Agilent 2200 TapeStation System

Troubleshooting Manual

Agilent Technologies

Notices

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Manual Part Number

G2964-90110

Edition

01/2015

Printed in Germany

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Contents

1
Essential Measurement Practices

Overview

This section lists all user relevant hints on handling the 2200 TapeStation instrument, tools, ScreenTape consumables and reagents.

For the latest information on 2200 TapeStation compatible assays, visit the Agilent website at: www.agilent.com/genomics/tapestation.

Intended use of the 2200 TapeStation System

The 2200 TapeStation system carries out electrophoretic separation of Nucleic Acids and Proteins. The system detects:

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

Performance Limitations of Use

The 2200 TapeStation System can analyze a maximum of 16 samples on a single ScreenTape device; more samples can be run using a 96-well plate and multiple ScreenTape.

The user is responsible for establishing performance characteristics necessary for upstream and downstream applications. Appropriate controls must be included in any upstream application requiring analysis on the 2200 TapeStation System.

Tools and Handling

- Always follow the GLP-rules established in the laboratory.
- Always wear gloves to prevent contamination.
- When pipetting sample, use pipette tips that are of adequate size. Pipette tips that are too large will lead to poor quantitation accuracy.
- Change pipette tips between steps to avoid cross-contamination.
- For RNA and High Sensitivity RNA assays use reverse pipetting procedure. When filling the pipette tip, push slightly past the first resistance. Empty the pipette tip only to the first resistance. This procedure ensures pipetting accuracy.

Mixing recommendations

Use a vortexer which is designed for mixing 8-way tube strips, or 96-well plates respectively.

- The IKA MS3 vortexer is recommended for use with the following applications:
  - D1000 ScreenTape Assay
  - High Sensitivity D1000 ScreenTape Assay
  - RNA ScreenTape Assay
  - High Sensitivity RNA ScreenTape Assay
- For these assays, vortex mix samples with sample buffer using the default vortex setting (1 minute).
- If an IKA MS3 vortexer is not available, please ensure thorough manual vortex mixing – 10 seconds on maximum speed.

Figure 1 An IKA MS3 Vortexer is bundled with 2200 TapeStation instruments.

NOTE TapeStation instruments (PN G2965AA or G2964AA) are supplied with an optional IKA MS3 vortexer which includes a 96-well plate adaptor suitable for both 96-well PCR plates and 8-way strips.

It is recommended that all current TapeStation users purchase the IKA MS3 for best results. Agilent Technologies will not sell these parts separately; this vortexer can be obtained directly from IKA (www.ika.com) by quoting the part number 4674100 and 'Agilent Technologies'. This part number is supplied only to Agilent customers and may not be listed on standard price lists.

NOTE For all other assays, ensure thorough mixing at each mixing step, following the recommendations in the assay Quick Guide and ensuring movement of the liquid within the sample tubes.

Reagents and Reagent Mixes

- Handle and store all reagents according to the instructions given in the appropriate Assay Quick Guide.
- Keep all reagents at the directed temperature when not in use. Reagents left at room temperature for a long period of time may decompose, leading to poor measurement results.
- Allow all reagents to equilibrate to room temperature for 30 minutes. 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 10 and appropriate Assay Quick Guide.
- 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 and sample buffer.

Samples

- Refer to the appropriate Assay Quick Guide for the recommended sizing and concentration ranges. Using samples outside the stated ranges can affect performance.
- For salt tolerance guidelines, please refer to the apporpriate Assay Quick Guide. Please ensure that sample buffers are below the maximum recommendation.
- Allow all samples to equilibrate to room temperature for 30 minutes. Mix and spin down prior to use.
- Pipette carefully
  - 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 assay instructions. Improper mixing can lead to quantification errors, see "Mixing recommendations" on page 10.
- Once mixed, briefly centrifuge to collect the contents at the base of tubes.
- Used sample strips and tips should be disposed of 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 2200 TapeStation will load a sample from a minimum of 3 µL onto ScreenTape.

CAUTION

Lids on sample tubes

Failure to remove lids can cause damage to the 2200 TapeStation and impact performance

➔ Ensure lids have been removed from the sample tubes before starting the run.

ScreenTape

- Details and specifications for each assay are available in the 2200 TapeStation User Manual, the specific assay quick guide, and online www.agilent.com/genomics/tapestation
- Protect the gel lanes of the ScreenTape from excessive force – do not bend or flex the ScreenTape
- Store the ScreenTape in the provided packaging between 2 – 8 °C
- ScreenTape can be used straight from the fridge with no equilibration time
- Handle ScreenTape carefully to avoid fingerprints or fibers, which can affect imaging
- Ensure that ScreenTape is *flicked* gently before inserting into the instrument, if there are any small bubbles present then this will move them to the top of the chamber, see Figure 2 on page 14.

NOTE

The presence of small bubbles within the buffer chamber of the ScreenTape is normal.

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.

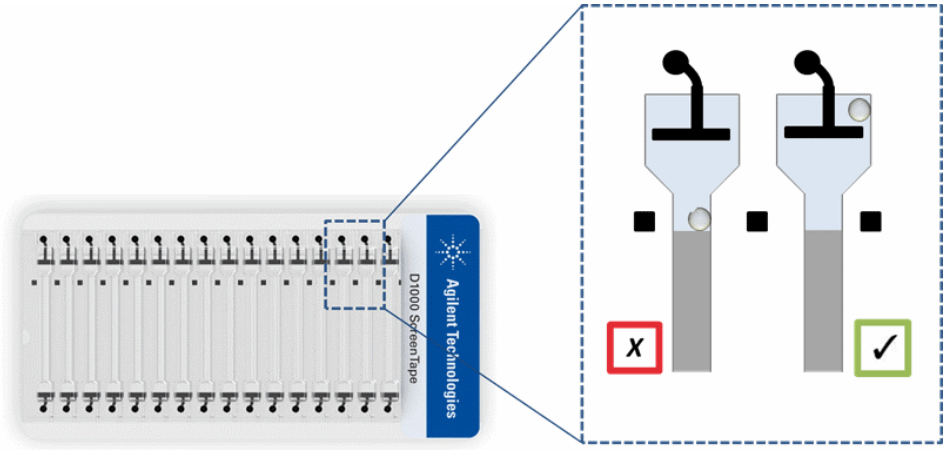


Figure 2 Flicking the ScreenTape consumable removes bubbles from the gel interface

Partially used ScreenTape (those that contain lanes run on previous occasions) should be returned to the box and stored vertically between 2 – 8 °C for a maximum of 2 weeks.

NOTE

The laptop utilized for performing any previous use(s) of the ScreenTape 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.

Used ScreenTape, sample strips and tips should be disposed of in accordance with local safety regulations.

2200 TapeStation Instrument

LED Status

- When powered up and idle, the instrument will have a blue LED visible on the front of the case.
- When running normally this blue LED will flash slowly.
- A quickly flashing LED indicates that the instrument has encountered an issue.

Operating Temperature

- Ensure that environmental conditions for the chosen assay are met
- Optimal Instrument operating temperature:
  - 20 °C (68 F)
- Assay specific operating temperatures:
  - 12 – 37 °C (54 – 99 F) for D1000 ScreenTape
  - 17 – 37 °C (63 – 99) for High Sensitivity D1000 ScreenTape
  - 15 – 30 °C (59 – 86 F) for Genomic DNA ScreenTape
  - 14 – 30 °C (57 – 86 F) for RNA and High Sensitivity RNA ScreenTape
  - 10 – 33 °C (50 – 91 F) for P200 ScreenTape

NOTE Instrument operating temperature may be higher than ambient lab temperature, especially after prolonged use.

If the instrument is out of the recommended temperature range for the ScreenTape inserted, the following error message will appear in the controller software:

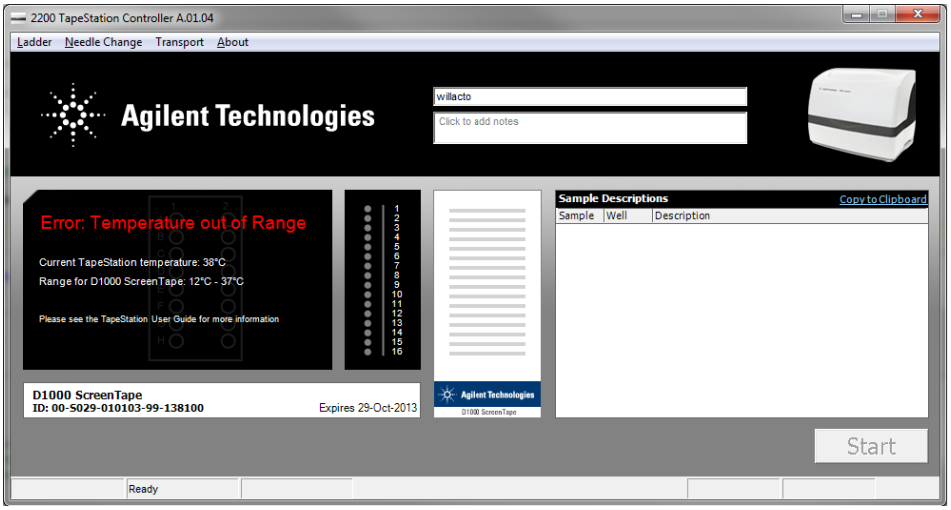


Figure 3 Error: Temperature out of Range

If the quoted current temperature is above the specified range, please move the system out of direct sunlight and away from any windows. Check that any air conditioning is functioning.

If the quoted current temperature is below the specified range please allow the instrument to equilibrate to the ambient temperature, and avoid using in a cooled area.

Transporting the TapeStation

- If transporting the TapeStation instrument, always allow it to acclimatize to the ambient temperature of the operating environment before use.
- Before transporting the TapeStation, ensure 'transport mode' has been enabled in the controller software using the drop down menu labelled **Transport**.
- Always remove the Sample plate, tip holder and ScreenTape from the instrument before transportation.
- When transporting the TapeStation ensure the correct packaging is used. Shipping boxes can be purchased using part number G2960-60100.

Consumables

CAUTION

Use of incorrect consumables

Damage to the TapeStation instrument and impact system performance

➔ • Ensure only Agilent 2200 TapeStation approved consumables are used

Consumables required for the 2200 TapeStation

- Loading tips (5067- 5152 or 5067- 5153)
- Optical Tube 8x Strip (401428) and Optical Cap 8x Strip (401425) or 96-well Sample Plates (5067- 5150) and 96-well Plate Foil Seal (5067-5154).
- Vortex mixer

Additional Material Required (Not Supplied)

- Volumetric pipettes
- Centrifuge suitable for 8 way strips or 96-well plates
- Heating block or PCR machine (for RNA and Protein assays)

Manual Marker Assignment

The 2200 TapeStation software assigns the most likely upper and lower marker using their run position. In the event that the incorrect markers are assigned, manually correcting this is possible using the simple procedure below.

NOTE

The following example corrects the lower marker in a DNA file. The same method can be used to manually assign upper markers.

RNA and Genomic DNA assays contain only lower markers.

1 Turn Alignment off, by pressing the **Aligned** button 

.

2 Un-aligning the gel image shows the run distance of each band, uncorrected by known marker sizes. In a fully functional file, markers will migrate to a similar position and have a similar shape.

Check the gel image to identify which bands are the correct upper and lower markers.

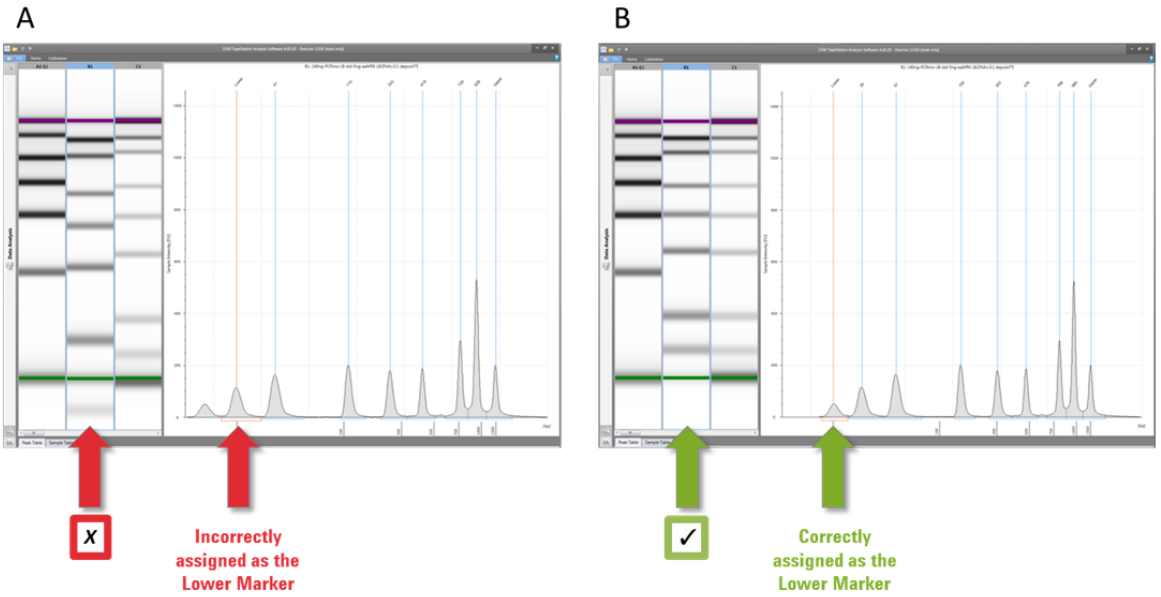


Figure 4 Manual lower marker assignment in the TapeStation Analysis Software. First identify the correct marker.

3 To add a Peak assignment, hover the mouse pointer above the unassigned peak, and right click. Then select **Add Peak** as shown in Figure 5 on page 20.

4 Next, assign the correct Marker peak by right clicking, and selecting **Assign as Upper Marker**/**Assign as Lower Marker** as shown in Figure 5 on page 20 .

