Warranty

The material contained in this document is provided “as is,” and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

If software is for use in the performance of a U.S. Government prime contract or subcontract, Software is delivered and licensed as “Commercial computer software” as defined in DFAR 252.227-7014 (June 1995), or as a “commercial item” as defined in FAR 2.101(a) or as “Restricted computer software” as defined in FAR 52.227-19 (June 1987) or any equivalent agency regulation or contract clause. Use, duplication or disclosure of Software is subject to Agilent Technologies’ standard commercial license terms, and non-DOD Departments and Agencies of the U.S. Government will receive no greater than Restricted Rights as defined in FAR 52.227-19(c)(1-2) (June 1987). U.S. Government users will receive no greater than Limited Rights as defined in FAR 52.227-14 (June 1987) or DFAR 252.227-7015 (b)(2) (November 1995), as applicable in any technical data.

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 4150 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 within the TapeStation software.

6 Operating Instructions
This chapter explains the operation of the 4150 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 4150 TapeStation system.

10 Troubleshooting
This chapter gives an overview about the troubleshooting features of the 4150 TapeStation system. It includes information about application troubleshooting as well.

11 Appendix
This chapter contains parts information.
Contents

1 Safety 9
   Symbols 10
   Safety Messages 12
   General Safety Information 13

2 Legal and Regulatory 15
   Sound Emission 16
   Waste Electrical and Electronic Equipment (WEEE) Directive 17

3 Product Description 19
   Intended use of the 4150 TapeStation System 20
   Overview of the System and ScreenTape Architecture 21
   Product Descriptions 25
   Physical Specification 32

4 Installation 33
   Site Requirements 34
   Bench Space Required 36
   Unpacking 38
   Initial Setup of the System 45
   Prepare the Working Area 48

5 TapeStation Software 51
   TapeStation Controller Software 52
   TapeStation Analysis Software 58
   Agilent Information Center 60
## Contents

### 9 Maintenance 123
- Maintenance Information 124
- Exchange the Piercing Needle 127
- Electrode Cartridge 130
- Cleaning the Instrument 146
- Preparing the Instrument for Transportation 147
- Boxing for Transportation 153
- Assembling Nest Covers 156
- Insertion of the Lower Nest Cover 158

### 10 Troubleshooting 165
- Software Messages 167
- Troubleshooting DNA Applications 178
- Troubleshooting RNA Applications 200
- Instrument Communication 212
- Collecting Troubleshooting Information 221

### 11 Appendix 223
- Accessories Shipment List 224
1 Safety

Symbols  10
Safety Messages  12
General Safety Information  13

This chapter provides information on safety symbols and safety messages.
## Symbols

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Symbol]</td>
<td>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.</td>
</tr>
<tr>
<td>![Symbol]</td>
<td>Indicates dangerous voltages.</td>
</tr>
<tr>
<td>![Symbol]</td>
<td>Indicates a protected ground terminal.</td>
</tr>
<tr>
<td>![Symbol]</td>
<td>Confirms that a manufactured product complies with all applicable European Community directives. The European Declaration of Conformity is available at: <a href="http://regulations.corporate.agilent.com/DoC/search.htm">http://regulations.corporate.agilent.com/DoC/search.htm</a></td>
</tr>
<tr>
<td>![Symbol]</td>
<td>Manufacturing date.</td>
</tr>
<tr>
<td>![Symbol]</td>
<td>Power symbol indicates On/Off. The apparatus is not completely disconnected from the mains supply when the power switch is in the Off position</td>
</tr>
<tr>
<td>![Symbol]</td>
<td>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.</td>
</tr>
<tr>
<td>![Symbol]</td>
<td>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.</td>
</tr>
</tbody>
</table>
### Table 1  Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>Indicates a pinching or crushing hazard</td>
</tr>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>Indicates a piercing or cutting hazard.</td>
</tr>
</tbody>
</table>
1 Safety
Safety Messages

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.
General Safety Information

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.
1 Safety
General Safety Information

**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.
2
Legal and Regulatory

Sound Emission 16
Waste Electrical and Electronic Equipment (WEEE) Directive 17

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 Lp <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

Intended use of the 4150 TapeStation System 20
Overview of the System and ScreenTape Architecture 21
  Overview of the System 21
  ScreenTape Architecture 24
Product Descriptions 25
Physical Specification 32

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

Intended use of the 4150 TapeStation System

Intended use of the 4150 TapeStation System

The 4150 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 4150 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 4150 TapeStation instrument offers automated sample processing of ScreenTape applications for DNA and RNA. It makes sample quality control (QC) within next-generation sequencing (NGS) and biobank workflows easier and more economical than ever.

![Overview of the 4150 TapeStation system](image)

The Agilent 4150 TapeStation instrument represents an entry-level system ideal for labs handling smaller number of samples. Up to 16 samples can be processed per run. The pre-installed TapeStation software on the TapeStation software laptop bundle (G2999AA) comprises functionality for instrument control and data analysis. It is intuitive and presents the automatically analyzed data as a familiar gel image and electropherogram for each sample.
The TapeStation software consists of three packages. It comes preinstalled with the laptops bundled to the TapeStation system. Else it needs to be installed, please see the readme.pdf for instructions on this.

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\textsuperscript{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 2  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.
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 4150 TapeStation instrument

Figure 4   Overview of the instrument

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Power button</td>
</tr>
<tr>
<td>2</td>
<td>Lid release button</td>
</tr>
<tr>
<td>3</td>
<td>Lid</td>
</tr>
<tr>
<td>4</td>
<td>Status LED</td>
</tr>
<tr>
<td>5</td>
<td>Serial number</td>
</tr>
</tbody>
</table>
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.

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 Magnet

Figure 5  Position of magnet in the instrument lid
The 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 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 6** Lid operation
3 Overview of the instrument workspace

Figure 7 Overview of the instrument workspace

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gantry cover</td>
</tr>
<tr>
<td>2</td>
<td>Upper nest cover</td>
</tr>
<tr>
<td>3</td>
<td>Lower nest cover</td>
</tr>
<tr>
<td>4</td>
<td>ScreenTape nest</td>
</tr>
<tr>
<td>5</td>
<td>Tip waste</td>
</tr>
<tr>
<td>6</td>
<td>Tip rack</td>
</tr>
<tr>
<td>7</td>
<td>Sample tube strip holder</td>
</tr>
</tbody>
</table>
4 Electrical connections

![Diagram of system with annotations](image)

**Figure 8** 1. Power line in, 2. USB-B to PC

Remaining connectors are blocked by plugs and reserved for factory use.
5 Status indicators (LED)

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>off</td>
<td>Idle</td>
</tr>
<tr>
<td>2</td>
<td>green</td>
<td>Run mode</td>
</tr>
<tr>
<td>3</td>
<td>yellow</td>
<td>Not-ready, waiting for a specific pre-run condition to be reached or completed</td>
</tr>
<tr>
<td>4</td>
<td>red</td>
<td>Error mode (restart required)</td>
</tr>
<tr>
<td>5</td>
<td>red blinking</td>
<td>Error mode (restart required) or ongoing firmware update</td>
</tr>
</tbody>
</table>

Figure 9 Status indicators
6 Status indicators (Lid)

Figure 10  Lid release button status light

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blinking: Note the input request from the TapeStation Controller software</td>
</tr>
<tr>
<td>2</td>
<td>Normal status</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Type</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>17.8 kg (39.2 lbs)</td>
</tr>
<tr>
<td>Dimensions (w×d×h)</td>
<td>254 x 510 x 415 mm (9.6 x 20.1 x 16.3 inches)</td>
</tr>
<tr>
<td>Line voltage</td>
<td>100 – 240 VAC ± 10 %</td>
</tr>
<tr>
<td>Line frequency</td>
<td>50 or 60 Hz ± 5 %</td>
</tr>
<tr>
<td>Power consumption</td>
<td>50 VA</td>
</tr>
<tr>
<td>Interface</td>
<td>USB2 (Instrument: USB-B; PC: USB-A)</td>
</tr>
<tr>
<td>Ambient operating temperature</td>
<td>10 – 40 °C (50 – 104 °F)</td>
</tr>
<tr>
<td>Operating humidity</td>
<td>15 – 80 %, non-condensing</td>
</tr>
<tr>
<td>Storage temperature</td>
<td>-40 to 70 °C (-40 to 158 °F)</td>
</tr>
<tr>
<td>Operating altitude</td>
<td>Up to 3000 m (9842 ft)</td>
</tr>
<tr>
<td>Safety standards</td>
<td>IEC, EN, CSA, UL, Installation category II, Pollution degree 2</td>
</tr>
<tr>
<td>ISM Classification</td>
<td>ISM Group 1 Class A, According to CISPR 11</td>
</tr>
<tr>
<td>Sound pressure</td>
<td>&lt;70 dB (A)</td>
</tr>
</tbody>
</table>

### Assay specific temperature range

- **D1000 ScreenTape**: 12 – 37 °C (54 – 99 °F)
- **High Sensitivity D1000 ScreenTape**: 17 – 37 °C (63 – 99 °F)
- **Genomic DNA ScreenTape**: 15 – 30 °C (59 – 86 °F)
- **RNA and High Sensitivity RNA ScreenTape**: 14 – 30 °C (57 – 86 °F)
- **D5000 ScreenTape**: 15 – 30 °C (59 – 86 °F)
- **High Sensitivity D5000 ScreenTape**: 15 – 30 °C (59 – 86 °F)
- **Cell-free DNA ScreenTape**: 16 – 30 °C (61 – 86 °F)

### NOTE

This is an ISM 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.

