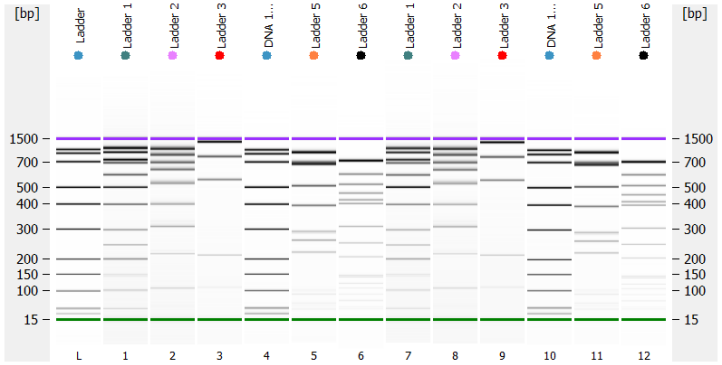
Bioanalyzer System

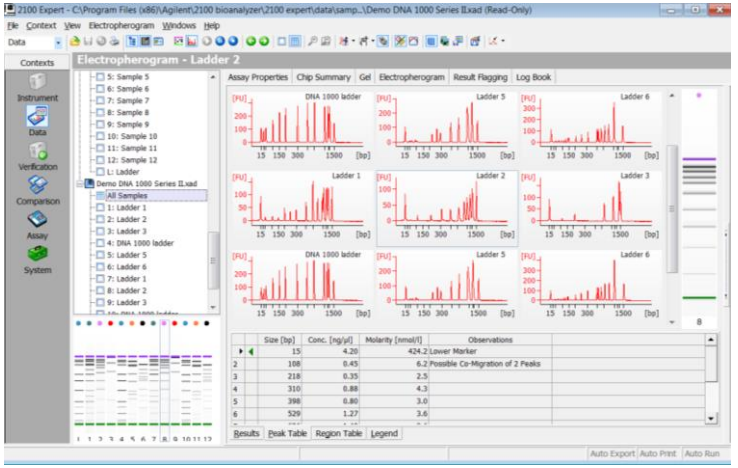
Troubleshooting Section

1

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?