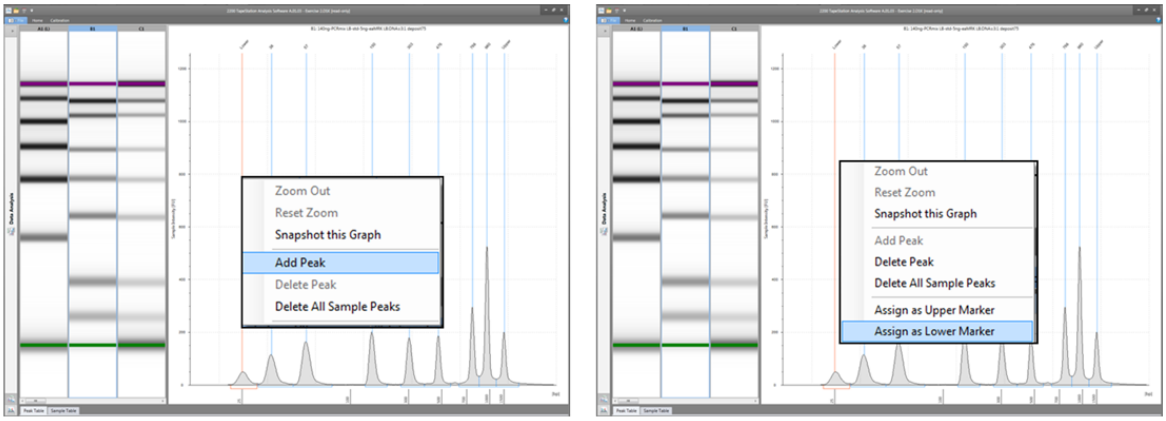


Figure 5 Manual lower marker assignment in the TapeStation Analysis Software. Assign the correct marker by right clicking.

5 Turn the alignment back on by pressing the **Aligned** button again. Check the updated gel and electropherogram images; the data should now be correctly aligned.

2
2200 TapeStation Software

This section gives an overview on the compatibility of the TapeStation Software.

The 2200 TapeStation Software can be downloaded from the Agilent Genomics website.

www.agilent.com/genomics/tapestation

NOTE

Important:

Ensure that the TapeStation instrument is not connected to the laptop during software installation or upgrade.

NOTE

For information regarding software installation, PC compatibility and known problems or limitations, please consult our latest Readme document.

This can be found using the link above.

ScreenTape Compatibility Matrix

The following matrix details the minimum allowable TapeStation software version required to run each ScreenTape assay.

Table 1 ScreenTape Compatibility Matrix

				ScreenTape					
			Product Number	**5067-5371**	**5067-5365**	**5067-5582**	**5067-5584**	**5067-5576**	**5067-5579**
			Name	P200 ScreenTape	Genomic DNA ScreenTape	D1000 ScreenTape	HS D1000 ScreenTape	RNA ScreenTape	HS RNA ScreenTape
TapeStation System	Current	**G2964AA**	2200 TapeStation	**A.01.01**	**A.01.03**	**A.01.04**	**A.01.04**	**A.01.04**	**A.01.04**
		G2965AA	2200 Nucleic Acid TapeStation	Not compatible	**A.01.03**	**A.01.04**	**A.01.04**	**A.01.04**	**A.01.04**
		G2966AA	2200 ScreenPlex TapeStation	Not compatible	**A.01.03**	**A.01.04**	**A.01.04**	**A.01.04**	**A.01.04**
	Legacy	**Lab901-ST007**	ScreenPlex TapeStation - Single Load	Not compatible	Not compatible	Not compatible	Not compatible	Not compatible	Not compatible
		Lab901-ST008	DNA TapeStation - Single Load	Not compatible	Not compatible	Not compatible	Not compatible	Not compatible	Not compatible
		Lab901-ST009	RNA TapeStation - Single Load	Not compatible	Not compatible	Not compatible	Not compatible	Not compatible	Not compatible
		Lab901-ST010	Combined TapeStation	**A.01.01**	**A.01.03**	**A.01.04**	**A.01.04**	**A.01.04**	**A.01.04**
		Lab901-ST017	ScreenPlex TapeStation	Not compatible	**A.01.03**	**A.01.04**	**A.01.04**	**A.01.04**	**A.01.04**
		Lab901-ST019	Nucleic Acid TapeStation	Not compatible	**A.01.03**	**A.01.04**	**A.01.04**	**A.01.04**	**A.01.04**

3 Instrument Communication

This section gives an overview on possible issues caused by instrument communication.

Instrument and Laptop

- Always power up the 2200 TapeStation System in this order:
  - **a** Laptop
  - **b** Instrument
  - **c** Controller
- Always power down laptops and instruments at night
- Always save run files to a local folder (on the TapeStation laptop)
- Always download anti-virus software onto the 2200 TapeStation laptops

USB Connection

2200 TapeStation instruments connect to bundled laptops via USB cable.

For instructions on connecting your 2200 TapeStation and laptop, please consult the readme document.

NOTE

Windows may display a Found New Hardware wizard once the software has loaded. In this instance, always perform the following steps:

1. Select **No, not this time** to prevent connecting to Windows Update and initiating a search for software.
2. In the next window select **Install the Software automatically**.
3. If a window appears, indicating the software did not pass the windows logo testing, click **Continue Anyway**.

   A window will then appear, indicating that the hardware has been successfully installed.

Updating USB Drivers

This section describes the procedure to update relevant USB drivers and identifies the device names specific to the 2200 TapeStation System.

If a laptop fails to connect to the instrument, or loses connection for any reason, first check that the power and USB cables are attached to the instrument, then if issues remain, re-install the USB drivers. This process is the same as for any external USB device.

If a connection failure occurs, a likely error message displayed in the controller software is as follows:

ERROR: 'Failed to connect to the barcode scanner or camera'

Each TapeStation instrument has three individual USB devices which are linked to the single USB connection on the back of the instrument.

The USB devices found within each instrument are:

1 The Barcode reader

The Barcode reader is generic across all instrument types and is identified in the list of **Ports** as:

- **5180 Area Imager**

2 The USB to serial converter

The USB to serial converter is generic across all instrument types and is identified in the **Universal Serial Bus controllers** list as:

- **USB↔Serial** Converter

3 The Camera

The Camera type listed depends on the instrument:

- Nucleic Acid 2200 TapeStation instruments (G2965AA) use a camera identified in the **Universal Serial Bus controllers** list as:

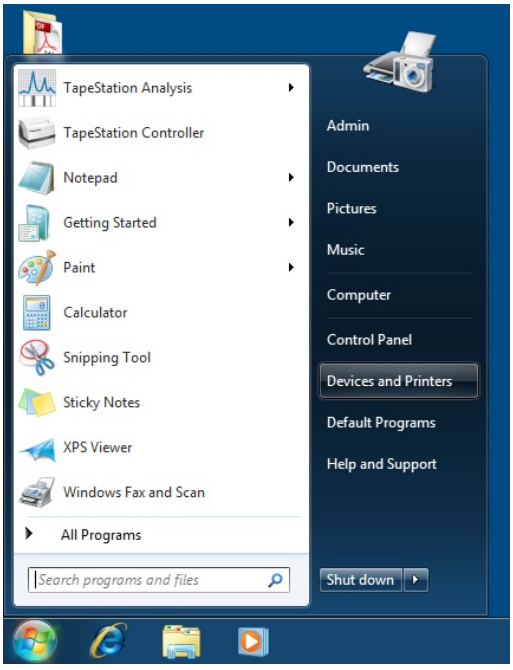
  uEye UI-154x Series

- 2200 TapeStation instruments (G2964AA) use a camera identified in the **Universal Serial Bus controllers** list as:

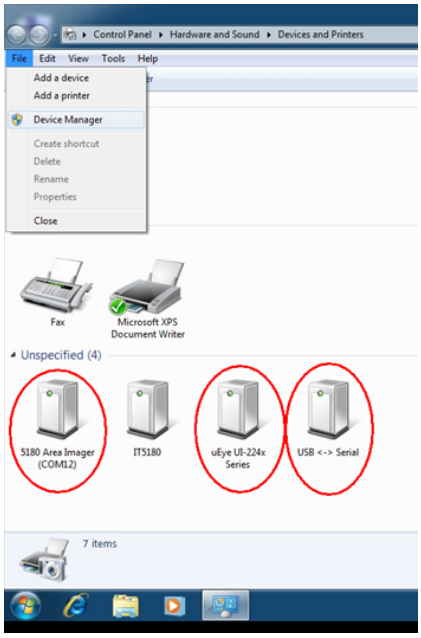
  uEye UI-224x Series

To update the USB Drivers, please use the following instructions.

1 Switch the instrument on and connect the USB cable. Open the Windows Start menu and click on **Devices and Printers**



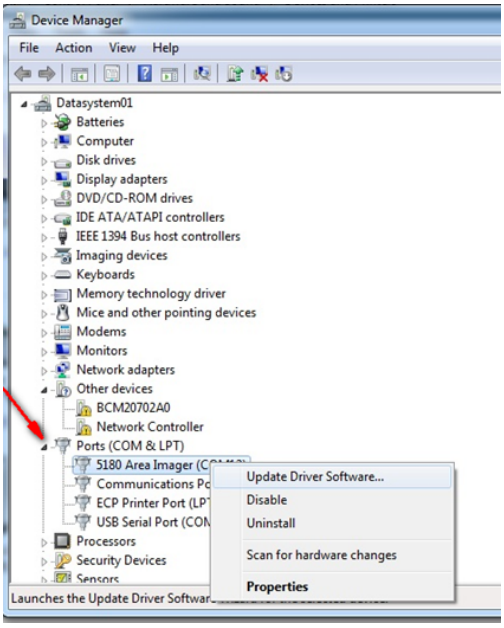
2 The Barcode reader (5180 Area Imager), the USB to Serial convertor, and the respective uEye UI camera should all be present in this list (see red circles). If one of the devices is not present, or is highlighted as having issues (with an exclamation mark), a driver update is required. To resolve this, first click on **File** and choose **Device Manager**.



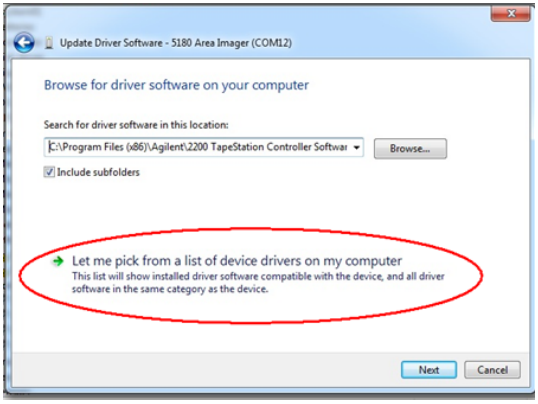
3 Make sure that all USB devices are enabled. Disabled devices show up with an exclamation mark. To enable disabled USB devices, right click on the device and click **Enable**.

Updating Barcode Reader and USB to Serial Convertor Drivers

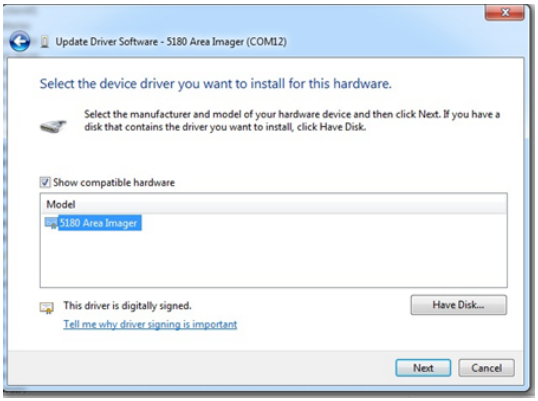
1 To locate the Barcode reader and USB to Serial Drivers, expand the **Ports** list. Right click on the device identified as malfunctioning in the previous step and select Update Driver software. The following instructions show updating the **5180 Area Imager** (barcode reader) driver.



2 If the following pop up window appears, choose **Browse my computer for driver software** and choose **let me pick from a list** on the next pop-up window.

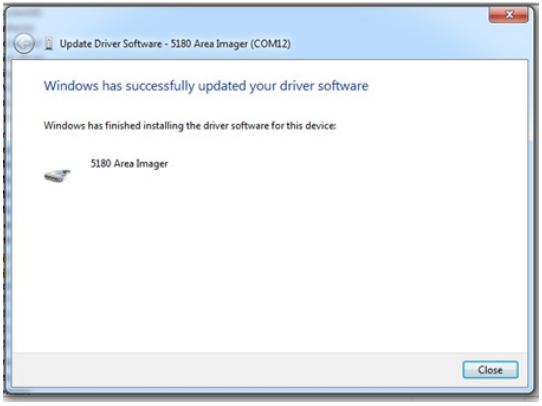


3 Click on the 5180 Area Imager' to mark it and click **Next**,



4 The Driver software will automatically update.

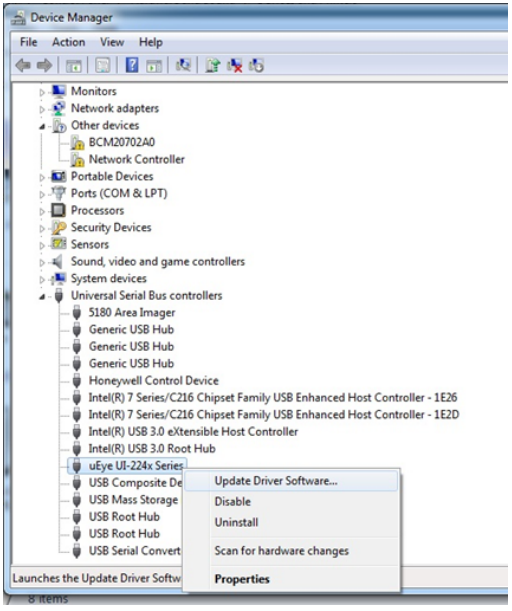
When complete, the following window will appear.



5 Make sure that all USB devices are enabled. Disabled devices show up with an exclamation mark. To enable disabled USB devices, right click on the device and click **Enable**.

Updating Camera Driver software

1 To re-install the camera on a protein capable 2200 TapeStation System (G2964AA) expand the Universal Serial Bus controller list and right click on the **uEye UI-224x Series**. For a Nucleic Acid TapeStation (G2965AA) right click on the **uEye UI-154x Series**. After identifying the camera driver identified as malfunctioning, update the appropriate drivers as described in the **Updating Barcode Reader and USB to Serial Convertor Drivers** section above.



2 Make sure that all USB devices are enabled. Disabled devices show up with an exclamation mark. To enable disabled USB devices, right click on the device and click **Enable**.

Barcode Reader

For correct function the instrument must read barcodes, both inside the instrument (when idle) and on the ScreenTape (when ScreenTape is inserted). Excess light can affect this procedure.

NOTE

Ensure that the 2200 TapeStation instrument is placed away from direct light sources.

Excess light can interfere with barcode recognition and result in a non-functioning instrument.

NOTE

When inserting a ScreenTape into the TapeStation, its orientation must be correct.

1 Insert the ScreenTape with the label towards the front of the instrument and the barcode facing right.

Retrieving log files

Log files can be retrieved using the TapeStation Analysis Software. These are often useful for troubleshooting purposes.

NOTE Please ensure that when communicating with the Technical Support Channel both the log files and the analysis file in question are sent for technical troubleshooting.