### NOTE

The assay specific temperature range is measured at the ScreenTape nest. Choose the location of the instrument and the ambient conditions to fit to the working range of the assay else the start of runs will be disallowed.
4

Installation

Site Requirements 34
Bench Space Required 36
Unpacking 38
Initial Setup of the System 45
Prepare the Working Area 48

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 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.

Identify the laboratory bench space based on the specifications table (see Table 2 on page 32).

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 required to control 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 32. 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**

**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 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. Place the instrument box on the floor and cut it open.
2  Pull the instrument out of the outer cardboard box.

3  Turn the instrument over to a side.
Unpacking

4. Remove the outer cartons and one part of the foam.
Heart pacemakers

**WARNING**
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.

---

5 Lift the instrument out of the remaining foam onto a workbench. See step on page 26 for the position of the magnet in the instrument lid.
4 Installation

Unpacking

6 Open the lid.
7 Remove the workspace foam.

8 Remove the Vision lock tape.
9 Carefully pull the gantry towards the front of the instrument.

**CAUTION**

**Damaged cables**

Instrument damage at pump cables.

➔ Handle the foam carefully without touching or damaging the cables.

10 Carefully lift the pump foam upwards.
11 Push the gantry carefully towards the back of the instrument.

12 Close the lid.

13 Keep all packaging material for later storage or shipment.
Initial Setup of the System

**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.

---

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.

Refer to “Preparing the Instrument” on page 65 for the required order when turning the instrument on.
4 Installation
Prepare the Working Area

Prepare the Working Area

1 Install the tip waste.

2 Install the sample tube strip holder.
3 Install the tip rack and remove the lid.
Prepare the Working Area

**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.

4 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.
5

TapeStation Software

TapeStation Controller Software 52
  TapeStation Controller Software User Interface 53
  TapeStation Controller Software Settings 55
  System Diagnostics 57
TapeStation Analysis Software 58
Agilent Information Center 60

This chapter gives an overview on the three packages within the TapeStation software.
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 63 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.
The TapeStation Controller software user interface consists of following items.

- **Ladder location**
- **Information field**
- **Sample tube strips**
- **Requirements**
- **ScreenTape image**
- **Start button**
- **Status messages**
- **Setting dialogue box**
- **Table functions**
- **Sample table**

**Figure 11**  TapeStation Controller software user interface for 4150 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 55)
- Sample locations on Sample tube strips can only be selected once a ScreenTape device is inserted. Up to 16 samples can then be selected by clicking and dragging across the tube strip. When samples are selected, the sample table populates the corresponding lines to allow you to enter sample descriptions.
- Sample 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 an optional second ScreenTape device, 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 the **Required For Run** field. Required additional ScreenTape devices appear in situations where the device in the ScreenTape nest has less unused lanes than samples were selected. In such case the TapeStation Controller software will prompt for insertion of the second ScreenTape during the run. A time window of 15 min for this applies.

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

Figure 12  TapeStation Controller software settings menu for the 4150 TapeStation instrument

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

• Access the Settings dialog box.

• **Maintenance** menu: Prepare the **Transportation lock** (see “Preparing the Instrument for Transportation” on page 147).

• Read diagnostic values from the instrument, see “Needle Pierce Counter” on page 124.

• Perform a needle exchange, see “Exchange the Piercing Needle” on page 127.

• Change the Electrode Cartridge, see “Exchange the Electrode Cartridge” on page 135.

• 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.

- **ScreenTape Settings**
- **RNA Setting Button**
- **File Name Configuration**
- **File Save Subdirectory Option**
- **File Save To Selection**

**Figure 13** TapeStation Controller software settings dialog box

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 122). By default result files from these test suites are collected into exported log files for remote support purposes (see “Collecting Troubleshooting Information” on page 221).

![System Diagnostics](image.png)

**Figure 14** TapeStation Controller software: **System Diagnostics** context
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\textsuperscript{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 60) 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 15  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 16  Agilent Information Center for the TapeStation system
This chapter explains the operation of the 4150 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 224). 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.

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

### Table 3  ScreenTape Assay portfolio

<table>
<thead>
<tr>
<th>ScreenTape Assay</th>
<th>Quick Guide Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent D1000 ScreenTape Assay</td>
<td>G2991-90031</td>
</tr>
<tr>
<td>Agilent High Sensitivity D1000 ScreenTape Assay</td>
<td>G2991-90131</td>
</tr>
<tr>
<td>Agilent RNA ScreenTape Assay</td>
<td>G2991-90021</td>
</tr>
<tr>
<td>Agilent High Sensitivity RNA ScreenTape Assay</td>
<td>G2991-90121</td>
</tr>
<tr>
<td>Agilent Genomic DNA ScreenTape Assay</td>
<td>G2991-90041</td>
</tr>
<tr>
<td>Agilent D5000 ScreenTape Assay</td>
<td>G2991-90051</td>
</tr>
<tr>
<td>Agilent High Sensitivity D5000 ScreenTape Assay</td>
<td>G2991-90151</td>
</tr>
<tr>
<td>Agilent Cell-free DNA ScreenTape Assay</td>
<td>G2991-90060</td>
</tr>
</tbody>
</table>

Please notice a documentation on “Good Measurement Practices” on page 81. Find information on “Troubleshooting DNA Applications” on page 178 and “Troubleshooting RNA Applications” on page 200.

The assay-specific Quick Guides contain important information on the ladder, see also “Ladder Considerations” on page 92. Please read this section carefully.
Preparing the Instrument

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

2  Turn On

Figure 17  Power switch. 1: On; 2: Off
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 212.

5 Open the lid by pressing the lid release button.
6 Empty the tip waste.

7 Install the tip rack and remove the lid. Ensure sufficient tips are present.
Preparing the Instrument

8 Flick the ScreenTape device. Trapped air bubbles will move up in the buffer chamber. For details, see “ScreenTape Devices” on page 87

**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.
9 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 90.

10 The TapeStation Controller software interface will change and displays the ScreenTape assay recognized. For details, see “TapeStation Controller Software” on page 52.

11 Centrifuge samples to remove spills and air bubbles. For details, see “Centrifugation Recommendations” on page 97.
CAUTION  Blockage of the robotic system

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

12 Insert sample tube strips. Remove the caps.
13 Ladder is exclusively provided from location A1 on the sample tube strip holder. For further details on handling the ladder within assays see the assay-specific Quick Guides or the section “Ladder Considerations” on page 92.

**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.
Prepare 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.

14 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.

15 Close the lid.
Starting the Analysis

1. Select samples for the analysis on the tube strip holder.

2. Enter sample descriptions.

3. Optional: Modify user name, notes, lot information and filename prefix. Add reagent lot and ScreenTape lot information.

4. Note required consumables: Make sure sufficient tips are present.
5 Note required consumables: A second ScreenTape device will be requested during the run if the first ScreenTape device has insufficient free lanes. Make sure a second ScreenTape device with sufficient lanes is available. A time frame of 15 minutes applies once the TapeStation Controller software prompts for this.

6 Make sure sufficient ladder is present.

7 Start the run.

8 Confirm tip waste is empty. Confirm tube strip lids are removed and proceed.

9 Presence of components such as tube strip lids, tips, and ScreenTape devices are checked.

**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, refer to “Assembling Nest Covers” on page 156, “Insertion of the Lower Nest Cover” on page 158 and “Software Messages” on page 167 for further information.
10 Instrument initialization and sample analysis take place. The remaining run time is displayed.

11 Sample analysis may be aborted immediately or stopped after sample analysis if desired. The lid button does blink while this dialog is displayed.
12 Insufficient lanes on a ScreenTape devices were present. Add a second ScreenTape device with sufficient unused lanes within 15 min to finalize the analysis.
Analysis End

Once the entire analysis is complete the instrument is reset and the lid unlocked. After acquisition of data from a first tape 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 two ScreenTape devices, the TapeStation Analysis Software will launch in read-only mode after data from the first ScreenTape device is available. Data from the additional ScreenTape device are added to this read-only file until the second ScreenTape device has been run; then the file is 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.
2 Empty the tip waste.

3 Remove the sample tube strip.
6 Operating Instructions

Follow Up

4 Cover the tip rack with lid until next use.

5 Close the lid.
This chapter summarizes information on good measurement practices for ScreenTape assays.
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:
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 94.
- 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 64).

- 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 94, “Good Pipetting Practice” on page 85, and the appropriate Quick Guide (Table 3 on page 64).

- 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.

**NOTE**
Failure to remove air bubbles at the gel/buffer interface might significantly affect results.
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 55. Resulting data are marked accordingly.
• 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 19](image)

**Figure 19** Different effects of bubbles in the gel matrix on TapeStation Analysis software results - Gel view
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.

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 64).
- 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 64).
- 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 85. Improper mixing can lead to quantification errors, see “Mixing Recommendations” on page 94 (Table 3 on page 64).
- 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 µL 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 64).

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 53.

**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 64).

![Figure 20](image)

**Figure 20** 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.

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.
Figure 22  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 23  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

<table>
<thead>
<tr>
<th></th>
<th>Measured concentration (ng/µL)</th>
<th>Theoretical concentration (ng/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vortex mixing then centrifugation</td>
<td>68.5</td>
<td>70</td>
</tr>
<tr>
<td>Pipette mixing only</td>
<td>21.2</td>
<td>70</td>
</tr>
<tr>
<td>No mixing</td>
<td>6.5</td>
<td>70</td>
</tr>
</tbody>
</table>

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.
1. Centrifuge samples to remove spills and air bubbles.

**Figure 24** 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 μL 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

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 200.

