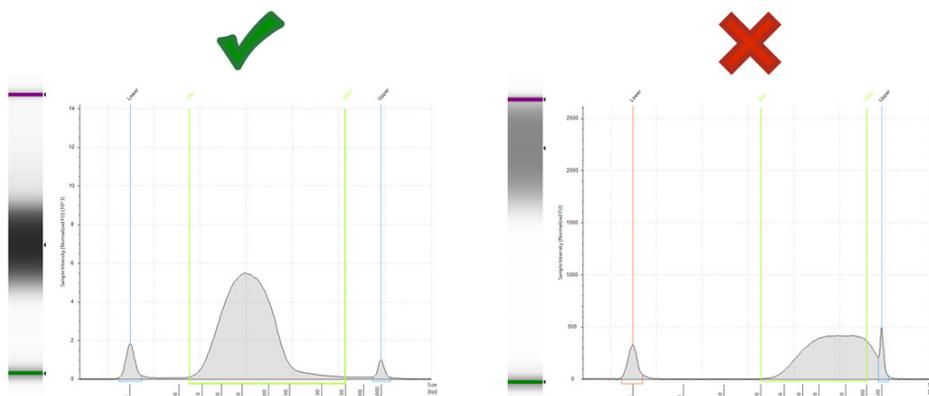


Figure 29 Example of a sample within the sizing range of the High Sensitivity D5000 assay (left) and the same sample merging with the upper marker (right) in the High Sensitivity D1000 assay.

Quantitative range

To achieve accurate results the samples used must be within the quantitative range as stated in the respective assay Quick Guide. Signal of samples with concentrations below then the lower limit of the quantitative range or below the limit of detection might not be visible. Signal of samples with concentrations higher than the upper limit of the quantitative range might be significantly higher than the marker peaks. Markers might not get assigned in this case (Table 3 on page 60).

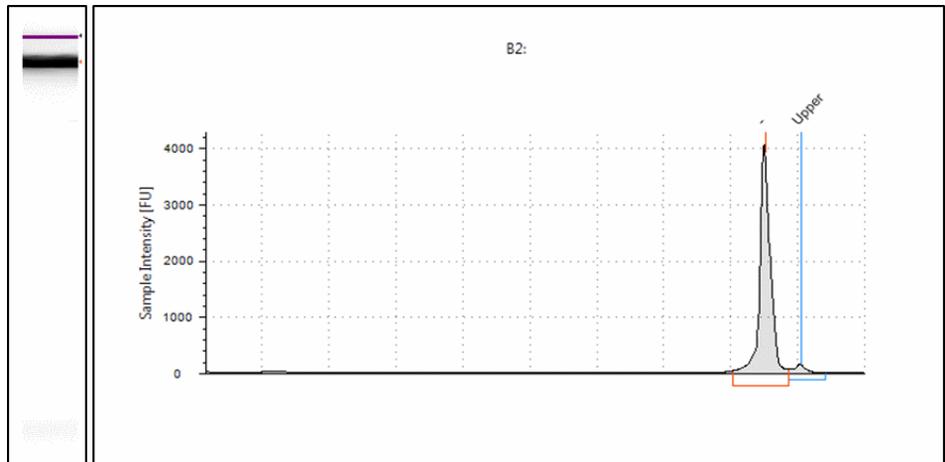


Figure 30 Example of missing marker peak due to too concentrated sample in the High Sensitivity D1000 assay in the **Gel** view (Left) and **Electropherogram** view (Right) of the TapeStation Analysis software.

7 Good Measurement Practices

Quantitative range

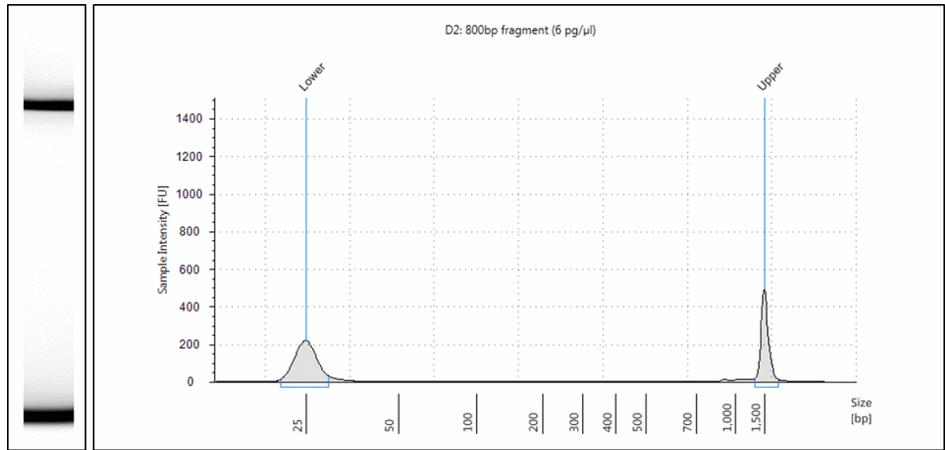


Figure 31 Example of missing sample peak due to too low concentrated sample in the High Sensitivity D1000 assay in the **Gel** view (Left) and **Electropherogram** view (Right) of the TapeStation Analysis software.

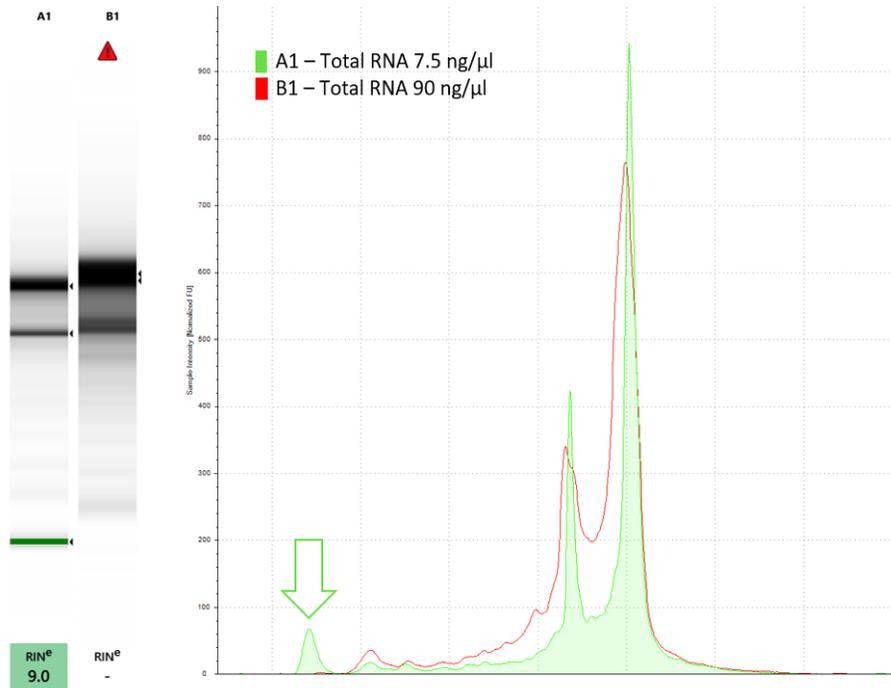


Figure 32 Example of a sample within (A1) and outside (B1) the quantitative range of the High Sensitivity RNA assay in the **Gel** view (Left) and **Comparison** view (Right) of the TapeStation Analysis software. The **Lower Marker** peak (green arrow) is missing in the overconcentrated sample.

Maximum Buffer Concentration in Sample

The *Maximum buffer concentration in sample* describes the maximum concentration of additional salt, namely ionic components in sample. ScreenTape assays are designed to compensate for negative effects salt introduces during electrophoresis. The buffer in which the nucleic acid sample is provided (that is, the sample matrix) must not exceed these maximum salt concentrations. Otherwise the performance is significantly affected. The *Maximum buffer concentration in sample* can be found in the respective Quick Guide ([Table 3](#) on page 60).

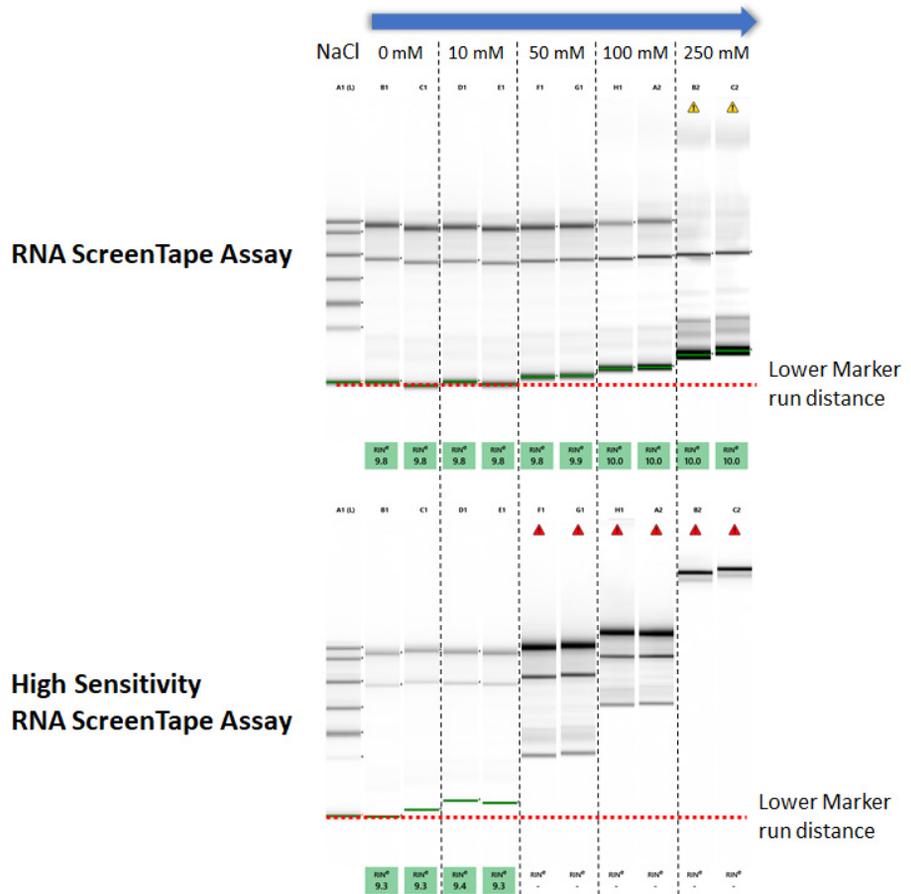


Figure 33 Effects of increasing salt concentrations in the sample matrix on the RNA and High Sensitivity RNA Screentape Assay in the unaligned Gel view of the TapeStation Analysis software.

RIN^e Functional Range

To achieve accurate RIN^e scores the samples used must be within the RIN^e functional range as stated in the respective assay Quick Guide. Samples with concentrations exceeding the RIN^e functional range can show altered electropherogram traces. Obtained RIN^e scores are significantly impacted by this (Table 3 on page 60).

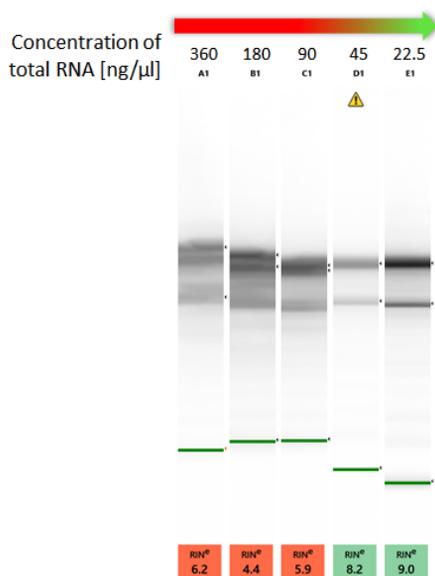
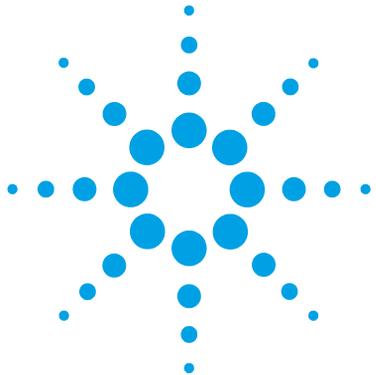


Figure 34 Effects of overconcentrated samples on run distance of the Lower Marker and RIN^e values in the High Sensitivity RNA ScreenTape assay in the Gel view of the TapeStation Analysis software.



8 Instrument Diagnosis

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This chapter contains information on instrument diagnosis, available tests, and reporting.



Instrument Diagnosis

The TapeStation Hardware Diagnostics and Functional Verification context (further referred to as "System Diagnostics") is designed to support continuous operation of the TapeStation system. It provides features for maintenance, routine self-tests and for service needs. This software includes diagnosis tests to verify proper performance of the mechanical, electrical, and optical components. It comes with tools supporting instrument maintenance. System Diagnostics is designed for users and trained Agilent service personnel.

System Diagnostics speeds up remote troubleshooting by providing detailed support information for any instrument error that may arise.

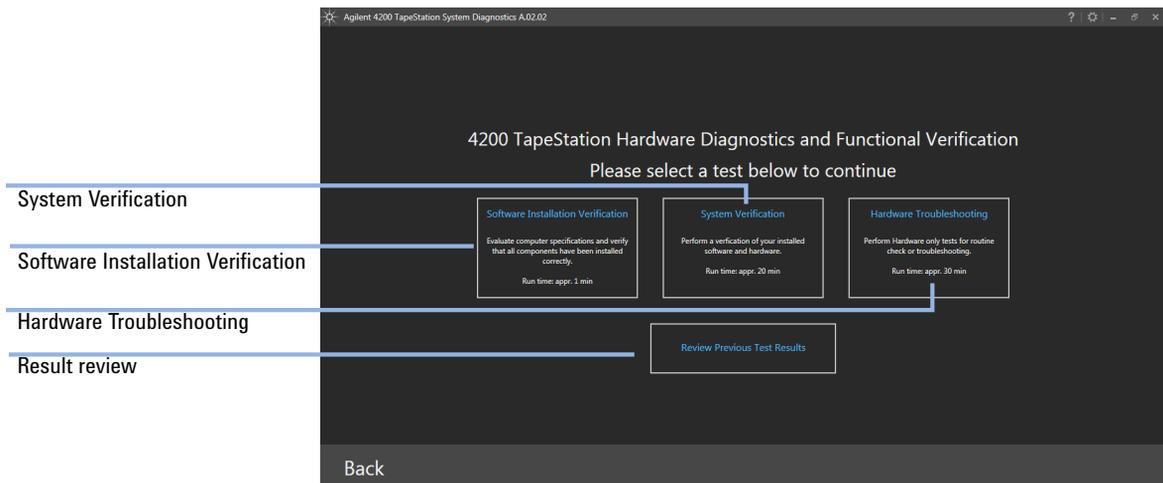


Figure 35 TapeStation Controller software: **System Diagnostics** context

Tests

Test suite groups

The System Verification and Hardware Troubleshooting consists of test groups:

Table 5 Test suite groups

Computer Environment	Evaluation of computer specification and installation status.
Setup	Prepares the instrument, requests insertion of material, checks status LED functionality. Information on current Maintenance counters, past Electrode Cartridges, past needle changes and current installed software is reported.
Vision	Camera and LED illumination are checked using the TapeStation Test Tape.
Electrophoresis	The voltage supply, current readout, and electrodes are checked using the TapeStation Test Tape.
Robotics	Robotics parts are checked by dedicated movements. Tip and ScreenTape device handling are checked using a full tip rack and one regular ScreenTape device.
Pipetting	This check procedure in the Hardware Troubleshooting visually checks pickup of liquid from 96-well sample plates and tube strips.
Cleanup	Resets the instrument to normal operational mode and requests removal of material.

NOTE

In case no valid TapeStation system is connected, only those tests belonging to the Software Installation Verification can be run.

Software Installation Verification

This evaluates computer specifications and verifies that all components have been installed correctly.

Please refer to the Agilent Information Center for details on failed tests.

Two procedures test the following items within 1 minute:

- Computer Specifications
- Software Specifications

NOTE

This test indicates if personal computers and laptops which are not matching the laptop bundle of the TapeStation System are suitable for running the TapeStation Analysis Software.

System Verification

The System Verification consists of tests to confirm functionality of various components of the system. See step by step instructions on this under [“Executing System Verification Tests”](#) on page 120. Test duration is approximately 20 minutes. Tests are organized in test groups for computer environment, robotics, electrophoresis, and vision system. The verification comes with predefined tests. Deselection of individual tests is possible. After entering the System Verification, the selection can be modified. Some tests are interdependent and require execution of others as prerequisite.

The System Verification runs automated and semi-automated tests which possibly require user input during execution.

The System Verification requires the following material upon start:

- TapeStation Test Tape (5067-5601)
- Full rack of Loading Tips (5067-5598)
- Pipet tip waste bin
- Empty 96-well plate (5042-8502)
- One regular ScreenTape device

During the test on 4200 TapeStation instruments, one regular ScreenTape device of any assay type needs to be inserted temporarily into the ScreenTape rack to conduct a tape handling test.

It is required to ensure the ScreenTape nest covers and the gantry cover are in place during execution of the System Verification.

Hardware Troubleshooting

The Hardware Troubleshooting tests for the 4200 TapeStation instrument consists of all tests present in the System Verification (see “[System Verification](#)” on page 116) plus additional tests. It creates comprehensive information for troubleshooting by service and support personnel. Test duration is approximately 30 minutes.

The optional liquid handling test within Hardware Troubleshooting requires the following additional material upon start:

- A foil sealed 96-well sample plate with:
 - 5 μL water in wells A1, H1, A12, H12,
 - 20 μL water in wells D6, D7, E6, E7.
- Two tube strips each with:
 - 5 μL water in wells A1, A2, H1, and H2.

Collect liquid at the bottom of the vial by centrifugation before starting the liquid handling check. Spilled liquid or trapped air bubbles affect the results. Refer to “[Centrifugation Recommendations](#)” on page 96.

TapeStation Test Tape

The Agilent TapeStation Test Tape (5067-5601) is a device designed to be used with the TapeStation system for hardware diagnosis. The TapeStation Test Tape can be used to check the performance of electrical and optical components.

It is part of the accessories shipped with the instrument, see “[Accessories Shipment List](#)” on page 204.