1 Open the TapeStation Analysis Software.

2 In the file menu, select '**Help**' then click on the **export error logs** button.

Export Error Logs

Export Error Logs from the Analysis and Controller applications to a zip file.

4
Instrument Troubleshooting

This section gives an overview on possible issues caused by the instrument.

Blank Lanes

There are a number of reasons blank lanes can occur in a TapeStation Analysis file. If, after checking each of the causes listed below, your results files continue to show blank lanes, please contact your local support representative.

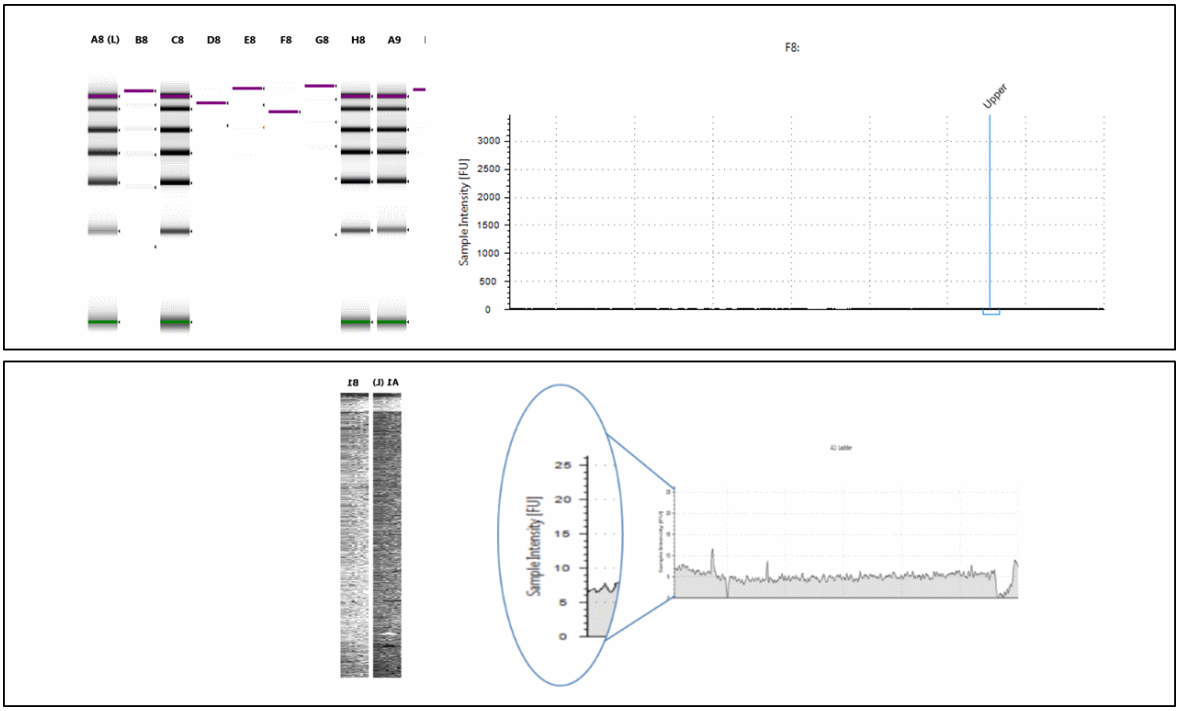


Figure 6 Examples of blank lanes in the TapeStation Analysis Software

Blank lanes can appear in either of the ways shown above. As a white lane with no visible peaks, or as a black grainy lane. A black lane happens when there is very little sample signal, causing any background noise to appear higher than normal. In these cases the sample intensity scale shows very small values.

Table 2 Possible causes of blank lanes in order of probability

Cause	Solution
Tips in the selected positions were missing from the tip rack. Without tips at the correct positions, sample cannot be picked up and loaded onto the ScreenTape.	Always ensure that all 16 tips are loaded into the tip rack at the start of each run.
A bubble in the sample tube prevented the sample from being picked up. During normal instrument function, tips pick up from the very bottom of the sample tubes. The presence of bubbles here can result in no sample being picked up.	Always ensure that sample strips or 96-well plates are centrifuged after preparation to remove any bubbles present at the bottom of wells.
Insufficient sample present.	Always ensure a minimum of 3 µL of solution is present in the sample tubes. Do not re-use sample strips from previous runs. Re-using samples can lead to poor results.
The samples were not selected correctly in the controller software.	Always ensure that the positions selected in the controller software match the location of the sample strips within the instrument.
The sample lids were not correctly removed before starting the run.	Always ensure that sample strip lids are removed before starting the run. Failure to do so can result in damage to the instrument.
Hardware fault	If the above causes have been ruled out, there may be a hardware fault. Please send all relevant data to your local support representative for investigation.

5 Troubleshooting DNA Applications

This section gives an overview on how to avoid problems with DNA Applications.

Good Measurement Practices for DNA

For reliable results, instructions regarding reagent preparation and instrument maintenance must be strictly followed. Important technical details are described in the TapeStation User Manual and other supporting documentation.

This section describes techniques for ensuring reliable quantification and sizing results using DNA assays on the 2200 TapeStation System, and includes advice on the following topics:

- Quantification
- Sizing
- Molarity
- Genomic DNA Assay

For more details please consult the Technical Overview using the link below:

www.chem.agilent.com/Library/technicaloverviews/Public/5991- 5153EN.pdf

Quantification

Sample mixing

- 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.
- The effects of insufficient mixing were investigated using D1000 ScreenTape and reagents, and results are presented in Figure 7 on page 41 and Figure 8 on page 42, as well as Table 3 on page 42.
- Poor mixing dramatically affects the reported sample concentration. Following the recommended procedure is the best way to attain accurate quantification results.
- Please see "Mixing recommendations" on page 10, or the appropriate assay Quick Guide for more details.

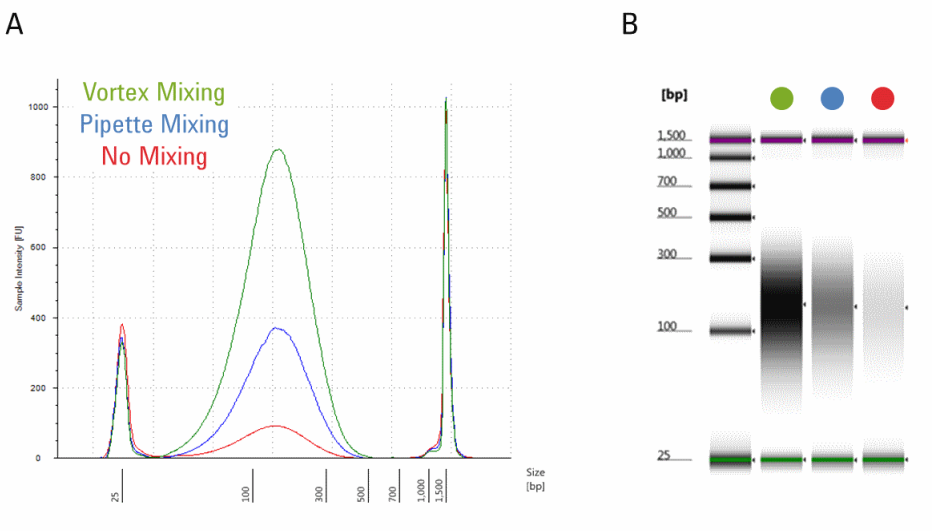


Figure 7 The electropherogram (A) and the gel image (B) of the ScreenTape mixing tests. In both panels the green trace shows the recommended protocol of vortex mixing using the IKA vortexer and adaptor at 2,000 rpm for 1 minute followed by brief centrifugation. Blue shows results for pipette mixing only. Red shows the effect of no mixing. Images were taken from the Agilent TapeStation Analysis Software

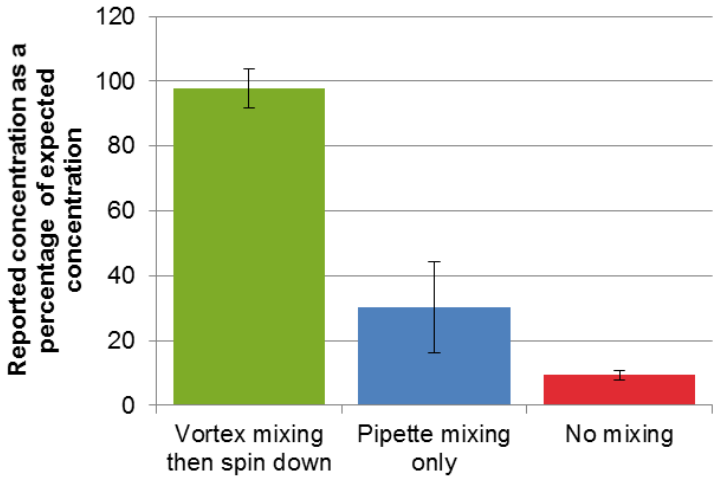


Figure 8 Chart of reported concentrations for the ScreenTape mixing tests, using the D1000 Assay. Concentrations are expressed as a percentage of the theoretical for the three mixing methods. As above, the green bar represents the recommended protocol of vortex mixing using the IKA vortexer and adaptor at 2,000 rpm for 1 minute followed by brief centrifugation; blue - pipette mixing only; red - no mixing.

Table 3 ScreenTape Reagent mixing tests. Quantification values 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 above

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

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. Figure 9 on page 43 shows examples of correct upper marker peak integration.

NOTE In assays with no upper marker (RNA, High Sensitivity RNA, Genomic DNA) quantification is taken from the lower marker.

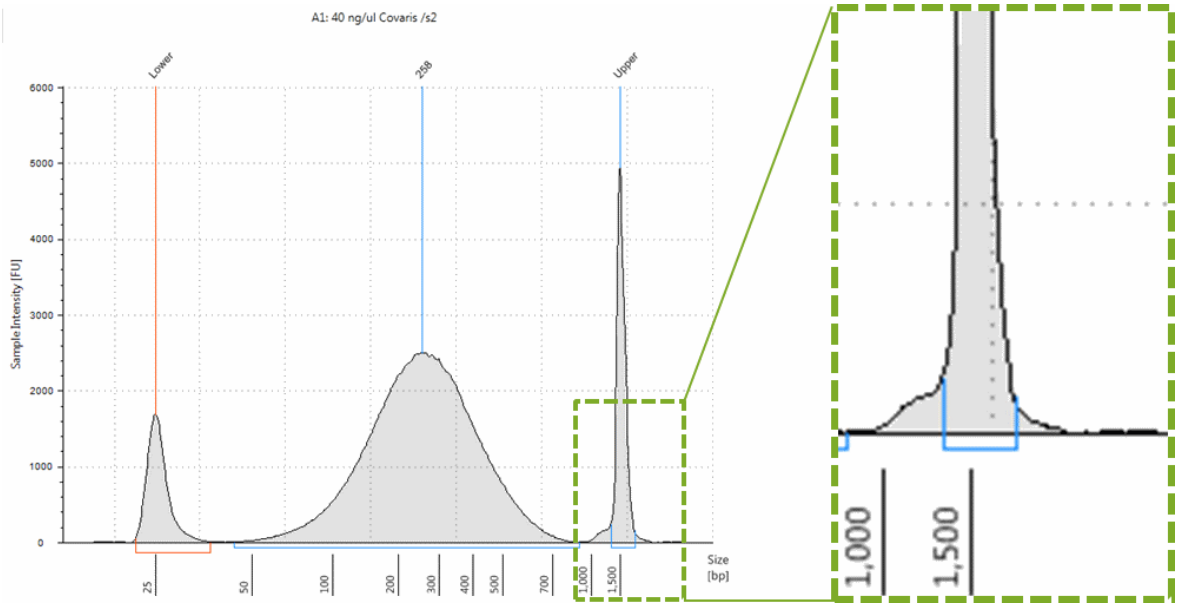


Figure 9 Correct upper marker integration for the 2200 TapeStation DNA Assays. Figure shows D1000 ScreenTape Assay.

Figure 10 on page 44 demonstrates the effect of peak integration on the quantitative results. Incorrect peak integration can significantly bias the determined DNA sample concentration.

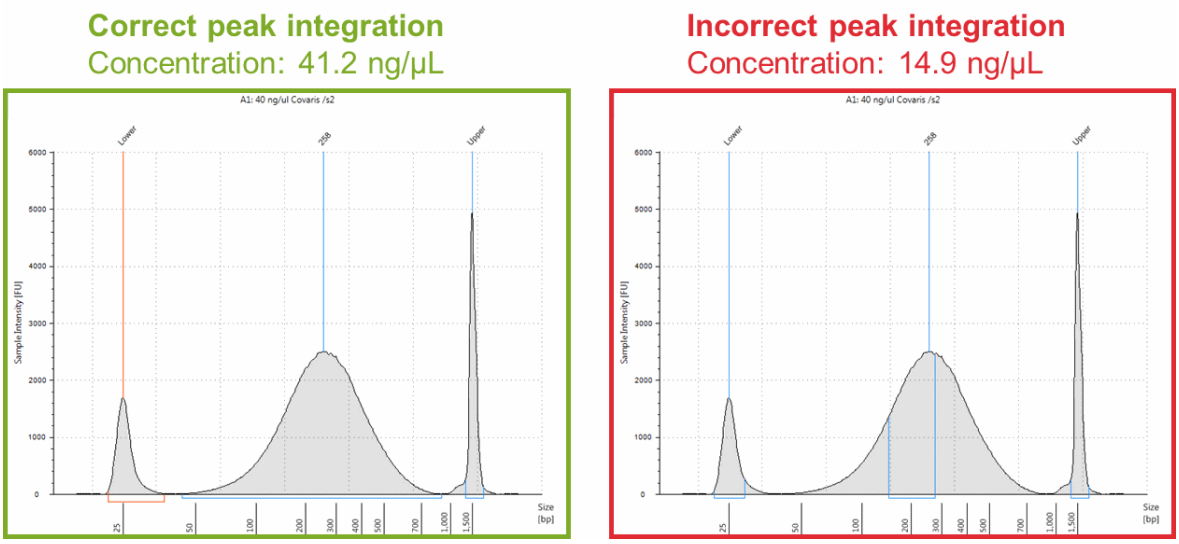


Figure 10 Example of correct (A) and incorrect (B) sample peak integration, and their effect on reported sample concentration.

Use the correct protocol

- Each ScreenTape type is designed for use with its corresponding Reagent kit.
- Ensure that sample and sample buffer volumes from the correct protocol are followed.
- 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 ranges, please refer to the User Manual, or the appropriate assay Quick Guide.

