

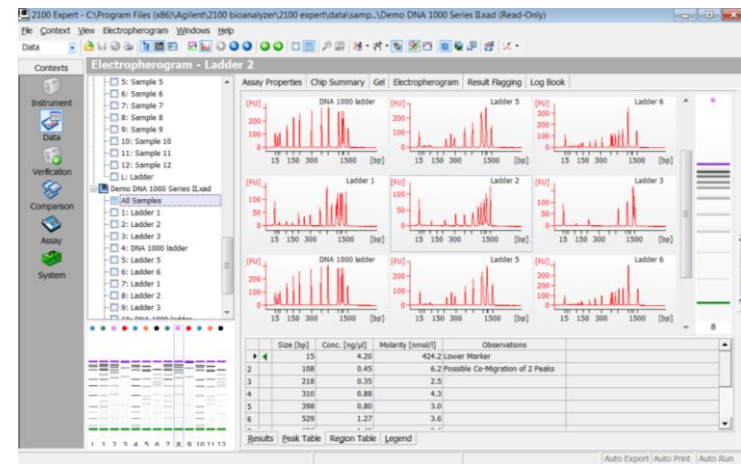
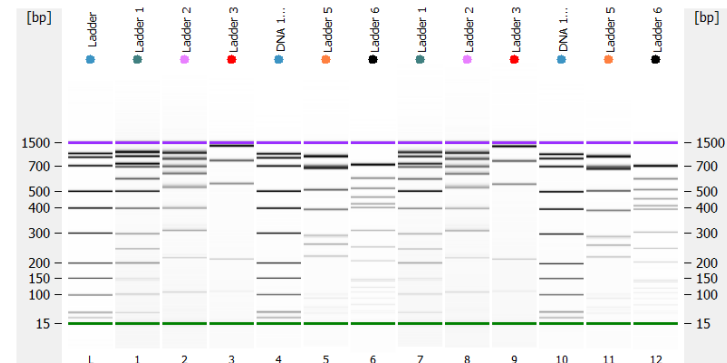
Bioanalyzer System

Troubleshooting Section



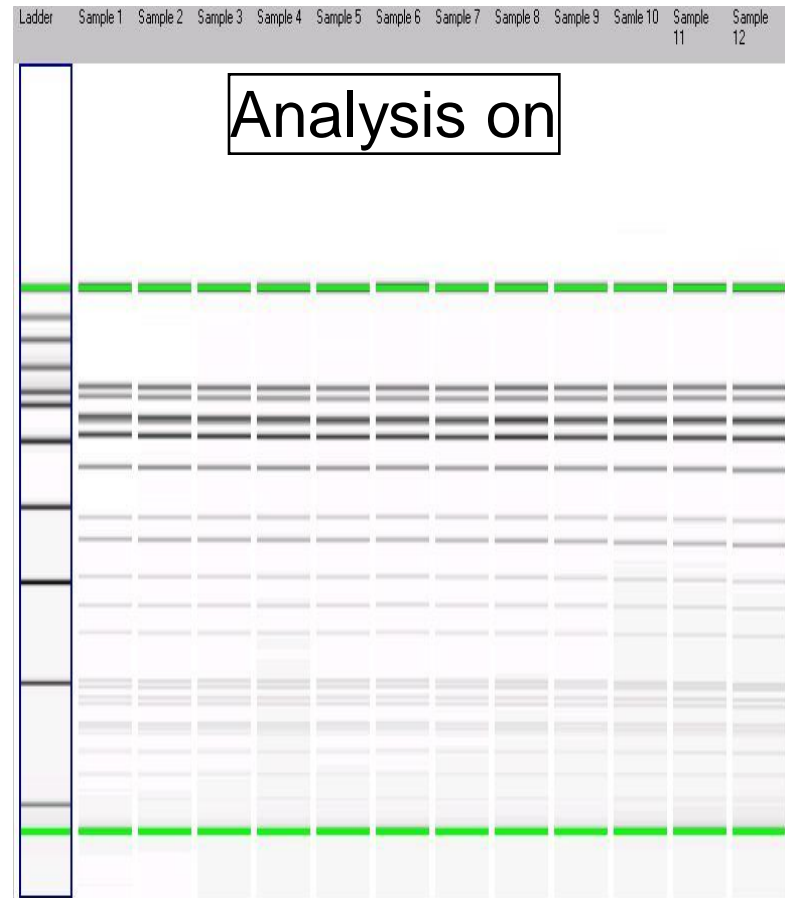
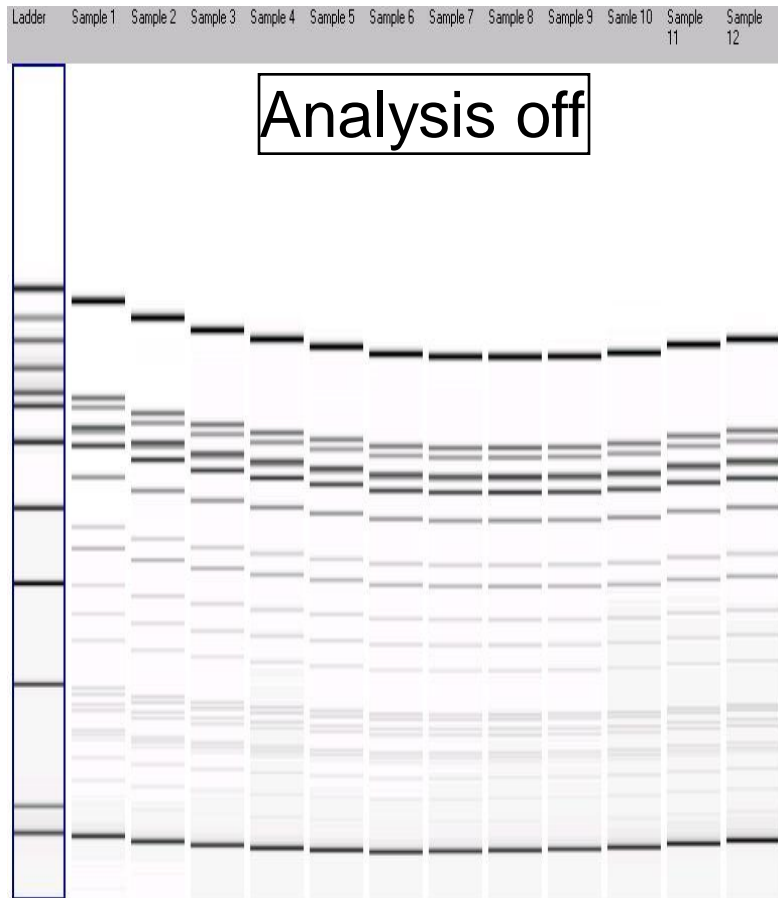
Where and what to look for when analyzing a run file

- Gel like image - overview
- Did the ladder run correctly?
- Are the markers present?
- Is this the profile I expected to see?



Data Analysis: Alignment

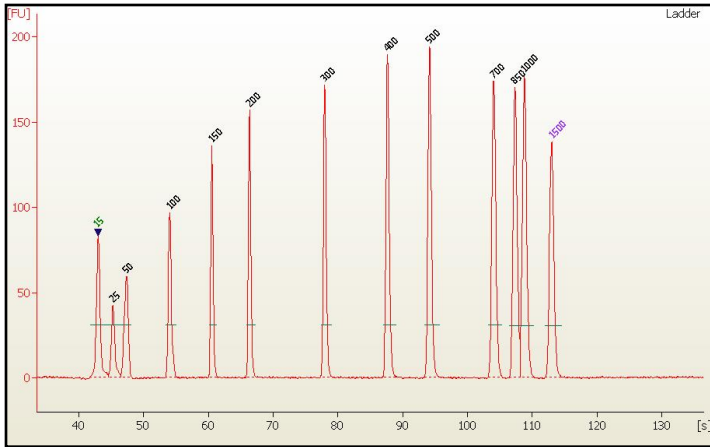
- For correct analysis: internal markers need to be identified correctly



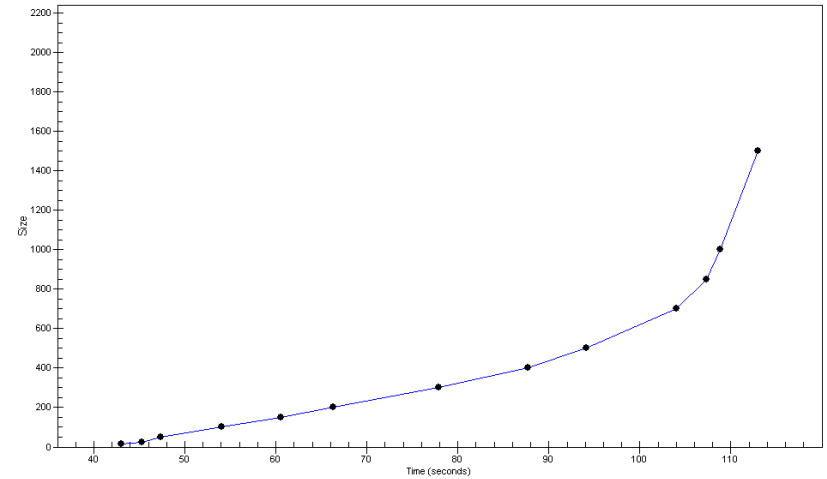
Data Analysis: DNA Ladder

Check if all DNA ladder fragments have been identified correctly:

DNA 1000 Ladder Electropherogram



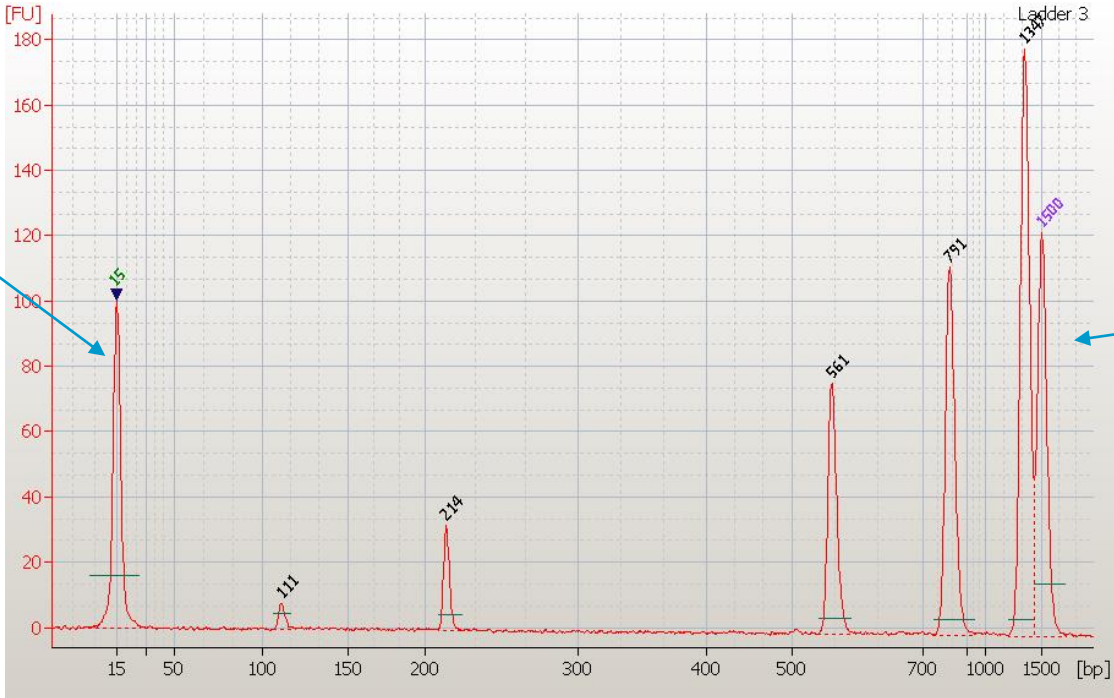
Standard Curve (Chip Summary tab)



- The standard curve is generated with a point to point fit using the migration time and sizes of the ladder peaks
- The size of each sample peak is calculated from the standard curve

Data Analysis: Samples

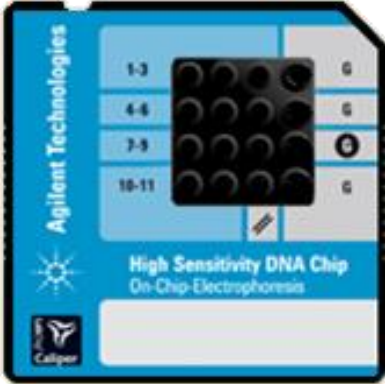
- Specified DNA fragments are used as Lower and Upper Markers
- The 2100 Expert SW automatically detects the Markers
- Lower and Upper Marker are used for the alignment -> Sizing
- Upper Marker is used for the quantitation



LM
15 bp

UM
1500 bp

DNA Chip Troubleshooting



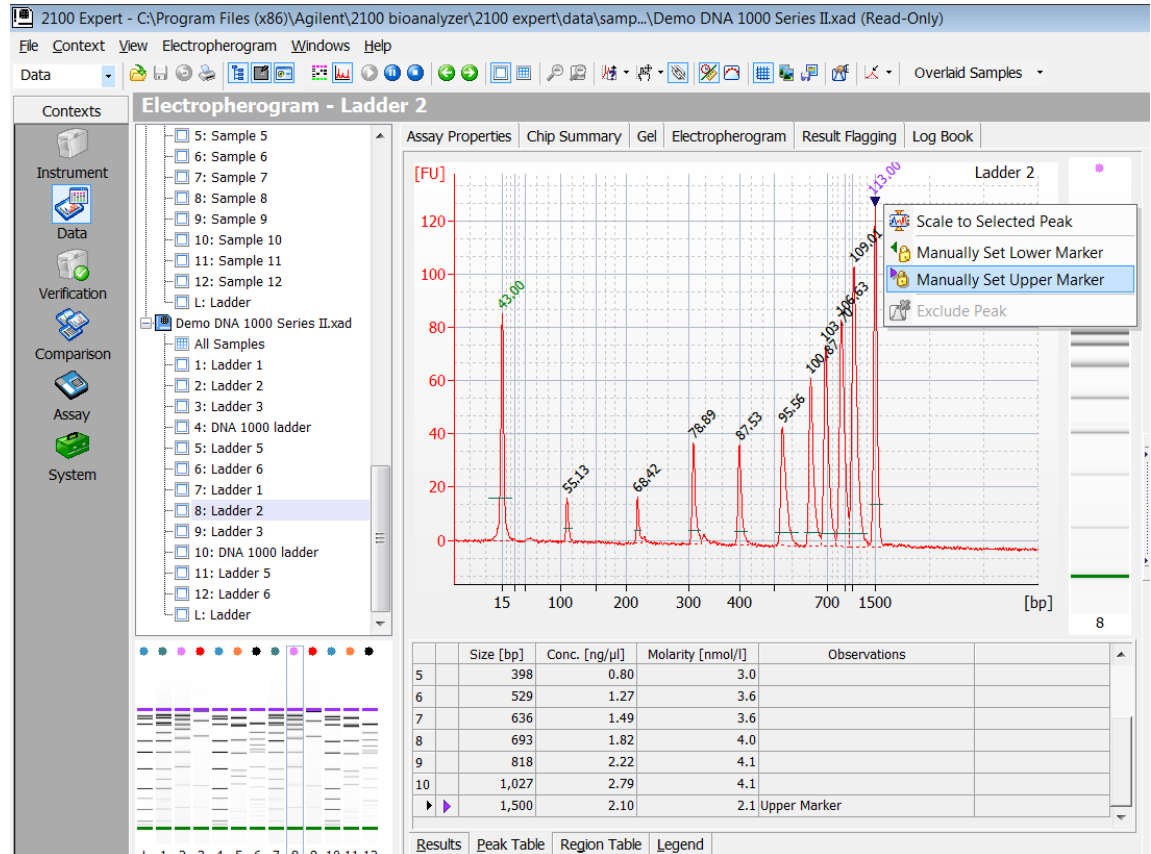
Why is it not possible to toggle the x-axis between time and size?

Hints:

- Look for Markers present in every well
- Check sample concentrations loaded onto the chip

Solutions

- Manually set Marker(s)
- Get peak recognized as a peak, use Manual Integration
- If one sample is missing its Upper Marker, go to File > Save Selected Sample > choose ladder and all samples except the affected sample. This will salvage the remaining samples.



Upper Marker not automatically recognized

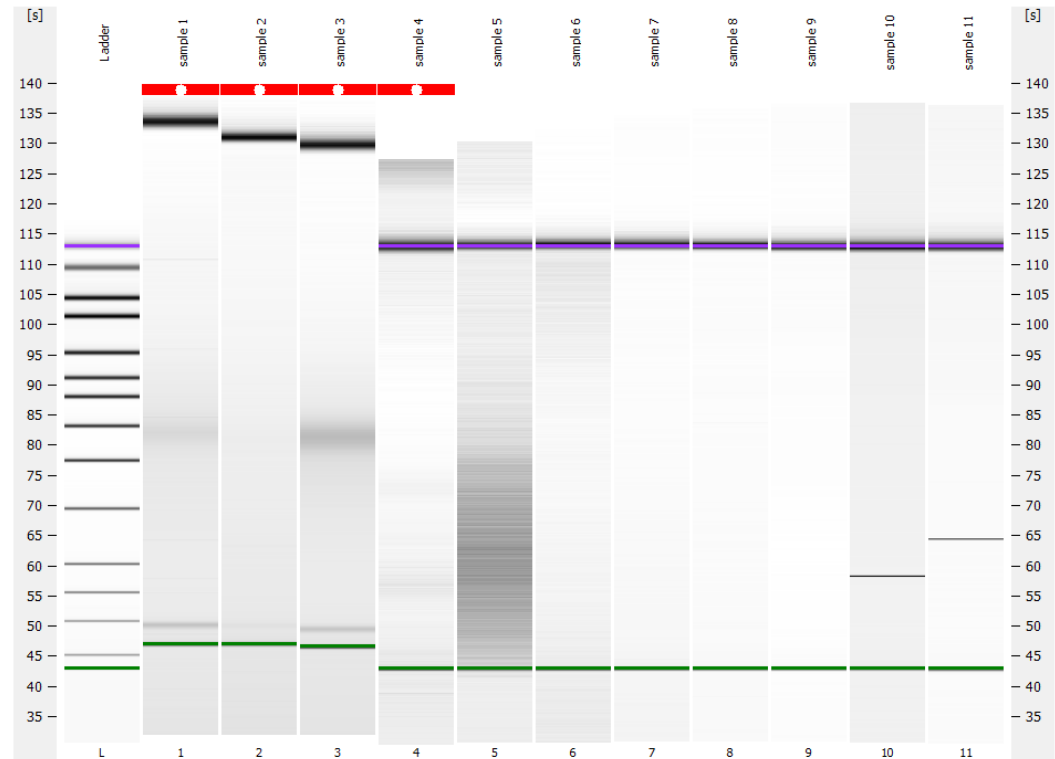
- Some samples show no sizing/quantitation information, red flags -

Issue:

Late migration - shifts the Upper Marker outside the expected time window

Solution:

Manually assign Upper Marker on Peak Table tab



Overloaded chip

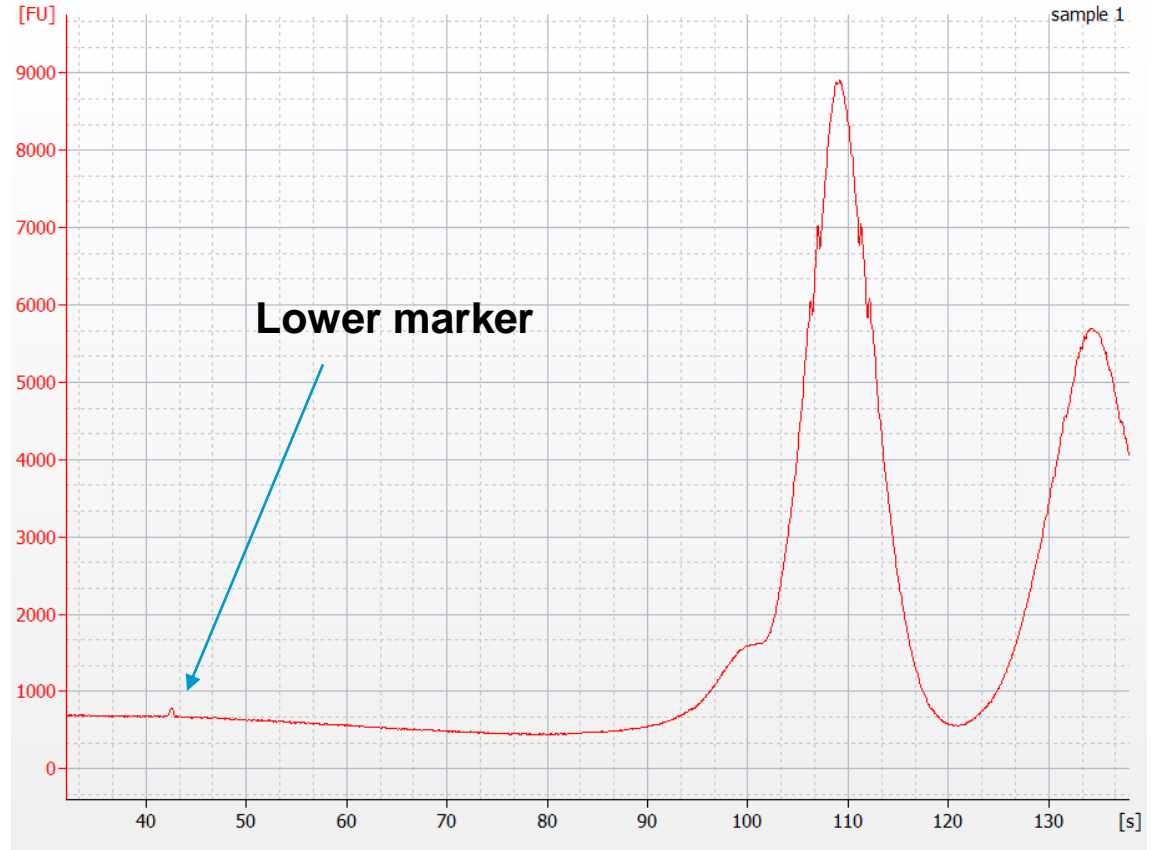
- No sizing/quantitation information, red flags, error message:
“optical signal too high” -

Issue:

Overloaded chip

Solution:

Adjust sample concentration
by dilution



No detectable peaks, distorted baseline

Hints:

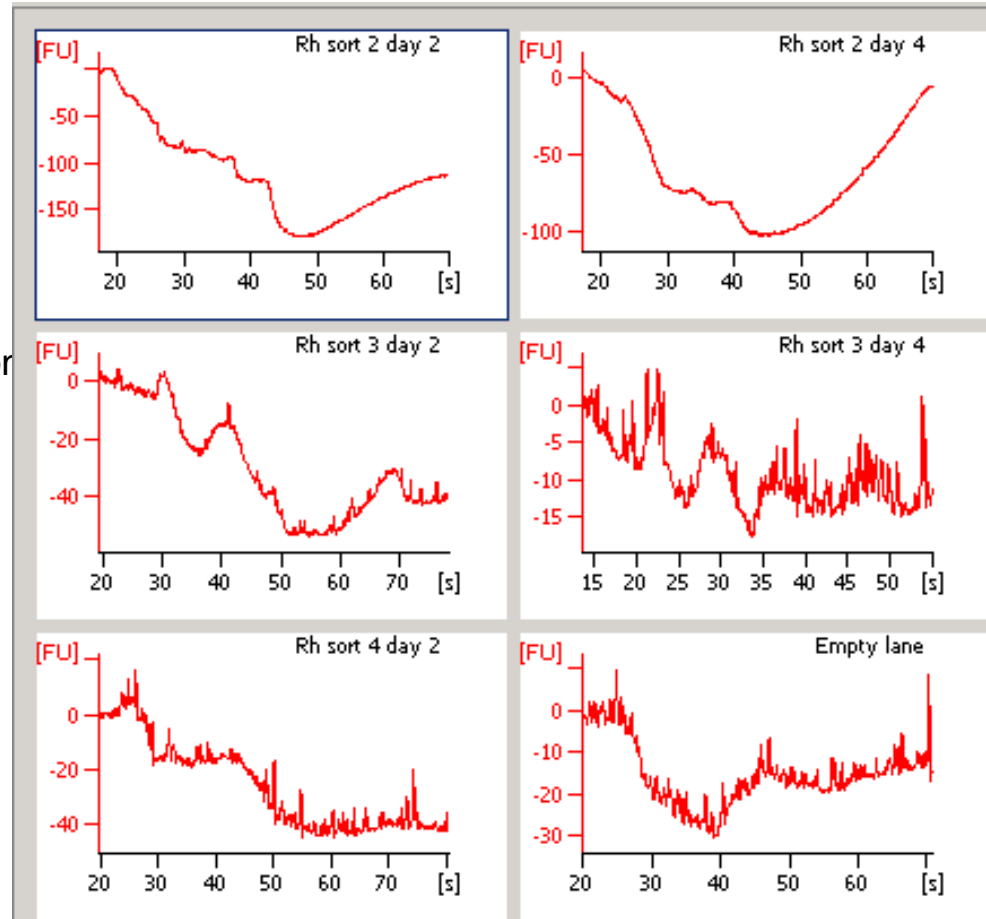
- Check for liquid spillage on chip surface
- Check chip priming station settings
- How much water was added to the cleaning chip
- When was maintenance for chip priming station last performed
- When was maintenance for pin set last performed

Issues for this particular case:

- Chip priming station channel blocked
- Incorrect pressure was applied - pressure not constant over time

Solution:

- Prepare a new chip and re-run the samples

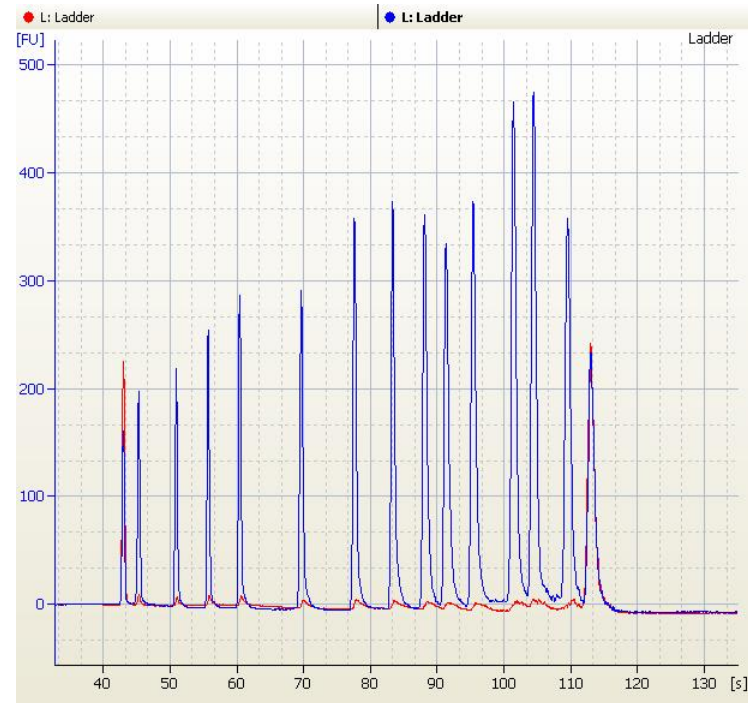


Low signal intensity (FU) with the HS DNA Ladder

HS DNA Ladder peaks are barely detectable

Solution:

Vortex ladder vial for 5 s before use



Broad dips in electropherogram (usually seen with HS DNA)

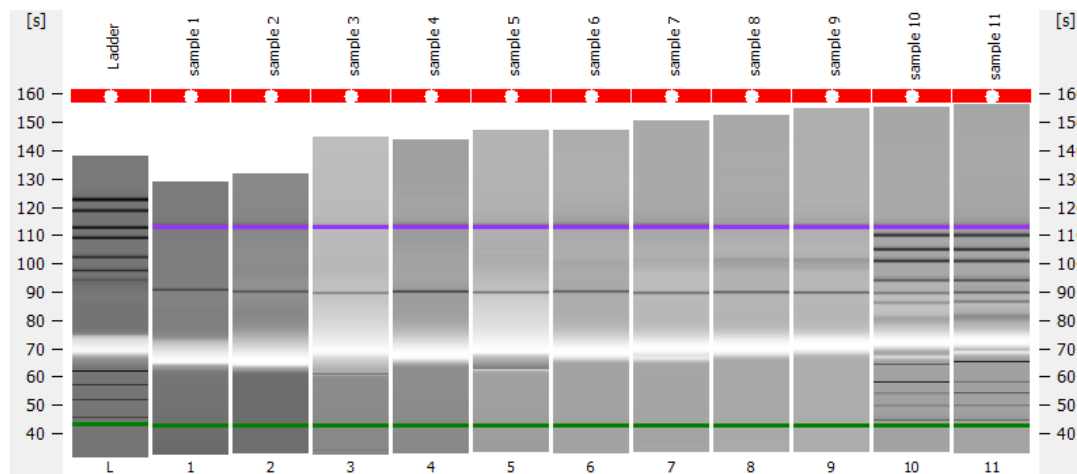
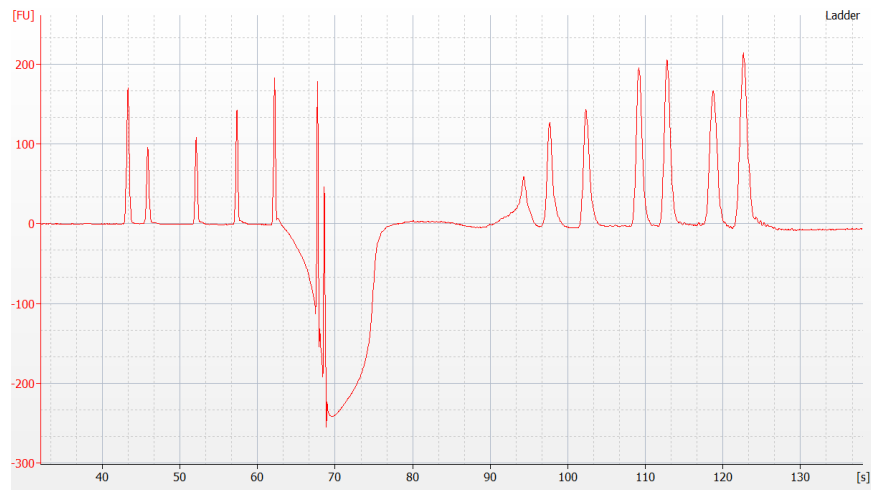
Broad dips in all/some samples and/or ladder