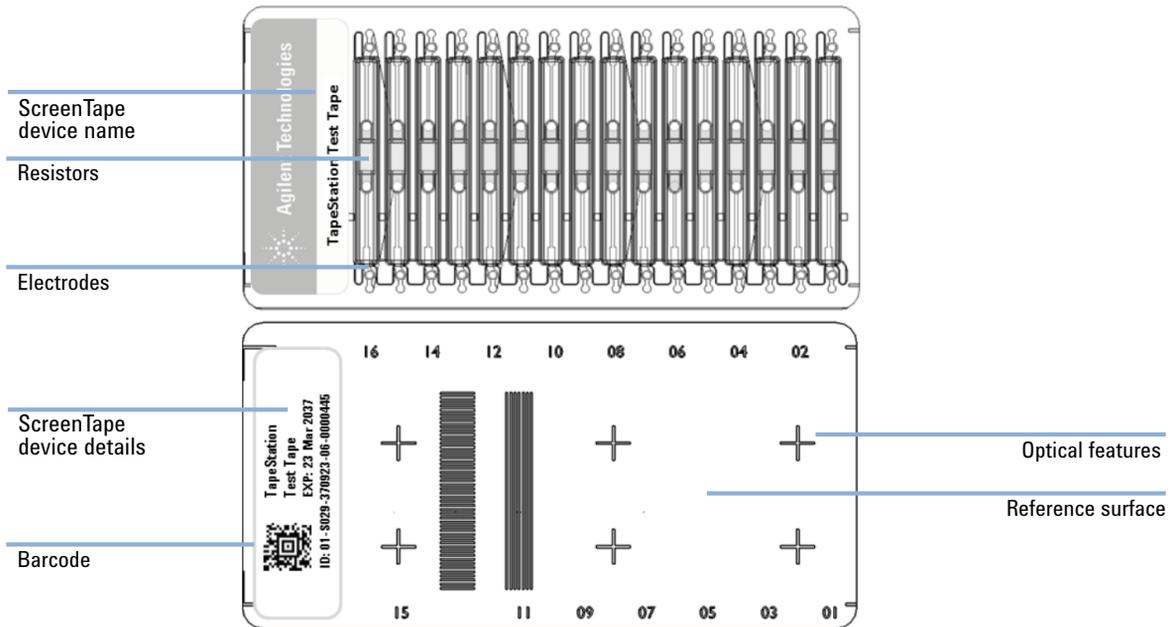


Figure 36 TapeStation Test Tape

Within the System Diagnostics, it can be used for routine confirmation of the system functionality in the System Verification (see “[System Verification](#)” on page 116) or for instrument troubleshooting in the extended Hardware Troubleshooting tests (see “[Hardware Troubleshooting](#)” on page 117) as directed by your support representative.

Dialogues during test suite execution (see “[Executing System Verification Tests](#)” on page 120) will request you to insert this TapeStation Test Tape to the ScreenTape nest.

CAUTION**Wrong insertion of TapeStation Test Tape**

The TapeStation Test Tape is a specialized test device that differs in physical properties from regular ScreenTape devices. Incorrectly inserting the TapeStation Test Tape can irreversibly damage it.

- Do not insert the TapeStation Test Tape to the ScreenTape rack for the ScreenTape handling test within the robotics test suite.
 - Only insert the TapeStation Test Tape to the ScreenTape nest.
 - Follow the instructions during the robotics test suite closely.
-

NOTE

The TapeStation Test Tape is designed for instrument diagnostic purposes only, and as such will not apply any changes to the TapeStation instrument.

Each TapeStation Test Tape has an operation life of 2 years.

NOTE

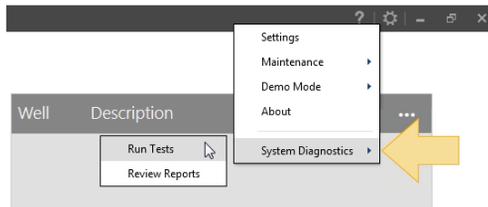
Protect the TapeStation Test Tape from excessive force. Do not bend or flex the Test Tape and store away from direct light and chemicals in the provided packaging when not in use.

The TapeStation Test Tape is inserted into the TapeStation systems in the same way as a regular ScreenTape device. The label with the barcode faces towards the back of the instrument.

Upon insertion of the TapeStation Test Tape to a TapeStation system that is connected to the TapeStation Controller software, the start of the System Diagnostics context is triggered. A dialog will guide you through this process.

Executing System Verification Tests

- 1 Turn on the laptop. Let the operating system start entirely.
- 2 Power on the TapeStation instrument with the instrument lid closed. Let it initialize entirely.
- 3 Start the TapeStation Controller software and allow the software to detect the instrument.
- 4 Select **System Diagnostics > Run Tests**.



- 5 Select one of the three test options, see [“System Diagnostics”](#) on page 53, for example the **System Verification** tests.

- 6** Select the default tests or omit tests according to your needs. Individual tests can be checked or unchecked.

Test selection

Test groups

Individual tests

Start button

NOTE

Some individual tests are depended on others. When a test is deselected, any dependent tests are also deselected.

- 7** Click **Start** and follow the on-screen instructions closely.

8 Instrument Diagnosis Tests

- 8 Upon request, insert material required for the test execution.
- For example, following material is required for a **System Verification**:
- a TapeStation Test Tape to the ScreenTape nest,
 - an empty 96-well plate,
 - a full rack of pipette tips, and
 - a regular ScreenTape device to the ScreenTape rack.

System Verification

Description: Perform a verification of your installed software and hardware.

Preparation

Description: Prepare for testing

Time Executed: -

Result: -

Test Execution 1 of 1

Start Time: -

Duration: -

Result: -

Procedure:

Please insert the following items into the instrument:

- TapeStation Test Tape (5067-3601)
- Empty 96 well plate (5042-8502)
- Full rack of pipette tips (5007-3398)
- Pipette tip waste bin
- Unused regular ScreenTape device into the tape rack:

Make sure the tape nest covers and the gantry cover are in place. Close the lid and click OK to continue.

OK Cancel

Test description

Test progress

On-screen instructions

Abort

- 9 Click **OK** to continue.
- 10 Upon request, monitor the status LED and lid indicator.
- 11 Confirm status LED and lid indicator functionality by clicking respective buttons in pop up windows.
- 12 Open the instrument lid and remove the TapeStation Test Tape from the ScreenTape nest upon request.
- 13 Click **OK** to continue.
- 14 Remove the regular ScreenTape device at the end of the tests from the ScreenTape nest and click **OK**.
- 15 Click **Back** to finalize tests, or select the print dialog.

NOTE

Results can be reviewed and printed at a later point in time, see [“Reviewing Test Results”](#) on page 124.

Any failed or aborted test remains selected even after the completion of the other tests. After all selected tests have been executed, **Start** reappears and allows you to specifically execute these tests again. All repetitions of a test are recorded and are subsequently visible for review.

For information on failed tests, refer to the Agilent Information Center (see [“Agilent Information Center”](#) on page 56) and search for details on root causes in the section on System Diagnostics. Consult the section [“Collecting Troubleshooting Information”](#) on page 202 before contacting the Agilent technical support experts.

Reporting

Reviewing Test Results

Test results can be printed to reports after the end of test execution or later by selecting the menu item **Review Previous Test Results** in the System Diagnostics context. Existing results can be selected and opened for review.

Result reports from test executions can be saved in PDF format.

The screenshot displays the Agilent 4200 TapeStation System Diagnostics software interface. The window title is "Agilent 4200 TapeStation System Diagnostics A.02.02". The interface is divided into several sections:

- Instrument serial number:** G2991A-PRDAA00061
- Test date/time:** 2016-12-08 16:44:14: System Verification (6 tests failed out of 29 tests executed) and 2016-12-08 16:40:35: System Verification (3 tests failed out of 8 tests executed).
- Report print dialog:** Computer:DATASYSTEM01
- Results overview:** 2016-12-08 15:44:39: Software Installation Verification (1 tests failed out of 3 tests executed)
- Results details:** The main test results are shown in a table with categories and individual test items.

Category	Test Item	Status	Executions
Computer Environment	Computer Specifications	✓	
Setup	Software environment	Executions: 2	✓
	Preparation	Executions: 2	✓
	Diagnostic Counters		✓
	Status LED		✓
Vision	Camera Movement		✓
	Barcode Read Test	Executions: 2	✗
	Filter Test		✓
	Focus Test		✓
	FOV Test		✓
	Uniformity Test		✓
Electrophoresis	Temperature Sensors		✓
	Read TapeStation Test Tape	Executions: 2	✓
	Electrophoresis Stability Test	Executions: 2	✗
Robotics	Remove Tape		✓
	Tapenest Movement		✓
	Gantry Axes		✓

Gantry Axes

Description: Check the movements of gantry
Time Executed: 4:47 PM
Result: All 1 test executions passed

Test Execution 1 of 1 ✓

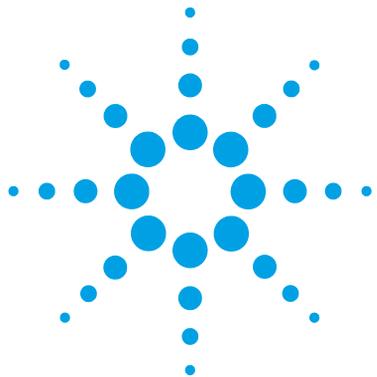
Start Time: 4:47 PM
Duration: 15 Seconds
Result: Passed

Procedure:

- 1: Move gantry axes ✓

NOTE

From the TapeStation Analysis software log files in ZIP format can be exported to be sent to Agilent technical support experts. See [“Collecting Troubleshooting Information”](#) on page 202 for details. These log files will automatically contain the latest test results.



9 Maintenance

Maintenance Information	126
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This chapter describes the maintenance of the 4200 TapeStation system.



Maintenance Information

Needle Pierce Counter

Each time a ScreenTape lane is pierced for sample application during the analysis the needle pierce counter is increased. The TapeStation Controller software displays the number of pierces. The piercing needle should be exchanged after 3840 pierces to ensure optimal instrument performance. The TapeStation Controller software displays a warning message when this threshold value is reached. Further analysis is inhibited if the maximum number of 4480 pierces is reached.

The command **Change Needle** (see [“Exchange the Piercing Needle”](#) on page 129) from the TapeStation Controller software leads to an exchange of the piercing needle and a reset of the needle pierce counter.

An unused Needle Change Cartridge (5067-5783) is prerequisite for the needle exchange and is recognized by the software. The Needle Change Cartridge is designed for one time use. An extracted piercing needle cannot be remounted.

Maintenance Counter

Each time a ScreenTape lane is used for electrophoresis the maintenance counter is increased. The TapeStation Controller software shows the counter and a respective warning when a threshold value of 50000 samples is reached. The electrodes in the ScreenTape nest contacting to the contact points on the ScreenTape devices should be exchanged after a defined number of analyses to ensure optimal instrument performance. Schedule planned preventive maintenance by Agilent Service at this point.

NOTE

Only in the 4150 TapeStation system the Electrode Cartridge can be changed either by a trained service engineer during a preventive maintenance or by the user. Scheduled a preventive maintenance service in the 4200 TapeStation system.

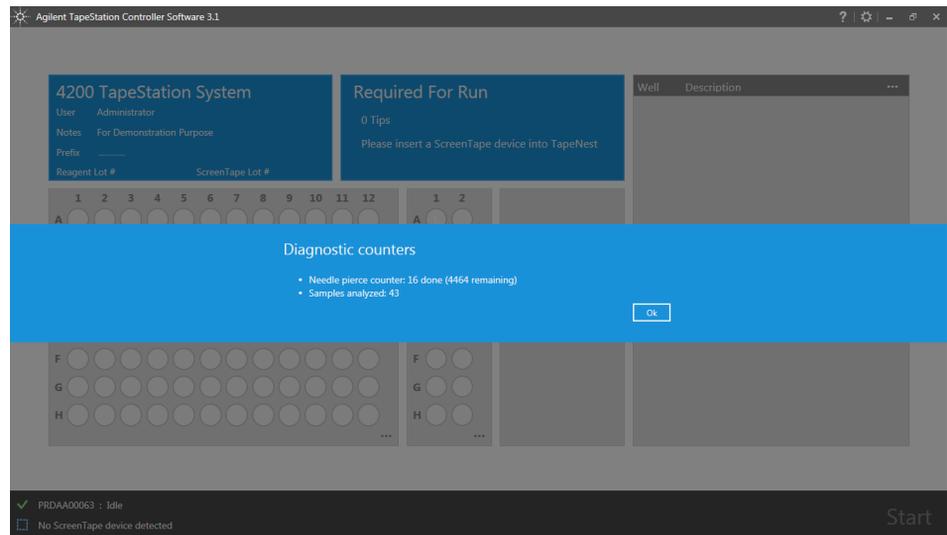


Figure 37 Diagnostic counters of the 4200 TapesStation System

Preventive Maintenance

An annual Preventive Maintenance is essential for the TapeStation system as it has many moving parts. By design a yearly Preventive Maintenance is planned, hence the Controller software will actively flag the due date based on the instruments' throughput and time elapsed.

A warning message appears after analysis of 50000 samples.

The preventive maintenance consists of:

- needle replacement,
- electrophoresis probe block replacement,
- firmware and software update if applicable,
- general cleaning of instrument,
- execution of functional hardware test,
- inspect the internal of the instrument for wear,
- check for foreign objects,
- do general cleaning of instrument inside and outside,
- apply dedicated sensor and positional recalibration steps,
- review dedicated log information.

The Preventive Maintenance service executed by Agilent certified personal does not include repair activities.

For customers with exceptionally high usage, the needle replacement procedure (see [“Exchange the Piercing Needle”](#) on page 129) can also be performed between annual Preventive Maintenance services.

Exchange the Piercing Needle

Parts required	p/n	Description
	5067-5783	Needle Change Cartridge

CAUTION

Damage to the cartridge or nest

The Needle Change Cartridge cannot be inserted into the ScreenTape nest in normal operation mode. It is required to start the needle exchange process by software first, this will open the ScreenTape nest.

- Do not use excessive force when inserting the Needle Change Cartridge into the ScreenTape nest.
- Insert Needle Change Cartridge to the ScreenTape nest only when requested by the software dialogue.

CAUTION

Analyses fail

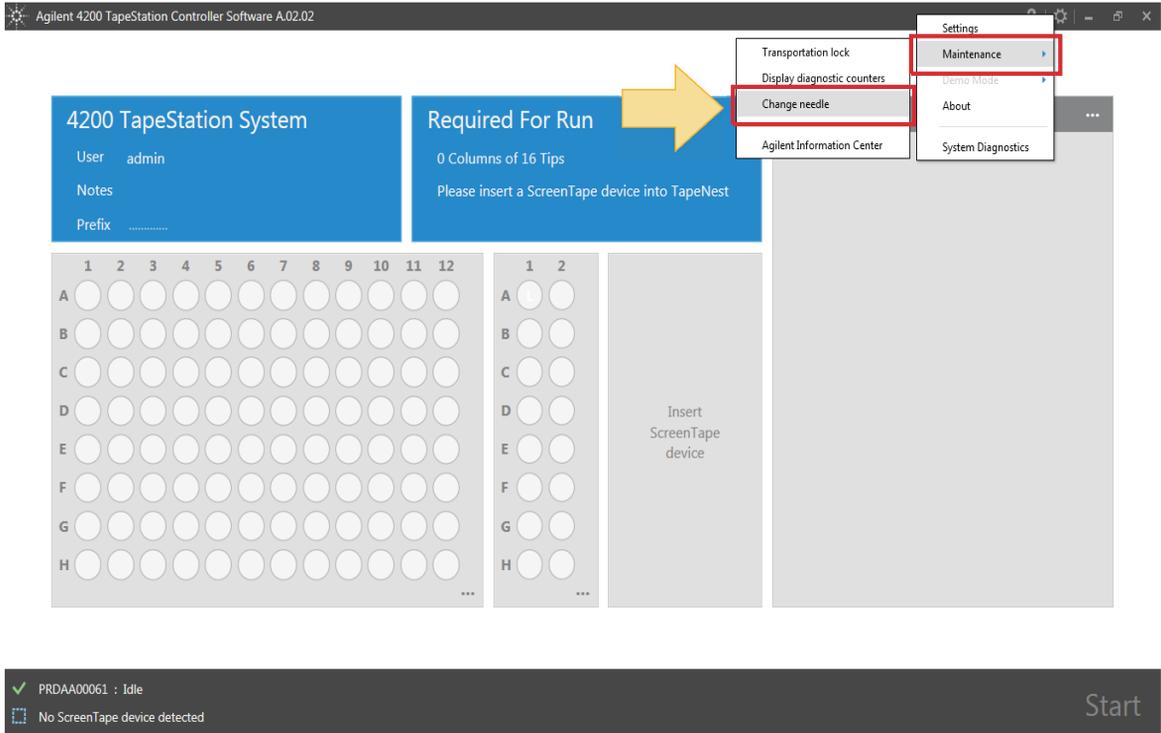
Turning the instrument off during the calibration procedure after the needle exchange will result in a fail of all subsequent analysis. Turning the instrument off during calibration will require a different new needle change cartridge.

- Do not turn the instrument off during the calibration procedure after needle exchange.

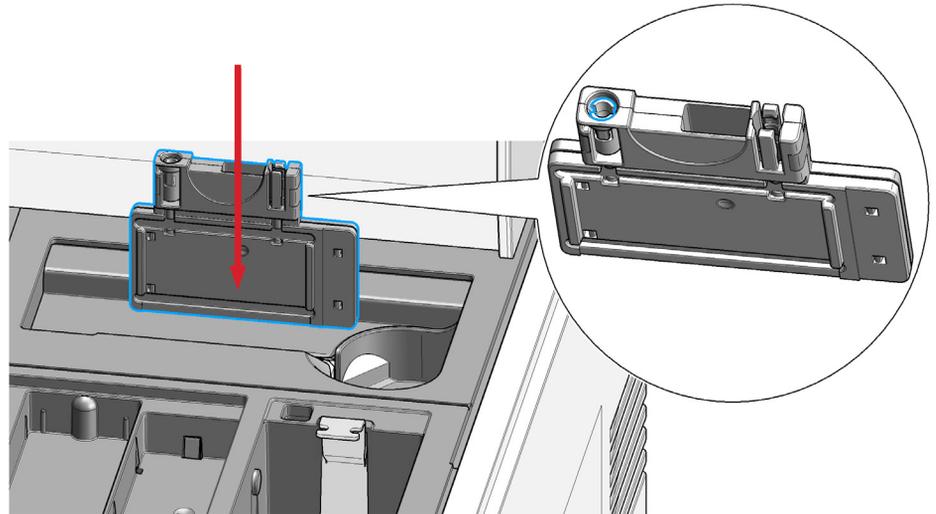
9 Maintenance

Exchange the Piercing Needle

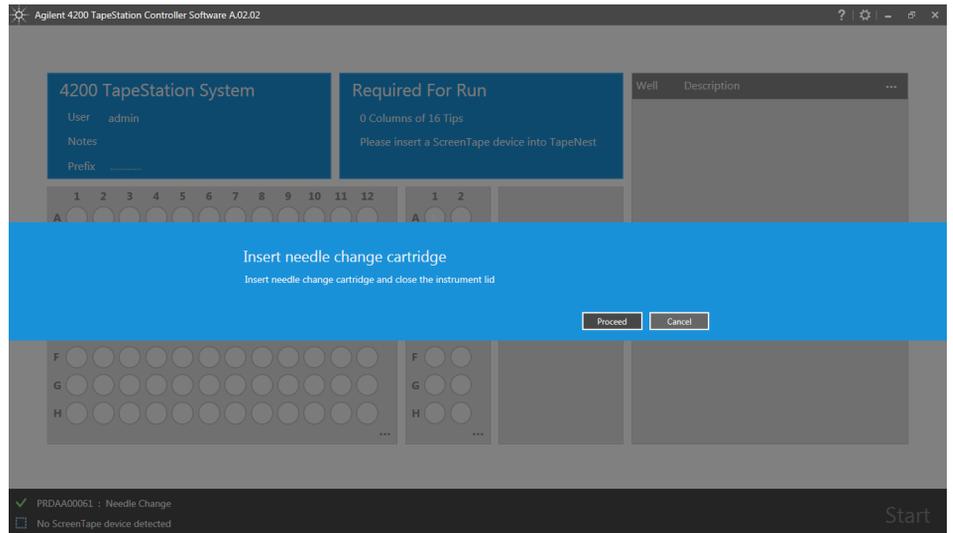
- 1 Prepare instrument by selecting **Tools > Maintenance > Change needle** from the TapeStation Controller software. The exchange process opens the ScreenTape nest to allow insertion of the Needle Change Cartridge.