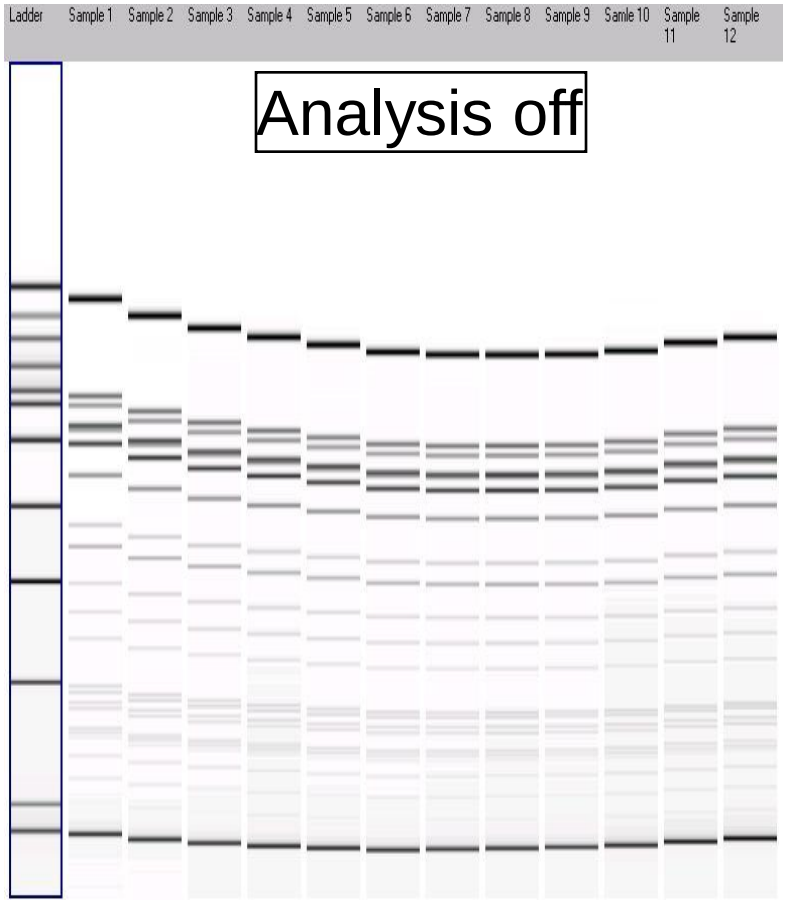


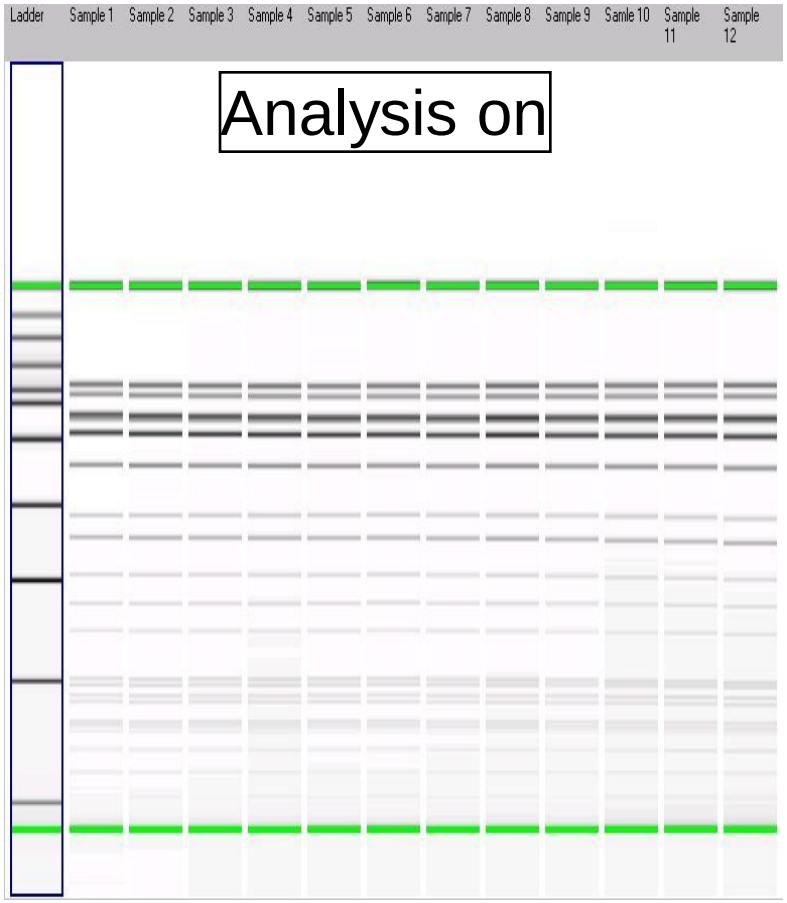


2

Data Analysis: Alignment

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

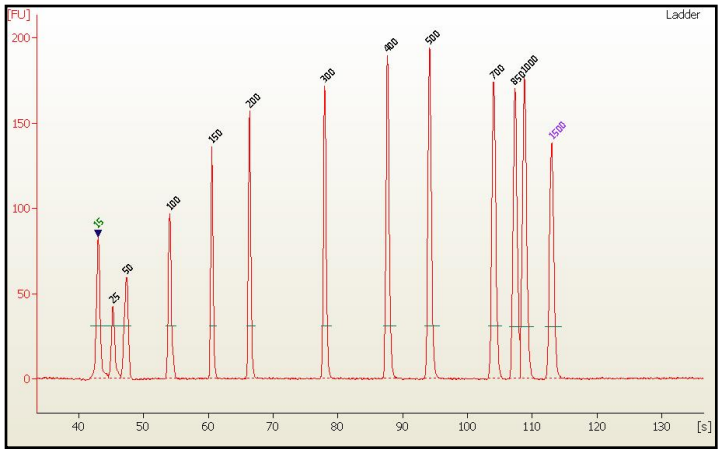




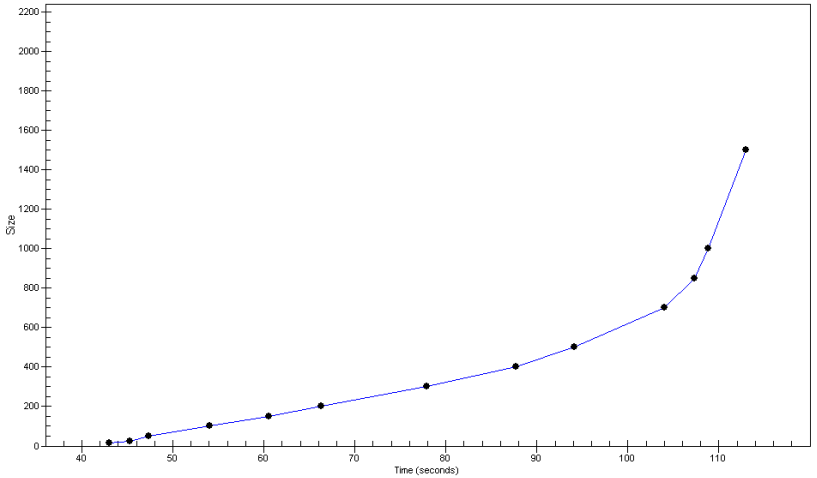
Data Analysis: DNA Ladder