Explanation:

Residual RNaseZap or SDS on the electrodes

Solution:

Additional washes with cleaning chip with H₂O (2x)



RNA Chip Troubleshooting



Data Analysis: RNA Ladders

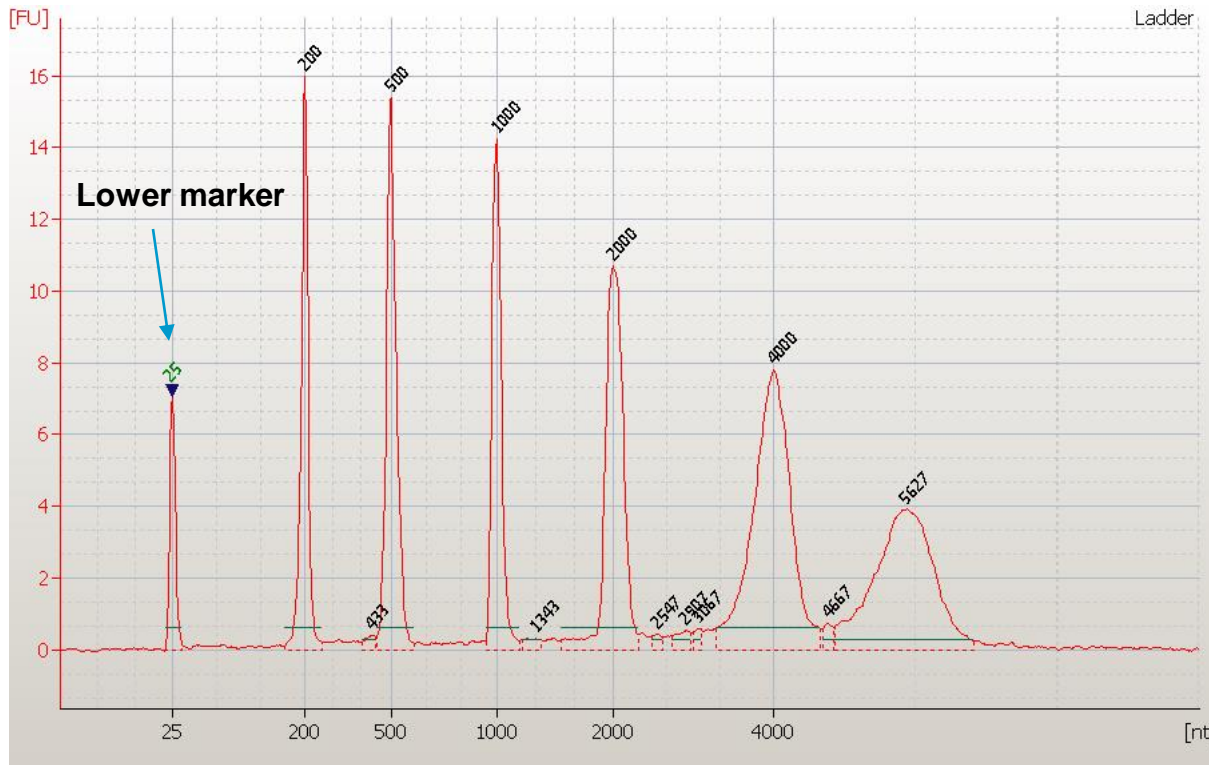
RNA Ladder is used for sizing *and* quantitation:

6 ladder peaks + Lower Marker

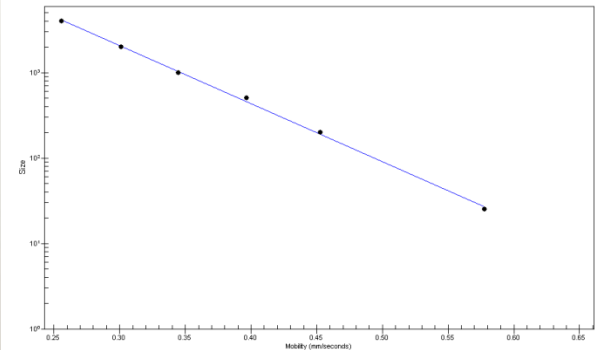
Ladder concentration: RNA Nano: 150 ng/ μ l

Small RNA ladder: 1000pg/ μ l

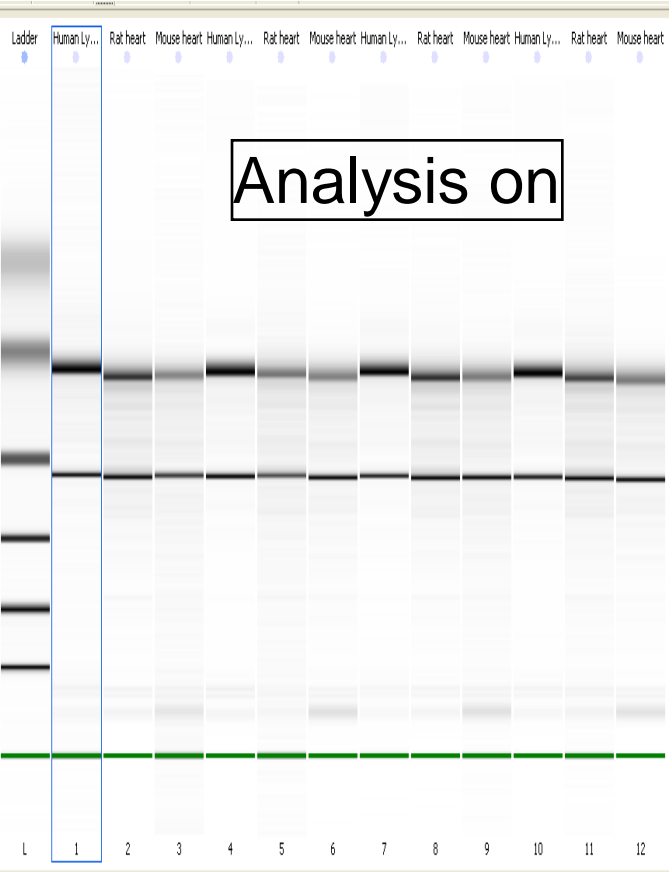
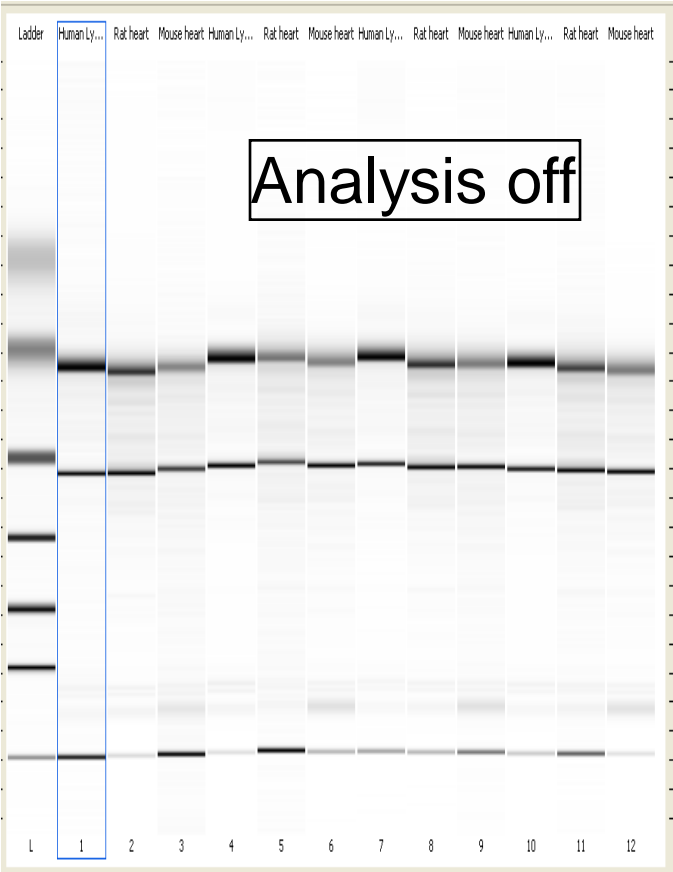
RNA Pico: 1000 pg/ μ l



Chip Summary >Standard Curve:

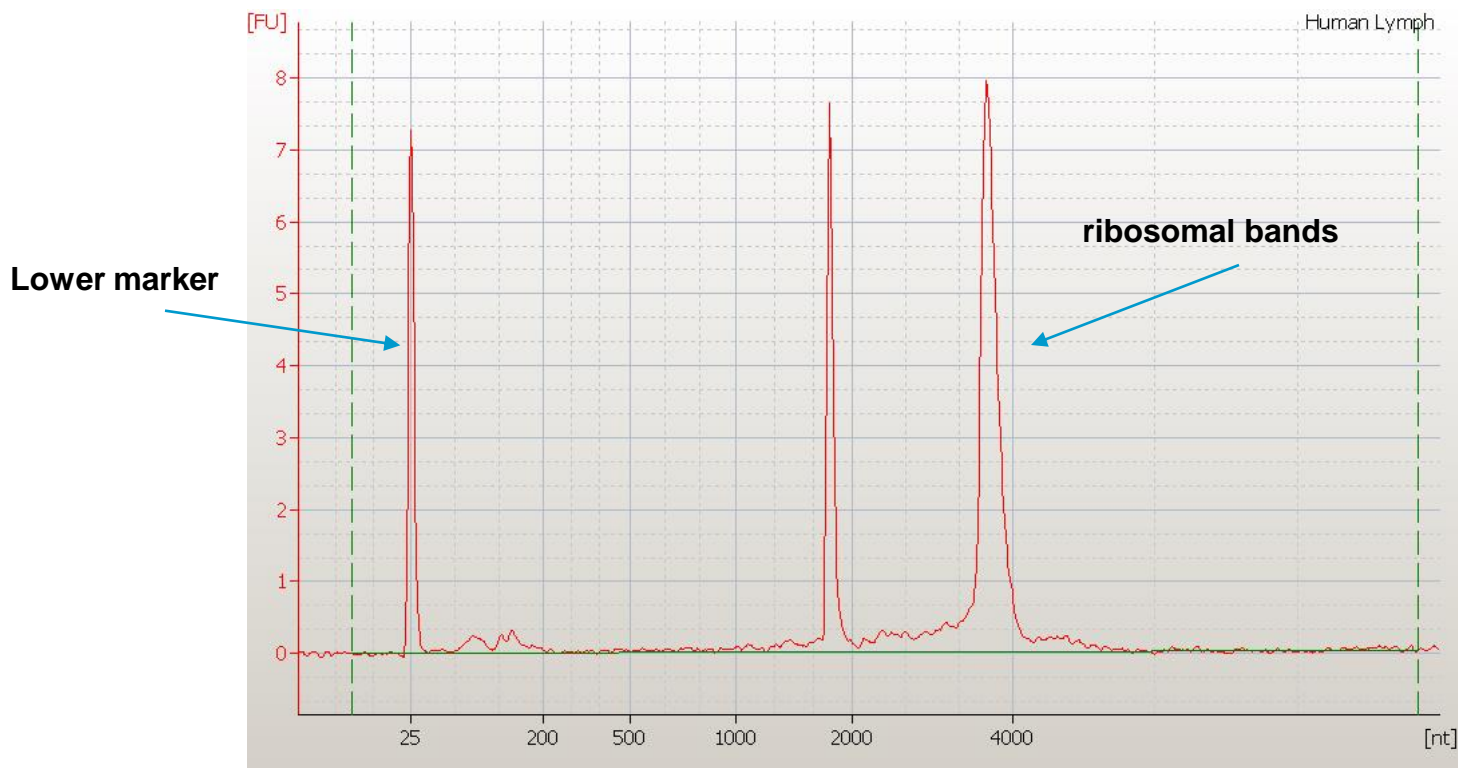


Use of Lower Marker for Alignment



Alignment

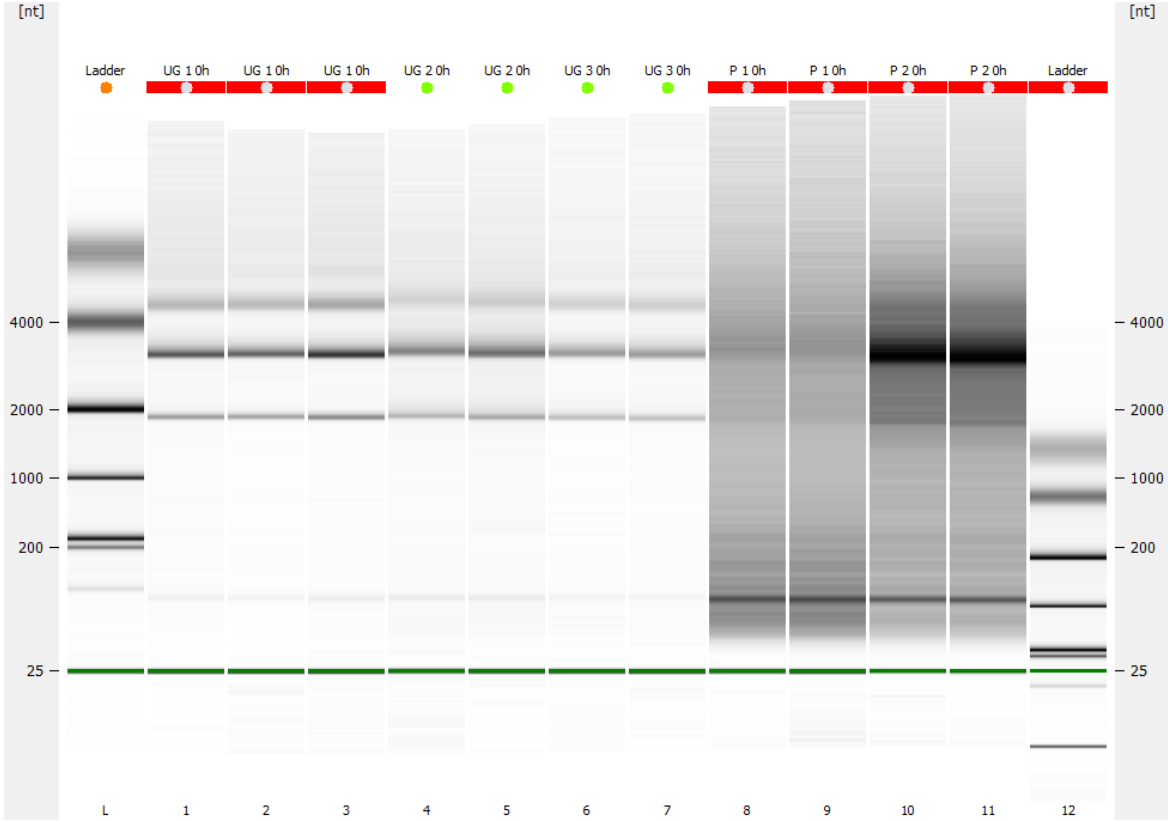
- A DNA Fragment (25 nt) is used as Lower Marker in RNA Nano/Pico Assay
- The 2100 Expert SW automatically detects the Lower Marker
- Lower Marker is used for the alignment



Why is RIN N/A?

Hints:

- Is the correct assay selected?
- Look for the red-flagged lanes on the gel-like image



Why is RIN N/A?

Solutions:

- Run new chip and select correct assay
- Manually assign peaks
- Clear critical error message(s) to get RIN displayed

The screenshot displays the Agilent software interface for a gel electropherogram analysis. The main window is titled "Gel - Sample 1" and shows a gel image with lanes labeled "Lad..." and "Sa...". The y-axis represents nucleotide length in [nt], ranging from 25 to 4000. A red circle highlights a specific region in the gel image, which is linked to an error message in the "Errors" tab at the bottom. The error message reads: "1 [X] 4,501 Unexpected signal in 5s region, Analysis".

The "Errors" tab is highlighted in red. The "RNA Integrity Number" section in the right-hand panel is also highlighted in red, indicating that the RIN value is N/A due to the error.

Code	Description	Category
1 [X]	4,501 Unexpected signal in 5s region, Analysis	Analysis

2100 Expert Software

Setpoint Explorer

Troubleshooting example: RIN N/A – adjust threshold(s) in the Setpoint Explorer to clear critical error(s) → RIN displayed

The screenshot displays the 'Setpoint Explorer' interface for 'Gel - Sample 1'. The central area shows a gel electrophoresis image with 11 lanes. A red circle highlights the 'RNA Integrity Number' section in the right-hand 'Global' settings panel. Below the gel image, a table shows an error: '1 4,501 Unexpected signal in 5s region. Analysis'. The 'Errors' tab is selected at the bottom.

Code	Description	Category
1	4,501 Unexpected signal in 5s region.	Analysis

Relax 5S Region Anomaly Threshold to 1 (most relaxed) will clear the critical error

2100 Expert Software

Setpoint Explorer

Troubleshooting example: RIN N/A – adjust threshold(s) in the Setpoint Explorer to clear critical error(s) => RIN displayed

The screenshot shows the 2100 Expert Software interface. The main window displays an electropherogram for 'Sample 2'. A yellow callout box with a red border is overlaid on the center of the screen, containing the following text:

IMPORTANT

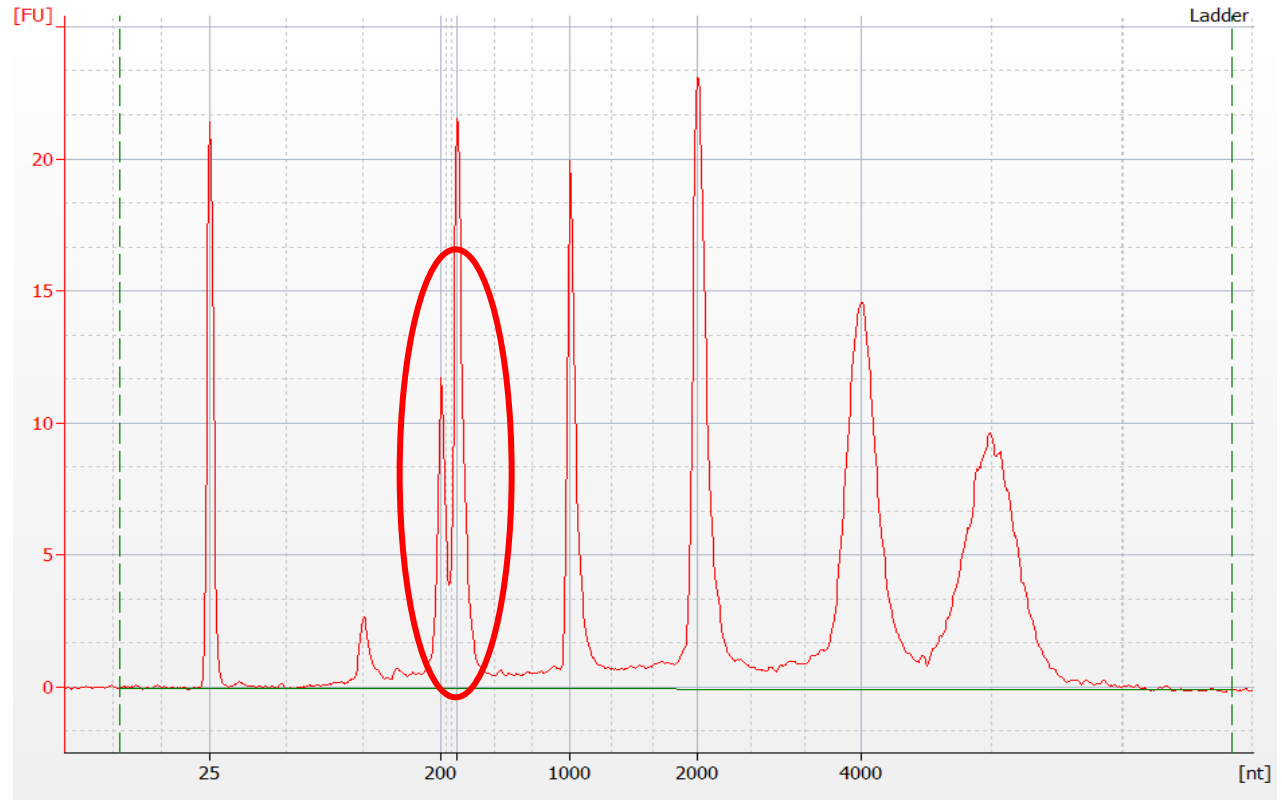
“RIN N/A” is a warning that the RIN may not be reliable for a particular sample (such as due to unusual noise/signals, ribosomal ratio, and other factors). Clearing the critical error message(s) may yield a RIN, but Agilent does not guarantee the accuracy of this value. It is recommended to also perform a visual inspection of the data.