- 2 Insert an unused Needle Change Cartridge to the ScreenTape nest. Insert the cartridge with the bar code facing towards the back of the instrument. An unused new cartridge has a new piercing needle in the left side and an empty slot on the right. The open slot on the right hand side has hooks.



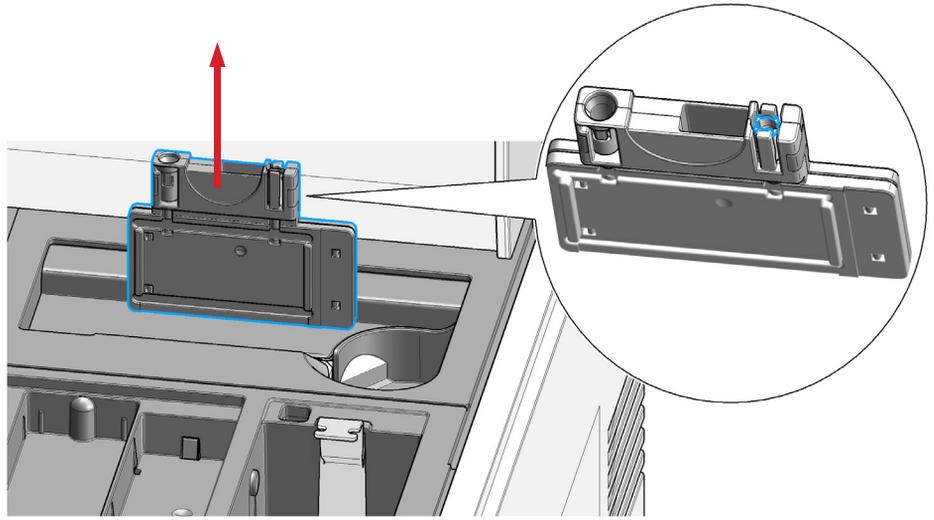
- 3 Click **Proceed** to continue.



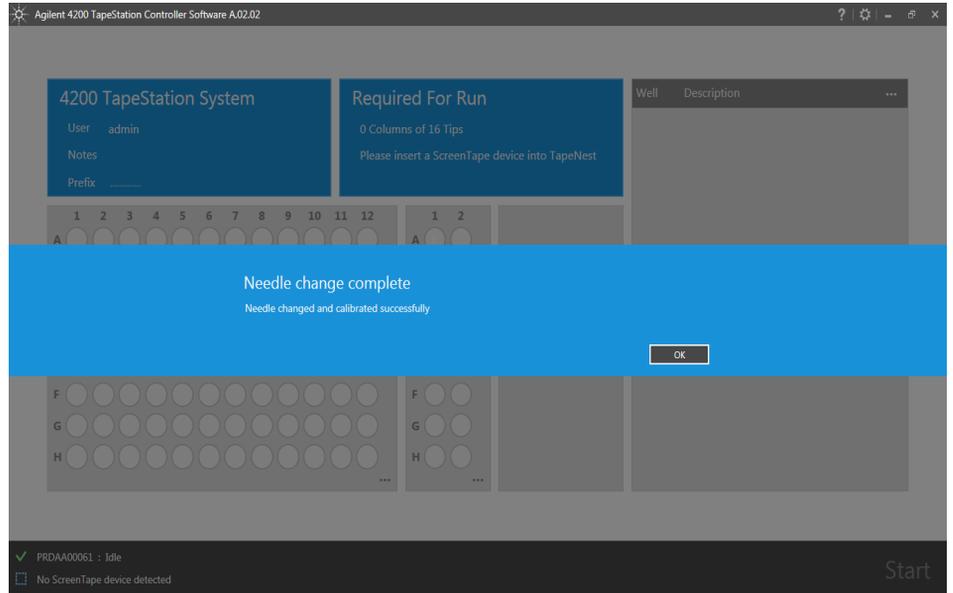
9 Maintenance

Exchange the Piercing Needle

- 4 After exchange: Remove Needle Change Cartridge from ScreenTape nest and discard. The used cartridge has an open slot on the left side and the old piercing needle in the slot on the right.



- 5 Wait approximately two minutes until the automated pierce needle calibration is complete. It is crucial not to turn off the instrument or terminate the software during the needle exchange; this could impact performance of all future analysis.



Cleaning the Instrument

The surfaces of the TapeStation instrument, the waste bucket, strip holder, ScreenTape rack, the Lower nest cover and the Upper nest cover can be cleaned with the following reagents:

- mild household detergents,
- mixture of 70% ethanol with 30% water,
- isopropanol, or
- RNaseZAP® (Ambion, Inc).

NOTE

Dislocation of Lower and Upper nest cover will create blockage errors during instrument operation.

Please see [“Assembling Nest Covers”](#) on page 143 and [“Insertion of the Lower Nest Cover”](#) on page 145 in case an issue arises.

CAUTION

Heat deformation of plastic components

Deformed plastic components will create blockage errors during instrument operation.

- Do not expose plastic components to heat.
 - Do not use a dishwasher.
-

Preparing the Instrument for Transportation

The 4200 TapeStation instrument must be brought into transport mode before being moved. Please follow the instructions below.

WARNING

A piercing needle is located behind the gantry cover.

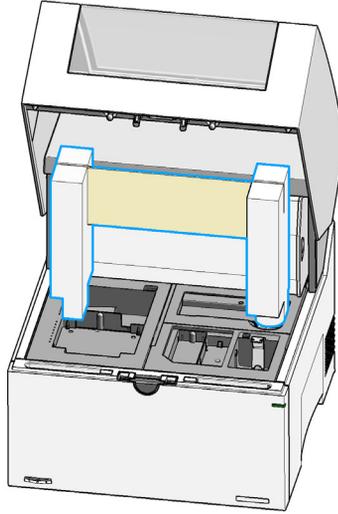
- Do not reach under the cover.
 - Do not remove the cover.
-

- 1 Remove all loose components such as tip waste, tip rack, tube strip holder, 96-well sample plate plate, and ScreenTape rack from the instrument.
- 2 Activate **Transportation lock**. The **Transportation lock** command from the TapeStation Controller software menu moves all robotic parts to a safe position.
- 3 Power down the instrument.

9 Maintenance

Preparing the Instrument for Transportation

- 4 Install the fixing foam. The foam has two differently shaped pillars. Align the foam in such way with the instrument that the round shaped pillar is located in the ScreenTape nest. The foam sits tight and holds the gantry cover in place during transportation once the lid is closed.



- 5 Close the lid.

Transportation Lock

The execution of the command Preparing Transportation from the TapeStation Controller software leads to locking of two moving mechanical parts of the 4200 TapeStation instrument. The robotics for liquid handling and the moving parts of the vision system are fixed to the chassis of the instrument. This locking is required prior to any transportation of the instrument (“[Engaging the Transportation Lock](#)” on page 138).

1 Liquid handling:

The transportation lock for the robotics for liquid handling can be exclusively released by pressing a dedicated button at the gantry of the instrument. The transportation lock for the robotics for liquid handling cannot be reverted by a software interaction (step on page 43).

2 Vision system:

The moving parts of the vision system are unlocked automatically at the next analysis start. There is no manual user interaction required to release the vision system lock.

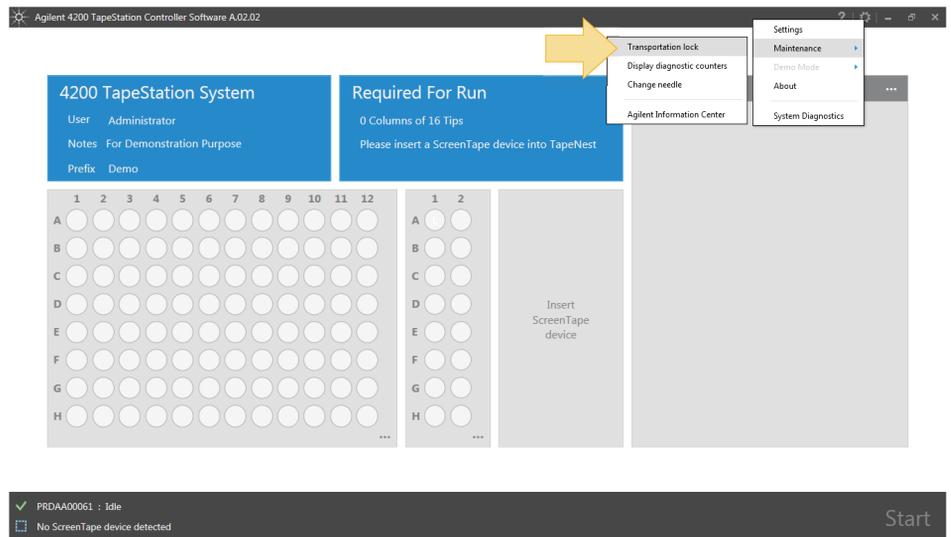
NOTE

If the unit needs to be transferred to another location, engage the transportation lock before moving it. Release the lock prior to next use, see release the Transportation lock (“[Engaging the Transportation Lock](#)” on page 138).

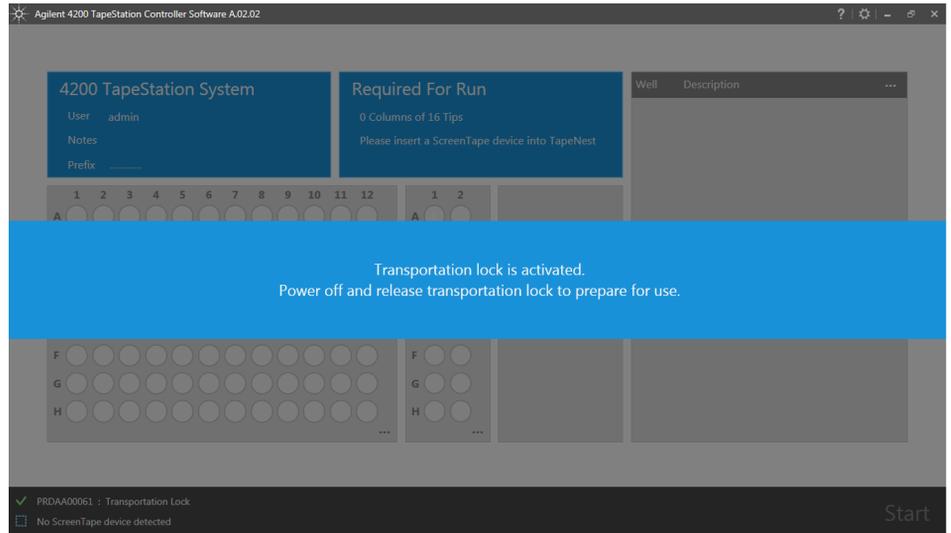
Engaging the Transportation Lock

Engaging the Transportation Lock by software is possible for an instrument that is powered on, successfully connected by USB, has a closed lid and functional robotics.

1 **Transportation lock** command moves all robotic parts to a safe position.



- 2 The transportation lock is now activated. Please close the TapeStation Controller software and power down the instrument.



NOTE

An instrument with engaged transportation lock cannot perform analysis. The TapeStation Controller software will prompt you for powercycling releasing the transportation lock in case of an analysis start.

Boxing for Transportation

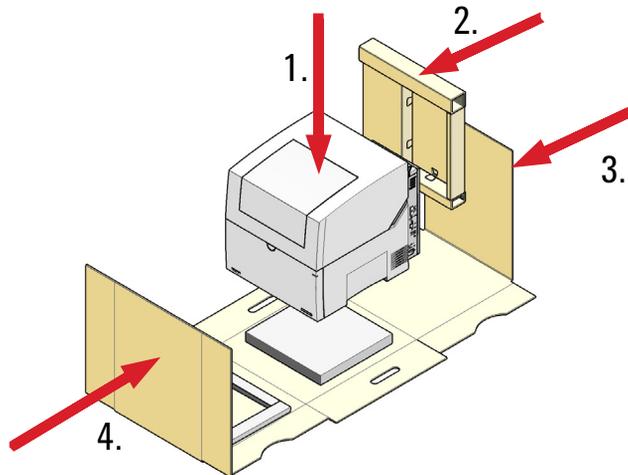
An instrument with activated transportation lock and inserted fixation foam (see “[Preparing the Instrument for Transportation](#)” on page 135) is ready for boxing.

WARNING

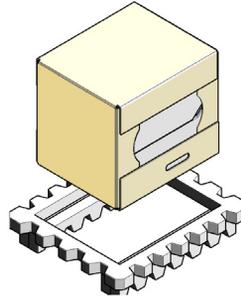
Be careful when lifting the instrument.

→ Because the instrument is heavy, lifting it will require the assistance of a second person to prevent injury.

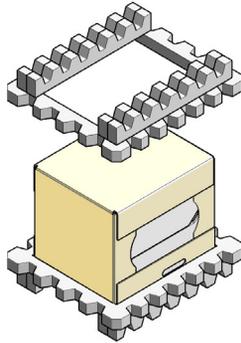
- 1 Place inner cardboard box on the floor and lift instrument with two persons onto it. Mount the cardboard box to the back.



- 2 Close inner cardboard box and place the instrument onto the bottom foam with two persons.



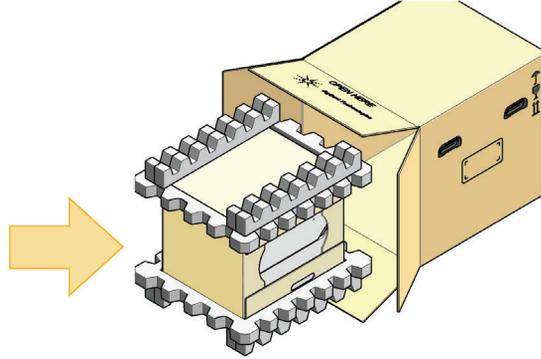
- 3 Add the top foam of the inner cardboard box.



9 Maintenance

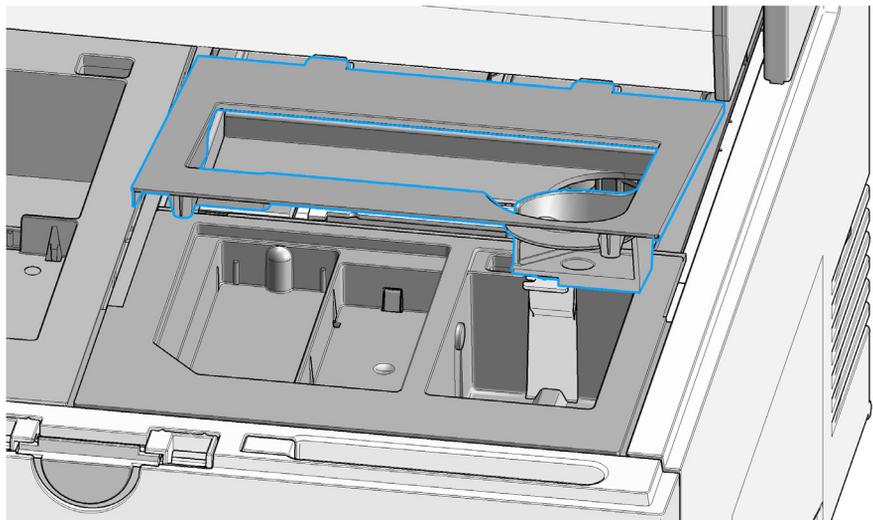
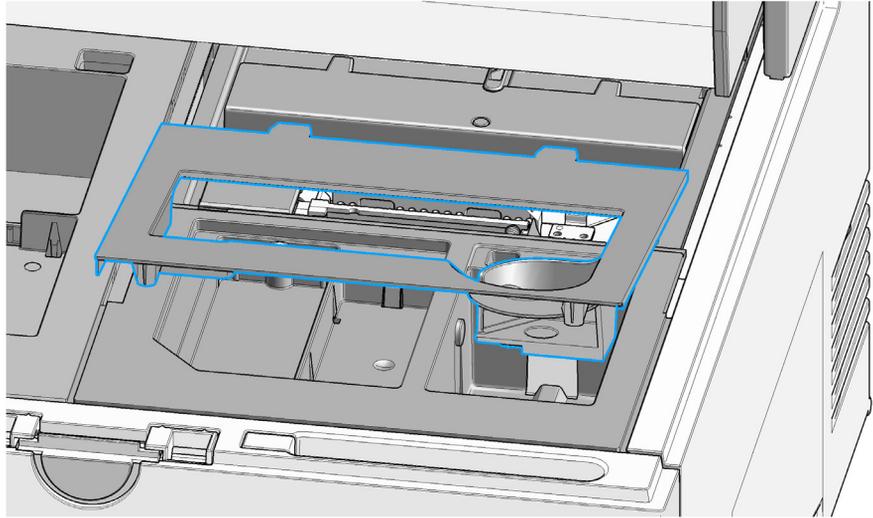
Boxing for Transportation

- 4 Slide the instrument into the outer cardboard box.

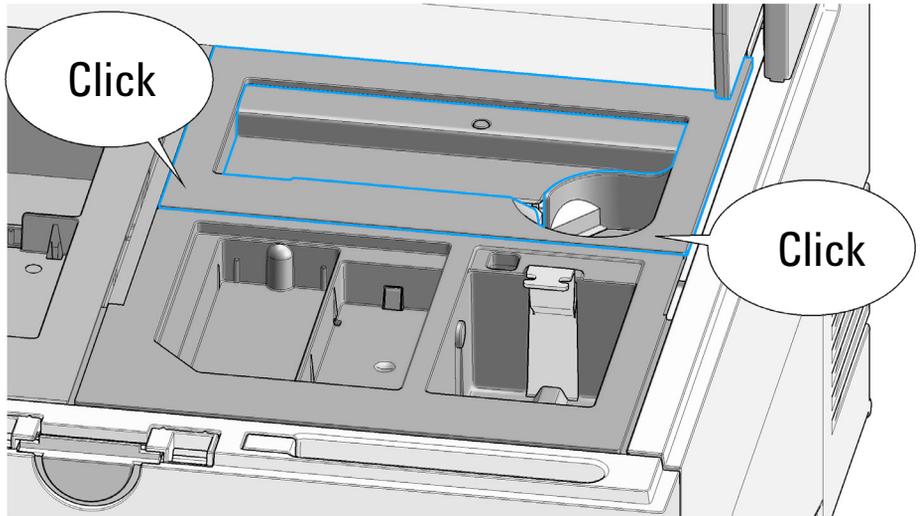
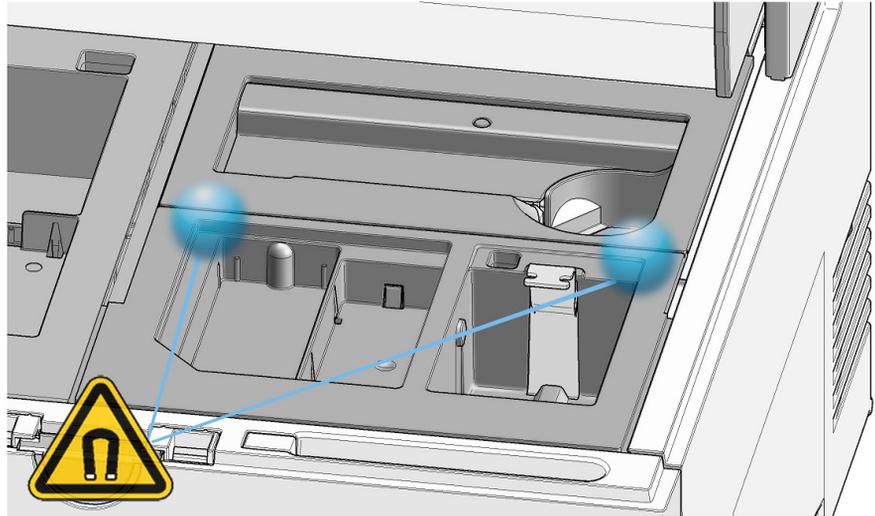


Assembling Nest Covers

- 1 Reinsert the upper nest cover: Guide the rear positioning extensions into the corresponding recesses under the gantry. Lower the cover until the magnets hold it in place.