Figure 25  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.
Good Measurement Practices
RNA Assays

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 26](image_url)

**Figure 26** 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 27](image) Correct upper marker integration for DNA ScreenTape assays. Figure shows D1000 ScreenTape assay.
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 28** 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 29** 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.
**Figure 30**  The sizing data obtained in Electropherogram and Region views of the TapeStation Analysis software.

**Figure 31**  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 64).

Figure 32 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 33 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 64).

Figure 34  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.
Figure 35  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 36  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 64).
Good Measurement Practices

Maximum Buffer Concentration in Sample

Figure 37  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\textsuperscript{e} Functional Range

To achieve accurate RIN\textsuperscript{e} scores, the samples used must be within the RIN\textsuperscript{e} functional range as stated in the respective assay Quick Guide. Samples with concentrations exceeding the RIN\textsuperscript{e} functional range can show altered electropherogram traces. Obtained RIN\textsuperscript{e} scores are significantly impacted by this (Table 3 on page 64).

**Figure 38** Effects of overconcentrated samples on run distance of the Lower Marker and RIN\textsuperscript{e} values in the High Sensitivity RNA ScreenTape assay in the Gel view of the TapeStation Analysis software.
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 39](image-url)  
*TapeStation Controller software: System Diagnostics context*
Test Suite Groups

The System Verification and Hardware Troubleshooting consists of test groups:

Table 5  Test suite groups

<table>
<thead>
<tr>
<th>Computer Environment</th>
<th>Evaluation of computer specification and installation status.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Setup</td>
<td>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.</td>
</tr>
<tr>
<td>Vision</td>
<td>Camera and LED illumination are checked using the TapeStation Test Tape.</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>The voltage supply, current readout, and electrodes are checked using the TapeStation Test Tape.</td>
</tr>
<tr>
<td>Robotics</td>
<td>Robotics parts are checked by dedicated movements. Tip detection is checked using a full tip rack.</td>
</tr>
<tr>
<td>Pipetting</td>
<td>This check procedure in the Hardware Troubleshooting visually checks pickup of liquid from tube strips.</td>
</tr>
<tr>
<td>Cleanup</td>
<td>Resets the instrument to normal operational mode and requests removal of material.</td>
</tr>
</tbody>
</table>

**NOTE**  
In case no valid TapeStation system is connected, only those tests belonging to the Software Installation Verification can be run.
8 Instrument Diagnosis
Tests

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

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 4150 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:

- Two tube strips each with:
  - 5 μL water in wells A1, A2, H1, and H2.
  - 20 μL water in wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2, G1, G2.

Please collect liquid at the bottom of the vial by centrifugation before starting the liquid handling check. Spilled liquid or trapped air bubbles will affect the results. Please refer to “Centrifugation Recommendations” on page 97.
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 224.

Figure 40  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.
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.

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.

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 57, 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.

**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 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, and
   - a full rack of pipette tips

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 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 122.

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 60) and search for details on root causes in the section on System Diagnostics. Consult the section “Collecting Troubleshooting Information” on page 221 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.

**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 221 for details. These log files will automatically contain the latest test results.
This chapter describes the maintenance of the 4150 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 127) 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

The electrodes in the ScreenTape nest contacting to the contact points on the ScreenTape devices can be replaced to ensure optimal instrument performance. This should happen after 50000 analyses or after a year of use, whatever comes first. Schedule planned preventive maintenance by Agilent Service at this point.

Each time a ScreenTape lane is used for electrophoresis the maintenance counter **Samples Analyzed** is increased. The planned use of the electrodes is 1 year, namely 365 days, starting with its insertion. The remaining time until an Electrode Cartridge change is due is shown in days. The TapeStation Controller software shows these maintenance counters and a respective warning when a threshold value of 50000 samples is reached or the Electrode Cartridge change is due.

**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 by a trained service engineer for the 4200 TapeStation system.

---

**Figure 41**  Diagnostic counters of the 4150 TapesStation System

---

**NOTE**
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 a year since last electrode exchange happened or 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.

For the 4150 TapeStation system most preventive maintenance tasks can be done by the user using information provided in the user guide. For instructions, see “Exchange the Electrode Cartridge” on page 135, “Exchange the Piercing Needle” on page 127, “Cleaning the Instrument” on page 146 and also “Electrode Cartridge” on page 130 for general information. In order to update software and firmware visit www.agilent.com and review the respective readme.pdf of any successor revision.

Your local Agilent trained service engineer will in addition to the tasks listed above do the following in a preventive maintenance service:
- 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 127) can also be performed between annual Preventive Maintenance services.
## Exchange the Piercing Needle

Beyond the descriptions in this user guide you will also find a detailed description within the Agilent Information Center (see “Agilent Information Center” on page 60).

### Parts required

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5067-5783</td>
<td>Needle Change Cartridge</td>
</tr>
</tbody>
</table>

**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.

**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.

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. Close Lid. Click **Proceed**.
9 Maintenance
Exchange the Piercing Needle

3 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.

4 Click **Proceed** to continue.

5 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.
6 Close Lid. Click **Removed**.

7 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 analyses.
Electrode Cartridge

The Electrode Cartridge is an essential part of the TapeStation system which facilitates electrophoresis. It possesses gold-coated metal pins which provide electrical contact to carbon electrodes printed onto ScreenTapes devices (“ScreenTape Architecture” on page 24). During analytical runs, contact is made and separation voltage is applied while the ScreenTape nest within the TapeStation System is closed.

Over time the Electrode Cartridge undergoes mechanical stress and is prone to contamination by spillage which impairs its function. As its function is crucial for the performance the TapeStation Controller software will notify users to replace the Electrode Cartridge after one year of use or after 50000 samples, whatever comes first.

In the 4200 TapeStation system, the Electrode Cartridge must be exchanged only by a trained service engineer during a scheduled preventive maintenance service. 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. For instructions, see “Exchange the Electrode Cartridge” on page 135.

See Figure 41 on page 125 within the TapeStation Controller software for the number of remaining days until the next Electrode Cartridge change is required. The current number of samples, which were run with the installed cartridge, is displayed as diagnostic counter as well. The same information is also shown in the respective System Diagnostic test, see “Tests” on page 115.

Information about past Electrode Cartridge changes is available from “Instrument Diagnosis” on page 114.

The figure below shows Electrode Cartridge (G2992-65818) for the 4150 TapeStation System with 32 electrode contact pins.

Positioning of the Electrode Cartridge inside the ScreenTape nest is crucial. Two components, the lever for latching and its pivot point, guide the Electrode Cartridge to its position. The correct position is reached when the lever is closed again. The lever for latching must point upward when the insertion begins and turns down while the Electrode Cartridge is lowered. Prerequisite to insert an Electrode Cartridge is that the ScreenTape nest is driven to a dedicated opening width. This is achieved when the TapeStation Controller software is used to assist the Electrode Cartridge Change process.

Follow the mounting instructions (“Exchange the Electrode Cartridge” on page 135) carefully. Prevent damage to the electrode pins. Avoid collision of electrode pins with any part of the ScreenTape nest. Do not touch pins or electronic parts.
Instrument or cartridge damage. Use the software to prepare the Electrode Cartridge exchange procedure. Remove the Electrode Cartridge only when prompted by a software dialog.

**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. Schedule a preventive maintenance service in case of the 4200 TapeStation system.
Additional Functionality of the Electrode Cartridge

Additional Functionality of the Electrode Cartridge

The Electrode Cartridge stores the following information in its internal memory:

- Electrode cartridge Serial number
- Type of construction
- Date of first use
- Sample counter

This information is accessed and displayed when diagnostic counters or results of the Electrode Cartridge History test are displayed.
Electrode Cartridge Exchange Process Detail

Electrode Cartridge exchange process detail

The Electrode Cartridge change process includes several background tasks such as:

- electronic deregistration of the old cartridge,
- electronic registration of the new cartridge
- validation of information stored on the internal memory
- short functionality test with the TapeStation Test Tape

After an Electrode Cartridge Change a banner shows results of the outcome. Users are subsequently guided to execute a System Validation test in any case. This additional system validation is not mandatory to continue working with the instrument but is recommended for good laboratory practice reasons.

The Electrode Cartridge Change is successful if the following criteria are fulfilled:

- The inserted Electrode Cartridge was successfully registered
- The date of first use is the current day
- No samples were analyzed with it
- The electrical functionality test with the TapeStation Test Tape passed
In case the electrical functionality test with the TapeStation Test Tape fails the entire Electrode Cartridge Change is labelled as failed. The Electrophoresis Cartridge Connectivity test from the System Validation test will indicate lanes that caused the fail. The fail might be caused by a wrongly inserted or defective Electrode Cartridge, by a defective TapeStation test tape or by defective instrument electronics. The instrument will remain inoperable on the respective lanes. Please correct the cartridge installation or mount a different valid cartridge in case all lanes were operational before. Please collect and provide troubleshooting information (“Collecting Troubleshooting Information” on page 221) and schedule a service visit if you cannot correct the error yourself.

In case the date of first use (“Used Electrode Cartridge Inserted” on page 175) is deviating from the current date the Electrode Cartridge Change is regarded failed as the insertion of a new and unused cartridge is expected. An Electrode Cartridge with a date of first use older than a year will trigger constantly a message in the TapeStation software user interface (“Electrode Cartridge Change Required” on page 174). An used Electrode Cartridge with a date of first use younger than a year will trigger no message in the TapeStation software user interface. Such used Electrode Cartridge might be used further if the System Validation test indicates its functionality.