The background interface includes a left-hand navigation pane with icons for Instrument, Data, Verification, Comparison, Assay, and System. The main area shows a plot of signal intensity over time (labeled 1 to 12). Below the plot, there are tabs for Results, Peak Table, Fragment Table, Errors, and Legend. A status bar at the bottom indicates 'Threshold Prerequisite ... 10'.

Troubleshooting: additional ladder fragments

Solutions:

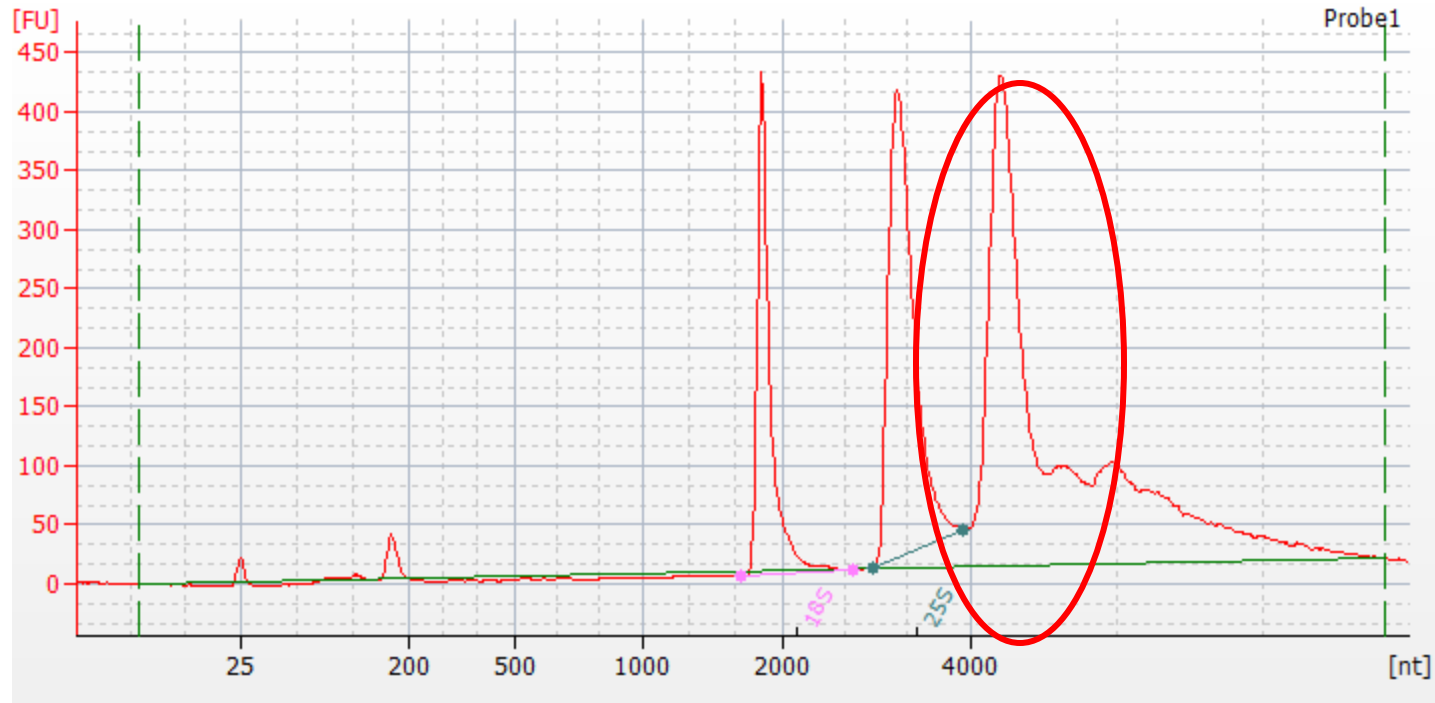
- Heat denature samples and ladder at 72°C for 2 min and immediately place on ice
- Aliquot the ladder after heat denaturation



Troubleshooting: additional RNA sample peak

Solution:

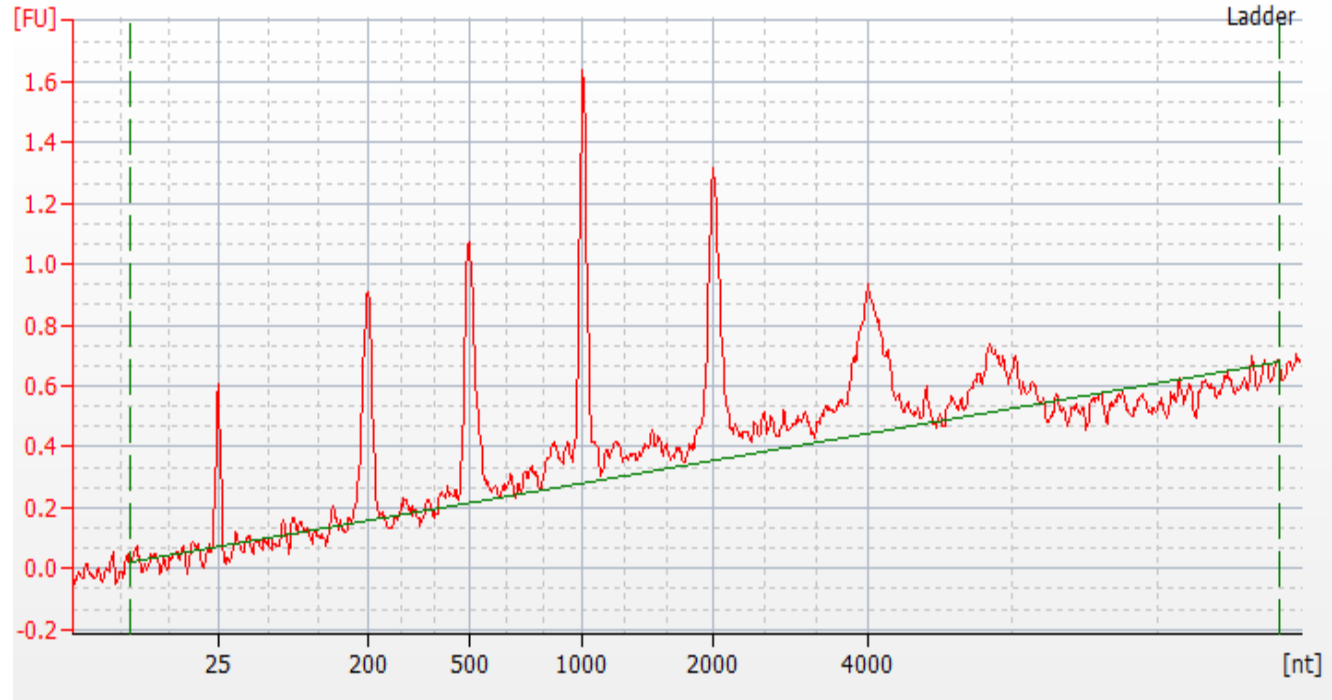
- DNase treatment
- Heat denature samples at 72°C for 2 min to resolve secondary structures



Troubleshooting: low signal intensity

Solutions:

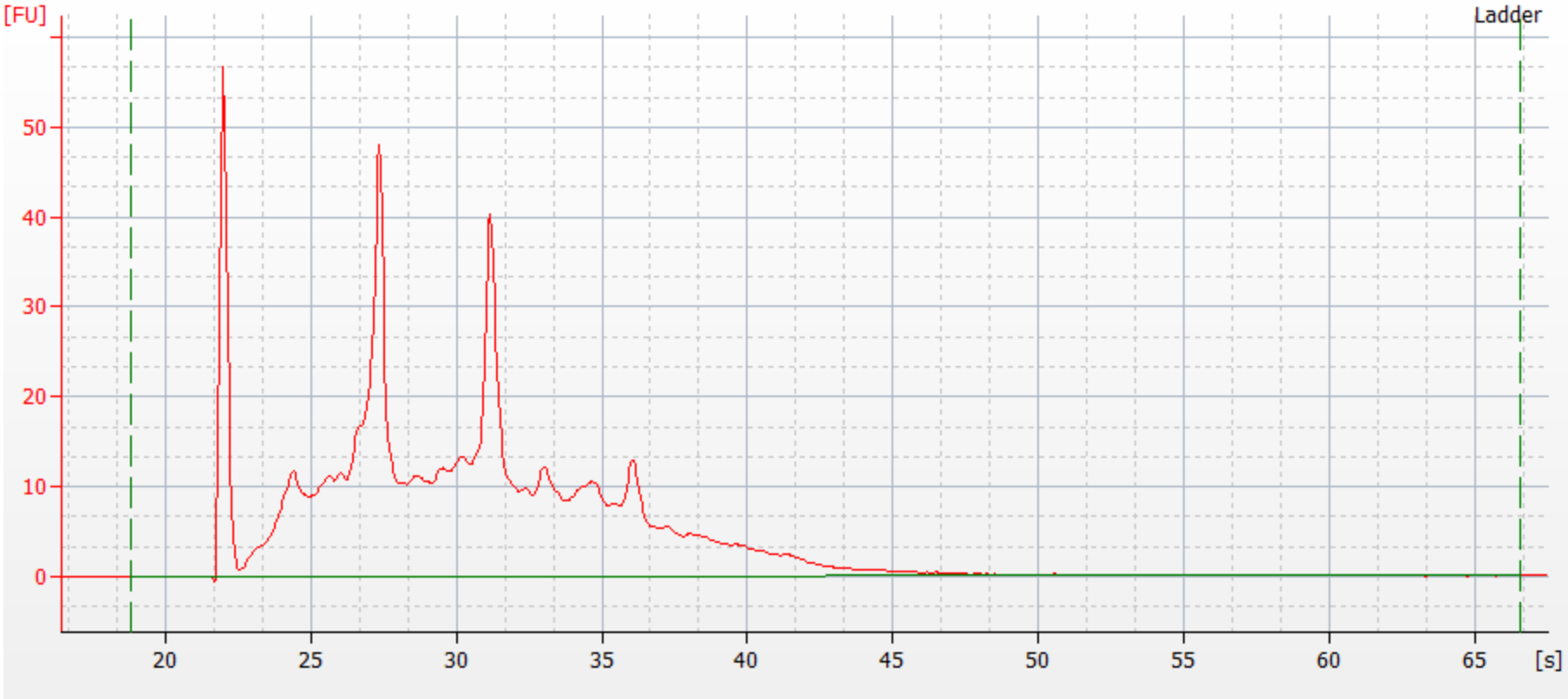
- Prepare a fresh gel-dye mix; protect dye and gel-dye mix from light
- Check kit (both chips and reagents) is within expiration
- Run full set of hardware diagnostic tests