9 Maintenance
Assembling Nest Covers



Insertion of the Lower Nest Cover

It is unnecessary to remove the lower nest cover. Follow the instructions in case this was done by accident.

CAUTION

Lower nest cover incorrectly mounted
Instrument malfunction and loss of material.

→ Do not touch the lower nest cover.

CAUTION

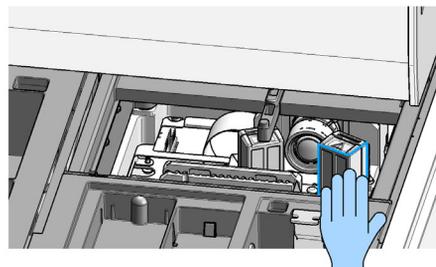
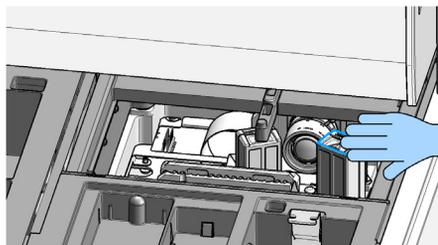
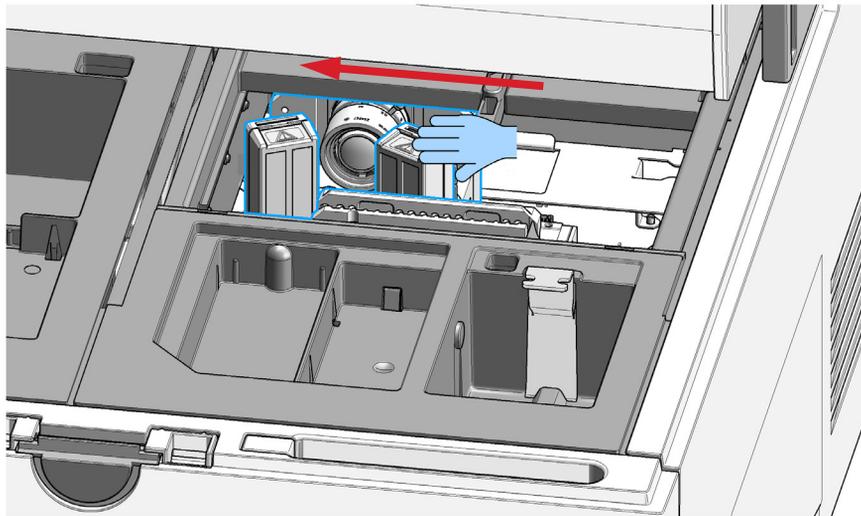
Unclean or dislocated LED module front
Analytical performance is impaired

→ Do not touch the LED module front.

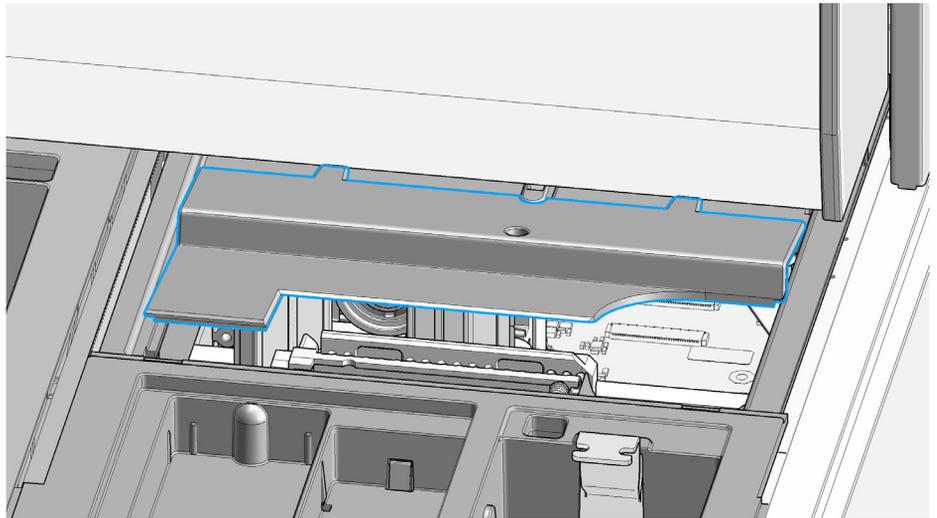
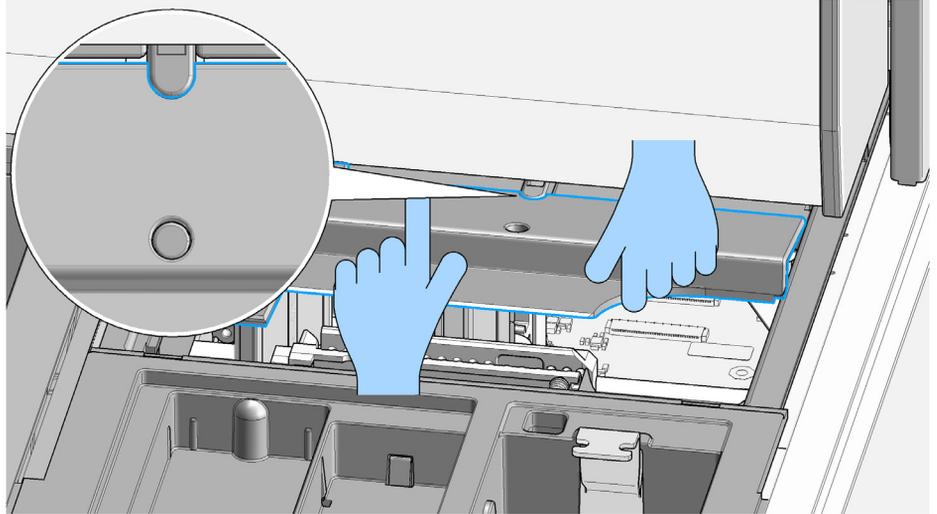
9 Maintenance

Insertion of the Lower Nest Cover

- 1 Carefully touch the LED module at its top and move the assembly to the left side.



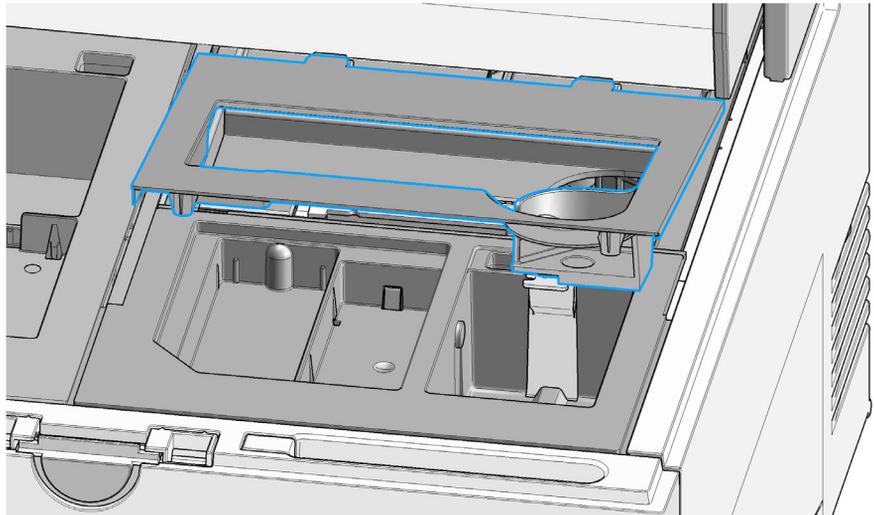
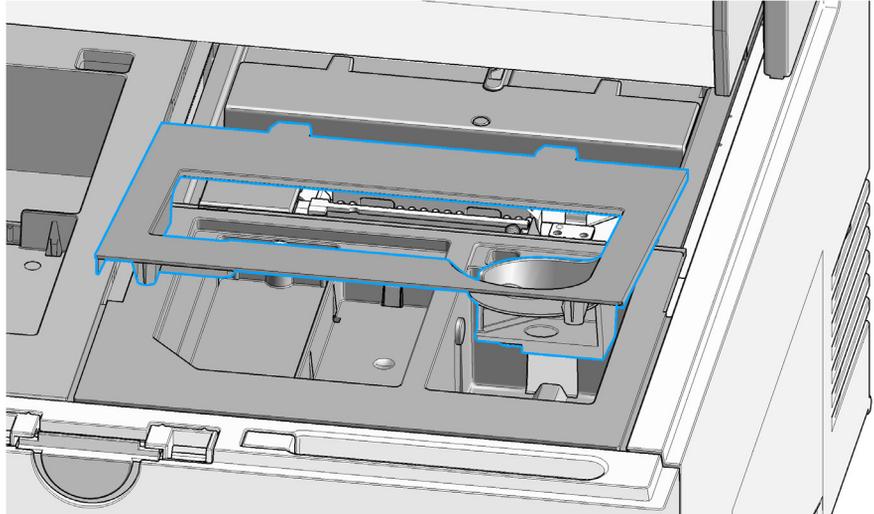
- 2 Mount the lower nest cover to the lever such that the half-round cutout meets its counter part on the lever.



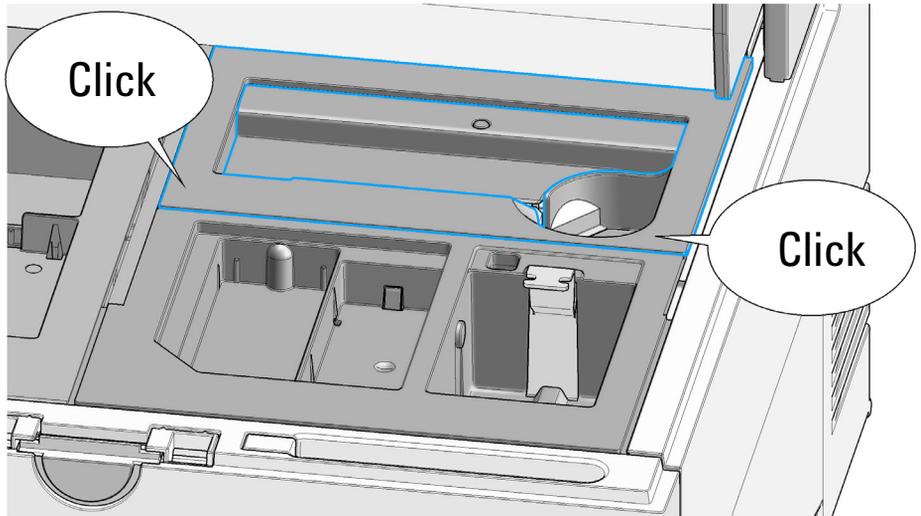
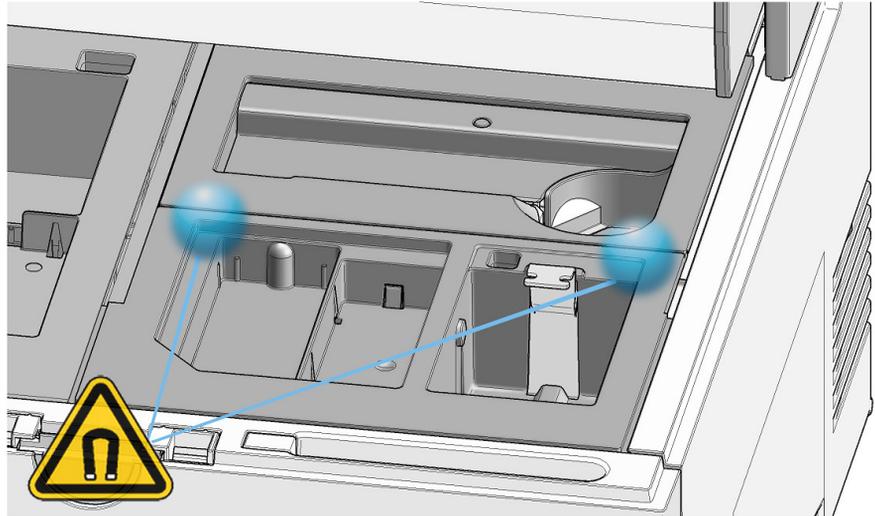
9 Maintenance

Insertion of the Lower Nest Cover

- 3 Reinsert the upper nest cover: Guide the rear positioning extensions into the corresponding recesses under the gantry. Lower the cover until the magnets hold it in place.



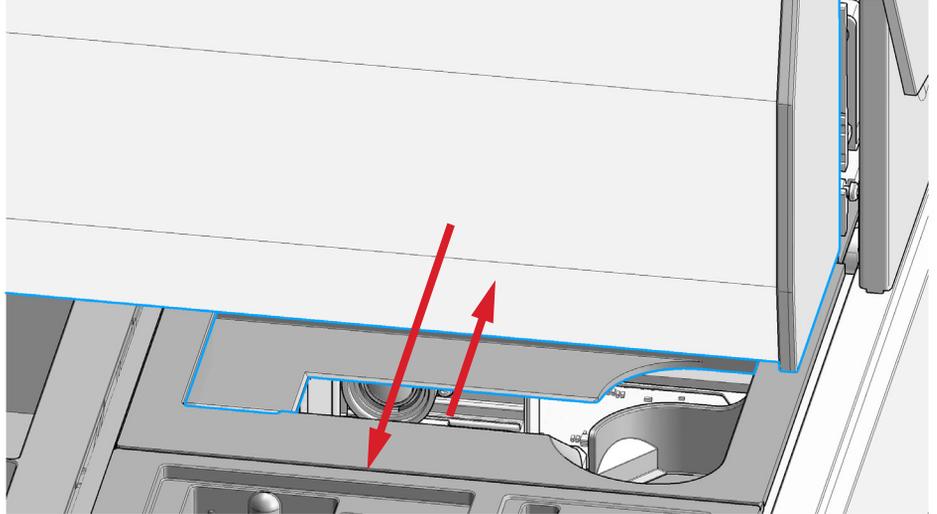
Insertion of the Lower Nest Cover



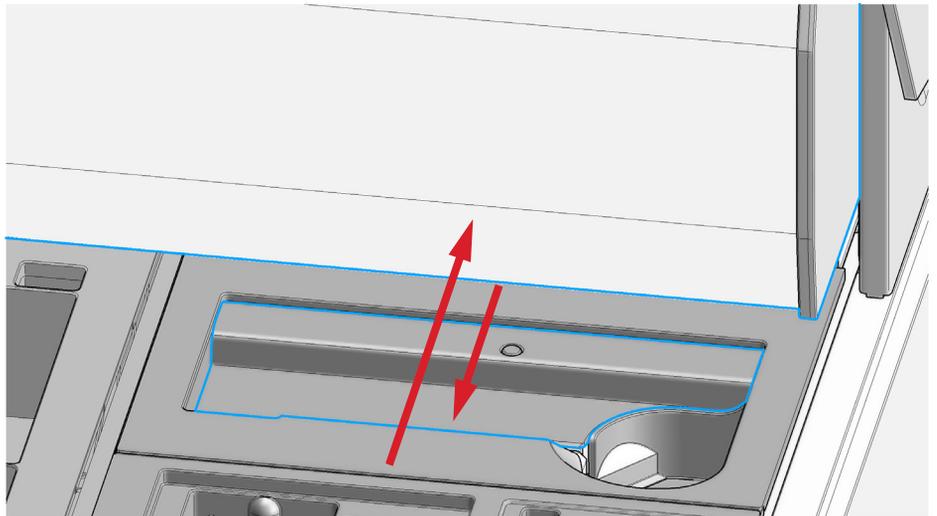
9 Maintenance

Insertion of the Lower Nest Cover

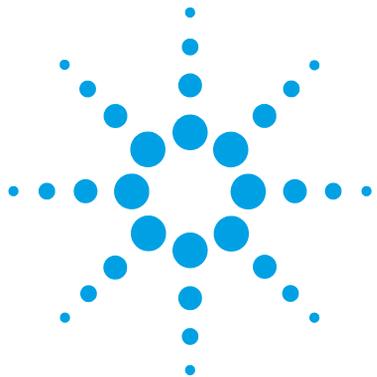
- 4 Move the gantry gently 5 cm towards the front. The lower nest cover moves backwards automatically by 3 cm.



- 5 Move the gantry gently back again. The lower nest cover moves forward.



- 6 The lower nest cover mounting is correct if it moves smoothly in opposite direction to the gantry cover.



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10 Troubleshooting

Insertion of the Lower Nest Cover

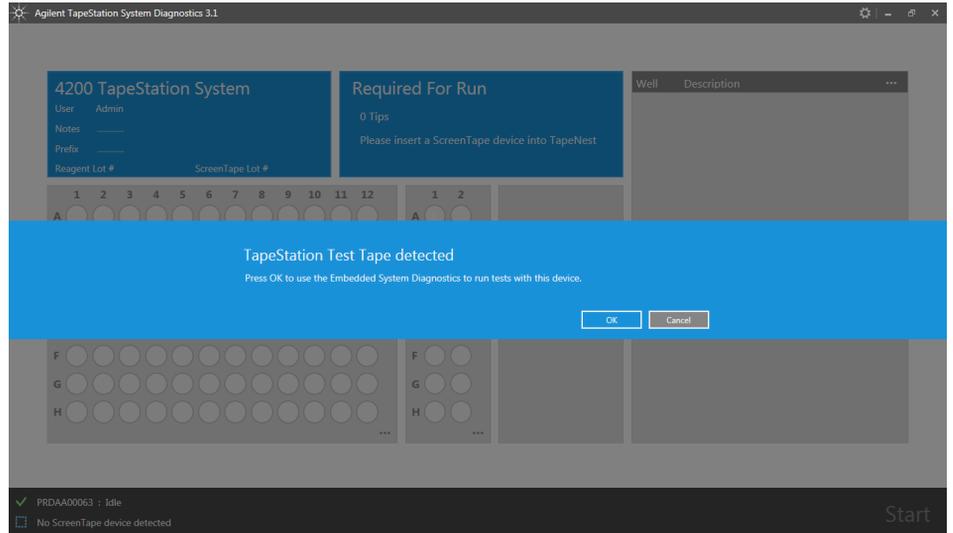
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This chapter gives an overview about the troubleshooting features of the 4200 TapeStation system. It includes information about application troubleshooting as well.