In case the Electrode Cartridge was already used for analysis (“Used Electrode Cartridge Inserted” on page 175) the Electrode Cartridge Change is regarded failed as the insertion of a new and unused cartridge is expected. Such used Electrode Cartridge might be used further if the System Validation test indicate its functionality.

In case electronic registration of the new cartridge was not successful the system will further on display the error Electrode cartridge not detected until the error is corrected. The instrument will remain inoperable until a correct and valid cartridge is installed. Please collect and provide troubleshooting information (“Collecting Troubleshooting Information” on page 221) and schedule a service visit if you cannot correct the error yourself.
Exchange the Electrode Cartridge

Beyond the descriptions in this user guide you will also find a detailed and animated description within the Agilent Information Center (see “Agilent Information Center” on page 60).

**CAUTION**
Wrong position of the ScreenTape nest
Instrument or cartridge damage can occur

➔ Use the TapeStation Controller software to prepare the Electrode Cartridge exchange procedure.

➔ Remove the Electrode Cartridge only when prompted by a software dialog.

1. During a preventive maintenance service the Electrode Cartridge is exchanged. For details, see “Electrode Cartridge” on page 130.

2. Prepare the instrument by navigating to Settings, Maintenance, and Change Electrode Cartridge. Follow further onscreen instructions.

3. Open the lid.
4 Insert a TapeStation Test Tape.

5 Close the lid.

6 Click Proceed.

7 The Electrode Cartridge is deactivated. The instrument remains inoperable until the following steps are performed successfully. Follow further onscreen instructions.
8 Open the lid.
9 Remove the TapeStation Test Tape.

10 Remove the upper nest cover.
9 Maintenance
Electrode Cartridge

CAUTION Lower nest cover incorrectly mounted
Instrument malfunction and loss of material.
➔ Do not touch the lower nest cover.

11 Do not alter the installed lower nest cover. Keep the lower nest cover in place.

12 Click Proceed. Follow further onscreen instructions.
13 Release the cartridge lever with a push and bring it to the upright position.

14 Pull the Electrode Cartridge out in a straight upward movement.

15 Click Proceed. Follow further onscreen instructions.
9 Maintenance
Electrode Cartridge

**CAUTION**

Damaged electrode pins
Collision of the electrode pins with any part of the ScreenTape nest can damage the Electrode Cartridge.

➔ Replace the Electrode Cartridge carefully.

16 Insert the new Electrode Cartridge carefully. Push downwards until it reaches a resistance.
17 Move the lever to the lock position.

An internal locking bolt positions the Electrode Cartridge correctly.

18 Click **Proceed**. Follow further onscreen instructions.
19 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.
20 Click **Proceed**. Follow further onscreen instructions.
21 Reinsert the TapeStation Test Tape.

22 Close the lid.

23 Click Proceed to start a short verification.

24 Click Proceed to start System Verification tests. If the Electrode Cartridge Change was not successful, please refer to “Troubleshooting” on page 165.
25 Execute System Verification tests and review the results to verify instrument functionality.

Please be aware of software notifications that are related to the Electrode Cartridge:

- “Electrode Cartridge Change Required” on page 174
- “Used Electrode Cartridge Inserted” on page 175
- “Electrode Cartridge Change has Failed” on page 176
- “Electrode Cartridge not Detected” on page 177
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 156 and “Insertion of the Lower Nest Cover” on page 158 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

**WARNING**

A piercing needle is located behind the gantry cover.

➔ Do not reach under the cover.

➔ Do not remove the cover.

1. Provide dedicated packaging material for later use.
9 Maintenance
Preparing the Instrument for Transportation

2 Remove all loose components such as tip waste, tip rack and tube strip holder from the instrument.

3 Close the lid.

4 Select Prepare Transportation from the TapeStation Controller software menu. The software guides you through steps listed below for fixation all robotic parts to a safe position.

5 Open the lid by pressing the lid release button.
6 Carefully pull the gantry towards the front of the instrument.

7 Move the pump to the middle of the axis. In case of defective instruments, this has to be done manually.
**CAUTION**

Damaged cables
Instrument damage at pump cables.

➔ Handle the foam carefully without touching or damaging the cables.

8  Install the pump foam carefully over the pump.

9  Push the gantry carefully towards the back of the instrument.
10 Click **Proceed**.

11 Insert the Vision lock tape to the ScreenTape Nest in a curved path. One part will point backwards through a cut-out.

**NOTE**

Insertion of the Vision lock tape is only possible if the camera is in its default position on the right side.

12 Click **Proceed**
9  Maintenance
Preparing the Instrument for Transportation

13 Insert the workspace foam.

14 Close the lid.

15 Close the TapeStation Controller software.

16 Power down the instrument.
Boxing for Transportation

**CAUTION**

Mechanical damage of the instrument

If the transport assembly is not prepared for transportation, the instrument could be damaged due to excessive shock of the shipping container during transport.

➔ Always prepare the unit for transportation before shipment, see “Preparing the Instrument for Transportation” on page 147.

➔ Protect the instrument by transport protection material, see step 13 on page 44

➔ Store the transport protection material in a safe place to use for later transport of the instrument.

1. Lift the instrument into one foam part.
2 Mount the second foam part.

3 Mount both cardboards.

4 Turn the instrument over to a side.
5 Slide the instrument into the 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.
Assembling Nest Covers

Click

Click
9 Maintenance
Insertion of the Lower Nest Cover

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.
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.
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.
9 Maintenance
Insertion of the Lower Nest Cover

Click
Click
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.
9 Maintenance
Insertion of the Lower Nest Cover
10 Troubleshooting

Software Messages  167
   TapeStation Test Tape Detected  167
   Camera Fault Detected  168
   Run Stopped During Initialization Process  169
   Block at Lower Nest Cover  170
   Insufficient Lanes Detected  171
   Premature End of Analysis  172
   Perform Needle Change  173
   Electrode Cartridge Change Required  174
   Used Electrode Cartridge Inserted  175
   Electrode Cartridge Change has Failed  176
   Electrode Cartridge not Detected  177

Troubleshooting DNA Applications  178
   Genomic DNA Assay  178
   Incorrect Quantification Results  180
   Incorrect Sizing Results  183
   Missing Marker Peaks  185
   Incorrect or Missing DIN Value  186
   Unexpected Migration Profile  188

Cell-Free DNA Assay  196
   TapeStation Analysis in the Agilent SureSelect and HaloPlex Workflow  198

Troubleshooting RNA Applications  200
   Incorrect Quantification Results  200
   Missing Marker Peaks  202
   Degraded RNA Ladder and/or Samples  203
   Incorrect or Missing RINe Value  204
   Incorrect or Missing Peak Annotation  205
   Unexpected Migration Profile  205
This chapter gives an overview about the troubleshooting features of the 4150 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.
1 Camera Fault Detected

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 212 for details.
Run Stopped During Initialization Process

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

Block at Lower Nest Cover

1 The lower nest cover did not move and requires proper assembly. See “Insertion of the Lower Nest Cover” on page 158 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 exchange is done. A warning message which recommends this appears already after 3840 samples. See “Exchange the Piercing Needle” on page 127 for further information.
Electrode Cartridge Change Required

1. An Electrode Cartridge Change is required after either 50000 samples or after a year of usage, whatever comes first. See further information “Electrode Cartridge” on page 130.
Used Electrode Cartridge Inserted

1 The Electrode Cartridge inserted was previously used. Check the values for first use date and number of samples analyzed. In case this is intentional, continue to verify functionality with the System Diagnostic tests. For further information see “Electrode Cartridge” on page 130.
Electrode Cartridge Change has Failed

1. An electrical functionality test with the TapeStation Test Tape failed on one or more lanes. Verify this result with the System Diagnostic tests and install a functional electrode cartridge. See further information “Electrode Cartridge” on page 130.
Electrode Cartridge not Detected

1 The instrument is inoperable because the Electrode Cartridge is removed, incorrectly installed or defective. Click Ok to start the installation process with a correct and valid cartridge. See further information “Electrode Cartridge” on page 130.
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 60).

**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 44**  
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 45** 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

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient Mixing Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.</td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information.</td>
<td></td>
</tr>
<tr>
<td>Sample concentration outside recommended range for application.</td>
<td>Linear concentration estimation cannot be achieved for samples exceeding the limits in the specification table.</td>
<td>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 64).</td>
</tr>
<tr>
<td>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.</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>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.</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Root cause</td>
<td>Explanation</td>
<td>Solution</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sample and the marker relevant for quantification have merged.</td>
<td>Additional sample overlaying with the marker will alter the known concentration of the marker that is used for concentration calculation.</td>
<td>Ensure that your sample is within the recommended sizing range for the applications, see “Sizing Range” on page 106.</td>
</tr>
<tr>
<td></td>
<td>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 198.</td>
<td>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.</td>
</tr>
<tr>
<td>Sample not collected at bottom of vial</td>
<td>Insufficient volume pipetted by the robotics and applied to the lane may affect migration behavior and alter the band shape.</td>
<td>Make sure to collect all liquid at the bottom of the vial by centrifugation. See “Centrifugation Recommendations” on page 97. 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.</td>
</tr>
<tr>
<td>Incorrect protocol used</td>
<td>Differences exist between Standard and High Sensitivity assay protocols.</td>
<td>Ensure that the correct sample protocol for the application is used, see “Use the Correct Protocol” on page 93.</td>
</tr>
<tr>
<td>Incorrect reagents used</td>
<td>Reagents from different assays may differ in dye and marker concentration. Furthermore markers used are different and upper markers may not be present.</td>
<td>Ensure that the correct reagent buffer for the application is used, see “Use the Correct Protocol” on page 93.</td>
</tr>
<tr>
<td>Sample prepared too long before analysis</td>
<td>Evaporation or settling may alter the sample to buffer ratio.</td>
<td>Ensure that your run is started immediately after sample preparation.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
<td>Always cover with the recommended foil seal (PN 5067-5154) and ensure tight sealing throughout the 96-well sample plate.</td>
</tr>
</tbody>
</table>
## Troubleshooting DNA Applications