Check if all DNA ladder fragments have been identified correctly:

DNA 1000 Ladder Electropherogram



Standard Curve (Chip Summary tab)



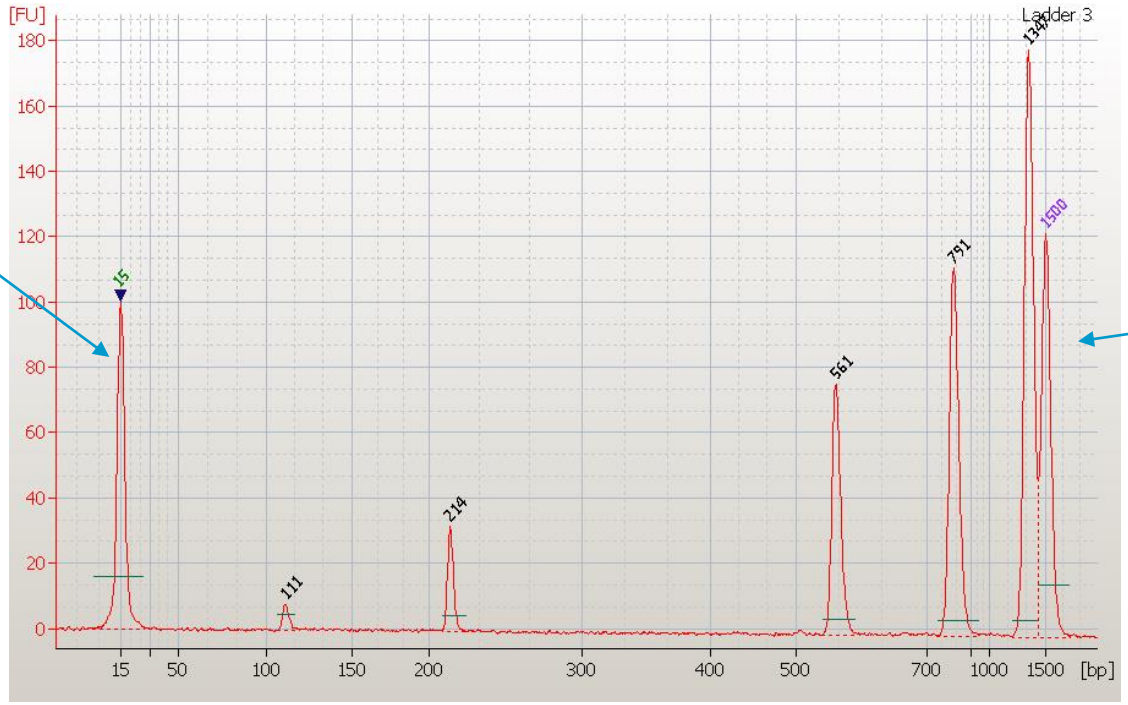
- **The standard curve is generated with a point to point fit using the migration time and sizes of the ladder peaks**