Software Messages

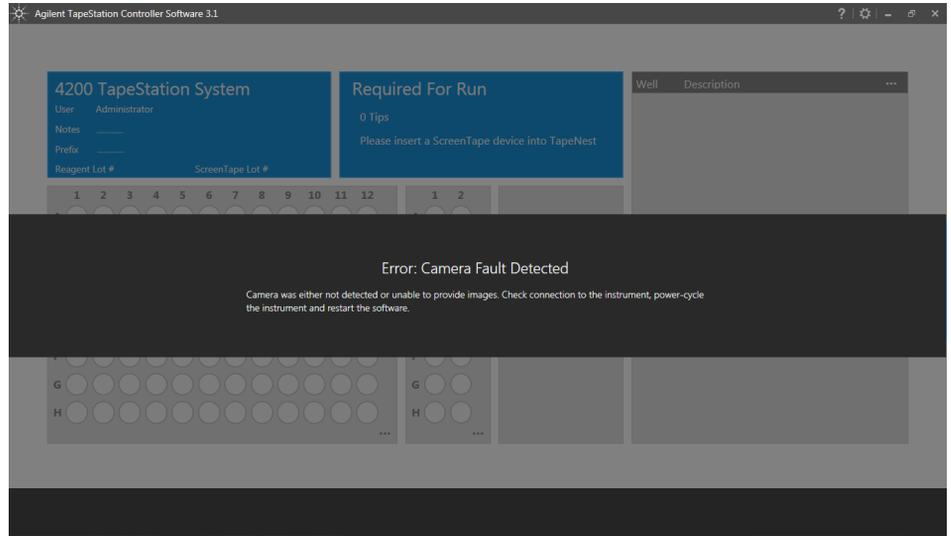
TapeStation Test Tape Detected

- 1 A TapeStation Test Tape (see “TapeStation Test Tape” on page 118) detected in the ScreenTape nest allows you to start the System Diagnostics.



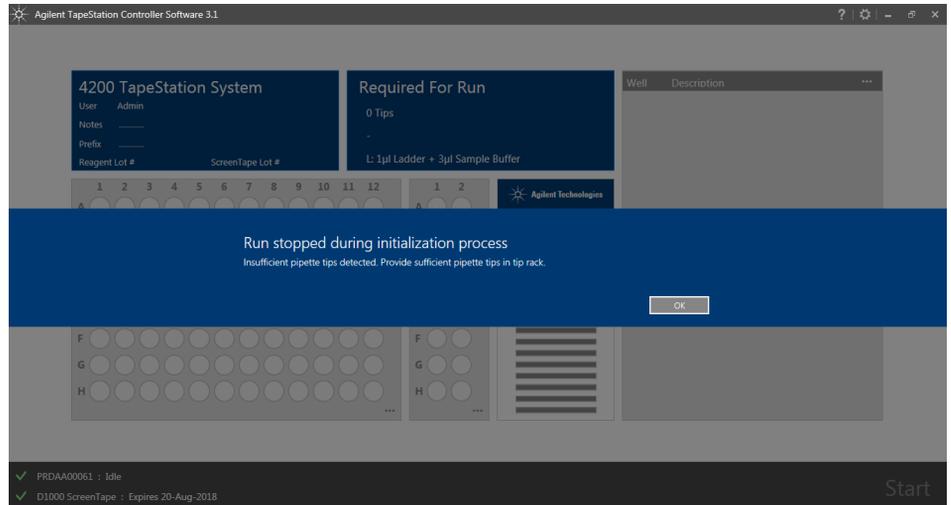
Camera Fault Detected

- 1 The TapeStation Controller software could not connect to the instruments' camera. Check USB connection to the instrument, power-cycle the instrument and restart the software. In case of recurrence reboot the laptop. See “[Instrument Communication](#)” on page 193 for details.



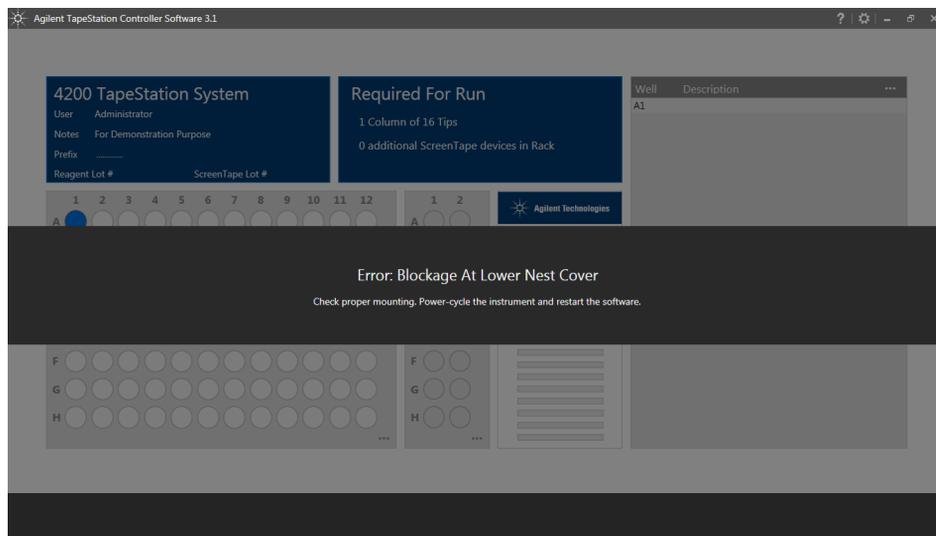
Run Stopped During Initialization Process

- 1 The consumables required for a run were not detected. Provide sufficient ScreenTape devices and loading tips in the tip rack. Remove tube strip caps, tip rack cover and empty the waste locations.



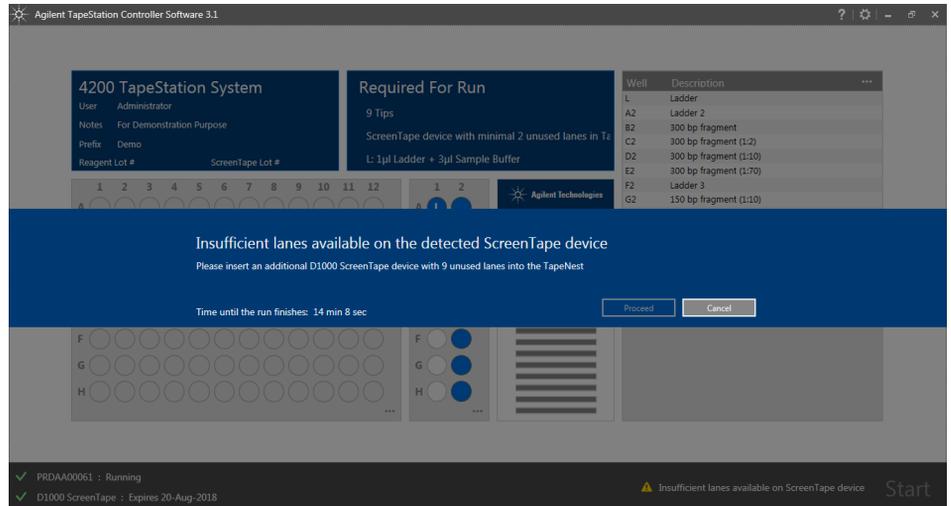
Block at Lower Nest Cover

- 1 The lower ScreenTape nest cover did not move and requires proper assembly. See “[Insertion of the Lower Nest Cover](#)” on page 145 for instructions.



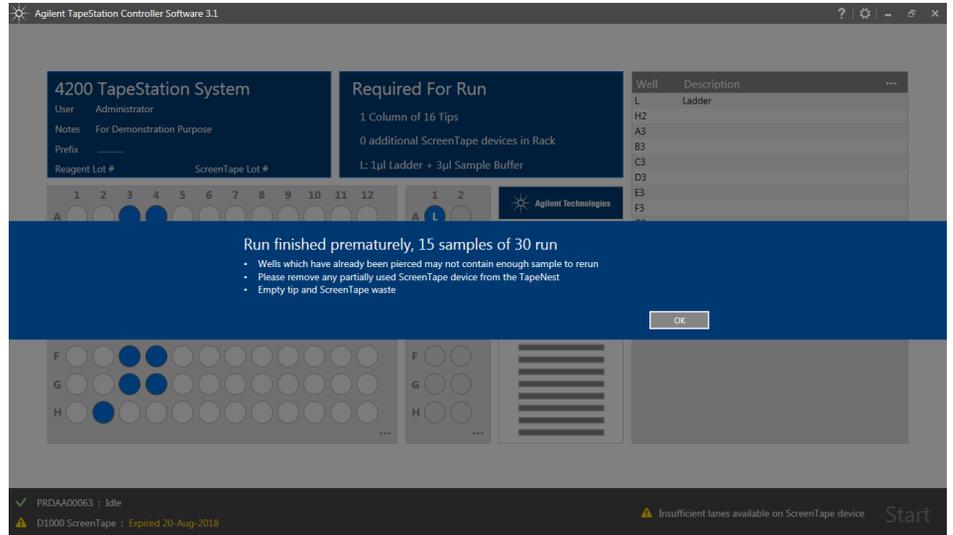
Insufficient Lanes Detected

- 1 Insufficient lanes on a ScreenTape devices were present. Add a second ScreenTape device with sufficient unused lanes within 15 min to finalize the analysis.



Premature End of Analysis

- 1 Insufficient number of correct ScreenTape devices in the rack leads to a premature end of the analysis. Consider that prepared samples which did not run due to an error might be aged and might not be analyzable.



Perform Needle Change

- 1 No further analysis is possible beyond 4480 samples until a Needle Change is done. A warning message will be displayed after 3840 samples. This allows to prepare for corrective actions. See “Needle Pierce Counter” on page 126 for further information.

The screenshot displays the Agilent TapeStation Controller Software 3.1 interface. At the top, there are two main panels: '4200 TapeStation System' and 'Required For Run'. The '4200 TapeStation System' panel shows user information (Administrator), notes (For Demonstration purposes), prefix (Demo), and reagent lot information. The 'Required For Run' panel indicates 8 tips and a screen tape device with minimal 2 unused lanes in the tape. Below these panels is a grid representing the tape wells (A-H, 1-12) and a 'D1000 ScreenTape' section. A yellow arrow points to a warning message at the bottom of the interface: 'Perform needle change'. The status bar at the bottom shows two green checkmarks for 'PRDAA00061 : Idle' and 'D1000 ScreenTape : Expires 20-Aug-2018', and a red triangle icon for the 'Perform needle change' warning. The 'Start' button is visible on the right side of the status bar.

Troubleshooting DNA Applications

NOTE

The troubleshooting information is complemented by Frequently Asked Questions, How To videos or animated graphics which are provided in the Agilent Information Center (see “Agilent Information Center” on page 56).

Genomic DNA Assay

Quantification

Incorrect quantification in the Genomic DNA assay can occur due to residual sample remaining at the top of the gel. This signal can be viewed in the TapeStation Analysis software using the **Scale to Molecular Weight** button (observation: Sample Well).

NOTE

Peaks that are designated as **well** are too large to have migrated into the gel; please be aware that an accurate concentration for the entire sample cannot be generated.

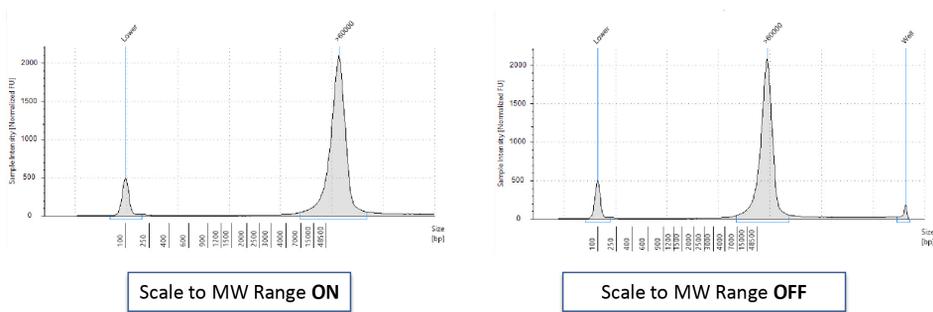


Figure 38 Visualizing a **well** peak in the Electropherogram view of the TapeStation Analysis software.

Sizing

Genomic DNA Ladder should be freshly prepared for each run. No electronic ladder is available for the Genomic DNA assay. The ladder vial should be handled carefully with only gentle vortexing before use. Shaking the Genomic DNA ladder vial can occasionally cause the largest fragment to degrade, since shaking results in different shearing forces than vortexing. This can lead to inaccurate sizing results. Where the degradation has resulted in failure of the software to assign the top ladder fragment, no DIN scores will be presented for the lanes in this file.

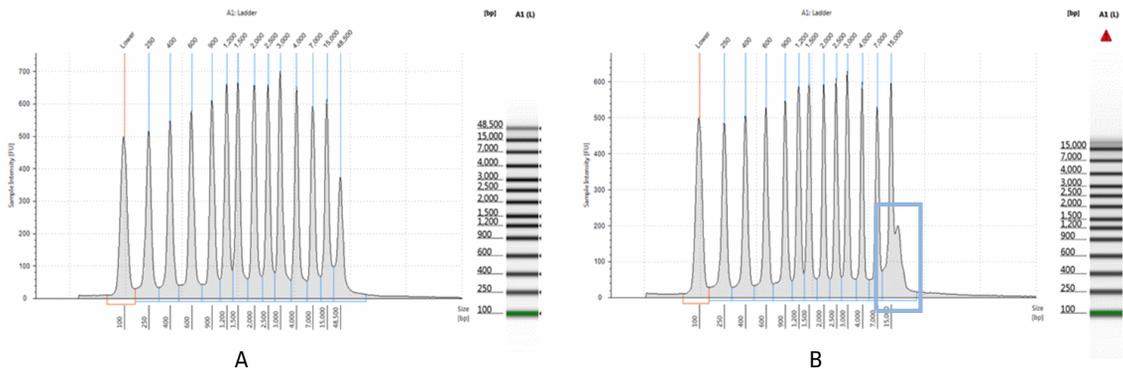


Figure 39 The effect of vigorous shaking of the Genomic DNA Ladder. A) Genomic DNA Ladder has been handled as directed prior to analysis on the Genomic DNA ScreenTape assay. B) The Genomic DNA Ladder vial was handled incorrectly - degradation of the top fragment (48500 bp) is clearly shown.

Incorrect Quantification Results

Quantification calculation is facilitated by comparing the area under a peak or region of interest in the electropherogram with the area under the Upper Marker. The Genomic DNA assay uses the Lower Marker for quantification calculations. Both Upper and Lower Marker have a defined concentration.

Incorrect quantification results can have multiple causes, please see the Agilent Information Center for further details. If after reviewing this technical overview and following the recommendations below your result files continue to show concentration discrepancies, please contact your local support representative.

Table 6 DNA - Possible causes of incorrect quantification results in order of probability

Root cause	Explanation	Solution
Insufficient Mixing	Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.	See " Mixing Recommendations " on page 93 and the Agilent Information Center for more information.
Sample concentration outside recommended range for application.	Linear concentration estimation cannot be achieved for samples exceeding the limits in the specification table.	Either dilute or concentrate your sample until it is within the recommended range for application as stated in the respective assay Quick Guide, then prepare a new run. Use the High Sensitivity assay if applicable (Table 3 on page 60).
Incorrect peak integration	The algorithm cannot establish a correct concentration estimation if marker or sample peaks are not completely encompassed as a signal from either marker or sample is left out of the calculation.	Ensure that all sample peaks and markers are integrated correctly in the TapeStation Analysis software, by clicking and dragging so that the whole peak is encompassed. See the Agilent Information Center for more information.
Incorrect analysis type	The Gel and Electropherogram view are designed to analyze discrete peaks within samples. The Region view is optimized for analyzing samples with broad peaks. Region view uses a different method to calculate concentration and sizing.	Use Gel view for samples with narrow peaks and the Region view for samples with broad peaks or smears such as libraries. See " Peak Maxima Versus Average Molecular Weight Sizing and Molarity " on page 104 for more information.

Table 6 DNA - Possible causes of incorrect quantification results in order of probability

Root cause	Explanation	Solution
Sample and the marker relevant for quantification have merged.	Additional sample overlaying with the marker will alter the known concentration of the marker that is used for concentration calculation.	Ensure that your sample is within the recommended sizing range for the applications, see “Sizing Range” on page 106.
	Contaminating beads can alter the run behavior of the sample and can introduce artifacts, see “TapeStation Analysis in the Agilent SureSelect and HaloPlex Workflow” on page 179.	If using the Sure Select Protocol, ensure that all AMPure beads are removed by increasing the time that the sample is on the magnetic plate.
Sample not collected at bottom of vial	Insufficient volume pipetted by the robotics and applied to the lane may affect migration behavior and alter the band shape.	Make sure to collect all liquid at the bottom of the vial by centrifugation. See “Centrifugation Recommendations” on page 96. Ensure that after removing strip caps and inserting the tube strips or 96-well sample plate into the TapeStation instrument, liquid is still located at the bottom of the well, and is not spilled.
Incorrect protocol used	Differences exist between Standard and High Sensitivity assay protocols.	Ensure that the correct sample protocol for the application is used, see “Use the Correct Protocol” on page 92.
Incorrect reagents used	Reagents from different assays may differ in dye and marker concentration. Furthermore markers used are different and upper markers may not be present.	Ensure that the correct reagent buffer for the application is used, see “Use the Correct Protocol” on page 92.
Sample prepared too long before analysis	Evaporation or settling may alter the sample to buffer ratio.	Ensure that your run is started immediately after sample preparation.
	The sample/buffer mix may evaporate over time if a 96-well sample plate was used which was not sealed with the recommended seal foil.	Always cover with the recommended foil seal (PN 5067-5154) and ensure tight sealing throughout the 96-well sample plate.
Incorrect pipetting technique or pipette calibration.	Incorrect pipetting technique or uncalibrated pipettes can lead to poor pipetting performance which in turn alter sample/buffer ratio in an unpredictable manner.	See “Good Pipetting Practice” on page 84 for more information or follow guidelines of the respective pipette vendor.
Incorrect Marker peaks picked up	Depending on the sample pattern, wrong peaks can get assigned as marker if running very close to the actual marker.	Use the Aligned button to detect the correct markers. Compare to aligned/unaligned data of good, for example the ladder, and questionable samples, then assign correct markers by right-clicking. See the Agilent Information Center for more information. Ensure that all sample peaks are within the recommended sizing range for the application.

Incorrect Sizing Results

Sizing calculation is facilitated by comparing the run distance of a sample peak or smear to the run distances of ladder peaks which originate from an electronic ladder or a known actual run ladder. Sizing information is obtained after aligning run distances. Both Lower and Upper Marker have a defined molecular weight and are required to be assigned correctly for this alignment.

Incorrect sizing results can have multiple causes. If after following the recommendations below, your result files continue to show sizing discrepancies, please contact your local support representative.

NOTE

For best sizing precision and accuracy, the user should run the appropriate ladder with the samples.