Use the correct tools for the job

- Use calibrated pipettes which are sufficient for the volume required.
- Ensure correct pipetting technique, so that volumes are precise, and that the concentrations can be calculated correctly.
- Use a vortexer designed for mixing 8-way tube strips, or 96-well plates respectively.
- For more details, please see “Mixing recommendations” on page 10.
- Use only the correct, Agilent supplied consumables including loading tips, 8-way strips and 96-well plates. See “Parts and Consumables” on page 109 for product codes.
- To run samples from a 96-well plate, also use the 2200 TapeStation foil cover (p/n 5067-5154) to prevent the sample from leaving the plate during vortexing.
- After vortexing, use an appropriate centrifuge for either 96-well plates, or 8-way strips to ensure that all of the samples are at the bottom of the tube before placing in the TapeStation.

TapeStation analysis in the Agilent Sure Select workflow

The 2200 TapeStation system has been verified for use within the Agilent Sure Select protocol. During the purification step however, residual AMPure XP beads can give signal which runs with the upper marker (See Figure 11 on page 46).

- Any signal under the upper marker 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.

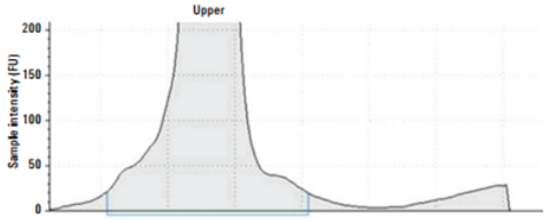


Figure 11 Enlarged image of the upper marker showing additional signal from AMPure beads.

Sizing

Peak maxima versus average molecular weight sizing

Within the 2200 TapeStation analysis software, sizing can be found in both electropherogram and region mode. The sizing information presented in these modes will be 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.

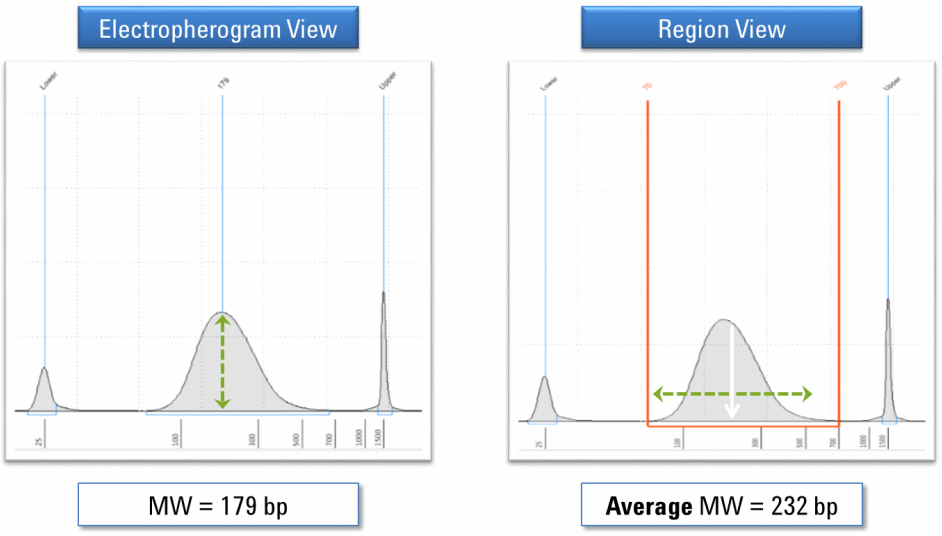


Figure 12 The sizing data obtained in Electropherogram and Region views of the Agilent TapeStation Analysis Software.

Identifying the correct markers

The markers are used as internal references to determine the molecular weight size of the sample. Incorrect identification will lead to miscalculations in reported sizing values.

NOTE

Always ensure that the upper and lower markers have been identified correctly.

See "Manual Marker Assignment" on page 18.

Flicking the ScreenTape

- The presence of small bubbles within the buffer chamber of the ScreenTape is normal.
- These bubbles often occur at the gel/buffer interface and need to be displaced by flicking prior to running.
- Failure to remove bubbles from the gel/buffer interface is detrimental to the performance of the ScreenTape.
- See "ScreenTape" on page 13 for more details.

Molarity

Molarity

Molarity

Molarity is determined from both *size and quantity*.

NOTE Errors in sizing and quantification will result in erroneous molarity calculations.

Always ensure that the good measurement practices for sizing and quantification have been followed to ensure accurate molarity values.

Genomic DNA Assay

When using the Genomic DNA Assay, it is important to note the following recommendations to ensure correct sizing.

Equilibrate reagents to room temperature

Genomic DNA Reagents must be equilibrated to room temperature for 30 minutes before use. Failure to do so can affect sizing results.

NOTE Cold reagents will overestimate the size of genomic DNA samples.

Table 4 The effect of room temperature (RT) equilibrated Genomic DNA reagents as well as cold reagents on the sizing accuracy of the Genomic DNA ScreenTape assay

	Expected	Reagents at RT	Reagents at 4 °C
MW	17000 bp	18867 bp	24369 bp
Accuracy	-	+11 %	+43 %

Always use fresh genomic DNA ladder

- Ladder must be prepared fresh for each run, and run in the first available position
- Run profile decreases as ladder warms up within the instrument
- Sizing results will be affected by adjusted ladder run profiles.

NOTE No software ladder is available for genomic DNA.

The effect of shaking the genomic DNA ladder vial

- Shaking the Genomic DNA ladder vial can degrade the top fragment (See Figure 13 on page 51).
- This can result in 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
- Prior to addition with sample buffer the ladder vial should be handled carefully
- Once mixed with sample buffer, the ladder should be gently vortexed for 5 seconds as normal.

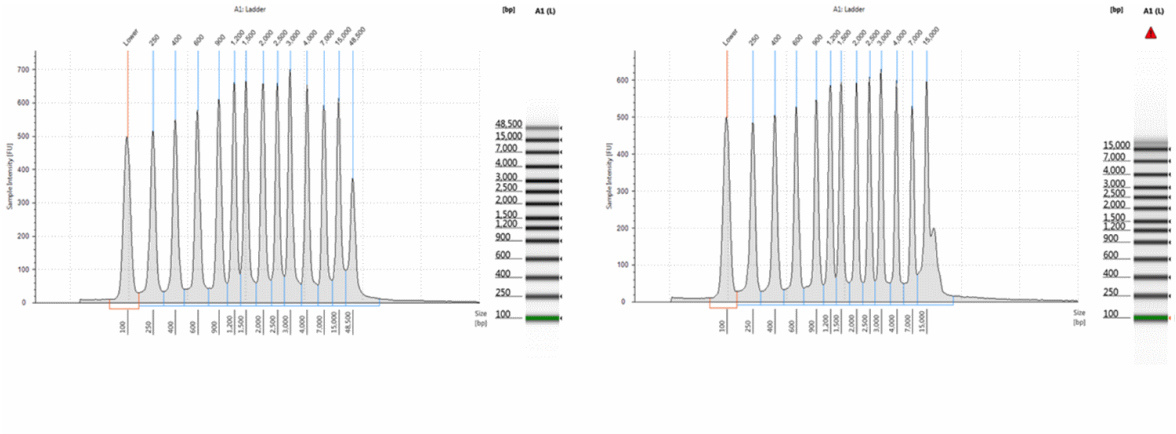


Figure 13 The effect of shaking the Genomic DNA Ladder. A) Genomic DNA Ladder has been vortex mixed for 5 seconds prior to analysis on the Genomic DNA ScreenTape assay. B) The Genomic DNA Ladder vial was shaken by manually inverting the tube 30 times. Degradation of the top fragment (48,500 bp) is clearly shown.

NOTE To minimize shaking during transit, the Genomic DNA reagents are shipped frozen, on dry ice. Once received, these should be kept at 2 – 8 °C in the refrigerator.

Incorrect Quantification Results

Incorrect Quantification results can have multiple causes, please read "Good Measurement Practices for DNA" on page 40 for further details. If, after reviewing this Technical overview and following the recommendations below your results files continue to show concentration discrepancies, please contact your local support representative.

Table 5 DNA - Possible causes of incorrect quantification results in order of probability

Cause	Solution
Insufficient Mixing	Ensure correct mixing by reading the mixing recommendations
	See "Good Measurement Practices for DNA" on page 40 and "Mixing recommendations" on page 10
Sample concentration outside recommended range for application	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
Incorrect Peak integration	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 "Good Measurement Practices for DNA" on page 40
Some sample has run concurrently with the Upper marker	Ensure your sample is within the recommended sizing range for the applications.
	If using Sure Select Protocol, ensure that all AMPure beads are removed by increasing the time your sample is on the magnetic plate
	See "Good Measurement Practices for DNA" on page 40
Incorrect protocol used	Ensure the correct sample protocol for the application is used. Differences exist between standard and High Sensitivity assay protocols, please consult the User Manual or appropriate Quick Guides for more detail.
	See "Good Measurement Practices for DNA" on page 40
Sample prepared too long before analysis	To avoid evaporation or settling, ensure that your run is started immediately after your sample preparation.
	If using a 96-well plate, always cover with the recommended foil seal, see "Consumables" on page 17
Incorrect pipetting technique or pipette calibration	Follow guidelines in the essential measurement practices section. ("Overview" on page 6)
Incorrect Marker peaks picked up	Use the **Aligned** button in the TapeStation Analysis Software to detect the correct markers, then assign them by right clicking.
	See "Manual Marker Assignment" on page 18
	Ensure that all sample peaks are within the recommended sizing range for the application.

Genomic DNA 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.

Any signal seen which is annotated **well** should be included in the analyzed region.

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

Incorrect Sizing Results

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

Incorrect sizing results can have multiple causes, please read the "Good Measurement Practices for DNA" on page 40. If, after reviewing this Technical overview and following the recommendations below, your results files continue to show sizing discrepancies, please contact your local support representative.

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

Cause	Solution
Incorrect Marker peaks picked up	Ensure the correct marker peaks are picked up in the TapeStation Analysis Software.
	See "Manual Marker Assignment" on page 18
	Ensure that all sample peaks are within the recommended sizing range for the application
Incorrect Analysis software mode	Reported sizing can differ between electropherogram and region modes. Select the correct mode for your samples.
	See "Good Measurement Practices for DNA" on page 40 for more details
Issues with the ladder lane	Any issues with the run ladder lane will be annotated within the TapeStation Analysis Software sample table, under **Observations**
	Manually insert or delete affected peaks, or insert a software saved ladder to regain sizing information (if available)
Insufficient migration due to presence of salt	Dilute your samples to ensure low levels of buffer salt
	True migration profiles can be seen by unaligning your gel image by pressing the **Aligned** button in the TapeStation Analysis Software
	Please refer to the salt tolerance guidelines for your assay in the User Manual or appropriate Quick Guide

Genomic DNA Sizing

Incorrect or inaccurate sizing can occur using the Genomic DNA ScreenTape assay when there has been an issue with the ladder lane.

For more details, please see the "Good Measurement Practices for DNA" on page 40.

Missing Marker Peaks

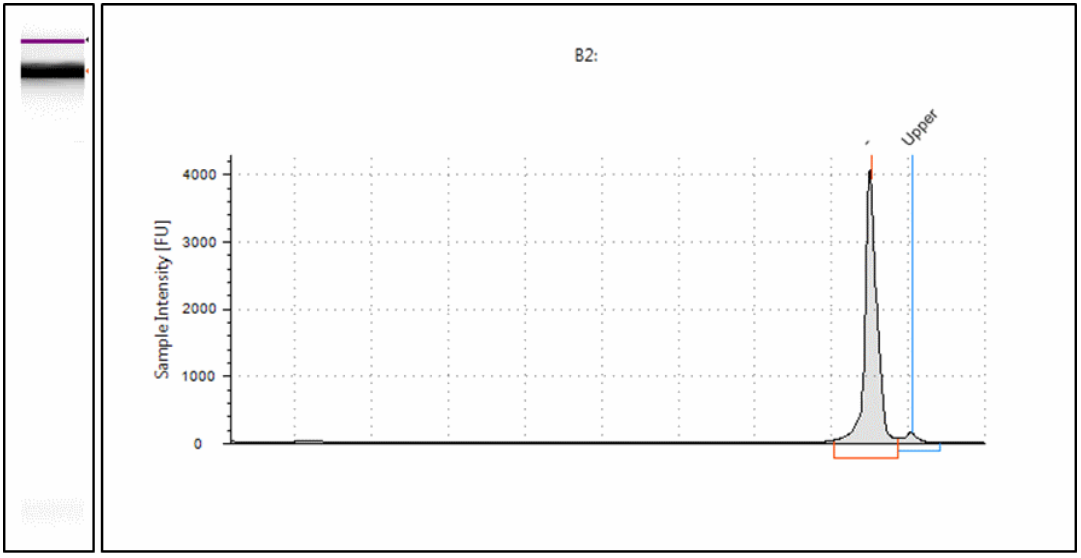


Figure 14 Example of missing marker peak

Table 7 DNA - Possible causes of missing marker peaks in order of probability

Cause	Solution
Insufficient Mixing	Ensure correct mixing by reading the mixing recommendations
	See "Good Measurement Practices for DNA" on page 40 and "Mixing recommendations" on page 10
Sample concentration outside recommended range for application	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
Blank lane	See Blank lane section
Insufficient migration	To check migration true distance, unalign the gel image using the **Aligned** button.
	See "Unexpected Migration Profile" on page 58

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 8 DNA - probable causes for unexpected migration profile

Problem	Likely Cause	Solution
The gel image looks distorted	Incorrect Marker peaks picked up	Ensure the correct marker peaks are picked up in the TapeStation Analysis Software.
		See "ScreenTape" on page 13 and "Manual Marker Assignment" on page 18
		Ensure that all sample peaks are within the recommended sizing range for the application

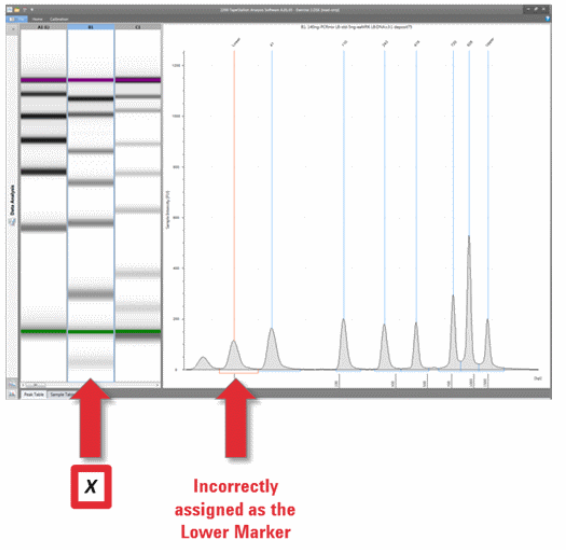


Figure 15 Identifying the correct lower marker

Table 8 DNA - probable causes for unexpected migration profile

Problem	Likely Cause	Solution
Slanted or smeared bands	A bubble could be located at the top of the ScreenTape	Always flick the ScreenTape before placing into the TapeStation. See "Good Measurement Practices for DNA" on page 40
	The sample may not have been mixed correctly with the sample buffer	Please follow the mixing recommendations for the assay. See "Good Measurement Practices for DNA" on page 40, and "Mixing recommendations" on page 10 for more details.
	The sample and sample buffer may have started to evaporate	Always ensure that samples are run on the TapeStation immediately after preparation with sample buffer.