### 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 85 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.

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incorrect pipetting technique or pipette calibration.</td>
<td>Incorrect pipetting technique or uncalibrated pipettes can lead to poor pipetting performance which in turn alter sample/buffer ratio in an unpredictable manner.</td>
<td>See “Good Pipetting Practice” on page 85 for more information or follow guidelines of the respective pipette vendor.</td>
</tr>
<tr>
<td>Incorrect Marker peaks picked up</td>
<td>Depending on the sample pattern, wrong peaks can get assigned as marker if running very close to the actual marker.</td>
<td>Use the <strong>Aligned</strong> 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.</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incorrect Marker peaks picked up</td>
<td>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.</td>
<td>Use the <strong>Aligned</strong> 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.</td>
</tr>
<tr>
<td>Incorrect analysis type</td>
<td>The <strong>Gel</strong> and <strong>Electropherogram</strong> view are designed to analyze discrete peaks within samples. The <strong>Region</strong> view is optimized for analyzing samples with broad peaks. <strong>Region</strong> view uses a different method to calculate concentration and sizing.</td>
<td>Use <strong>Gel</strong> view for samples with narrow peaks and the <strong>Region</strong> 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.</td>
</tr>
<tr>
<td>Issues with the ladder lane</td>
<td>Any issues with the run ladder lane will be annotated within the TapeStation Analysis software sample table under <strong>Observations</strong>.</td>
<td>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 178.</td>
</tr>
<tr>
<td>Insufficient migration due to presence of salt</td>
<td>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.</td>
<td>Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by unaligning your gel image by pressing the <strong>Aligned</strong> 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 64).</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample concentration outside recommended range for application</td>
<td>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.</td>
<td>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 64).</td>
</tr>
<tr>
<td>Insufficient migration</td>
<td>Marker peak may not be completely resolved from a sample peak or is not at an expected run distance.</td>
<td>To check migration true distance, unalign the gel image using the aligned button. See “Unexpected Migration Profile” on page 188, “Maximum Buffer Concentration in Sample” on page 110, and “Quantitative range” on page 107.</td>
</tr>
<tr>
<td>Blank lane</td>
<td></td>
<td>See the Agilent Information Center for more information.</td>
</tr>
</tbody>
</table>
# 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

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too few ladder peaks have been detected</td>
<td>In order to calculate a DIN value a correct sizing is needed which can only be established with a correctly run and integrated ladder.</td>
<td>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 178. Assign a different lane as the ladder (if applicable).</td>
</tr>
<tr>
<td>Incorrect marker peak picked up or no marker peaks detected</td>
<td>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.</td>
<td>Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by unaligning your gel image by pressing the <strong>Aligned</strong> 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 64). Rerun samples with fresh reagents.</td>
</tr>
<tr>
<td>Due to sensitivity, artifacts might be erroneously assigned as Lower Marker.</td>
<td></td>
<td>Use the <strong>Aligned</strong> 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.</td>
</tr>
</tbody>
</table>
Troubleshooting DNA Applications

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

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ladder run on ScreenTape device</td>
<td>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.</td>
<td>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.</td>
</tr>
<tr>
<td>Sample concentration outside functional DIN range</td>
<td>The DIN algorithm only allows linear estimation of sample integrity within the given concentration range.</td>
<td>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 64).</td>
</tr>
<tr>
<td>Sample with multiple peaks in lane</td>
<td>A DIN value is not presented in sample lanes containing more than 14 peaks as this potentially represents the ladder.</td>
<td>Check the electropherogram trace for incorrectly assigned peaks and judge if these are genuine peaks. Rerun samples with fresh reagents if in doubt.</td>
</tr>
</tbody>
</table>
**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>
<thead>
<tr>
<th>Problem</th>
<th>Likely cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>The gel image looks distorted</td>
<td>Incorrect Marker peaks picked up</td>
<td>Depending on the sample pattern wrong peaks can get assigned as marker if running very close to the actual marker.</td>
<td>Use the <strong>Aligned</strong> 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 &quot;<strong>Peak Integration</strong>&quot; on page 101.</td>
</tr>
</tbody>
</table>
### Troubleshooting DNA Applications

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slanted or smeared bands</td>
<td>A bubble could be located at the top of the ScreenTape device</td>
<td>A bubble in the buffer chamber distorts the initial entry of a sample into the gel.</td>
<td>Always flick the ScreenTape device before placing into the TapeStation instrument. See “ScreenTape Devices” on page 87.</td>
</tr>
<tr>
<td>Sample not collected at bottom of vial</td>
<td>Insufficient volume pipetted by the robotics and applied to the lane may affect the migration behavior and alter the band shape.</td>
<td></td>
<td>Make sure to collect all liquid at the bottom of the vial by centrifugation. See “Centrifugation Recommendations” on page 97. 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.</td>
</tr>
<tr>
<td>The sample may not have been mixed correctly with the sample buffer</td>
<td>Ionic strength of the sample/buffer mix may not reach expected optimal conditions and thus electrophoresis is impaired.</td>
<td>Follow the mixing recommendations for the assay. See “Mixing Recommendations” on page 94 for more details.</td>
<td></td>
</tr>
<tr>
<td>The sample and sample buffer may have started to evaporate</td>
<td>Ionic strength of the sample/buffer mix may exceed the maximum buffer strength due to water loss and thus electrophoresis is impaired.</td>
<td>Always ensure that samples are run on the TapeStation instrument immediately after preparation with sample buffer.</td>
<td></td>
</tr>
<tr>
<td>A bubble could be located within the gel matrix</td>
<td>A bubble in the gel matrix introduces significant artifacts in the data. Those bubbles cannot be flicked away.</td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>
## Troubleshooting DNA Applications

**Table 10** DNA - probable causes for unexpected migration profile

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>The markers have appeared in the sample lane, but the sample has not</td>
<td>The sample analyzed is too diluted</td>
<td>The sample concentration is below the limit of detection.</td>
<td>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.</td>
</tr>
<tr>
<td>Insufficient Mixing</td>
<td>Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.</td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information.</td>
<td></td>
</tr>
</tbody>
</table>
### Table 10  DNA - probable causes for unexpected migration profile

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra bands have appeared to those expected in the sample lane</td>
<td>There may be a contaminant peak that is caused by dust or dirt on the ScreenTape device</td>
<td>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.</td>
<td>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.</td>
</tr>
<tr>
<td>A bubble could be located within the gel matrix</td>
<td>A bubble in the gel matrix introduces significant artifacts in the data. Those bubbles cannot be flicked away.</td>
<td></td>
<td>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 87 for more information.</td>
</tr>
<tr>
<td>The genomic DNA sample has degraded</td>
<td>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.</td>
<td></td>
<td>This is not an analytical artifact but shows the true state of the genomic DNA sample.</td>
</tr>
<tr>
<td>Reagent Buffer or sample contaminated with ladder</td>
<td>Failure to change pipette tips in between pipetting steps might lead to Reagent Buffer or sample being contaminated with ladder.</td>
<td></td>
<td>Use new Reagent Buffer vial and make sure to change pipette tips in between pipetting steps. See “Sample Preparation” on page 91 for more information.</td>
</tr>
</tbody>
</table>
## Troubleshooting DNA Applications

### Table 10  DNA - probable causes for unexpected migration profile

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>The upper and lower markers appear unusually intense in the gel image.</td>
<td>This effect might appear during normal alignment and scaling.</td>
<td>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.</td>
<td>To get an idea about raw fluorescence intensities, unalign the image using the <strong>Aligned</strong> button in the TapeStation Analysis software.</td>
</tr>
<tr>
<td>Sample prepared too long before analysis</td>
<td>Evaporation or settling may alter the sample to buffer ratio.</td>
<td></td>
<td>Ensure that your run is started immediately after sample preparation.</td>
</tr>
<tr>
<td>Insufficient Mixing</td>
<td>Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.</td>
<td></td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information.</td>
</tr>
<tr>
<td>The sample analyzed is too diluted</td>
<td>The sample concentration is below the limit of detection.</td>
<td></td>
<td>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.</td>
</tr>
</tbody>
</table>

![Figure 46](image.png)  
**Figure 46**  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.
Troubleshooting DNA Applications

Table 10  DNA - probable causes for unexpected migration profile

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher than normal background noise</td>
<td>Insufficient Mixing</td>
<td>Buffer and sample were not sufficiently vortexed. The dye could not incorporate completely into the sample.</td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information.</td>
</tr>
<tr>
<td>Incorrect ScreenTape device or reagent storage</td>
<td>Incorrect storage (too cold or too warm)</td>
<td>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.</td>
<td>Follow the storage conditions that are specified for the assay (see Table 3 on page 64).</td>
</tr>
</tbody>
</table>
Troubleshooting DNA Applications

Table 10  DNA - probable causes for unexpected migration profile

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low signal intensity of sample or marker</td>
<td>Insufficient Mixing</td>
<td>Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.</td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information.</td>
</tr>
<tr>
<td>peaks (one or other, not both)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorrect ScreenTape device or reagent</td>
<td>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.</td>
<td>Follow the storage conditions that are specified for the assay (see Table 3 on page 64).</td>
<td></td>
</tr>
<tr>
<td>storage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample concentration outside recommended</td>
<td>Linear concentration estimation cannot be achieved for samples exceeding the limits in the specification table.</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>range for application</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 10  DNA - probable causes for unexpected migration profile

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low signal intensity of sample and marker peaks (both) or of ladder peaks</td>
<td>Incorrect reagent storage</td>
<td>Incorrect storage of the reagents may induce degradation of the dye. This can lead to a higher than normal background noise.</td>
<td>Follow the storage conditions that are specified for the assay (see Table 3 on page 64).</td>
</tr>
<tr>
<td>Incorrect reagents used</td>
<td>Components of High Sensitivity Assay Reagents are too diluted to be properly detected by Standard Sensitivity Assays, see “Reagents and Reagent Mixes” on page 86.</td>
<td></td>
<td>Use the correct protocol and consumables</td>
</tr>
<tr>
<td>Incorrect migration - samples have not reached the lower end of the gel lane</td>
<td>Salt concentration</td>
<td>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.</td>
<td>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 64).</td>
</tr>
<tr>
<td>Partial electrophoresis failure caused by bubbles at gel-buffer interface</td>
<td>Air bubbles at the gel-buffer buffer interface may disrupt current flow between the electrodes.</td>
<td></td>
<td>Ensure ScreenTape device is flicked to remove any bubbles before use. See the Agilent Information Center and “ScreenTape Devices” on page 87 for more information.</td>
</tr>
</tbody>
</table>
### 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 64).