Table 7 DNA - Possible causes of incorrect sizing results in order of probability

Root cause	Explanation	Solution
Incorrect Marker peaks picked up	Depending on the sample that is used, wrong peaks can get assigned as marker if the sample size is very close to the actual marker.	Use the Aligned button in the TapeStation Analysis software to detect the correct markers. Compare to aligned/unaligned data of good and questionable samples; then assign correct markers by right-clicking. See the Agilent Information Center for more information. Ensure that all sample peaks are within the recommended sizing range for the application.
Incorrect analysis type	The Gel and Electropherogram view are designed to analyze discrete peaks within samples. The Region view is optimized for analyzing samples with broad peaks. Region view uses a different method to calculate concentration and sizing.	Use Gel view for samples with narrow peaks and the Region view for samples with broad peaks or smears such as libraries. See “Peak Maxima Versus Average Molecular Weight Sizing and Molarity” on page 104 for more information.
Issues with the ladder lane	Any issues with the run ladder lane will be annotated within the TapeStation Analysis software sample table under Observations .	Manually insert or delete affected peaks. In assays where an electronic ladder is available (D1000, High Sensitivity D1000, and Cell-free DNA Assay), insert the electronic ladder to regain sizing information, see the Agilent Information Center for more information. In assays where an electronic ladder is not available (D5000, High Sensitivity D5000, and Genomic DNA Assay), re-run affected samples and ensure that Good Measurement Practices are followed, see “Good Measurement Practices” on page 81. For Genomic DNA Ladder considerations, see “Genomic DNA Assay” on page 160.
Insufficient migration due to presence of salt	Sample matrices exceeding the maximum buffer strength specifications may alter currents in an unpredictable manner leading to short running lanes, see “Maximum Buffer Concentration in Sample” on page 110.	Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by unaligning your gel image by pressing the Aligned button in the TapeStation Analysis software. Please refer to the salt tolerance guidelines for your assay in the appropriate Quick Guide (Table 3 on page 60).

Sizing in Assays with Mandatory Ladder

No sizing and quantification results can be obtained in the Genomic DNA, D5000 and D5000 High Sensitivity assays if the correct number of ladder peaks was not assigned.

Missing Marker Peaks

Table 8 DNA - Possible causes of missing marker peaks in order of probability

Root cause	Explanation	Solution
Sample concentration outside recommended range for application	The majority of the dye binds to the sample so that the signal from the marker is too weak to be detected if the sample is too concentrated.	Dilute your sample until it is within the recommended range for the application as stated in the assay Quick Guide; then prepare a new run (Table 3 on page 60).
Insufficient migration	Marker peak may not be completely resolved from a sample peak or is not at an expected run distance.	To check migration true distance, unalign the gel image using the Aligned button. See “Unexpected Migration Profile” on page 169, “Maximum Buffer Concentration in Sample” on page 110, and “Quantitative range” on page 107.
Blank lane		See the Agilent Information Center for more information.

Incorrect or Missing DIN Value

The DNA Integrity Number (DIN) is an automated numerical software assessment which allows integrity determination of genomic DNA samples from the distribution of signal across the size range.

Table 9 Genomic DNA - Probable causes for missing or incorrect DIN results

Root cause	Explanation	Solution
Too few ladder peaks have been detected	In order to calculate a DIN value a correct sizing is needed which can only be established with a correctly run and integrated ladder.	Check the electropherogram trace of the ladder for incorrectly or not assigned peaks; then manually assign ladder peaks. Make use of the demo file to identify correct peaks. See the Agilent Information Center for more information. In case the biggest ladder fragment is degraded see "Genomic DNA Assay" on page 160. Assign a different lane as the ladder (if applicable).
Incorrect marker peak picked up or no marker peaks detected	Sample matrices exceeding the maximum buffer strength specifications may alter currents in an unpredictable manner leading to short running lanes, see "Maximum Buffer Concentration in Sample" on page 110.	Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by unaligning your gel image by pressing the Aligned button in the TapeStation Analysis Software. Please refer to the salt tolerance guidelines for your assay in the appropriate Quick Guide (Table 3 on page 60). Rerun samples with fresh reagents.
	Due to sensitivity, artifacts might be erroneously assigned as Lower Marker.	Use the Aligned button in the TapeStation Analysis software to detect the correct markers. Compare to aligned/unaligned data of good and questionable samples; then assign correct markers by right-clicking. See the Agilent Information Center for more information. Ensure that all sample peaks are within the recommended sizing range for the application.

10 Troubleshooting

Troubleshooting DNA Applications

Table 9 Genomic DNA - Probable causes for missing or incorrect DIN results

Root cause	Explanation	Solution
No ladder run on ScreenTape device	For Genomic DNA application, one ladder per ScreenTape must be run in order to ensure correct sizing and DIN calculation. The ladder should be run in the first available position on the ScreenTape device.	Use the Assign button to use the correct ladder lane if a ladder has mistakenly been run in a position other than position A1 when using tube strips. Rerun samples if no ladder was used at all. In case a 96-well sample plate was used a ladder always has to be provided in position A1 from the tube strip location.
Sample concentration outside functional DIN range	The DIN algorithm only allows linear estimation of sample integrity within the given concentration range.	Either dilute or concentrate your sample until it is within the recommended range for the application as stated in the assay Quick Guide; then prepare a new run (Table 3 on page 60).
Sample with multiple peaks in lane	A DIN value is not presented in sample lanes containing more than 14 peaks as this potentially represents the ladder.	Check the electropherogram trace for incorrectly assigned peaks and judge if these are genuine peaks. Rerun samples with fresh reagents if in doubt.

Unexpected Migration Profile

There are a number of issues which can affect migration and sample peak profile. If the issues below do not describe your analysis file, please contact your support representative.

Table 10 DNA - probable causes for unexpected migration profile

Problem	Likely cause	Explanation	Solution
The gel image looks distorted	Incorrect Marker peaks picked up	Depending on the sample pattern wrong peaks can get assigned as marker if running very close to the actual marker.	Use the Aligned button in the TapeStation Analysis software to detect the correct markers. Compare to aligned/unaligned data of good and questionable samples; and then assign correct markers by right-clicking. See the Agilent Information Center for more information. Ensure that all sample peaks are within the recommended sizing range for the application, see " Peak Integration " on page 101.

10 Troubleshooting

Troubleshooting DNA Applications

Table 10 DNA - probable causes for unexpected migration profile

Problem	Likely cause	Explanation	Solution
Slanted or smeared bands	A bubble could be located at the top of the ScreenTape device	A bubble in the buffer chamber distorts the initial entry of a sample into the gel.	Always flick the ScreenTape device before placing into the TapeStation instrument. See “ScreenTape Devices” on page 86.
	Sample not collected at bottom of vial	Insufficient volume pipetted by the robotics and applied to the lane may affect the migration behavior and alter the band shape.	Make sure to collect all liquid at the bottom of the vial by centrifugation. See “Centrifugation Recommendations” on page 96. Ensure that after removing strip caps and inserting the tube strips or 96-well sample plate into the TapeStation instrument liquid is still located at the bottom of the well, and is not spilled.
	The sample may not have been mixed correctly with the sample buffer	Ionic strength of the sample/buffer mix may not reach expected optimal conditions and thus electrophoresis is impaired.	Follow the mixing recommendations for the assay. See “Mixing Recommendations” on page 93 for more details.
	The sample and sample buffer may have started to evaporate	Ionic strength of the sample/buffer mix may exceed the maximum buffer strength due to water loss and thus electrophoresis is impaired.	Always ensure that samples are run on the TapeStation instrument immediately after preparation with sample buffer.
	A bubble could be located within the gel matrix	A bubble in the gel matrix introduces significant artifacts in the data. Those bubbles cannot be flicked away.	Always inspect ScreenTape devices for bubbles in the lanes. Lanes in question cannot be used for analysis. Such bubbles can form after the expiration date or upon wrong storage. See the Agilent Information Center for more information.

Table 10 DNA - probable causes for unexpected migration profile

Problem	Likely cause	Explanation	Solution
The markers have appeared in the sample lane, but the sample has not	The sample analyzed is too diluted	The sample concentration is below the limit of detection.	Concentrate your sample until it is within the recommended range for the application; and then prepare a new run, see “Quantitative range” on page 107. Use the High Sensitivity assay if applicable.
	Insufficient Mixing	Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.	See “Mixing Recommendations” on page 93 and the Agilent Information Center for more information.

10 Troubleshooting

Troubleshooting DNA Applications

Table 10 DNA - probable causes for unexpected migration profile

Problem	Likely cause	Explanation	Solution
Extra bands have appeared to those expected in the sample lane	There may be a contaminant peak that is caused by dust or dirt on the ScreenTape device	Peaks caused by contaminants appear as sharp strong bands which are often slanted or uneven in the gel image, and are usually easily distinguishable from sample peaks.	Unalign the image using the Aligned button in the TapeStation Analysis software. Contaminant peaks can be unassigned by right-clicking on either the gel image or electropherogram peak. Rerun samples if needed.
A bubble could be located within the gel matrix		A bubble in the gel matrix introduces significant artifacts in the data. Those bubbles cannot be flicked away.	Always inspect ScreenTape devices for bubbles in the lanes. Lanes in question cannot be used for analysis. Such bubbles can form after the expiration date or upon wrong storage. See the Agilent Information Center and " ScreenTape Devices " on page 86 for more information.
The genomic DNA sample has degraded		Genomic DNA might be subject to apoptotic laddering. It is the result of natural DNA fragmentation processes occurring during apoptosis. See the Agilent Information Center for more information.	This is not an analytical artifact but shows the true state of the genomic DNA sample.
Reagent Buffer or sample contaminated with ladder		Failure to change pipette tips in between pipetting steps might lead to Reagent Buffer or sample being contaminated with ladder.	Use new Reagent Buffer vial and make sure to change pipette tips in between pipetting steps. See " Sample Preparation " on page 90 for more information

Table 10 DNA - probable causes for unexpected migration profile

Problem	Likely cause	Explanation	Solution
The upper and lower markers appear unusually intense in the gel image.	This effect might appear during normal alignment and scaling.	DNA lower markers are normalized to appear for all lanes the same in the electropherogram, which can result in sample peaks appearing smaller than expected.	To get an idea about raw fluorescence intensities, unalign the image using the Aligned button in the TapeStation Analysis software.
	Sample prepared too long before analysis	Evaporation or settling may alter the sample to buffer ratio.	Ensure that your run is started immediately after sample preparation.
	Insufficient Mixing	Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.	See “Mixing Recommendations” on page 93 and the Agilent Information Center for more information.
	The sample analyzed is too diluted	The sample concentration is below the limit of detection.	Concentrate your sample until it is within the recommended range for the application; and then prepare a new run, see “Quantitative range” on page 107. Use the High Sensitivity assay if applicable.

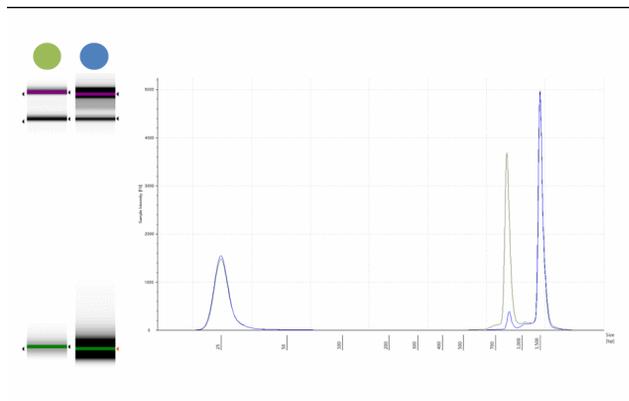


Figure 40 Example of intense upper and lower markers seen in the blue gel image. Lower markers are normalized in electropherogram view and appear identical in signal.

10 Troubleshooting

Troubleshooting DNA Applications

Table 10 DNA - probable causes for unexpected migration profile

Problem	Likely cause	Explanation	Solution
Higher than normal background noise	Insufficient Mixing	Buffer and sample were not sufficiently vortexed. The dye could not incorporate completely into the sample.	See “ Mixing Recommendations ” on page 93 and the Agilent Information Center for more information.
	Incorrect ScreenTape device or reagent storage	Incorrect storage (too cold or too warm) may accelerate aging of the ScreenTape device and reduce the shelf life. Storing reagents incorrectly may induce degradation of the dye. Both can lead to a higher than normal background noise.	Follow the storage conditions that are specified for the assay (see Table 3 on page 60).

Table 10 DNA - probable causes for unexpected migration profile

Problem	Likely cause	Explanation	Solution
Low signal intensity of sample or marker peaks (one or other, not both)	Insufficient Mixing	Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.	See “ Mixing Recommendations ” on page 93 and the Agilent Information Center for more information.
	Incorrect ScreenTape device or reagent storage	Incorrect storage (too cold or too warm) may accelerate aging of the ScreenTape device and reduce the shelf life. Storing reagents incorrectly may induce degradation of the dye. Both can lead to a higher than normal background noise.	Follow the storage conditions that are specified for the assay (see Table 3 on page 60).
	Sample concentration outside recommended range for application	Linear concentration estimation cannot be achieved for samples exceeding the limits in the specification table.	Either dilute or concentrate the sample until it is within the recommended range for the application as stated in the respective assay Quick Guide; and then prepare a new run, see “ Quantitative range ” on page 107. Use the High Sensitivity assay if applicable.

10 Troubleshooting

Troubleshooting DNA Applications

Table 10 DNA - probable causes for unexpected migration profile

Problem	Likely cause	Explanation	Solution
Low signal intensity of sample and marker peaks (both) or of ladder peaks	Incorrect reagent storage	Incorrect storage of the reagents may induce degradation of the dye. This can lead to a higher than normal background noise.	Follow the storage conditions that are specified for the assay (see Table 3 on page 60).
	Incorrect reagents used	Components of High Sensitivity Assay Reagents are too diluted to be properly detected by Standard Sensitivity Assays, see “Reagents and Reagent Mixes” on page 85.	Use the correct protocol and consumables
Incorrect migration - samples have not reached the lower end of the gel lane	Salt concentration	High salt concentrations can cause short running within the gel lane which can subsequently cause incorrect identification of Lower marker peaks, see “Maximum Buffer Concentration in Sample” on page 110.	Unalign the image using the Aligned button in the TapeStation Analysis software. This will show the true position of all peaks present, see “Peak Integration” on page 101. Refer to the salt tolerance guidelines for the assay (see Table 3 on page 60).
	Partial electrophoresis failure caused by bubbles at gel-buffer interface	Air bubbles at the gel-buffer interface may disrupt current flow between the electrodes.	Ensure ScreenTape device is flicked to remove any bubbles before use. See the Agilent Information Center and “ScreenTape Devices” on page 86 for more information.

Cell-Free DNA Assay

High EDTA Concentration in Sample

A high concentration of EDTA in the sample might result in a sizing offset. This is caused by a decreased run distance of the lower marker. EDTA may be a contaminant in the sample as a remnant from sample preparation. Please refer to the maximum buffer concentration in sample in the Quick Guide (Table 3 on page 60).

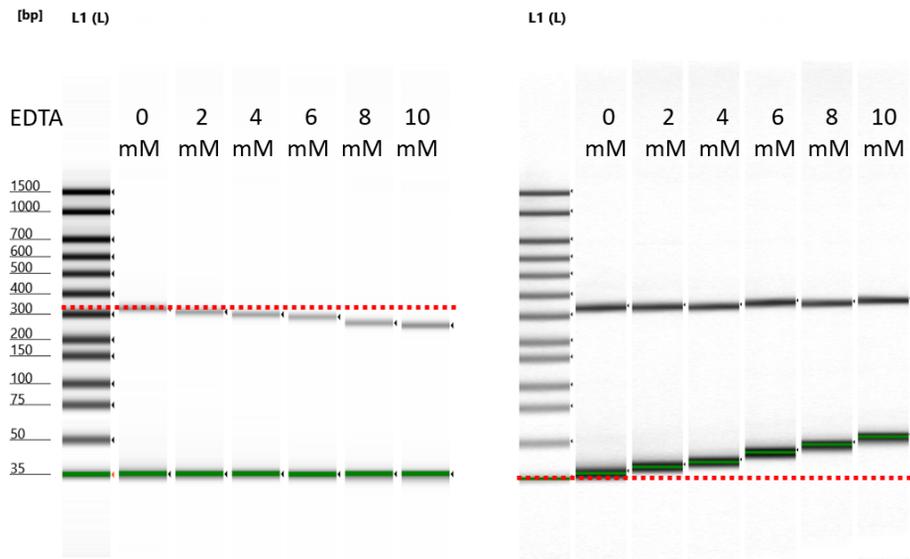


Figure 41 Effect of increasing EDTA concentration on lower marker run distance in aligned (left) and unaligned (right) gel view in the TapeStation Analysis software.

Split Genomic DNA Peak

A genomic DNA contamination with a DIN <6 in a cell-free DNA sample might erratically show a split peak instead of a single peak. Performance such as quantification or %cfscore is not impaired in such a case.

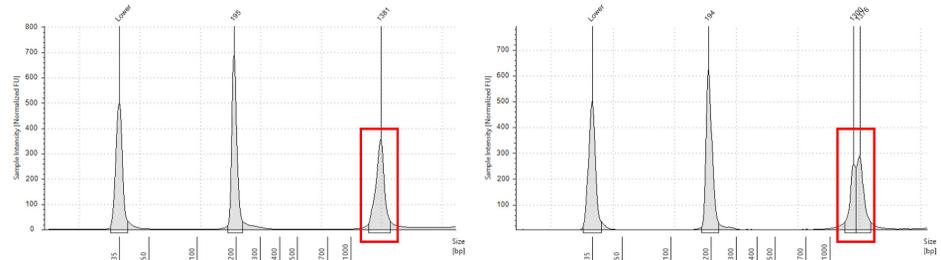


Figure 42 Genomic DNA contamination in a cell-free DNA sample as single peak (left) and split peak (right) in the electropherogram view in the TapeStation Analysis software.

TapeStation Analysis in the Agilent SureSelect and HaloPlex Workflow

The TapeStation system has been verified for use within the Agilent SureSelect and HaloPlex protocols.

- During the purification step however, residual AMPure XP beads can result in a signal which runs with the upper marker peak.
 - Any additional signal under the upper marker peak causes a lower reported value of sample concentration.
 - This artifact can be avoided by increasing the time for which the samples are incubated on the magnetic plate to 10 minutes, thereby removing a higher percentage of the beads.

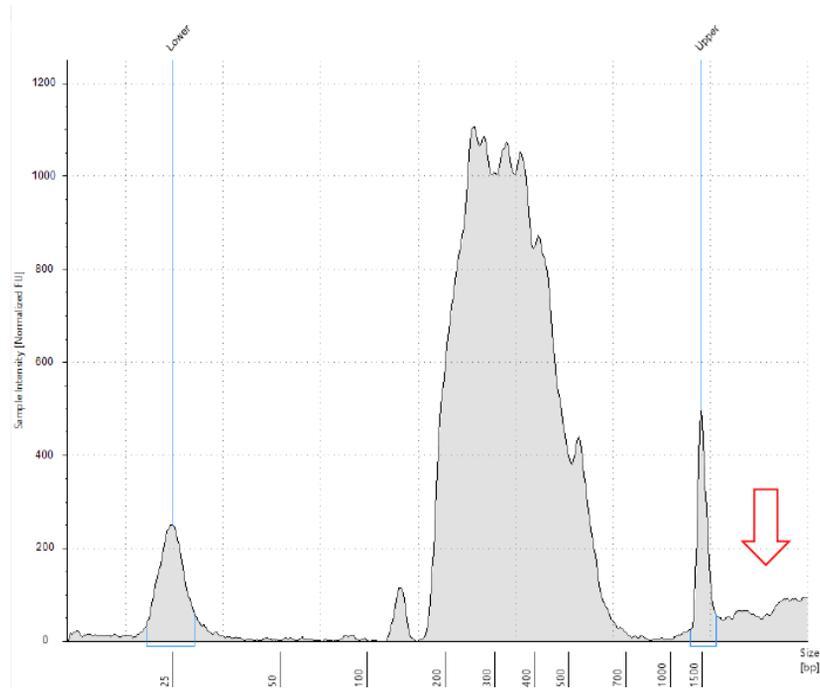


Figure 43 Electropherogram image with additional signal from AMPure beads above the Upper Marker peak (arrow)

10 Troubleshooting

Troubleshooting DNA Applications

- An additional peak within the library smear appears in the analysis file.
 - This peak is due to a concentration-dependent stacking effect and represents no contamination in the library.
 - This peak fronting can occur in Low Input libraries with medium to low concentrations.
 - Sizing and quantification results obtained in the Region View is still within the given specifications.
 - For examples with expected results within an Agilent SureSelect or HaloPlex workflow please see the demo files available in the TapeStation Analysis software.