Troubleshooting: degraded RNA Ladder

Improper handling, preparation, and/or storage

RNA Ladders are available separately



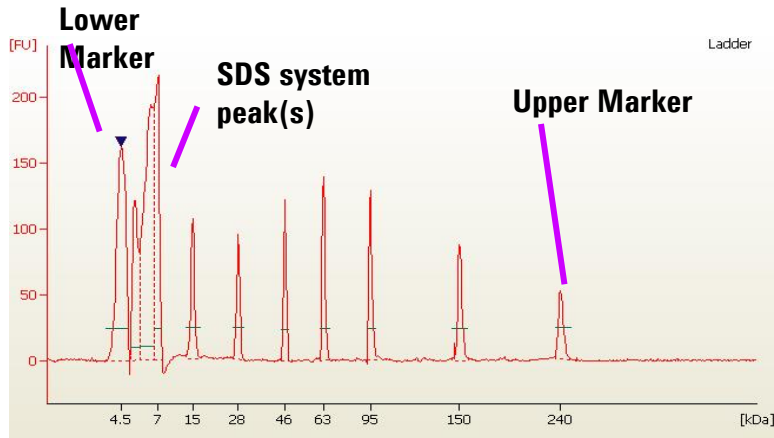
Protein Chip Troubleshooting



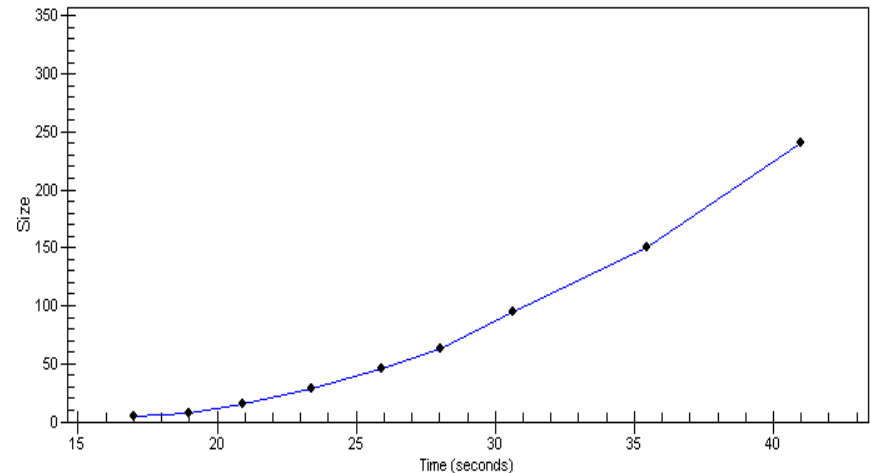
Data Analysis: Protein Ladder

Check if all Protein Ladder fragments have been identified correctly:

Protein 230 Ladder Electropherogram



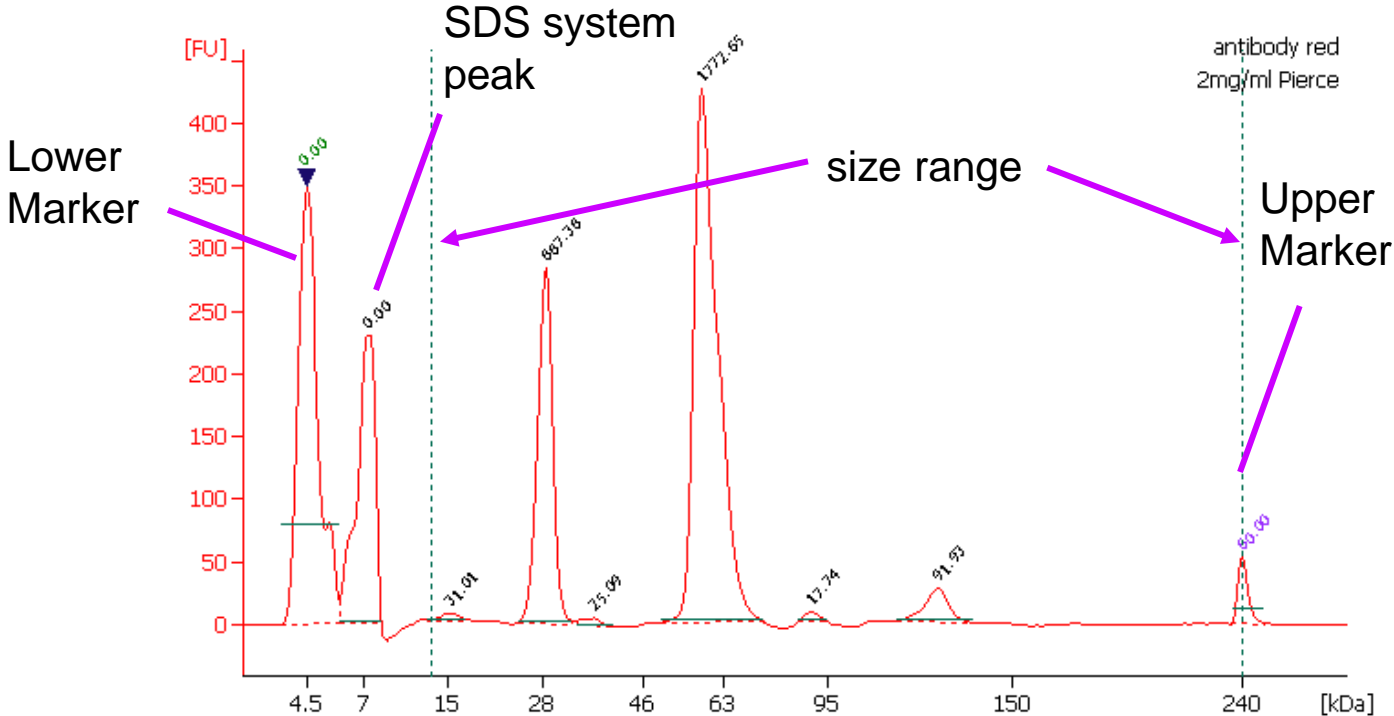
Standard Curve (Chip Summary tab)



- The standard curve is generated with a point to point fit using the migration times and sizes of the ladder peaks
- The size of each sample peak is calculated from the standard curve

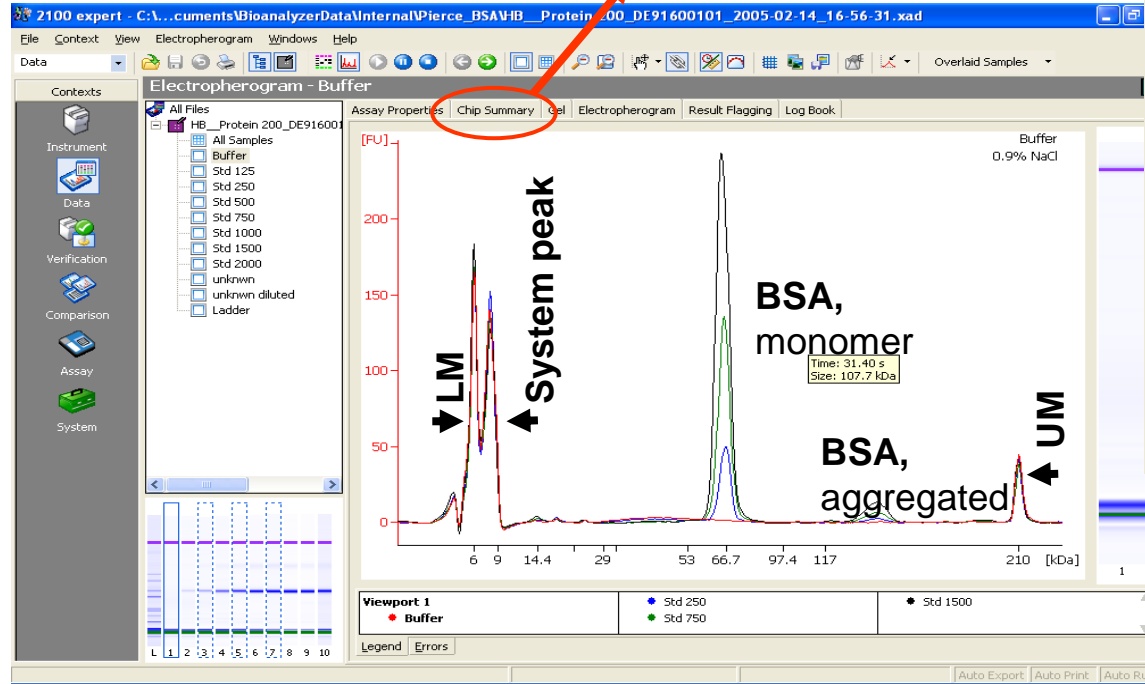
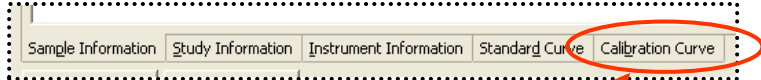
Data Analysis: Protein Samples