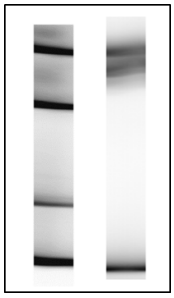


Figure 16 Effect of bubbles at the gel interface on TapeStation Analysis Software results - Gel view.

Table 8 DNA - probable causes for unexpected migration profile

Problem	Likely Cause	Solution
The markers have appeared in the sample lane, but the sample has not	The sample used is too dilute	Concentrate your sample until it is within the recommended range for application, then prepare a new run.
	Insufficient Mixing	Ensure correct mixing by reading the mixing recommendations. See "Good Measurement Practices for DNA" on page 40

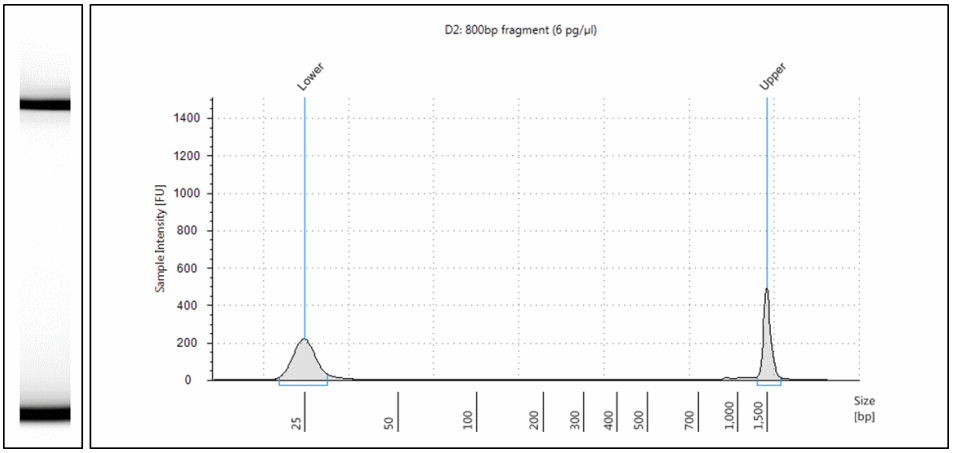


Figure 17 Example of missing sample peak

Table 8 DNA - probable causes for unexpected migration profile

Problem	Likely Cause	Solution
Additional bands have appeared to those expected in the sample lane	There may be a contaminant peak caused by dust or dirt on the ScreenTape	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. Peaks caused by contaminants present sharp strong bands which are often slanted or uneven in the gel image, and are usually easily distinguishable from sample peaks.
The upper and lower markers appear unusually intense in the gel image. DNA lower markers are normalised to appear the same in the electropherogram, which can result in sample peaks looking smaller than expected.	Sample prepared too long before analysis	To avoid evaporation or settling, ensure that your run is started immediately after sample preparation. If using a 96-well plate, always cover with the recommended foil seal.
	Insufficient Mixing	Ensure correct mixing by reading the mixing recommendations. See "Good Measurement Practices for DNA" on page 40
	The sample used is too dilute	Concentrate your sample until it is within the recommended range for application, then prepare a new run.

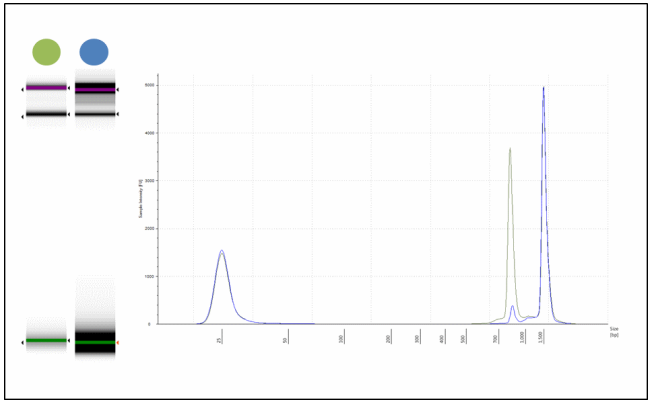


Figure 18 Example of intense upper and lower markers seen in the blue gel image. Lower markers are normalised in electropherogram view and appear identical in signal.

Table 8 DNA - probable causes for unexpected migration profile

Problem	Likely Cause	Solution
Higher than normal background noise	Insufficient Mixing	Ensure correct mixing by reading the mixing recommendations. See "Good Measurement Practices for DNA" on page 40
	Incorrect ScreenTape or reagent storage	Follow the storage conditions specified for the assay.

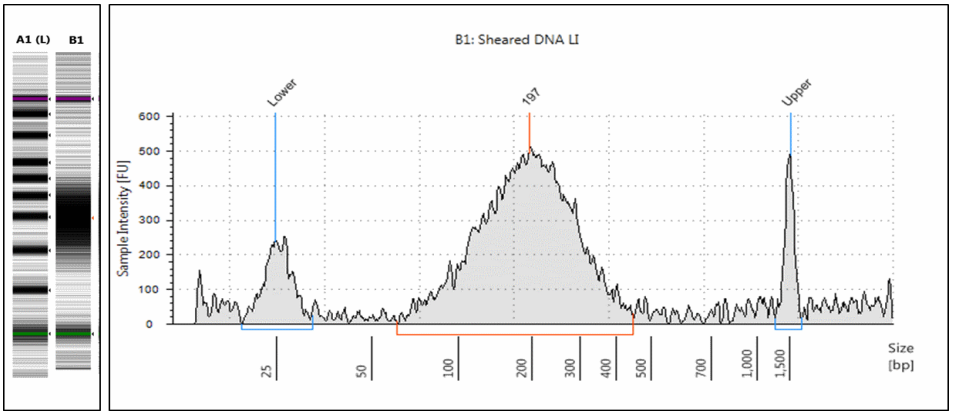


Figure 19 Example of high background noise

Table 8 DNA - probable causes for unexpected migration profile

Problem	Likely Cause	Solution
Low signal intensity of sample or marker peaks (one or other, not both)	Insufficient Mixing	Ensure correct mixing by reading the mixing recommendations. See "Good Measurement Practices for DNA" on page 40
	Incorrect reagent storage conditions	Follow the storage conditions specified for the assay.
	Sample concentration outside recommended range for application	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.

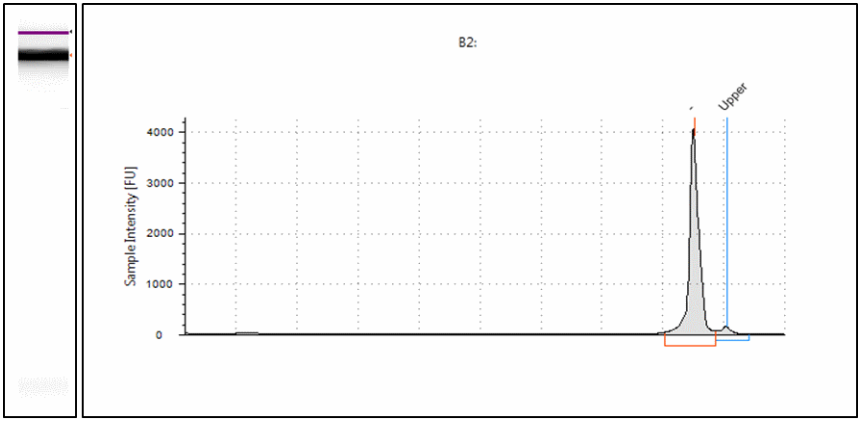


Figure 20 Example of low signal intensity of the marker peaks

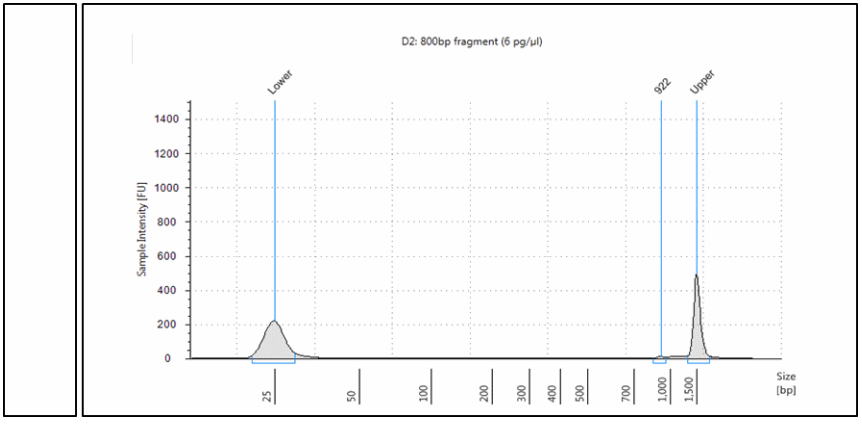


Figure 21 Example of low signal intensity of the sample peaks

Table 8 DNA - probable causes for unexpected migration profile

Problem	Likely Cause	Solution
Low signal intensity of sample and marker peaks (Both) or of ladder peaks	Incorrect reagent storage conditions	Follow the storage conditions specified for the assay. Prepare a new run with fresh reagents.

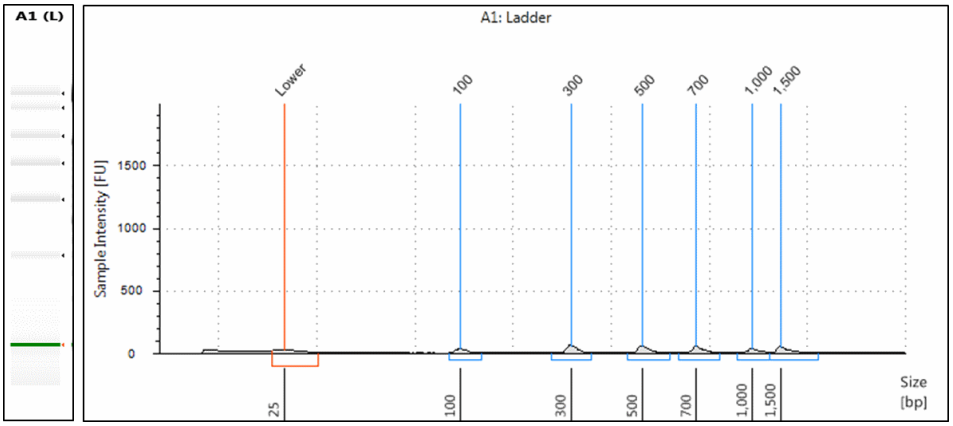


Figure 22 Example of low signal intensity in the High Sensitivity D1000 Ladder

Table 8 DNA - probable causes for unexpected migration profile

Problem	Likely Cause	Solution
Incorrect migration - samples have not reached the lower end of the gel lane	Salt concentration	Unalign the image using the **Aligned** button in the TapeStation Analysis Software. This will show the true position of all peaks present. High salt concentrations can cause short running within the gel lane which can cause incorrect identification of Lower marker peaks. Please refer to the salt tolerance guidelines for the assay.
	Partial electrophoresis failure caused by bubbles at gel-buffer interface	Ensure ScreenTape is *flicked* to remove any bubbles prior to use

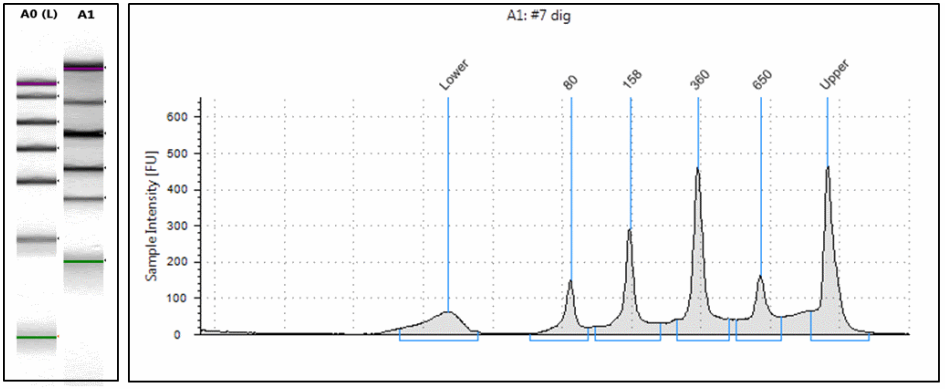


Figure 23 Example of shortened migration caused by high salt concentration

6
Troubleshooting RNA Applications

This section gives an overview on how to avoid problems with RNA Applications.

Incorrect Quantification Results

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 9 RNA - Possible causes of incorrect quantification in order of probability.

Cause	Solution
Sample concentration out with recommended range for application	Either dilute or concentrate your sample until it is within the recommended range for the application as seen in the appropriate assay Quick Guide, then prepare a new run.
Incorrect Peak integration	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.
Some sample has run concurrently with the Lower marker	Ensure your samples are within the recommended sizing range for the applications.
Incorrect protocol used	Ensure the correct sample protocol for the application is used. Differences exist between standard and High Sensitivity assay protocols, please consult the User Manual or appropriate Quick Guides for more detail.
Insufficient Mixing	Ensure correct mixing, see "Mixing recommendations" on page 10.
Incorrect heating procedure	Ensure that the samples are heat denatured according to the assay instructions before running. Both over and under heating the samples can affect concentration values.
Sample prepared too long before analysis	To avoid evaporation or settling, ensure that the run is started immediately after sample preparation. If using a 96-well plate, always cover with the recommended foil seal.
Incorrect pipetting technique or pipette calibration	Ensure correct pipetting technique and up to date pipette calibration. Reverse pipetting technique is advised for RNA and High Sensitivity RNA assays.
Incorrect Marker peaks picked up	Use the **Aligned** button in the TapeStation Analysis Software to detect the correct markers, then assign them by right clicking, see "Manual Marker Assignment" on page 18. Ensure that all sample peaks are within the recommended sizing range for the application.
Insufficient migration due to presence of salt	Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by pressing the **Aligned** button in the TapeStation Analysis Software. Please refer to the salt tolerance guidelines for your assay in the User Manual or appropriate Quick Guide.