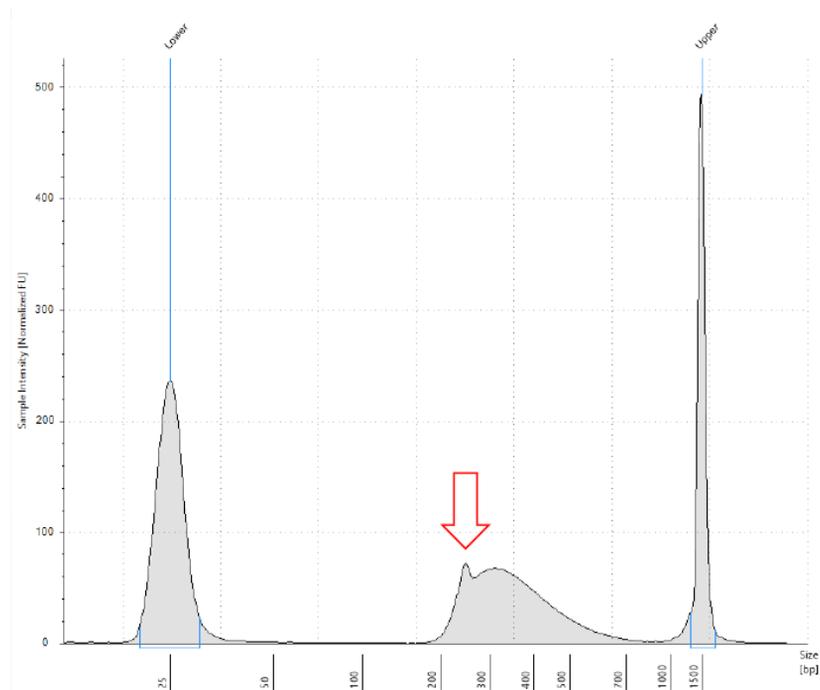


Figure 44 Electropherogram image with additional peak in a library smear (arrow)

Troubleshooting RNA Applications

NOTE

The troubleshooting information is complemented by How To videos or animated graphics which are provided in the Agilent Information Center (see “[Agilent Information Center](#)” on page 56).

Incorrect Quantification Results

Quantification calculation is facilitated by comparing the area under a peak or region of interest in the electropherogram with the area under the Lower Marker. The Lower Marker has a defined concentration.

Incorrect quantification results can have multiple causes. If, after checking each of these causes, your results files continue to show concentration discrepancies, please contact your local support representative.

Table 11 RNA - Possible causes of incorrect quantification in order of probability.

Root cause	Explanation	Solution
Insufficient Mixing	Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.	See “ Mixing Recommendations ” on page 93 and the Agilent Information Center for more information.
Sample concentration outside recommended range for application	Linear concentration estimation cannot be achieved for samples exceeding the limits in the specification table.	Either dilute or concentrate the sample until it is within the recommended range for the application as stated in the respective assay Quick Guide; then prepare a new run. Use the High Sensitivity assay if applicable (“ Quantitative range ” on page 107, Table 3 on page 60).
Incorrect pipetting technique or pipette calibration	Incorrect pipetting technique or uncalibrated pipettes can lead to poor pipetting performance which in turn alter sample/buffer ratio in an unpredictable manner.	See “ Good Pipetting Practice ” on page 84 and follow guidelines of the respective pipette vendor.

10 Troubleshooting

Troubleshooting RNA Applications

Table 11 RNA - Possible causes of incorrect quantification in order of probability.

Root cause	Explanation	Solution
Sample not collected at bottom of vial	Insufficient volume pipetted by the robotics and applied to the lane may affect the migration behavior and alter the band shape.	Make sure to collect all liquid at the bottom of the vial by centrifugation. See “Centrifugation Recommendations” on page 96. Ensure that after removing strip caps and inserting the tube strips or 96-well sample plate into the TapeStation instrument liquid is still located at the bottom of the well, and is not spilled.
Incorrect peak integration	The algorithm cannot establish a correct concentration estimation if marker or sample peaks are not completely encompassed because the signal from either marker or sample is left out of the calculation.	Ensure that all sample peaks and markers are integrated correctly in the TapeStation Analysis software, by clicking and dragging so that the whole peak is encompassed, see “Peak Integration” on page 101. See the Agilent Information Center for more information.
Incorrect Marker peaks picked up	Depending on the sample pattern wrong peaks can get assigned as marker if running very close to the actual marker.	Use the Aligned button to detect the correct markers. Compare to aligned/unaligned data of good, for example the ladder, and questionable samples, then assign correct markers by right-clicking. See the Agilent Information Center for more information. Ensure that all sample peaks are within the recommended “Sizing Range” on page 106 for the application.
Incorrect heating procedure	Both over and under-heating the samples can affect concentration values due to differences in the migration behavior of RNA still trapped in secondary structure.	Ensure that the samples are heat denatured according to the assay instructions before running.
Sample and the marker relevant for quantification have merged.	Additional sample overlaying with the marker will alter the known concentration of the Lower Marker.	For these samples an accurate quantification cannot be provided. Highly concentrated samples may be diluted in order to minimize the amount of sample merging with the Lower Marker.
	Contaminating beads can alter the run behavior of the sample and can introduce artifacts.	If using the Sure Select Protocol, ensure that all AMPure beads are removed by increasing the time that the sample is on the magnetic plate.

Table 11 RNA - Possible causes of incorrect quantification in order of probability.

Root cause	Explanation	Solution
Incorrect protocol used	Differences exist between Standard and High Sensitivity assay protocols.	Ensure that the correct sample protocol for the application is used, “ Reagents and Reagent Mixes ” on page 85.
Sample prepared too long before analysis	Evaporation or settling may alter the sample to buffer ratio.	Ensure that your run is started immediately after sample preparation.
	The sample/buffer mix may evaporate over time if a 96-well sample plate was used which was not sealed with the recommended seal foil.	Always cover with the recommended foil seal (PN 5067-5154) and ensure tight sealing throughout the 96-well sample plate.
Incorrect reagents used	Reagents from different assays may differ in dye and marker concentration. Furthermore markers used are different and upper markers may not be present.	Ensure that the correct reagent buffer for the application is used, see “ Reagents and Reagent Mixes ” on page 85.

Missing Marker Peaks

Table 12 RNA - Possible causes of missing marker peaks in order of probability

Cause	Explanation	Solution
Sample concentration outside recommended range for application	The majority of the dye binds to the sample so that the signal from the marker is too weak to be detected if the sample is too concentrated.	Dilute your sample until it is within the recommended range for the application as stated in the assay Quick Guide, see Table 3 on page 60; then prepare a new run, see “ Quantitative range ” on page 107.
Insufficient migration	Marker peak may not be completely resolved from a sample peak or is not at an expected run distance.	To check migration true distance, unalign the gel image using the Aligned button. See “ Unexpected Migration Profile ” on page 186, “ Maximum Buffer Concentration in Sample ” on page 110, and “ Quantitative range ” on page 107.
Blank lane		See the Agilent Information Center for more information.

Degraded RNA Ladder and/or Samples

Table 13 RNA - Possible causes of degraded sample in order of probability

Root cause	Explanation	Solution
Incorrect heating step	If heat denaturation was too long and/or too high temperatures were used the sample will degrade upon this treatment	Ensure the correct Heat Denaturations for the assay are followed as described in the appropriate Quick Guide, then prepare a new run (“ Heat Denaturation ” on page 98, Table 3 on page 60)
Sample concentration outside recommended range for application	A too concentrated sample will significantly alter the electropherogram	Either dilute or concentrate your sample until it is within the recommended range for application as stated in the assay Quick Guide, then prepare a new run (“ Quantitative range ” on page 107)
Insufficient migration due to presence of salt	Sample matrices exceeding the maximum buffer strength specifications may alter currents in an unpredictable manner leading to short running lanes. See “ Maximum Buffer Concentration in Sample ” on page 110.	Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by unaligning your gel image by pressing the Aligned button in the TapeStation Analysis software. Please refer to the salt tolerance guidelines for your assay in the appropriate Quick Guide (Table 3 on page 60)
RNase contamination of the reagents	RNase contamination will lead to degradation of the sample	Ensure Good Measurement Practices when working with RNA assays; then prepare a new run using new reagents (“ Good Measurement Practices ” on page 81).
RNase contamination of the plasticware	RNase contamination will lead to degradation of the sample.	Ensure Good Measurement Practices when working with RNA assays; then prepare a new run using new reagents (“ Good Measurement Practices ” on page 81).

Incorrect or Missing RIN^e Value

The RNA Integrity Number equivalent (RIN^e) is an automated numerical software assessment which allows determination of sample integrity. It was validated to match the RNA Integrity Number (RIN) of the Agilent 2100 Bioanalyzer system. The algorithm takes into account the peak height of the 18S peak in relation to the background signal to calculate a RIN^e value for total RNA.

Table 14 RNA - Possible causes for missing or incorrect RIN^e results in order of probability.

Root cause	Explanation	Solution
Sample concentration outside recommended range for application	An overconcentrated sample will alter the electropherogram significantly. A sample below the detection limit may also not get an assigned RIN ^e	Either dilute or concentrate the sample until it is within the recommended range for the application as stated in the respective assay Quick Guide (see also “RIN^e Functional Range” on page 112); then prepare a new run. Use the High Sensitivity assay if applicable (Table 3 on page 60)
Incorrect heating step	The 18S peak may have partially reverted back to its original, non-denatured conformation which results in characteristic double peaks and influences RIN ^e calculation	Ensure the correct denaturation conditions during RNA sample preparation, according to the assay Quick Guide. Samples should be kept on ice after preparation, and run within 2 hours of denaturation (see “Heat Denaturation” on page 98) (Table 3 on page 60)
Genomic DNA contamination	Samples that are contaminated with genomic DNA contain additional signals migrating in and beyond the region of the 18S and 28S. Occasionally this peak can be mistaken for the 18S or 28S peak	Ensure that all peaks are annotated correctly and correct if this is appropriate. Treat samples with DNase to eliminate the genomic DNA peak then prepare a new run
There are 2 bands present at the 18S peak position	There may have been insufficient sample denaturation prior to analysis.	Ensure the correct denaturation conditions during the RNA sample preparation, according to the assay Quick Guide (Table 3 on page 60).
	The 18S peak may have partially reverted back to its original, non-denatured conformation.	Samples should be kept on ice after preparation, and run within 2 hours of denaturation, see “Heat Denaturation” on page 98 for more information.

Incorrect or Missing Peak Annotation

Table 15 RNA - Possible causes of incorrect or missing peak annotation in order of probability.

Root cause	Explanation	Solution
Peak is not a ribosomal RNA or Lower Marker peak	Only specific RNA peaks (16, 18, 23, 28S) and Lower marker peak will be annotated by the software	Manually add any important peaks by right-clicking on either the gel or electropherogram and selecting add other peak. See the Agilent Information Center for more information
Extremely degraded sample	The TapeStation Analysis software will only recognize ribosomal RNA sample peaks at expected positions. If no distinct peak is found at that position, no peak will be assigned	The TapeStation Analysis software can occasionally fail to recognize peaks extremely degraded RNA samples. Ensure that Good Measurement Practices are followed as detailed in "Heat Denaturation" on page 98; then prepare a new run ("Good Measurement Practices" on page 81)
Incorrect heating step	The 18S peak may have partially reverted back to its original, non-denatured conformation which results in characteristic double peaks and influences RIN ^e calculation	Ensure the correct denaturation conditions during the RNA sample preparation, according to the assay Quick Guide. Samples should be kept on ice after preparation, and run within 2 hours of denaturation, see "Heat Denaturation" on page 98 (Table 3 on page 60)
Genomic DNA contamination	Samples that are contaminated with genomic DNA contain a third peak which migrates in the region of the 18S and 28S. Occasionally this peak can be mistaken for the 18S or 28S peak	Ensure that all peaks are annotated correctly. Treat samples with DNase to eliminate the genomic DNA peak; then prepare a new run

Unexpected Migration Profile

There are a number of issues which can affect migration and sample peak profile. If the issues below do not describe your analysis file, please contact your support representative.

Table 16 RNA - Possible causes of unexpected migration profile.

Problem	Likely Cause	Explanation	Solution
Additional bands are present in the region of the 18S and 28S peak	Incorrect heating step	The 18S peak may have partially reverted back to its original, non-denatured conformation which results in characteristic double peaks and influences RIN ^e calculation	Ensure the correct denaturation conditions during the RNA sample preparation, according to the assay Quick Guide. Samples should be kept on ice after preparation, and run within 2 hours of denaturation (see “Heat Denaturation” on page 98) (Table 3 on page 60)
	Genomic DNA contamination	Samples that are contaminated with genomic DNA contain additional signals migrating in and beyond the region of the 18S and 28S. Occasionally this peak can be mistaken for the 18S or 28S peak	Ensure that all peaks are annotated correctly and correct if this is appropriate. Treat samples with DNase to eliminate the genomic DNA peak then prepare a new run. See the Agilent Information Center for more information.
	Contamination with prokaryotic total RNA	Ribosomal RNA peaks, namely 16S and 23S, are smaller than eukaryotic 18S and 28S rRNA peaks and as such can be seen in the analysis. See the Agilent Information Center for more information.	If not intended or expected ensure that sample tissue is free of prokaryotic contaminations.
	There may be a contaminant peak that is caused by dust or dirt on the ScreenTape device	Peaks caused by contaminants appear as sharp strong bands which are often slanted or uneven in the gel image, and are usually easily distinguishable from sample peaks	Unalign the image using the Aligned button in the TapeStation Analysis software. Contaminant peaks can be unassigned by right-clicking on either the gel image or electropherogram peak. Rerun samples if needed
	A bubble could be located within the gel matrix	A bubble in the gel matrix will introduce significant artifacts in the data. Those bubbles cannot be flicked away	Always inspect ScreenTape devices for bubbles in the lanes. Lanes in question cannot be used for analysis. Such bubbles can form after the expiration date or upon wrong storage. See the Agilent Information Center or “ScreenTape Devices” on page 86 for more information

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Troubleshooting RNA Applications

Table 16 RNA - Possible causes of unexpected migration profile.

Problem	Likely Cause	Explanation	Solution
A split or additional unexpected peak is evident in the RNA ladder	Insufficient denaturation of the RNA ladder	Some peaks may have partially reverted back to its original, non-denatured conformation which results in characteristic double peaks	Ensure the correct denaturation conditions when preparing the ladder. Ladder and samples should be kept on ice, and run within 2 hours of denaturation (see “Heat Denaturation” on page 98)
	Wrong peak assigned	In rare cases, shoulders of peaks can get assigned as ladder peaks	Delete the wrong peaks and assign correct ladder peaks if needed. See the Agilent Information Center for more information
Additional bands are present at the top of the gel image	Incorrect ScreenTape device storage	This peak is comparable to the 'Well' peak in the Genomic DNA assay (“Genomic DNA Assay” on page 160) and can occur if the ScreenTape device stored incorrectly	Follow the specified storage conditions for the assay (see Table 3 on page 60). Do not leave ScreenTape devices for prolonged period of time at room temperature. See the Agilent Information Center for more information
	The sample may be too concentrated for the application	A too high concentrated sample can result in artifacts in the gel	Either dilute or concentrate your sample until it is within the recommended range for application as stated in the assay Quick Guide (see “Quantitative range” on page 107); then prepare a new run
The gel image looks distorted	Incorrect Marker peaks picked up	Depending on the sample pattern wrong peaks can get assigned as marker if running very close to the actual marker	Use the Aligned button in the TapeStation Analysis software to detect the correct markers. Compare to aligned/unaligned data of good and questionable samples; then assign correct markers by right-clicking. See the Agilent Information Center for more information

Table 16 RNA - Possible causes of unexpected migration profile.

Problem	Likely Cause	Explanation	Solution
Slanted or smeared bands	A bubble could be located at the top of the ScreenTape device	A bubble in the buffer chamber distorts the initial entry of a sample into the gel	Always flick the ScreenTape device before placing into the TapeStation instrument. See “ScreenTape Devices” on page 86
	Sample not collected at the bottom of the vial	Insufficient volume pipetted by the robotics and applied to the lane may affect the migration behavior and alter the band shape	Make sure to collect all liquid at the bottom of the vial by centrifugation. See “Centrifugation Recommendations” on page 96. Ensure that after removing strip caps and inserting the tube strips or 96-well sample plate into the TapeStation instrument liquid is still located at the bottom of the well and is not spilled
	The sample may not have been mixed correctly with the sample buffer	Ionic strength of the sample/buffer mix may not reach expected optimal conditions and thus electrophoresis is impaired	Please follow the mixing recommendations for the assay. See “Mixing Recommendations” on page 93 for more details
	The sample and sample buffer may have started to evaporate	Ionic strength of the sample/buffer mix may exceed the maximum buffer strength due to water loss and thus electrophoresis is impaired	Always ensure that samples are run on the TapeStation instrument immediately after preparation with sample buffer
	A bubble could be located within the gel matrix	A bubble in the gel matrix will introduce significant artifacts in the data. Those bubbles cannot be flicked away	Always inspect ScreenTape devices for bubbles in the lanes. Lanes in question cannot be used for analysis. Such bubbles can form after the expiration date or upon wrong storage. See the Agilent Information Center and “ScreenTape Devices” on page 86 for more information
The markers have appeared in the sample lane, but the sample has not	The sample analyzed is too diluted	The sample concentration is below the limit of detection	Concentrate your sample until it is within the recommended range for the application (see “Quantitative range” on page 107); then prepare a new run. Use the High Sensitivity assay if applicable
	Insufficient Mixing	Buffer and sample were not sufficiently vortexed. The dye could not incorporate completely into the sample	See “Mixing Recommendations” on page 93 and the Agilent Information Center for more information

10 Troubleshooting

Troubleshooting RNA Applications

Table 16 RNA - Possible causes of unexpected migration profile.