**Figure 47** 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 48  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 49  Electropherogram image with additional signal from AMPure beads above the Upper Marker peak (arrow)
• 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 50  Electropherogram image with additional peak in a library smear (arrow)
Troubleshooting RNA Applications

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.

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient Mixing</td>
<td>Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample.</td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information.</td>
</tr>
<tr>
<td>Sample concentration outside recommended range</td>
<td>Linear concentration estimation cannot be achieved for samples exceeding the limits in the specification table.</td>
<td>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 64).</td>
</tr>
<tr>
<td>for application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorrect pipetting technique or pipette</td>
<td>Incorrect pipetting technique or uncalibrated pipettes can lead to poor pipetting performance which in turn alter sample/buffer ratio in an unpredictable manner.</td>
<td>See “Good Pipetting Practice” on page 85 and follow guidelines of the respective pipette vendor.</td>
</tr>
</tbody>
</table>
### Table 11  RNA - Possible causes of incorrect quantification in order of probability.

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample not collected at bottom of vial</td>
<td>Insufficient volume pipetted by the robotics and applied to the lane may affect the migration behavior and alter the band shape.</td>
<td>Make sure to collect all liquid at the bottom of the vial by centrifugation. See “Centrifugation Recommendations” on page 97. 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.</td>
</tr>
<tr>
<td>Incorrect peak integration</td>
<td>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.</td>
<td>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.</td>
</tr>
<tr>
<td>Incorrect Marker peaks picked up</td>
<td>Depending on the sample pattern wrong peaks can get assigned as marker if running very close to the actual marker.</td>
<td>Use the <strong>Aligned</strong> 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.</td>
</tr>
<tr>
<td>Incorrect heating procedure</td>
<td>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.</td>
<td>Ensure that the samples are heat denatured according to the assay instructions before running.</td>
</tr>
<tr>
<td>Sample and the marker relevant for quantification have merged.</td>
<td>Additional sample overlaying with the marker will alter the known concentration of the Lower Marker.</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Contaminating beads can alter the run behavior of the sample and can introduce artifacts.</td>
<td>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.</td>
</tr>
</tbody>
</table>
Table 11  RNA - Possible causes of incorrect quantification in order of probability.

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incorrect protocol used</td>
<td>Differences exist between Standard and High Sensitivity assay protocols.</td>
<td>Ensure that the correct sample protocol for the application is used, “Reagents and Reagent Mixes” on page 86.</td>
</tr>
<tr>
<td>Sample prepared too long before analysis</td>
<td>Evaporation or settling may alter the sample to buffer ratio.</td>
<td>Ensure that your run is started immediately after sample preparation.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
<td>Always cover with the recommended foil seal (PN 5067-5154) and ensure tight sealing throughout the 96-well sample plate.</td>
</tr>
<tr>
<td>Incorrect reagents used</td>
<td>Reagents from different assays may differ in dye and marker concentration. Furthermore markers used are different and upper markers may not be present.</td>
<td>Ensure that the correct reagent buffer for the application is used, see “Reagents and Reagent Mixes” on page 86.</td>
</tr>
</tbody>
</table>

Missing Marker Peaks

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

<table>
<thead>
<tr>
<th>Cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample concentration outside recommended range for application</td>
<td>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.</td>
<td>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 64; then prepare a new run, see “Quantitative range” on page 107.</td>
</tr>
<tr>
<td>Insufficient migration</td>
<td>Marker peak may not be completely resolved from a sample peak or is not at an expected run distance.</td>
<td>To check migration true distance, unalign the gel image using the Aligned button. See “Unexpected Migration Profile” on page 205, “Maximum Buffer Concentration in Sample” on page 110, and “Quantitative range” on page 107.</td>
</tr>
<tr>
<td>Blank lane</td>
<td></td>
<td>See the Agilent Information Center for more information.</td>
</tr>
</tbody>
</table>
## Degraded RNA Ladder and/or Samples

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

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incorrect heating step</td>
<td>If heat denaturation was too long and/or too high temperatures were used the sample will degrade upon this treatment</td>
<td>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 99, Table 3 on page 64)</td>
</tr>
<tr>
<td>Sample concentration outside recommended range for application</td>
<td>A too concentrated sample will significantly alter the electropherogram</td>
<td>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)</td>
</tr>
<tr>
<td>Insufficient migration due to presence of salt</td>
<td>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.</td>
<td>Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by unaligning your gel image by pressing the <strong>Aligned</strong> 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 64)</td>
</tr>
<tr>
<td>RNAse contamination of the reagents</td>
<td>RNAse contamination will lead to degradation of the sample</td>
<td>Ensure Good Measurement Practices when working with RNA assays; then prepare a new run using new reagents (“Good Measurement Practices” on page 81).</td>
</tr>
<tr>
<td>RNAse contamination of the plasticware</td>
<td>RNAse contamination will lead to degradation of the sample</td>
<td>Ensure Good Measurement Practices when working with RNA assays; then prepare a new run using new reagents (“Good Measurement Practices” on page 81).</td>
</tr>
</tbody>
</table>
Troubleshooting RNA Applications

Incorrect or Missing RIN<sup>e</sup> Value

The RNA Integrity Number equivalent (RIN<sup>e</sup>) 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<sup>e</sup> value for total RNA.

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample concentration outside</td>
<td>An overconcentrated sample will alter the electropherogram significantly. A sample below the detection limit may also not get an assigned RIN&lt;sup&gt;e&lt;/sup&gt;</td>
<td>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&lt;sup&gt;e&lt;/sup&gt; Functional Range” on page 112); then prepare a new run. Use the High Sensitivity assay if applicable (Table 3 on page 64)</td>
</tr>
<tr>
<td>recommended range for application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorrect heating step</td>
<td>The 18S peak may have partially reverted back to its original, non-denatured conformation which results in characteristic double peaks and influences RIN&lt;sup&gt;e&lt;/sup&gt; calculation</td>
<td>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 99) (Table 3 on page 64)</td>
</tr>
<tr>
<td>Genomic DNA contamination</td>
<td>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</td>
<td>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</td>
</tr>
<tr>
<td>There are 2 bands present at the 18S</td>
<td>There may have been insufficient sample denaturation prior to analysis.</td>
<td>Ensure the correct denaturation conditions during the RNA sample preparation, according to the assay Quick Guide (Table 3 on page 64).</td>
</tr>
<tr>
<td>peak position</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The 18S peak may have partially reverted back to its original, non-denatured conformation.</td>
<td>Samples should be kept on ice after preparation, and run within 2 hours of denaturation, see “Heat Denaturation” on page 99 for more information.</td>
</tr>
</tbody>
</table>
Troubleshooting RNA Applications

Incorrect or Missing Peak Annotation

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

<table>
<thead>
<tr>
<th>Root cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak is not a ribosomal RNA or Lower Marker peak</td>
<td>Only specific RNA peaks (16, 18, 23, 28S) and Lower marker peak will be annotated by the software</td>
<td>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</td>
</tr>
<tr>
<td>Extremely degraded sample</td>
<td>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</td>
<td>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 99; then prepare a new run (“Good Measurement Practices” on page 81)</td>
</tr>
<tr>
<td>Incorrect heating step</td>
<td>The 18S peak may have partially reverted back to its original, non-denatured conformation which results in characteristic double peaks and influences RIN® calculation</td>
<td>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 99 (Table 3 on page 64)</td>
</tr>
<tr>
<td>Genomic DNA contamination</td>
<td>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</td>
<td>Ensure that all peaks are annotated correctly. Treat samples with DNase to eliminate the genomic DNA peak; then prepare a new run</td>
</tr>
</tbody>
</table>

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.
## Troubleshooting RNA Applications