Incorrect Sizing Results

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

Table 10 RNA - Possible causes of incorrect sizing results in order of probability

Cause	Solution
Genomic DNA contamination	Samples which are contaminated with genomic DNA contain a third peak which migrates in the region of the 18 and 28S. Occasionally this can be mistaken for the 18 or 28S peak. For correct sizing, ensure that all peaks are annotated correctly. Treat samples with DNAse and rerun to eliminate the genomic DNA peak.
Issues with ladder lane	Any issues with the run ladder lane will be annotated within the TapeStation Analysis Software sample table, under **Observations** Manually insert or delete affected peaks, or insert a software saved ladder to regain sizing information For best sizing results, rerun to ensure a functioning ladder lane.
Incorrect Marker peaks picked up	Ensure the correct marker peaks are picked up in the TapeStation Analysis Software. See "Manual Marker Assignment" on page 18. Ensure that all sample peaks are within the recommended sizing range for the application.
Insufficient migration due to presence of salt	Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by pressing the **Aligned** button in the TapeStation Analysis Software. Please refer to the salt tolerance guidelines for your assay in the User Manual or appropriate Quick Guide.

Missing Marker Peaks

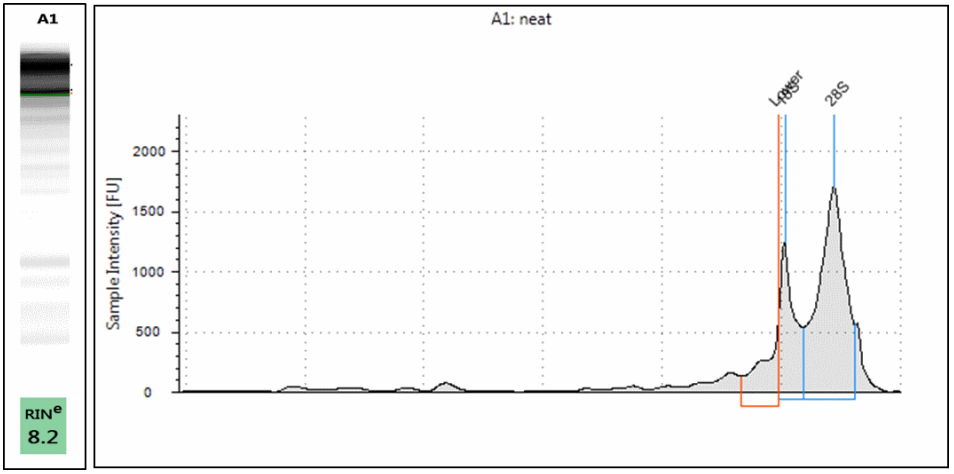


Figure 24 Example of missing marker peaks in an RNA assay

Table 11 RNA - Possible causes of missing marker peaks. In order of probability.

Cause	Solution
Insufficient Mixing	Ensure correct mixing, see "Mixing recommendations" on page 10
Sample concentration outside recommended range for application	Either dilute or concentrate your sample until it is within the recommended range for the application as seen in the appropriate assay Quick Guide, then prepare a new run.

Degraded RNA Ladder and/or samples

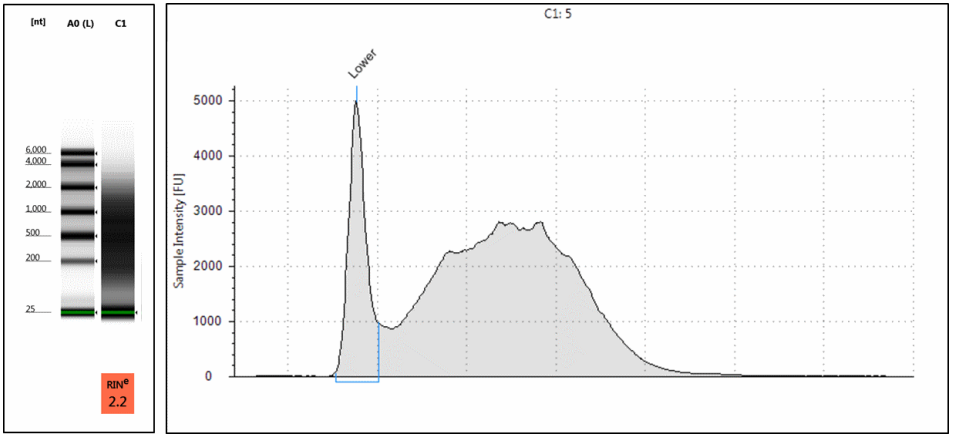


Figure 25 Example of degraded RNA resulting in missing peak identification.

Table 12 RNA - Possible causes of degraded sample in order of probability.

Cause	Solution
Incorrect heating step	Ensure the correct heating recommendations for the assay are followed as described in the User Manual and appropriate Quick Guide
RNAse contamination of the reagents	Ensure Good Laboratory Practices are followed as detailed in the RNA section of the TapeStation user manual Prepare a new run using new reagents
RNAse contamination of the plasticware	Ensure Good Laboratory Practices are followed as detailed in the RNA section of the TapeStation user manual

Incorrect or missing RIN^e value

Table 13 RNA - Possible causes for missing or incorrect RIN^e results in order of probability

Cause	Solution
There are 2 bands present at the 18S peak position	There may have been insufficient sample denaturation prior to ScreenTape analysis. The 18S peak may have partially reverted back to its original, non-denatured conformation. 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.
Extremely degraded sample	The TapeStation Analysis Software can occasionally fail to recognize extremely degraded RNA sample peaks.
Sample concentration outside recommended range for application	Either dilute or concentrate your sample until it is within the recommended range for the application as seen in the appropriate assay Quick Guide, then prepare a new run.
Genomic DNA contamination	Samples contaminated with genomic DNA contain a third peak which migrates in the region of the 18 and 28S. Occasionally this peak can be mistaken for the 18 or 28S peak. Ensure that all peaks are annotated correctly. Treat samples with DNAse to eliminate the genomic DNA peak then prepare a new run.

Incorrect or Missing Peak Annotation

Table 14 RNA - Possible causes of incorrect or missing peak annotation in order of probability.

Cause	Solution
Only specific RNA peaks (16, 18, 23, 28S) and Lower marker peak will be annotated by the software	Manually add any important peaks by right clicking on either the gel or electropherogram and selecting add other peak.
Extremely degraded sample	The TapeStation Analysis Software can occasionally fail to recognize extremely degraded RNA sample peaks. Prepare samples again, ensuring Good Laboratory practices are followed.
There are 2 bands present at the 18S peak position	There may have been insufficient sample denaturation prior to ScreenTape analysis. The 18S peak may have partially reverted back to its original, non-denatured conformation. Ensure the correct denaturation conditions during RNA sample preparation, according to the assay sample preparation guide. Samples should be kept on ice after preparation, and run within 2 hours of denaturation.
Genomic DNA contamination	Samples contaminated with genomic DNA contain a third peak which migrates in the region of the 18 and 28S. Occasionally this peak can be mistaken for the 18 or 28S peak. Ensure that all peaks are annotated correctly. Treat samples with DNAse to eliminate the genomic DNA peak then prepare a new run.

Unexpected migration profile

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

Table 15 RNA - Possible causes of unexpected migration profile

Problem	Likely Cause	Solution
There are two bands evident in the 28S peak opposition	The 28S peak may have partially reverted back to its original, non-denatured conformation	There may have been insufficient sample denaturation prior to ScreenTape analysis. Ensure the correct denaturation conditions during RNA sample preparation. Samples should be kept on ice, and run within 2 hours of denaturation.
There are two bands present at the 18 or 28S peak position	The peak may have partially reverted back to its original, non-denatured conformation	There may have been insufficient sample denaturation prior to ScreenTape analysis. Ensure the correct denaturation conditions during RNA sample preparation. Samples should be kept on ice, and run within 2 hours of denaturation.

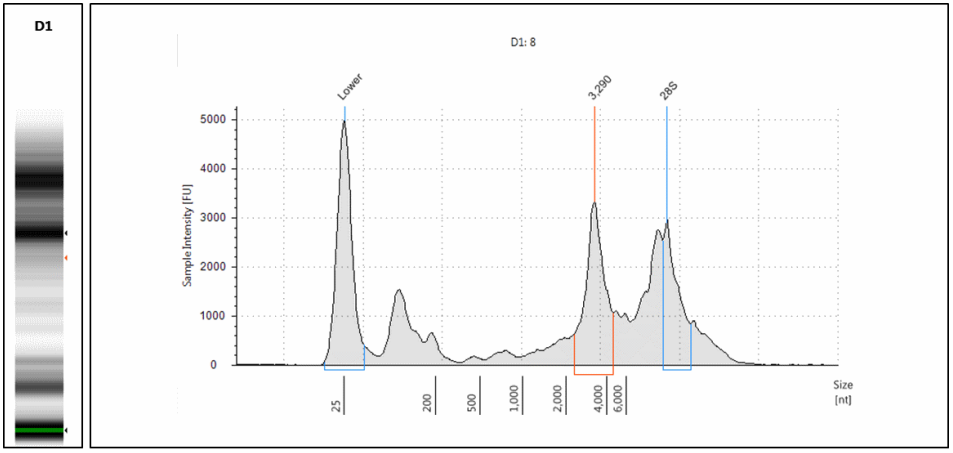


Figure 26 Example of extra peaks being picked up at the 28S position

Table 15 RNA - Possible causes of unexpected migration profile

Problem	Likely Cause	Solution
A split peak or additional unexpected band is evident in the RNA Ladder	Denaturation of the RNA Ladder may not have been sufficient	Ensure the correct denaturation conditions when preparing the ladder Ladder and samples should be kept on ice, and run within 2 hours of denaturation
Additional bands are present in the region of the 18 and 28S peak	Genomic DNA contamination	Samples contaminated with genomic DNA contain a third peak which migrates in the region of the 18 and 28S. Occasionally this peak can be mistaken for the 18 or 28S peak. Ensure that all peaks are annotated correctly. Treat samples with DNAse to eliminate the genomic DNA peak then prepare a new run.
	There may be a contaminant peak caused by dust or dirt on the ScreenTape	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. Peaks caused by contaminants present sharp strong bands which are often slanted or uneven in the gel image, and usually easily distinguishable from sample peaks.

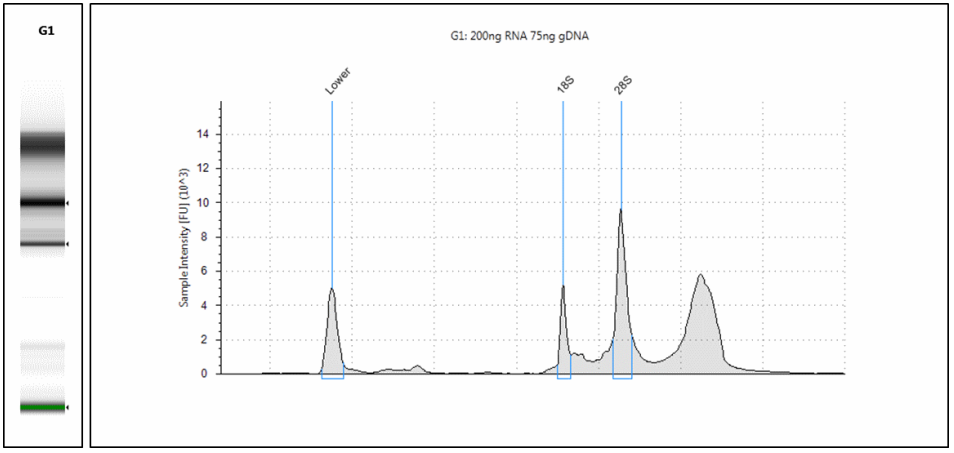


Figure 27 Example of genomic DNA contamination of RNA sample

Table 15 RNA - Possible causes of unexpected migration profile

Problem	Likely Cause	Solution
Additional bands are present at the top of the gel image	The sample may be too concentrated for the application	Dilute or concentrate your sample until it is within the recommended range for the application, then prepare the sample again and rerun on ScreenTape. If necessary, use the High Sensitivity RNA ScreenTape assay.

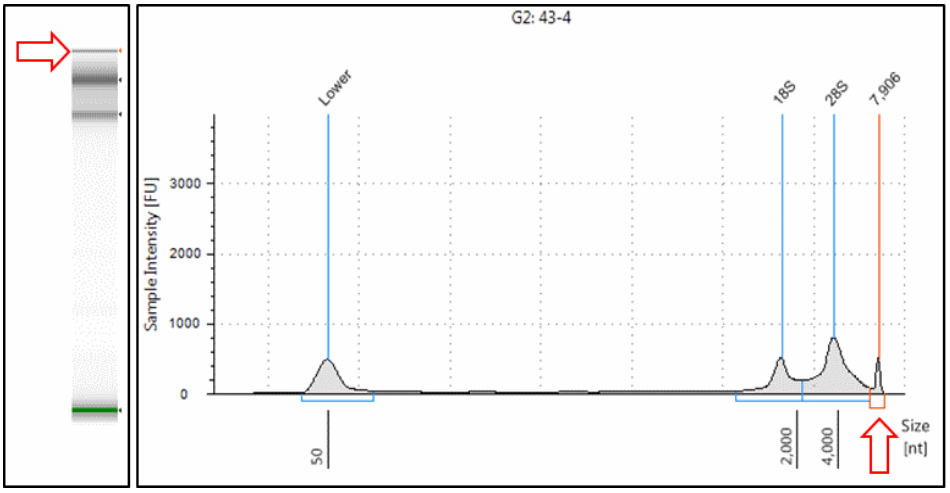


Figure 28 Example of highly concentrated RNA sample presenting as additional bands (presented with arrows) above the 28S peak

Table 15 RNA - Possible causes of unexpected migration profile

Problem	Likely Cause	Solution
The markers have appeared in the sample lane, but the sample has not	The sample may be too dilute for the application	Concentrate your sample until it is within the recommended range for application, then prepare the sample again and rerun on ScreenTape
	Insufficient Mixing	Ensure correct mixing by reading the "Mixing recommendations" on page 10

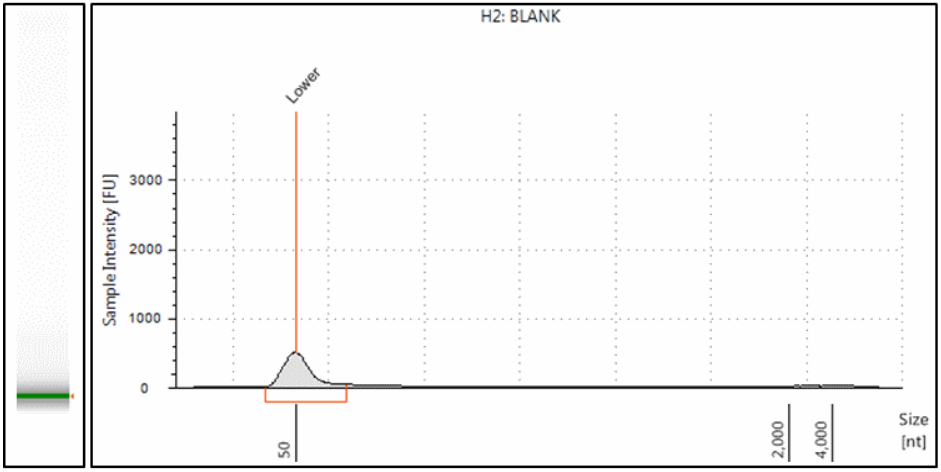


Figure 29 Example of missing sample peaks in an RNA run

Higher than normal background noise	Insufficient Mixing	Ensure correct mixing by reading the "Mixing recommendations" on page 10
Low signal intensity	Insufficient Mixing	Ensure correct mixing by reading the "Mixing recommendations" on page 10
	Incorrect reagent storage conditions	Follow the storage conditions specified for the assay as stated in the user manual and appropriate Quick Guide.