Problem	Likely Cause	Explanation	Solution
Additional bands have appeared to those expected in the sample lane	There may be a contaminant peak that is caused by dust or dirt on the ScreenTape device	Peaks caused by contaminants appear as sharp strong bands which are often slanted or uneven in the gel image, and are usually easily distinguishable from sample peaks	Unalign the image using the Aligned button in the TapeStation Analysis software. Contaminant peaks can be unassigned by right-clicking on either the gel image or electropherogram peak. Rerun samples if needed
	A bubble could be located within the gel matrix	A bubble in the gel matrix will introduce significant artifacts in the data. Those bubbles cannot be flicked away	Always inspect ScreenTape devices for bubbles in the lanes. Lanes in question cannot be used for analysis. Such bubbles can form after the expiration date or upon wrong storage. See the Agilent Information Center and “ScreenTape Devices” on page 86 for more information
	Fragmented genomic DNA contamination	Genomic DNA is a common contamination in total RNA samples. Genomic DNA might be subject to apoptotic laddering. It is the result of natural DNA fragmentation processes occurring during apoptosis. See the Agilent Information Center for more information.	Treat the RNA sample with DNase to remove contaminating DNA from the sample
	Reagent Buffer or sample contaminated with ladder	Failure to change pipette tips in between pipetting steps might lead to Reagent Buffer or sample being contaminated with ladder.	Use new Reagent Buffer vial and make sure to change pipette tips in between pipetting steps. See “Sample Preparation” on page 90 for more information

Table 16 RNA - Possible causes of unexpected migration profile.

Problem	Likely Cause	Explanation	Solution
The Lower Marker appears unusually intense in the gel image	This effect might appear in the course of normal alignment and scaling.	Lower markers are normalized to appear the same in the electropherogram, which can result in sample peaks looking smaller than expected	In order to get an idea about raw fluorescence intensities unalign the image using the Aligned button in the TapeStation Analysis software
	Sample prepared too long before analysis.	Evaporation or settling may alter the sample to buffer ratio	Ensure that your run is started immediately after sample preparation. Samples should be kept on ice after preparation, and run within 2 hours of denaturation
	Insufficient Mixing	Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample	See " Mixing Recommendations " on page 93 and the Agilent Information Center for more information
	The sample analyzed is too diluted	The sample concentration is below the limit of detection	Concentrate your sample until it is within the recommended range for application (see " Quantitative range " on page 107); then prepare a new run. Use the High Sensitivity assay if applicable
Higher than normal background noise	Insufficient Mixing	Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample	See " Mixing Recommendations " on page 93 and the Agilent Information Center for more information
	Incorrect ScreenTape device or reagent storage	Incorrect storage (too cold or too warm) may accelerate aging of the ScreenTape device and reduce the shelf life. Storing reagents incorrectly may induce degradation of the dye. Both can lead to a higher than normal background noise	Follow the storage conditions that are specified for the assay (see Table 3 on page 60)

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Troubleshooting RNA Applications

Table 16 RNA - Possible causes of unexpected migration profile.

Problem	Likely Cause	Explanation	Solution
Low signal intensity of sample or marker peaks (one or other, not both)	Insufficient Mixing	Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample	See “ Mixing Recommendations ” on page 93 and the Agilent Information Center for more information
	Incorrect ScreenTape device or reagent storage	Incorrect storage (too cold or too warm) may accelerate aging of the ScreenTape device and reduce the shelf life. Storing reagents incorrectly may induce degradation of the dye. Both can lead to a higher than normal background noise	Follow the storage conditions that are specified for the assay (see Table 3 on page 60)
	Sample concentration outside recommended range for the application	Linear concentration estimation cannot be achieved for samples exceeding the limits in the specification table	Either dilute or concentrate the sample until it is within the recommended range for the application as stated in the respective assay Quick Guide (see “ Quantitative range ” on page 107); then prepare a new run. Use the High Sensitivity assay if applicable
Low signal intensity of sample and marker peaks (both) or of ladder peaks	Incorrect reagent storage	Storing reagents incorrectly may induce degradation of the dye. This can lead to a higher than normal background noise	Follow the storage conditions that are specified for the assay (see Table 3 on page 60)
Incorrect migration - samples have not reached the lower end of the gel lane	Salt concentration	High salt concentrations can cause short running within the gel lane which can cause incorrect identification of Lower marker peaks (see “ Maximum Buffer Concentration in Sample ” on page 110)	Unalign the image using the Aligned button in the TapeStation Analysis software. This will show the true position of all peaks present. Please refer to the salt tolerance guidelines for the assay (see Table 3 on page 60)
	Partial electrophoresis failure that is caused by bubbles at gel-buffer interface	Air bubbles at the gel-buffer interface may disrupt current flow between the electrodes	Ensure ScreenTape device is flicked to remove any bubbles before use. See the Agilent Information Center and “ ScreenTape Devices ” on page 86 for more information

Instrument Communication

Instrument and Laptop

This section gives an overview on good practices which help to mitigate instrument communication issues originating from the control laptop and instrument.

- Always power up the TapeStation System in this order:
 - a** Laptop - allow computer to boot up entirely.
 - b** Instrument - keep lid closed during powering it on.
 - c** TapeStation Controller software

See also [“Preparing the Instrument”](#) on page 61
- Ensure that all data files are stored to a local folder on the laptop first. Copy data only later to network drives. Network connection stability might be insufficient to store data during analytical runs. This can cause data loss and application crashes.
- Operating system regional settings must be set to English (US).
- Do not install additional software applications onto laptops containing the TapeStation Controller software. Review the latest ReadMe.pdf for the TapeStation system.
- Do not use other USB devices while using the TapeStation instrument. The imaging module in the TapeStation instrument is a high resolution device that can utilize the entire bandwidth of the USB link during imaging. Use of other USB devices at the same time can cause timing issues with the imaging module that causes the data acquisition to fail. The use of a USB mouse is not critical.
- All users should always log off entirely from the laptop in case more than one user is set up in the operating system. Processes from other users remain active in the background and can contribute to communication issues. If in doubt, reboot the laptop.
- Power down the laptop and instrument when not in use. Powering down the laptop terminates active processes that might become disturbing. These processes might originate from other applications that were started.
- Always download antivirus software onto the TapeStation laptops. See the latest ReadMe.pdf for known problems or limitations.
- Keep your system up to date with Windows updates.

USB Connection

The TapeStation instrument connects to bundled laptops via a specific USB cable (USB cable A to B, 2 m (5188-8047)). Do not exchange this cable for a different one. Undesired connection timeout due to different transfer rates can occur if using a different cable.

For instructions on connecting your TapeStation instrument and laptop, please consult the section “[Initial Setup of the System](#)” on page 39 and additionally the latest Readme.pdf.

- Do not install other USB devices.
- Review the section “[Windows Device Manager](#)” on page 194 for further details on how to verify a proper USB connection.

NOTE

In case you swap USB ports, give the operating system sufficient time to register this internally. Wait 1 minute after unplugging before plugging the USB connection into a different USB slot.

NOTE

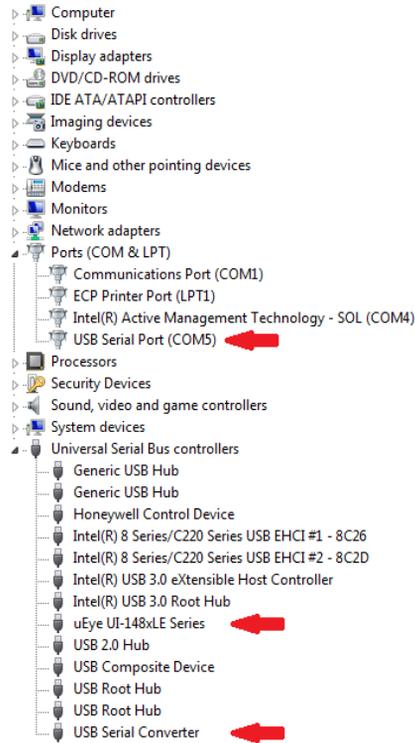
The use of an additional USB mouse is not critical.

Windows Device Manager

Upon connecting the TapeStation instrument to the laptop the device manager of your operating system will populate entries specific for the TapeStation instrument. Please refer to the figure below showing conditions for a correctly established instrument connection. The example shows the HP 650G1 laptop which is one model supplied as bundled laptop with 4200 TapeStation systems. Other bundled laptops will vary in details.

The three relevant items associated with the connection of the TapeStation instrument are:

- 1 USB Serial Port (COM x)
- 2 uEye UI-148xLE Series
- 3 USB Serial Converter



NOTE

The COM port number (x) itself is variable and not relevant. It is assigned by the operating system.

1 To access the device manager, go to **Start > Control Panel > Device Manger**.

In case one or more of the entries marked are missing or a problem is reported with one of them by your operating system, follow the steps provided in [“Universal corrective actions”](#) on page 196.

NOTE

Executing the System diagnosis ([“Instrument Diagnosis”](#) on page 114) to verify the suitability of your laptop and proper functions of your instrument assists you to identify and correct connection issues of the system.

Universal corrective actions

This compilation of universal corrective actions will help to eliminate instrument communication issues.

NOTE

Executing the System diagnosis (“[Instrument Diagnosis](#)” on page 114) to verify the suitability of your laptop and proper functions of your instrument assists you to identify and correct connection issues of the system.

NOTE

Ensure that the TapeStation instrument is NOT connected to the laptop during software installation or update. Else device drivers might be malfunctioning.

In case you encounter intermittent communication issues when connecting the instrument to the laptop, follow these steps:

- 1 Check if power and USB cables are plugged into the instrument.
- 2 Try a different USB port. Wait 1 minute between unplugging and re-plugging to another USB port. Check if the original issue persists.
- 3 Restart the instrument with closed lid. Check if the original issue persists.
- 4 Restart the computer and the instrument. Check if the original issue persists.
- 5 Try a different USB port after the computer was restarted and the instrument was initialized again. Check if the original issue persists.
- 6 Make sure all required Windows updates are present. See the latest [ReadMe.pdf](#) for reference.
- 7 Uninstall and reinstall the latest TapeStation software from the original media. Make sure all required Windows updates are installed.

In case above steps did not resolve the original issue, the laptop might need to be re-imaged. This process is supported by the recovery media supplied with the system.

- 1 Carefully backup important data first.
- 2 Reimaging is done with the media provided in the shipment of the TapeStation system.
- 3 Set up the Windows operating system with regional settings English-US.
- 4 Apply Windows Updates through a connection to the internet. This process might be time consuming.

- 5 For HP650G1 computers models: Prior to installation of the TapeStation Software, install at least the Windows Security update KB3033929 for driver authentication.

NOTE

Collect support information (see [“Collecting Troubleshooting Information”](#) on page 202) and contact your local support.

Error Scenario: Instrument Not Connecting

In case an instrument is not connecting properly to the TapeStation Controller software, the user interface remains for 3 minutes with the animated message “Scanning...”. If no connection was established within this time frame, the following message appears: “Instrument detection timeout. Check instrument connection, power-cycle the instrument and restart the software. If the error persists, power-cycle the laptop.”

NOTE

Ensure that the TapeStation instrument is NOT connected to the laptop during software installation or update. Else device drivers might be malfunctioning.

- 1 Follow the instructions provided by the software.
- 2 Execute the **System Diagnostics** ([“Instrument Diagnosis”](#) on page 114) to verify the suitability of your laptop and proper functions of your instrument.
- 3 Review failed tests and correct detected issues, if feasible.

The following table lists information for further corrective actions:

Possible reasons for connection fails	Corrective actions
Instrument is turned off.	Connect power supply cable and turn the instrument on. Verify power cord is the one supplied with the instrument.
USB cable is unplugged or defective.	Connect the supported USB cable (5188-8047) properly or exchange it.
The laptop shows a malfunction in USB connection protocol. The operating system fails to enable USB or COM functionality.	Restart the computer. See also “ Windows Device Manager ” on page 194.
Instrument is not initializing correctly or defective.	Turn the instrument on following all guidelines, see “ Initial Setup of the System ” on page 39 and “ Preparing the Instrument ” on page 61. If the error persists, execute System Verification . Collect support information (see “ Collecting Troubleshooting Information ” on page 202) and contact your local support.
TapeStation Controller software is corrupt or inhibited by other conflicting software.	Power-cycle the laptop to terminate any disturbing application. If the error persists: reinstall the TapeStation Controller software and remove any additional software.
Drivers of other USB devices corrupted or inhibited TapeStation USB Device drivers.	Disconnect and uninstall foreign devices and software.
TapeStation USB Device drivers are not installed or malfunctioning.	Reinstall the TapeStation software package. Make sure all required Windows updates are installed. See the latest ReadMe.pdf for reference.

NOTE

Refer to “[Collecting Troubleshooting Information](#)” on page 202 and contact your local support.

Error Scenario: Unexpected Termination of the TapeStation Controller Software

Unexpected termination of the TapeStation Controller software might happen under the following conditions: A connection to the instrument is established and this is either idle or an analytical run is ongoing.

Termination of the TapeStation Controller software might lead to error messages containing **Unexpected Error** or **Instrument Lost Connection** in the user interface. Be aware in case such termination happens during an analytical run, a truncated data file might contain some results. Navigate with the file explorer of your data system to the data folder and double click on the truncated file.

NOTE

In case a run with multiple tapes is terminated prematurely only that data is available that was recorded until the last completely processed ScreenTape device.

10 Troubleshooting

Instrument Communication

The following table lists information for corrective actions:

Possible reasons for the above error	Corrective actions
Instrument was turned off erroneously.	Verify the instrument has no loose power connection. Put plugs in firmly. Avoid touching the cables while the instrument performs analytical runs. Avoid setups in which the power button can be touched accidentally. Check general power supply for instabilities or open fuses.
Instrument went into error mode (= resident).	Avoid the error conditions. Restart laptop and instrument. The instrument will initialize and clear the error.
USB cable is unplugged; physical connection is instable.	Verify the USB connection has no loose cables. Put plugs in firmly. Exchange cable if appropriate. Avoid touching the cables during analytical runs.
Issue caused by other activities on laptop such as by further USB devices or increased USB traffic on the laptop or busy network connections.	Disconnect and uninstall other USB devices. Avoid activities using the network during analytical runs.
Issue caused by anti-virus scans dragging resources.	Avoid scheduled virus scans during analytical runs. If applicable, disconnect from the world wide web.
Communication issues with unknown root cause while using the laptop differently.	Avoid activities that include installing/uninstalling other applications, drivers, or use of the laptop which create extra USB/LAN data traffic.
Laptop is in power save mode for USB connections or the screen.	Do not activate additional power save settings or sleep modes for the laptop.
Error during startup of the TapeStation Analysis software after ScreenTape run.	Ensure the TapeStation Analysis software has no open dialogue or is in error mode itself. Close the software entirely if in doubt; it will automatically reopen at the end of a tape run to display results.
Unforeseen activities or clicks in TapeStation Controller software.	Reduce activities such as changing preferred settings within the TapeStation Controller software to a minimum.

NOTE

Refer to [“Collecting Troubleshooting Information”](#) on page 202 and contact your local support.

Error scenario: ScreenTape barcode not detected

In case the TapeStation Controller software graphical user interface doesn't display information of inserted tapes or even the assay:

- 1 Execute the **System Diagnostics** to verify the suitability of your laptop and proper functions of your instrument.
- 2 Review failed tests and correct detected issues, if feasible.

Review the following information for further corrective actions:

Possible reasons for the above errors	Corrective actions
ScreenTape device inserted incorrectly.	The barcode must be on the right hand side and facing backwards. Push the ScreenTape device down and ensure it is horizontal.
ScreenTape type not supported.	Do not use ScreenTape device dedicated to other systems.
Barcode is damaged, smeared or absent.	Ensure the barcode region of the ScreenTape device is not exposed to mechanical stress. Do not write on labels or expose them to dyes. Do not remove and reattach the barcode label.
Camera is not in default position after an instrument error.	If the instrument ran into an error earlier allow it to recover from that error by power cycling and restarting the TapeStation Controller software. Keep the lid closed to allow the instrument to fully re-initialize.
The flashing green barcode illumination is absent.	Power cycle the laptop. Power cycle the instrument and restart TapeStation Controller software. Keep the lid closed to allow the instrument to fully re-initialize.
Data transfer issue of camera due to general communication issues.	Follow the " Universal corrective actions " on page 196 for communication troubleshooting.

NOTE

Refer to "[Collecting Troubleshooting Information](#)" on page 202 and contact your local support.

Collecting Troubleshooting Information

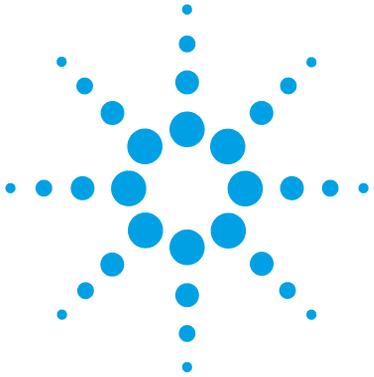
A dedicated feature within the TapeStation Analysis software assists you in your dialog with Agilent technical support experts. For troubleshooting in a support case you might be asked to run the “[Hardware Troubleshooting](#)” on page 117 and then export log files using the menu **File > Help > Export Log Files**.



Export Log Files

Export log files from the Analysis and Controller applications to a zip file.

The collected files contain internal log files generated by your TapeStation system, diagnostic information and result files from the past Instrument Diagnostic. For your convenience, this complete set is collected from various folder locations and provided as a ZIP file on the desktop to be sent out to Agilent technical support experts. Be aware that this package of log files does not include data files, experimental information or sample information. To properly describe your issue completely, you need to collect and compile this information in addition.



11 Appendix

Accessories Shipment List 204

This chapter contains parts information.



Accessories Shipment List

The following table lists accessories shipped together with the Agilent 4200 TapeStation system.

Printed Quick Guides for 4200 TapeStation system

p/n	Description
G2991-90021	Agilent RNA ScreenTape Quick Guide for TapeStation Systems
G2991-90031	Agilent D1000 ScreenTape Quick Guide for TapeStation Systems
G2991-90051	Agilent D5000 ScreenTape Quick Guide for TapeStation Systems
G2991-90041	Agilent Genomic DNA ScreenTape Quick Guide for TapeStation Systems
G2991-90121	Agilent High Sensitivity RNA ScreenTape Quick Guide for TapeStation Systems
G2991-90131	Agilent High Sensitivity D1000 ScreenTape Quick Guide for TapeStation Systems
G2991-90151	Agilent High Sensitivity D5000 ScreenTape Quick Guide for TapeStation Systems

Instrument Accessories

p/n	Description
G2964-64030	Software and User Information, USB stick and Booklet, Box
5067-5601	TapeStation Test Tape
5188-8047	USB cable A to B, 2 m
G2991-40007	4200 TapeStation ScreenTape Rack
G2992-40042	TapeStation Tube Strip Holder, tray for 16 tubes
G2992-40046	TapeStation Tip Waste Bucket, 1 bucket

Additional Material

p/n	Description
401425	Optical Cap 8x Strip, Box of 120, 0.2 mL
401428	Optical Tube 8x Strip, Box of 120, 0.2 mL
5042-8502	96-well plate
5067-5154	96-well Plate Foil Seal, Pack of 100 foils
5067-5598	Loading Tips

NOTE

The initial shipment comes with 4 boxes of loading tips. This is typically suitable for running 4 boxes of 7 ScreenTape devices.

Please order further packs of Loading Tips (5067-5598) or a set of 10 packs of Loading Tips (5067-5599) in order to run more ScreenTape assays.

Other orderable equipment:

p/n	Description
5067-5783	Needle Change Cartridge
5067-5599	Loading Tips 10 packs of 112 tips

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In This Book

The manual describes the following:

- Introduction to the system
- Site requirements and specifications
- Installation
- Software
- Operation of the system
- Instrument diagnosis
- Maintenance
- Troubleshooting
- Product notices

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