### Table 16  RNA - Possible causes of unexpected migration profile.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely Cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additional bands are present in the region of the 18S and 28S peak</td>
<td>Incorrect heating step</td>
<td>The 18S peak may have partially reverted back to its original, non-denatured conformation which results in characteristic double peaks and influences RIN&lt;sup&gt;e&lt;/sup&gt; calculation</td>
<td>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 &quot;Heat Denaturation&quot; on page 99) (Table 3 on page 64)</td>
</tr>
<tr>
<td>Genomic DNA contamination</td>
<td>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</td>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Contamination with prokaryotic total RNA</td>
<td>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.</td>
<td></td>
<td>If not intended or expected ensure that sample tissue is free of prokaryotic contaminations.</td>
</tr>
<tr>
<td>There may be a contaminant peak that is caused by dust or dirt on the ScreenTape device</td>
<td>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</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>A bubble could be located within the gel matrix</td>
<td>A bubble in the gel matrix will introduce significant artifacts in the data. Those bubbles cannot be flicked away</td>
<td>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 &quot;ScreenTape Devices&quot; on page 87 for more information</td>
<td></td>
</tr>
</tbody>
</table>
Table 16  RNA - Possible causes of unexpected migration profile.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely Cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A split or additional unexpected peak is evident in the RNA ladder</td>
<td>Insufficient denaturation of the RNA ladder</td>
<td>Some peaks may have partially reverted back to its original, non-denatured conformation which results in characteristic double peaks</td>
<td>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 99)</td>
</tr>
<tr>
<td>Wrong peak assigned</td>
<td>In rare cases, shoulders of peaks can get assigned as ladder peaks</td>
<td>Delete the wrong peaks and assign correct ladder peaks if needed. See the Agilent Information Center for more information</td>
<td></td>
</tr>
<tr>
<td>Additional bands are present at the top of the gel image</td>
<td>Incorrect ScreenTape device storage</td>
<td>This peak is comparable to the 'Well' peak in the Genomic DNA assay (“Genomic DNA Assay” on page 178) and can occur if the ScreenTape device stored incorrectly</td>
<td>Follow the specified storage conditions for the assay (see Table 3 on page 64). Do not leave ScreenTape devices for prolonged period of time at room temperature. See the Agilent Information Center for more information</td>
</tr>
<tr>
<td>The sample may be too concentrated for the application</td>
<td>A too high concentrated sample can result in artifacts in the gel</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>The gel image looks distorted</td>
<td>Incorrect Marker peaks picked up</td>
<td>Depending on the sample pattern wrong peaks can get assigned as marker if running very close to the actual marker</td>
<td>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</td>
</tr>
</tbody>
</table>
### Table 16  RNA - Possible causes of unexpected migration profile.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely Cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slanted or smeared bands</td>
<td>A bubble could be located at the top of the ScreenTape device</td>
<td>A bubble in the buffer chamber distorts the initial entry of a sample into the gel</td>
<td>Always flick the ScreenTape device before placing into the TapeStation instrument. See “ScreenTape Devices” on page 87</td>
</tr>
<tr>
<td>Sample not collected at the bottom of the vial</td>
<td>Insufficient volume pipetted by the robotics and applied to the lane may affect the migration behavior and alter the band shape</td>
<td>Make sure to collect all liquid at the bottom of the vial by centrifugation. See “Centrifugation Recommendations” on page 97. 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</td>
<td></td>
</tr>
<tr>
<td>The sample may not have been mixed correctly with the sample buffer</td>
<td>Ionic strength of the sample/buffer mix may not reach expected optimal conditions and thus electrophoresis is impaired</td>
<td>Please follow the mixing recommendations for the assay. See “Mixing Recommendations” on page 94 for more details</td>
<td></td>
</tr>
<tr>
<td>The sample and sample buffer may have started to evaporate</td>
<td>Ionic strength of the sample/buffer mix may exceed the maximum buffer strength due to water loss and thus electrophoresis is impaired</td>
<td>Always ensure that samples are run on the TapeStation instrument immediately after preparation with sample buffer</td>
<td></td>
</tr>
<tr>
<td>A bubble could be located within the gel matrix</td>
<td>A bubble in the gel matrix will introduce significant artifacts in the data. Those bubbles cannot be flicked away</td>
<td>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 87 for more information</td>
<td></td>
</tr>
<tr>
<td>The markers have appeared in the sample lane, but the sample has not</td>
<td>The sample analyzed is too diluted</td>
<td>The sample concentration is below the limit of detection</td>
<td>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</td>
</tr>
<tr>
<td>Insufficient Mixing</td>
<td>Buffer and sample were not sufficiently vortexed. The dye could not incorporate completely into the sample</td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information</td>
<td></td>
</tr>
</tbody>
</table>
### Troubleshooting RNA Applications

Additional bands have appeared to those expected in the sample lane

- **Likely Cause:** There may be a contaminant peak that is caused by dust or dirt on the ScreenTape device.
- **Explanation:** 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.
- **Solution:** 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

- **Likely Cause:** A bubble in the gel matrix will introduce significant artifacts in the data. Those bubbles cannot be flicked away.
- **Solution:** 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 87 for more information.

Fragmented genomic DNA contamination

- **Likely Cause:** 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.
- **Solution:** Treat the RNA sample with DNAse to remove contaminating DNA from the sample.

Reagent Buffer or sample contaminated with ladder

- **Likely Cause:** Failure to change pipette tips in between pipetting steps might lead to Reagent Buffer or sample being contaminated with ladder.
- **Solution:** Use new Reagent Buffer vial and make sure to change pipette tips in between pipetting steps. See “Sample Preparation” on page 91 for more information.

### Table 16 RNA - Possible causes of unexpected migration profile.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely Cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additional bands have appeared to those</td>
<td>There may be a contaminant peak that is caused by dust or dirt on the</td>
<td>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.</td>
<td>Unalign the image using the Aligned button in the TapeStation Analysis software. Contaminant</td>
</tr>
<tr>
<td>expected in the sample lane</td>
<td>ScreenTape device</td>
<td></td>
<td>peaks can be unassigned by right-clicking on either the gel image or electropherogram peak.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rerun samples if needed</td>
</tr>
<tr>
<td>A bubble could be located within the gel</td>
<td>A bubble in the gel matrix will introduce significant artifacts in the data. Those bubbles cannot be flicked away.</td>
<td></td>
<td>Always inspect ScreenTape devices for bubbles in the lanes. Lanes in question cannot be used</td>
</tr>
<tr>
<td>matrix</td>
<td></td>
<td></td>
<td>for analysis. Such bubbles can form after the expiration date or upon wrong storage. See the</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Agilent Information Center and “ScreenTape Devices” on page 87 for more information.</td>
</tr>
<tr>
<td>Fragmented genomic DNA contamination</td>
<td>Genomic DNA is a common contamination in total RNA samples. Genomic DNA</td>
<td>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.</td>
<td>Treat the RNA sample with DNAse to remove contaminating DNA from the sample.</td>
</tr>
<tr>
<td>Reagent Buffer or sample contaminated with</td>
<td>Failure to change pipette tips in between pipetting steps might lead to</td>
<td></td>
<td>Use new Reagent Buffer vial and make sure to change pipette tips in between pipetting steps.</td>
</tr>
<tr>
<td>ladder</td>
<td>Reagent Buffer or sample being contaminated with ladder.</td>
<td></td>
<td>See “Sample Preparation” on page 91 for more information.</td>
</tr>
</tbody>
</table>
### Troubleshooting RNA Applications

#### 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 94 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

Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample. See “Mixing Recommendations” on page 94 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 64).

---

**Table 16: RNA - Possible causes of unexpected migration profile.**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely Cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Lower Marker appears unusually intense in the gel image</td>
<td>This effect might appear in the course of normal alignment and scaling.</td>
<td>Lower markers are normalized to appear the same in the electropherogram, which can result in sample peaks looking smaller than expected</td>
<td>In order to get an idea about raw fluorescence intensities unalign the image using the <strong>Aligned</strong> button in the TapeStation Analysis software.</td>
</tr>
<tr>
<td>Sample prepared too long before analysis.</td>
<td>Evaporation or settling may alter the sample to buffer ratio</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Insufficient Mixing</td>
<td>Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample</td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information.</td>
<td></td>
</tr>
<tr>
<td>The sample analyzed is too diluted</td>
<td>The sample concentration is below the limit of detection</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Higher than normal background noise</td>
<td>Insufficient Mixing</td>
<td>Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample</td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information.</td>
</tr>
<tr>
<td>Incorrect ScreenTape device or reagent storage</td>
<td>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</td>
<td>Follow the storage conditions that are specified for the assay (see Table 3 on page 64).</td>
<td></td>
</tr>
</tbody>
</table>
### Table 16  RNA - Possible causes of unexpected migration profile.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Likely Cause</th>
<th>Explanation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low signal intensity of sample or marker peaks</td>
<td>Insufficient Mixing</td>
<td>Buffer and sample was not sufficiently vortexed. The dye could not incorporate completely into the sample</td>
<td>See “Mixing Recommendations” on page 94 and the Agilent Information Center for more information</td>
</tr>
<tr>
<td>(one or other, not both)</td>
<td></td>
<td>-------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Incorrect ScreenTape device or reagent storage</td>
<td>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</td>
<td>Follow the storage conditions that are specified for the assay (see Table 3 on page 64)</td>
<td></td>
</tr>
<tr>
<td>Sample concentration outside recommended range for the application</td>
<td>Linear concentration estimation cannot be achieved for samples exceeding the limits in the specification table</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Low signal intensity of sample and marker peaks</td>
<td>Incorrect reagent storage</td>
<td>Storing reagents incorrectly may induce degradation of the dye. This can lead to a higher than normal background noise</td>
<td>Follow the storage conditions that are specified for the assay (see Table 3 on page 64)</td>
</tr>
<tr>
<td>(both) or of ladder peaks</td>
<td></td>
<td>-------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Incorrect migration - samples have not reached the lower end of the gel lane</td>
<td>Salt concentration</td>
<td>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)</td>
<td>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 64)</td>
</tr>
<tr>
<td>Partial electrophoresis failure that is caused by bubbles at gel-buffer interface</td>
<td>Air bubbles at the gel-buffer interface may disrupt current flow between the electrodes</td>
<td>Ensure ScreenTape device is flicked to remove any bubbles before use. See the Agilent Information Center and “ScreenTape Devices” on page 87 for more information</td>
<td></td>
</tr>
</tbody>
</table>
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 65
- 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 45 and additionally the latest Readme.pdf.