- Lower and Upper Markers are included in the Sample Buffer
- The concentration of the Upper Marker is known [60 ng/μl]
- The ratio between sample and Sample Buffer as well as sample preparation are fixed in the protocol
- Comparing TIME-CORRECTED PEAK areas between Upper Marker and sample peak returns a relative sample peak concentration



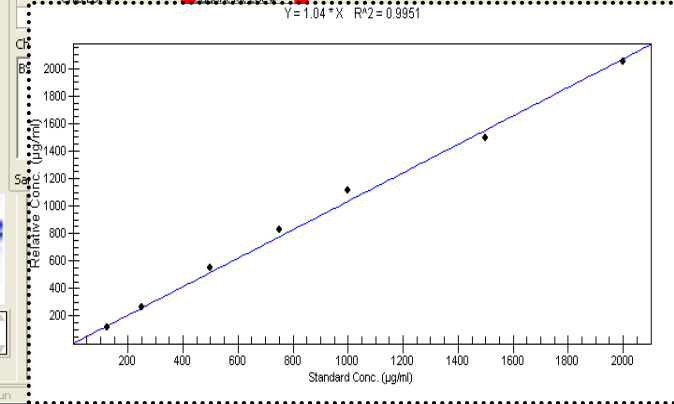
Absolute Quantification Using the Calibration Curve

- Use internal standards (user defines number of wells to be used as standard)
- To select samples for generation of calibration curve, check "Use for Calibration" box next to these samples
- Enter each standard concentration in $\mu\text{g/ml}$



A screenshot of the 'Assay Properties' and 'Chip Summary' window. It displays metadata for a data file: 'HB_Protein 200_DE91600101_2005-02-14_16-56-31.xad'. The location is 'C:\...ttings\habrunne\My Documents\BioanalyzerData\Internal\Pierce_BSA'. The file was created on February 14, 2005, and modified on February 18, 2005. The software version is 'Created by version B.01.02.SI136, modified by B.01.02.SI136'. An image of a 'Protein LabChip' is also shown.

Sample Name	Sample Conc.	Use For Calibration	Conc. [$\mu\text{g/ml}$]	Status	Observation	Result Label	Re
1	Buffer	<input type="checkbox"/>	0.9% NaCl	0	✓		
2	Std 125	<input checked="" type="checkbox"/>	125	✓			
3	Std 250	<input checked="" type="checkbox"/>	250	✓			
4	Std 500	<input checked="" type="checkbox"/>	500	✓			
5	Std 750	<input checked="" type="checkbox"/>	750	✓			
6	Std 1000	<input checked="" type="checkbox"/>	1000	✓			
7	Std 1500	<input checked="" type="checkbox"/>	1500	✓			
8	Std 2000	<input checked="" type="checkbox"/>	2000	✓			
9	unkwn (2mg BSA)	<input type="checkbox"/>	0	✓			
10	unkwn dilu... with 0.9%	<input type="checkbox"/>	0	✓			

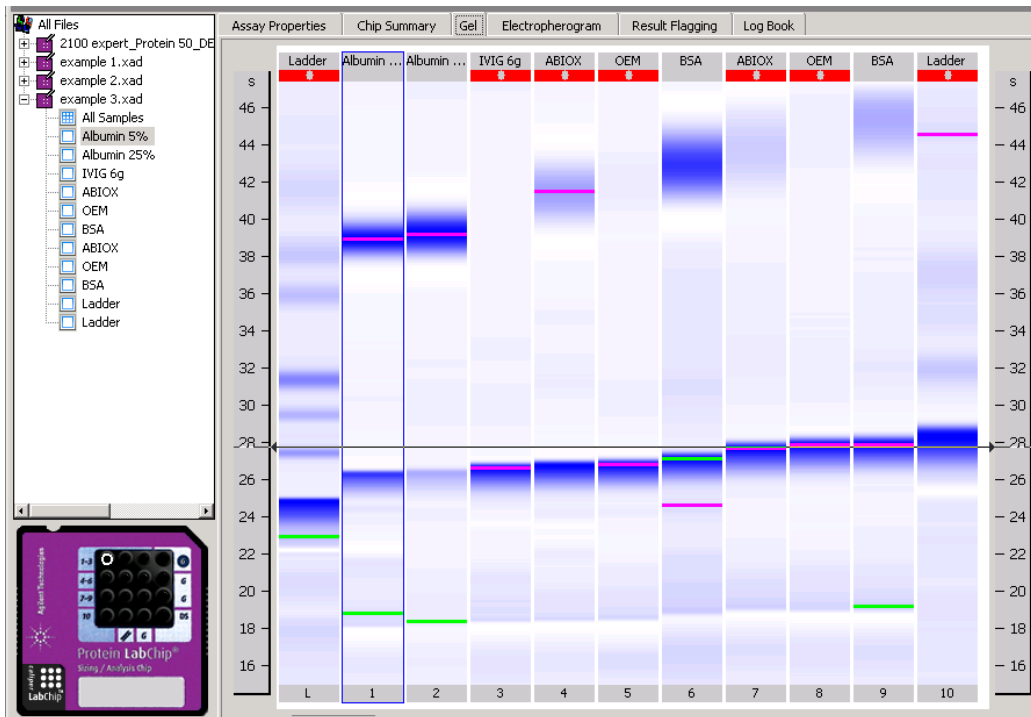


Troubleshooting: clogged priming station

Issue: Plastic adapter of priming station clogged

Symptom: Broad and fuzzy peaks

Solutions: Routine maintenance of priming station; avoid spillage of gel



Recommended after chip priming step:

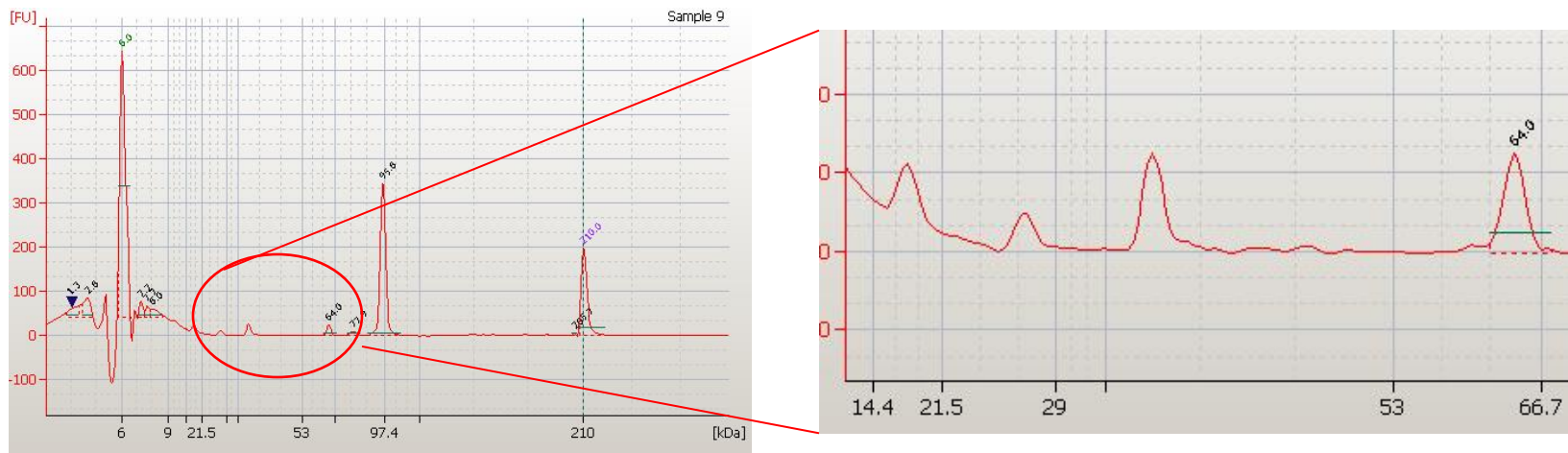
Quick view if residual gel is visible on the adapter/gasket;

If yes: use Kimwipe to clean adapter, flush out adapter with syringe and water. If dried gel in hole, pick out with a needle.

Troubleshooting: peak not detected

Issue: A sample peak is not detected by the software automatically

Example:



Solutions:

- Use Setpoint Explorer to modify integration limits e.g. height threshold
- Activate Manual Integration and choose 'Add peak'

Want to know more?



- The 2100 Expert software has many features and a very extensive Help menu
- For the latest version of the Maintenance & Troubleshooting Guide:
<http://www.chem.agilent.com/Library/usermanuals/Public/G2946-90003.pdf>

Agilent 2100 Bioanalyzer Help Desk

Agilent 2100 Bioanalyzer Help Desk
Agilent 2100 Bioanalyzer 2100 Expert Guide
Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Help
License Administration Utility
Related Documents
Help on Help

Agilent 2100 Bioanalyzer

Maintenance and Troubleshooting Guide

Agilent Technologies

Need help?

Are you stuck? Do you need our help? Please see the following slides for details on the support process.



Need to get in contact with us?



- Email contact:
bioanalyzer@agilent.com
- Phone: 1-800-227-9770 options
3x4x1

Need to get in contact with us?



Please provide the following information to help us with troubleshooting:

- Error description, in your words. Also, the expected result in your words.
- Kit used - lot #s and expiration dates of chips and reagents used.
- Sample information:
 - DNA/RNA or Protein?
 - Which species, which tissue organ are the samples extracted from?
 - Extraction method, post-extraction processing (e.g. DNase digest), purification method
 - What kind of buffer are the samples in?
 - What is the expected concentration range (and sizing range, if applicable) of the samples?
- The data file (**.xad**) from the run that had problems. Also, any data file from a recent successful run.

Data file: e.g. 2100expert_EukaryoteTotalRNAPico_12345_2013-05-17_13-24-56.xad

Location: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\data\

Questions?