Table 15 RNA - Possible causes of unexpected migration profile

Problem	Likely Cause	Solution
Incorrect migration - samples have not reached the lower end of the gel lane	Insufficient migration due to presence of salt	Dilute your samples to ensure low levels of buffer salt True migration profiles can be seen by unaligning your gel image as described in "Manual Marker Assignment" on page 18
	Partial electrophoresis failure caused by bubbles at gel-buffer interface	Ensure ScreenTape is *flicked* to remove any bubbles prior to use, see "ScreenTape" on page 13.

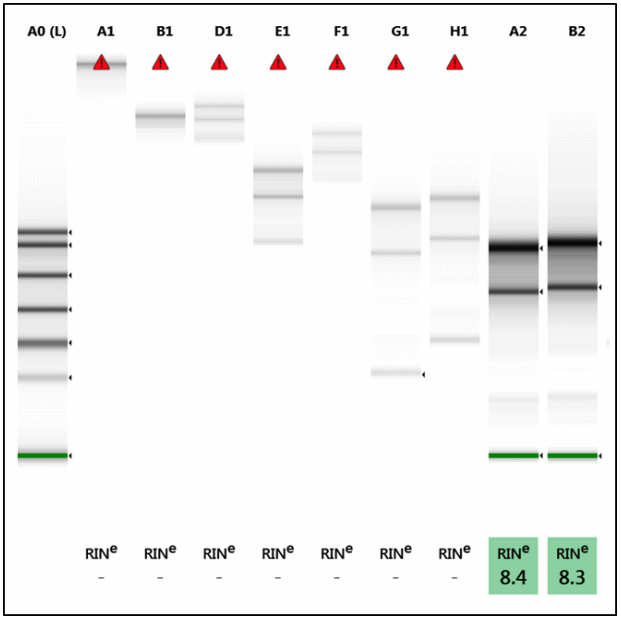


Figure 30 Example of short migration caused by high salt concentration. Lanes A1- B2 show the same sample in buffers of decreasing salt concentrations, allowing migration further through the gel lane.

7
Troubleshooting Protein Applications

This section gives an overview on how to avoid problems with Protein Applications.

Buffer compatibility

Please refer to the technical overview "Understanding the effects of proteins and buffers on staining, denaturation and electrophoresis when analyzing proteins with Agilent P200 ScreenTape" www.chem.agilent.com/library/technicaloverviews/Public/5990-9603EN.pdf.

Quantification of Protein Samples

Quantification of protein samples in the TapeStation Analysis Software is currently customized by the user. This means that when a file first loads, no concentration values are displayed.

In order to see this information, a calibration curve must be constructed manually, by running samples of known concentrations.

It is possible to manually enter concentrations for known sample peaks in the electropherogram tab by following these instructions.

1 Right click on the sample peak with known concentration.

2 Select **Assign Concentration**.

This will automatically take you to the concentration field for that peak in the peak data table.

3 Manually type in the concentration and press enter.

4 Repeat this process with as many known peaks as possible.

The more values entered, the better the calibration curve constructed will be.

A calibration curve will now have been created using the manually entered data, which can be viewed in the file menu under **Run Properties**.

This new curve will be used to fill in concentration data for all remaining sample peaks.

Samples used to construct the calibration curve show two concentration values:

- The manually entered value in red in brackets follows the value back calculated from the curve, which is displayed in black
- For example 29.6(30)

5 Manually entered values can be edited or deleted in the electropherogram view by clicking on the concentration field in the data table.

The calibration curve will then be corrected to use the updated or remaining values.

Incorrect Quantification results

Table 16 Protein - Possible causes for incorrect quantification results in order of probability.

Cause	Solution
Insufficient Mixing	Ensure correct mixing, see "Mixing recommendations" on page 10
Sample concentration outside recommended range for application	Either dilute or concentrate the sample until it is within the recommended concentration range for application, then prepare a new run.
Incorrect Peak integration	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.
Incorrect heating procedure	Ensure that the samples are heated denatured before running according to the assay instructions. Over and under heating can affect concentration values.
Incorrect protocol used	Ensure the correct sample protocol for the application is followed.
Sample prepared too long before the run is performed	To avoid evaporation or settling, ensure that your run is started immediately after sample preparation. If using a 96-well plate, always cover with the approved foil seal.
Pipetting technique or pipette calibration	Follow guidelines in the Essential Measurement Practices section, see "Essential Measurement Practices" on page 5.
Incorrect ScreenTape or reagent storage	Follow the storage conditions specified for the assay, as stated in the User Manual and appropriate Quick Guide Use stain solution within 1 week of preparation

Incorrect Sizing Results

Table 17 Protein - Possible causes of incorrect sizing results in order of probability.

Cause	Solution
Incorrect sample buffer used. P200 Reagent kits contain both reducing and non-reducing buffer.	Ensure the correct buffer is used, Reducing or non-reducing conditions will affect the size of fragments analyzed.
Issues with the ladder lane	Any issues with the ladder lane will be annotated within the TapeStation Analysis Software sample table, under **Observations**. Manually insert or delete affected peaks, or insert a software saved ladder to regain sizing information.
Incorrect migration - samples have not reached the lower end of the gel lane	Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by unaligning your gel image by pressing the **Aligned** button in the TapeStation Analysis Software. Please refer to the P200 Buffer compatibility section and make the necessary adjustments to the sample buffer.
Incorrect storage of ScreenTape or reagents	Store reagent kit according to assay Quick Guide Use stain solution within 1 week of preparation.

Unexpected Migration Profile

Table 18 Protein - Causes of unexpected migration profile

Problem	Likely Cause	Solution
Additional sharp bands are present at the top of the gel image	The sample may be too concentrated for the application	Dilute your sample until it is within the recommended concentration range for application, then prepare a new run.
The markers have appeared in the sample lane, but the sample has not	The sample used is below the concentration range recommended to the assay	Concentrate your sample until it is within the recommended concentration range for application, then prepare a new run.
	Insufficient Mixing	Ensure correct mixing, see "Mixing recommendations" on page 10
Higher than normal background noise	Insufficient Mixing	Please follow the mixing recommendations for the assay.
	Incorrect ScreenTape and reagent storage conditions	Follow the storage conditions specified for the assay.
Low signal intensity	Insufficient Mixing	Ensure correct mixing, see "Mixing recommendations" on page 10
	Incorrect reagent storage conditions	Follow the storage conditions specified for the assay.
Incorrect migration - samples have not reached the lower end of the gel lane	Salt concentration	Dilute your samples to ensure low levels of buffer salt. True migration profiles can be seen by unaligning your gel image by pressing the **Aligned** button in the TapeStation Analysis Software. Please refer to the P200 Buffer compatibility section and make the necessary adjustments to the sample buffer.

8
Instrument Maintenance

This section gives an overview on the importance of Preventive Maintenance.

General Information

For continuous reliable operation the TapeStation system requires a defined set of Preventative Maintenance (PM) operations to be performed every 16000 samples or on an annual basis. This maintenance must be performed by a trained Agilent Field Service Engineer and can be scheduled by contacting your local Agilent Service representative.

During the PM the following components within the TapeStation system will be replaced:

- Fan filters
- Piercing Needles
- Electrophoresis Probes

The instruments tip sensor will also be re-adjusted, all moving axis will be lubricated, optics cleaned and the any foreign objects will be removed.

In addition to the Annual Preventative Maintenance service, users with a high throughput of samples will also need to perform a Needle Change procedure every 8320 samples. This simple procedure is explained in the ‘Changing the needle’ section, and can be performed by the end user with the aid of a disposable cartridge.

Changing the Needle

It is important to know which TapeStation system you have before changing the needle(s), in order to purchase the correct needle cartridge.

Table 19 Overview of TapeStation Configuration - Needle Cartridge

Product Number	TapeStation Configuration	Pump	Needle Cartridge Ordering Code
ST007	TapeStation for ScreenPlex	Single	G2960-60062
ST008	TapeStation for DNA		
ST009	TapeStation for Nucleic acids		
ST017	TapeStation for ScreenPlex	Twin	G2960-60063
ST019	TapeStation for Nucleic acids		
ST010	TapeStation for Protein / Combined TapeStation		
G2960A	2200 TapeStation System	Twin	G2960-60063
G2961A	2200 TapeStation Nucleic Acid System		
G2964AA	2200 TapeStation System		
G2965AA	2200 TapeStation Nucleic Acid System		
G2966AA	2200 TapeStation ScreenPlex System		

Needle change intervals:

- After 7680 lanes (3840 pierces in a Single loading system), the controller software will inform the user that a needle change is pending. The word **Needle** will appear in the bottom of the controller software inside a yellow box.
- After 8320 lanes (4160 pierces in a Single loading system), a needle change is recommended. The box around the word **Needle** will change from yellow to red.
- After 8960 lanes (4480 pierces in a Single loading system), the needle has completed its lifetime and must be changed before the TapeStation will start.

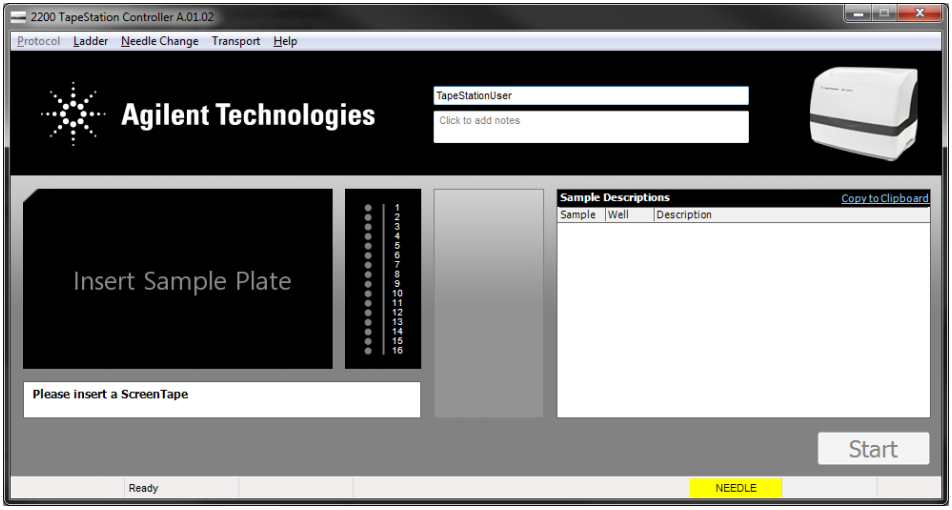


Figure 31 Controller software indicating a **Needle** change is recommended

Parts required	#	p/n	Description
	1	G2960-60062	Needle cartridge (for use in single pump systems) For use with product numbers ST007, ST008 and ST009
OR	1	G2960-60063	Needle cartridge (for use in dual pump systems) For use with product numbers ST017, ST019, ST010, G2960A, G2961A, G2964AA, G2965AA and G2966AA

NOTE

The needle replacement procedure as detailed below is performed during the annual Preventative Maintenance procedure.

Customers with high throughput may require additional needle changes between PM services, and should use the table above to order the correct parts for their instrument.

NOTE

New needle cartridges can be ordered at any time from Agilent Technologies by contacting your local sales agent.

For details on the correct needle cartridge for your TapeStation model, refer to Table 19 on page 87.

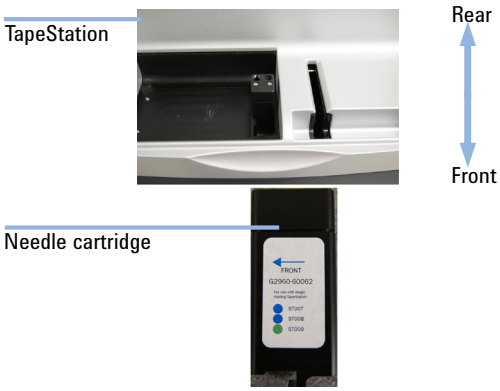
Changing the needle cartridge

1 Remove the sample plate and tip holder.

2 Remove the foil tab from the top of the needle cartridge.

NOTE Care must be taken to keep the needle cartridge level after removing the foil tab

3 Insert the needle cartridge into the tip holder space, using the label for orientation. The cartridge should be placed so that the label faces to the right, and the printed arrow points to the front of the TapeStation.



4 Close the lid.

5 Go to **Needle Change** on the Controller software toolbar and select **Run**.

Preventative Maintenance Interval

After 16000 samples, the controller software will inform the user that Preventative Maintenance is required. The word **Maintenance** will appear in the bottom of the controller software inside a yellow box. The Preventative Maintenance service must be performed by a trained Agilent Field Service Engineer and can be scheduled by contacting your local Agilent Service representative.