- **The size of each sample peak is calculated from the standard curve**

Data Analysis: Samples

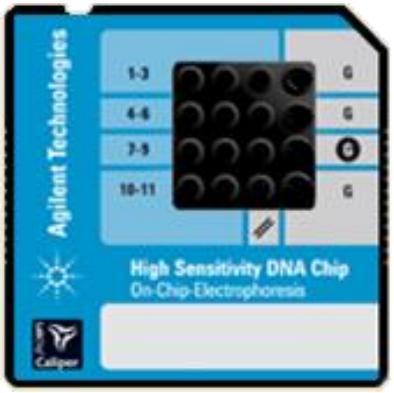
- Specified DNA fragments are used as Lower and Upper Markers
- The 2100 Expert SW automatically detects the Markers
- Lower and Upper Marker are used for the alignment -> Sizing
- Upper Marker is used for the quantitation

LM
15 bp



UM
1500 bp

DNA Chip Troubleshooting



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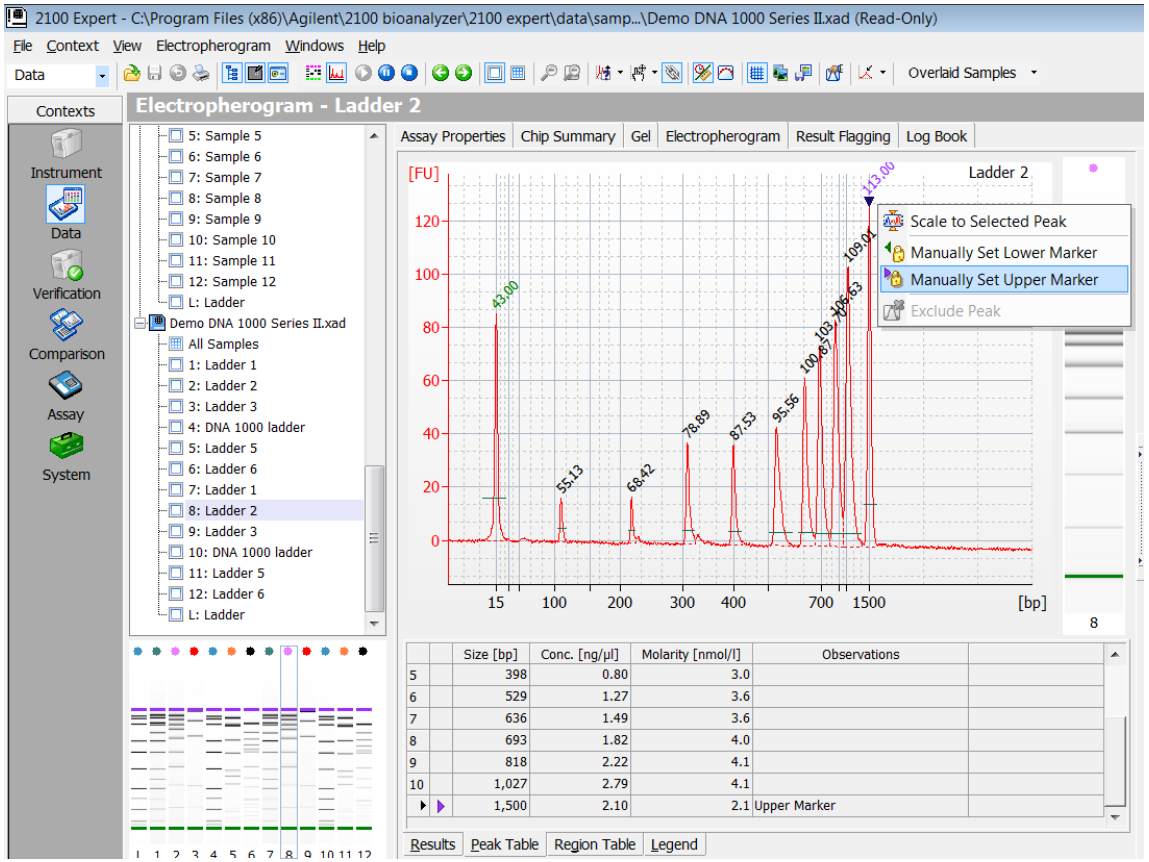
Why is it not possible to toggle the x-axis between time and size?