- Do not install other USB devices.
- Review the section “Windows Device Manager” on page 213 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
To access the device manager, go to **Start > Control Panel > Device Manager**.

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 215.

**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.

The COM port number (x) itself is variable and not relevant. It is assigned by the operating 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 221) 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:

<table>
<thead>
<tr>
<th>Possible reasons for connection fails</th>
<th>Corrective actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument is turned off.</td>
<td>Connect power supply cable and turn the instrument on. Verify power cord is the one supplied with the instrument.</td>
</tr>
<tr>
<td>USB cable is unplugged or defective.</td>
<td>Connect the supported USB cable (5188-8047) properly or exchange it.</td>
</tr>
<tr>
<td>The laptop shows a malfunction in USB connection protocol. The operating system fails to enable USB or COM functionality.</td>
<td>Restart the computer. See also “Windows Device Manager” on page 213.</td>
</tr>
<tr>
<td>Instrument is not initializing correctly or defective.</td>
<td>Turn the instrument on following all guidelines, see “Initial Setup of the System” on page 45 and “Preparing the Instrument” on page 65. If the error persists, execute System Verification. Collect support information (see “Collecting Troubleshooting Information” on page 221) and contact your local support.</td>
</tr>
<tr>
<td>TapeStation Controller software is corrupt or inhibited by other conflicting software.</td>
<td>Power-cycle the laptop to terminate any disturbing application. If the error persists: reinstall the TapeStation Controller software and remove any additional software.</td>
</tr>
<tr>
<td>Drivers of other USB devices corrupted or inhibited TapeStation USB Device drivers.</td>
<td>Disconnect and uninstall foreign devices and software.</td>
</tr>
<tr>
<td>TapeStation USB Device drivers are not installed or malfunctioning.</td>
<td>Reinstall the TapeStation software package. Make sure all required Windows updates are installed. See the latest ReadMe.pdf for reference.</td>
</tr>
</tbody>
</table>

NOTE
Refer to “Collecting Troubleshooting Information” on page 221 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.
The following table lists information for corrective actions:

<table>
<thead>
<tr>
<th>Possible reasons for the above error</th>
<th>Corrective actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument was turned off erroneously.</td>
<td>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.</td>
</tr>
<tr>
<td>Instrument went into error mode (= resident).</td>
<td>Avoid the error conditions. Restart laptop and instrument. The instrument will initialize and clear the error.</td>
</tr>
<tr>
<td>USB cable is unplugged; physical connection is unstable.</td>
<td>Verify the USB connection has no loose cables. Put plugs in firmly. Exchange cable if appropriate. Avoid touching the cables during analytical runs.</td>
</tr>
<tr>
<td>Issue caused by other activities on laptop such as by further USB devices or increased USB traffic on the laptop or busy network connections.</td>
<td>Disconnect and uninstall other USB devices. Avoid activities using the network during analytical runs.</td>
</tr>
<tr>
<td>Issue caused by anti-virus scans dragging resources.</td>
<td>Avoid scheduled virus scans during analytical runs. If applicable, disconnect from the world wide web.</td>
</tr>
<tr>
<td>Communication issues with unknown root cause while using the laptop differently.</td>
<td>Avoid activities that include installing/uninstalling other applications, drivers, or use of the laptop which create extra USB/LAN data traffic.</td>
</tr>
<tr>
<td>Laptop is in power save mode for USB connections or the screen.</td>
<td>Do not activate additional power save settings or sleep modes for the laptop.</td>
</tr>
<tr>
<td>Error during startup of the TapeStation Analysis software after ScreenTape run.</td>
<td>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.</td>
</tr>
<tr>
<td>Unforeseen activities or clicks in TapeStation Controller software.</td>
<td>Reduce activities such as changing preferred settings within the TapeStation Controller software to a minimum.</td>
</tr>
</tbody>
</table>

**NOTE** Refer to “Collecting Troubleshooting Information” on page 221 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:

<table>
<thead>
<tr>
<th>Possible reasons for the above errors</th>
<th>Corrective actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ScreenTape device inserted incorrectly.</td>
<td>The barcode must be on the right hand side and facing backwards. Push the ScreenTape device down and ensure it is horizontal.</td>
</tr>
<tr>
<td>ScreenTape type not supported.</td>
<td>Do not use ScreenTape device dedicated to other systems.</td>
</tr>
<tr>
<td>Barcode is damaged, smeared or absent.</td>
<td>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.</td>
</tr>
<tr>
<td>Camera is not in default position after an instrument error.</td>
<td>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.</td>
</tr>
<tr>
<td>The flashing green barcode illumination is absent.</td>
<td>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.</td>
</tr>
<tr>
<td>Data transfer issue of camera due to general communication issues.</td>
<td>Follow the &quot;Universal Corrective Actions&quot; on page 215 for communication troubleshooting.</td>
</tr>
</tbody>
</table>

**NOTE**
Refer to “Collecting Troubleshooting Information” on page 221 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.

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.
10 Troubleshooting
Collecting Troubleshooting Information
11 Appendix

Accessories Shipment List 224

This chapter contains parts information.
The following table lists accessories shipped together with the Agilent 4150 TapeStation system.

Printed Quick Guides of the entire ScreenTape Assay portfolio, see Table 3 on page 64.

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2991-90021</td>
<td>Agilent RNA ScreenTape Quick Guide for TapeStation Systems</td>
</tr>
<tr>
<td>G2991-90031</td>
<td>Agilent D1000 ScreenTape Quick Guide for TapeStation Systems</td>
</tr>
<tr>
<td>G2991-90041</td>
<td>Agilent Genomic DNA ScreenTape Quick Guide for TapeStation Systems</td>
</tr>
<tr>
<td>G2991-90051</td>
<td>Agilent D5000 ScreenTape Quick Guide for TapeStation Systems</td>
</tr>
<tr>
<td>G2991-90060</td>
<td>Agilent Cell-free DNA ScreenTape Quick Guide for TapeStation Systems</td>
</tr>
<tr>
<td>G2991-90121</td>
<td>Agilent High Sensitivity RNA ScreenTape Quick Guide for TapeStation Systems</td>
</tr>
<tr>
<td>G2991-90131</td>
<td>Agilent High Sensitivity D1000 ScreenTape Quick Guide for TapeStation Systems</td>
</tr>
<tr>
<td>G2991-90151</td>
<td>Agilent High Sensitivity D5000 ScreenTape Quick Guide for TapeStation Systems</td>
</tr>
</tbody>
</table>

**Instrument Accessories**

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2964-64030</td>
<td>Software and User Information, USB stick and Booklet, Box</td>
</tr>
<tr>
<td>5067-5601</td>
<td>TapeStation Test Tape</td>
</tr>
<tr>
<td>5188-8047</td>
<td>USB cable A to B, 2 m</td>
</tr>
<tr>
<td>G2992-40042</td>
<td>TapeStation Tube Strip Holder, tray for 16 tubes</td>
</tr>
<tr>
<td>G2992-40046</td>
<td>TapeStation Tip Waste Bucket, 1 bucket</td>
</tr>
</tbody>
</table>
## Additional Material

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>401425</td>
<td>Optical Cap 8x Strip, Box of 120, 0.2 mL</td>
</tr>
<tr>
<td>401428</td>
<td>Optical Tube 8x Strip, Box of 120, 0.2 mL</td>
</tr>
<tr>
<td>5067-5598</td>
<td>Loading Tips</td>
</tr>
</tbody>
</table>

**NOTE**

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

Please order further packs of single boxes 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:

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5067-5783</td>
<td>Needle Change Cartridge</td>
</tr>
<tr>
<td>5067-5599</td>
<td>Loading Tips 10 packs of 112 tips</td>
</tr>
<tr>
<td>5067-5786</td>
<td>32-Pin Electrode Cartridge</td>
</tr>
<tr>
<td>G2999AA</td>
<td>TapeStation laptop with Pre-installed TapeStation software, bundle</td>
</tr>
</tbody>
</table>
### R
- **reagents**
  - reagent mixes 86
- **recommendations**
  - centrifugation 97
- **RINe**
  - incorrect 204
  - missing 204
- **RNA assays** 99
- **RNA**
  - degraded ladder 203
  - degraded samples 203
  - incorrect peak annotation 205
  - missing marker peaks 202
  - missing peak annotation 205
  - troubleshooting 200
  - unexpected migration profile 205

### S
- **sample preparation** 91
  - sample preparation 64
- **ScreenTape architecture** 24
- **ScreenTape devices**
  - partially used 90
- **ScreenTape** 87
- **settings**
  - controller software 55
- **software installation verification** 116
- **software**
  - analysis 58
  - controller software settings 55
  - controller software user interface 53
  - controller 52
- **symbols** 10
- **system verification tests** 120
- **system verification** 116

### T
- **test results** 122
- **tests** 120
- **tools** 83, 84
- **troubleshooting**
  - collecting information 221
  - DNA 178
  - RNA 200

### U
- **USB**
  - cable 224
  - communication troubleshooting 215
- **user interface**
  - controller software 53

### W
- **waste**
  - electrical and electronic equipment 17
- **WEEE directive** 17
- **workflow**
  - HaloPlex 198
  - SureSelect 198
In This Book

This manual contains...

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