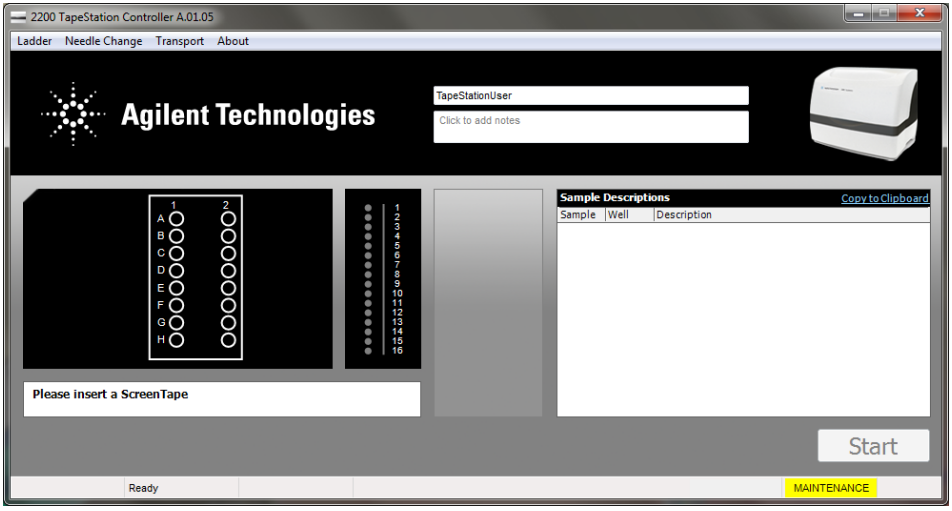


Figure 32 Controller software indicating a Preventative Maintenance is due

If both the **Maintenance** message and the **Needle** message are being displayed simultaneously then only a Preventative Maintenance should be performed. The Preventative Maintenance procedure includes a Needle Change and following PM service both messages will disappear.

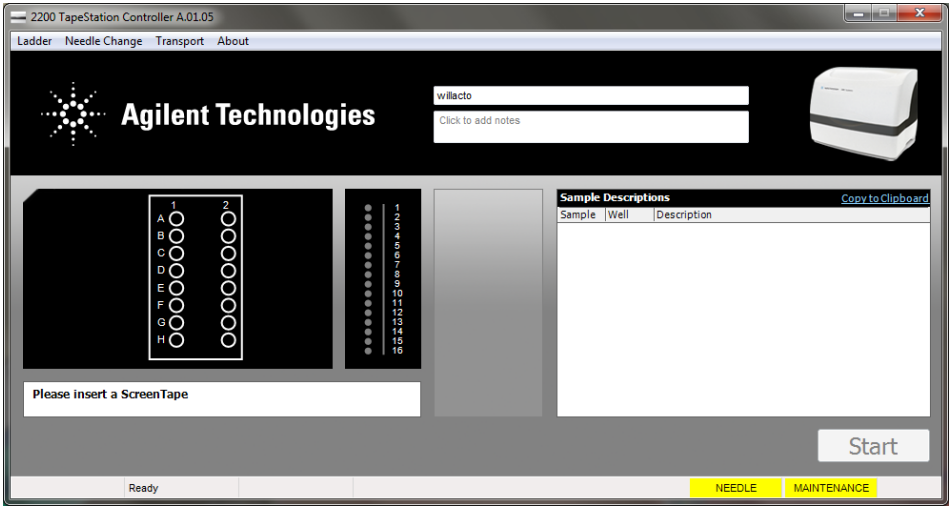


Figure 33 Controller software indicating a Preventative Maintenance is due. Schedule Preventative Maintenance, do not perform a Needle Change

9 Error Messages

This section gives an overview on Error Messages.

Analysis Software Warning Messages

Warning messages are displayed in the TapeStation Analysis Software within the sample table. Each lane which has a warning is flagged by colored triangles containing an exclamation mark which are detailed in the **Observations** column.

Warning flags can be yellow or red.

- A yellow warning indicates an abnormal lane where the results may not be reliable.
- A red warning indicates a significant problem with that lane where some data may be missing.

NOTE These warning messages can also be found in the TapeStation Analysis Software online help by clicking on the question mark **?** anywhere within the software.

Table 20 Analysis Software Warning Messages

Affected Assay	Observation	Detailed description	Corrective action
All Assays	Caution! Expired ScreenTape	ScreenTape was used after its expiration date.	Re-run with in-date ScreenTape and consumables.
	Caution! Expired ScreenTape (used after two weeks of first use)	ScreenTape was used after two weeks of the first usage.	Re-run with in-date ScreenTape and consumables.
	Marker(s) not detected	The lower and/or upper marker has not been identified by the software. The image is unaligned and no sizing information provided.	Manually assign the upper and/or lower marker. See "Manual Marker Assignment" on page 18 for details. If markers are not present, see "Missing Marker Peaks" on page 57 for DNA or "Missing Marker Peaks" on page 70 for RNA Applications as appropriate.
	Markers outside standard running position	The lower and/or upper marker is running outside the expected detection window for the assay. This may affect sizing, though the image will not be unaligned.	Manually assign the upper and/or lower marker. See "Manual Marker Assignment" on page 18 for details.
	Issue with ladder peak detection (too few peaks detected)	Less than the expected number of ladder peaks have been identified. This may affect sizing. For gDNA samples no DIN will be displayed in this file.	If possible, manually add extra peaks by right clicking on the affected peak and selecting **add**. For gDNA assay prepare a new run with Genomic DNA Ladder in the first position. Please see "Good Measurement Practices for DNA" on page 40 for advice on handling the Genomic DNA Ladder.
	Issue with ladder peak detection (too many peaks detected)	More than the expected number of ladder peaks have been identified. This may affect sizing. For Genomic DNA files, DIN results in this file may not be reliable.	Where possible, manually delete extra peaks by right clicking on the affected peak and selecting **delete**. For Genomic DNA files, please see "Good Measurement Practices for DNA" on page 40 for advice on handling the Genomic DNA Ladder.

Table 20 Analysis Software Warning Messages

Affected Assay	Observation	Detailed description	Corrective action
Genomic DNA	DIN edited (Marker position changed)	The User has manually changed the position of a marker peak. DIN may have changed as a result	A DIN will be displayed. To recover the original DIN value assigned by the software, use the **Restore Default Settings** button on the main ribbon.
	DIN edited (Ladder sizing changed)	User has manually changed the position of a ladder peak. DIN may have changed as a result	A DIN will be displayed. To recover the original DIN assigned by the software, use the **Restore Default Settings** button on the main ribbon.
	File does not have ladder assigned	Genomic DNA file where either no ladder has been run, or the ladder has been unassigned.	The Genomic DNA ScreenTape assay always requires a ladder. If a ladder has been run, assign it using the Assign button in the main ribbon. Otherwise prepare a new run with Genomic DNA Ladder in the first position.
	Ladder run as sample	For Genomic DNA files, a ladder has been assigned as a sample. No DIN will be displayed.	Re-assign this lane as a ladder using the **Assign** button in the main ribbon.
	Sample concentration outside functional range for DIN	The gDNA sample concentration is outside the recommended functional range for DIN. DIN values may not be present or reliable for this file	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
	Sample concentration outside recommended range	The concentration of the sample is out of the concentration specified for the assay. Concentration values are not reliable.	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.
	The original ladder for this lane had too many peaks	For gDNA comparison files: The ladder of the original file had too many peaks. This may affect sizing.	Where possible, manually delete extra peaks by right clicking on the affected peak and selecting **delete**. Please see "Good Measurement Practices for DNA" on page 40 for advice on handling the Genomic DNA Ladder.

Table 20 Analysis Software Warning Messages

Affected Assay	Observation	Detailed description	Corrective action
RNA High Sensitivity RNA	RIN^e edited	User has manually changed the position of the 18S fragment. RIN^e may have changed as a result.	An edited RIN^e will be displayed. To recover the original RIN^e assigned by the software, use the **Restore Default Settings** button on the main ribbon.
	RNA concentration outside recommended range for RIN^e	RNA Sample concentration lies outside the functional range for RIN^e. RIN^e values are not reliable.	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.
	Sample concentration outside recommended range	The concentration of the sample is out of the concentration specified for the assay. Concentration values are not reliable.	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.

Communication Error Messages

Should you encounter an error message, please work through the suggested actions below.

If, after completing the suggested actions, the error message persists, or instrument function does not return to normal, please contact your local support representative.

Found new hardware

A **Found New Hardware** pop-up launched when the USB cable of the TapeStation was inserted into the laptop.

Probable cause	Suggested actions
1 The USB cable has been inserted into a new port	Re-install software using the software Readme

Failed to connect to the barcode scanner or camera

Probable cause	Suggested actions
1 The drivers have not been installed correctly	• Power cycle instrument leaving 60 seconds after power up before re-launching controller. • If the message persists, try a different USB port on the laptop for the cable to connect to the TapeStation.

Camera error – Freeze Image returned failure code: 122

Probable cause	Suggested actions
1 Camera connection error	Power cycle instrument leaving 60 seconds after power up before re-launching controller

The instrument has failed to respond in a timely fashion

Probable cause	Suggested actions
1 Incorrect consumables have been used	Check the correct consumables have been used, and that the lids are removed from sample tubes before use. Contact your Agilent support team with details of error and instrument log files.

The last stepper move of the (Shuttle / Z – Axis / X – Axis) finished (- xxx) steps from the home flag

Probable cause	Suggested actions
1 Incorrect consumables have been used	Check the correct consumables have been used, and that the lids are removed from sample tubes before use. Contact your Agilent support team with details of error and instrument log files.

A hardware device associated with the last command is missing or malfunctioning

Probable cause	Suggested actions
1 The instrument temperature sensor is malfunctioning	Contact your Agilent support team with details of error and instrument log files.

Failed to connect to the barcode reader/Camera

Probable cause	Suggested actions
1 There could be a connection issue with the USB port	• Ensure no other USB devices are connected. • Power cycle the instrument by switching off, waiting 2 minutes, then switching back on again. • If the error message remains, connect the instrument using a different USB Port.

The tape just inserted was not recognized, please re-insert, ensuring barcode is near the front of the instrument and facing right.

Probable cause	Suggested actions
1 The idle barcode is not being read correctly	• Ensure that the ScreenTape is inserted correctly, with the bar code is facing away from the sample block and towards the front of the instrument. • Ensure the TapeStation is facing away from any direct light sources which could interfere with barcode reading • Power cycle the TapeStation by switching off, waiting 2 minutes, then switching back on again, and restart the laptop. If the problem persists, contact Agilent support
2 The ScreenTape barcode is not being read correctly	• Ensure that the ScreenTape is inserted correctly, with the bar code is facing away from the sample block and towards the front of the instrument. • Ensure the TapeStation is facing away from any direct light sources which could interfere with barcode reading • Power cycle the TapeStation by switching off, waiting 2 minutes, then switching back on again, and restart the laptop. If the problem persists, contact Agilent support

Consumable Error Messages

The licensed application of your system does not support the use of the tape just inserted

A pop-up window states **The licensed application of your system does not support the use of the tape just inserted**

Probable cause	Suggested actions
1 The wrong ScreenTape type has been inserted for the analysis software mode	• Ensure the correct software and ScreenTape type are used together. Please see the note below. • Ensure that software versions installed are the most recent available.

NOTE

TapeStation Analysis Software:

ScreenTape types:

- D1000, High Sensitivity D1000, Genomic DNA
- RNA, High Sensitivity RNA
- P200

TapeStation ScreenPlex Software:

ScreenTape types:

- DS12

Pipette bin full

One or more pipette tips may still be in the pipette-tip bin from a previous run. Empty the pipette tip bin before continuing.

Probable cause	Suggested actions
1 The tip discard section has not been emptied after the last run	Remove all discarded tips and replace the tip holder into the instrument.

Pipette pump affixed to the pump

A pipette pump may be affixed to the pump. Please insert an empty tip holder into the instrument, close the lid then click OK to continue

Probable cause	Suggested actions
1 It is likely that the TapeStation has lost power during a run, and needs to discard any tips which have been picked up before starting again.	Insert an EMPTY tip holder into the instrument then follow the on screen instructions. The instrument will automatically discard any tips into the tip bin. Remember to empty the tip bin before starting the next run.

10
ScreenTape Products, Parts and Consumables

This section gives an overview on ScreenTape Products, Parts and Consumables.

ScreenTape Products

Kit Components (High Sensitivity D1000 ScreenTape Assay)

Part Number	Name	Color	Amount
5067-5584	High Sensitivity D1000 ScreenTape		7 ScreenTape
5067-5585	High Sensitivity D1000 Reagents		2 vials
	• High Sensitivity D1000 Ladder		20 µL
	• High Sensitivity D1000 Sample Buffer		300 µL
5067-5587	High Sensitivity D1000 Ladder		1 vial
			20 µL

Kit Components (D1000 ScreenTape Assay)

Part Number	Name	Color	Amount
5067-5582	D1000 ScreenTape		7 ScreenTape
5067-5583	D1000 Reagents		2 vials
	• D1000 Ladder		10 µL
	• D1000 Sample Buffer		400 µL
5067-5586	D1000 Ladder		1 vial
			10 µL

Kit Components (Genomic DNA ScreenTape Assay)

Part Number	Name	Color	Amount
5067-5365	Genomic DNA ScreenTape		7 ScreenTape
5067-5366	Genomic DNA Reagents		2 vials
	• Genomic DNA Ladder		75 µL
	• Genomic DNA Sample Buffer		1350 µL

Kit Components (High Sensitivity RNA ScreenTape Assay)

Part Number	Name	Color	Amount
5067-5579	High Sensitivity RNA ScreenTape		7 ScreenTape
5067-5580	High Sensitivity RNA ScreenTape Sample Buffer		1 vial 250 µL
5067-5581	High Sensitivity RNA ScreenTape Ladder		1 vial 10 µL

Kit Components (RNA ScreenTape Assay)

Part Number	Name	Color	Amount
5067-5576	RNA ScreenTape		7 ScreenTape
5067-5577	RNA ScreenTape Sample Buffer		1 vial 600 µL
5067-5578	RNA ScreenTape Ladder		1 vial 10 µL

Kit Components (P200 ScreenTape Assay)

Part Number	Name	Color	Amount
5067-5371	P200 ScreenTape		7 ScreenTape
5067-5372	P200 Reagents		
	• P200 5X Labeling Dye		70 µL
	• P200 Labeling Buffer		350 µL
	• P200 Reducing Sample Buffer		550 µL
	• P200 pH Buffer	clear	1000 µL
	• P200 Non-Reducing Sample Buffer		550 µL
	• P200 Markers (pre-stained)		270 µL
	• P200 Ladder		40 µL

Parts and Consumables

Table 21 Parts and Consumables

Product	Contents	Catalogue Number
Filtered loading tips (10 pk)	10 x 384 tips	5067-5152
Filtered loading tips (1 pk)	1 x 384 tips	5067-5153
Optical Tube, 8x Strip	Box of 120	401428
Optical Cap, 8x Strip	Box of 120	401425
Sample block (for tube strips)	1	5067-5155
96-well sample plates	10	5067-5150
Sample block (for 96-well plate)	1	5067-5156
96-well Plate Foil seal	1 x 100	5067-5154
Loading Tip Holder	1	5067-5158
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- 2200 TapeStation Software
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- Instrument Troubleshooting
- Troubleshooting DNA Applications
- Troubleshooting RNA Applications
- Troubleshooting Protein Applications
- Instrument Maintenance
- Error Messages

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Printed in Germany
01/2015

G2964-90110

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