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.



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Upper Marker not automatically recognized

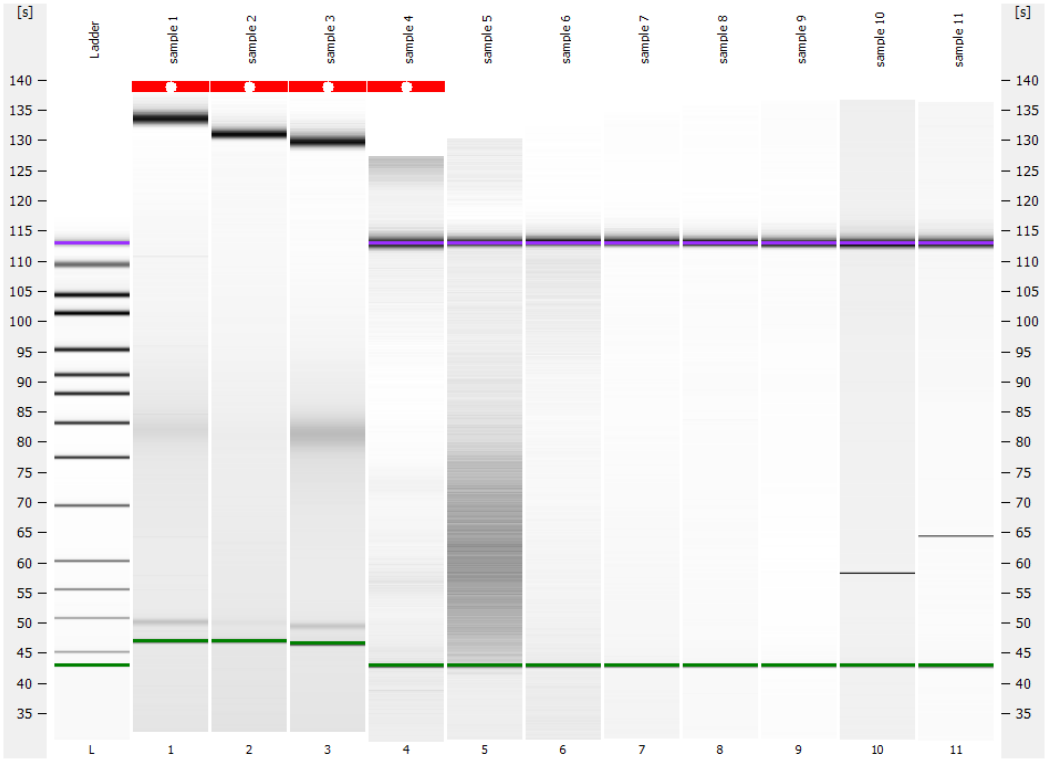
- Some samples show no sizing/quantitation information, red flags -

Issue:

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

Solution:

Manually assign Upper Marker on Peak Table tab



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Overloaded chip

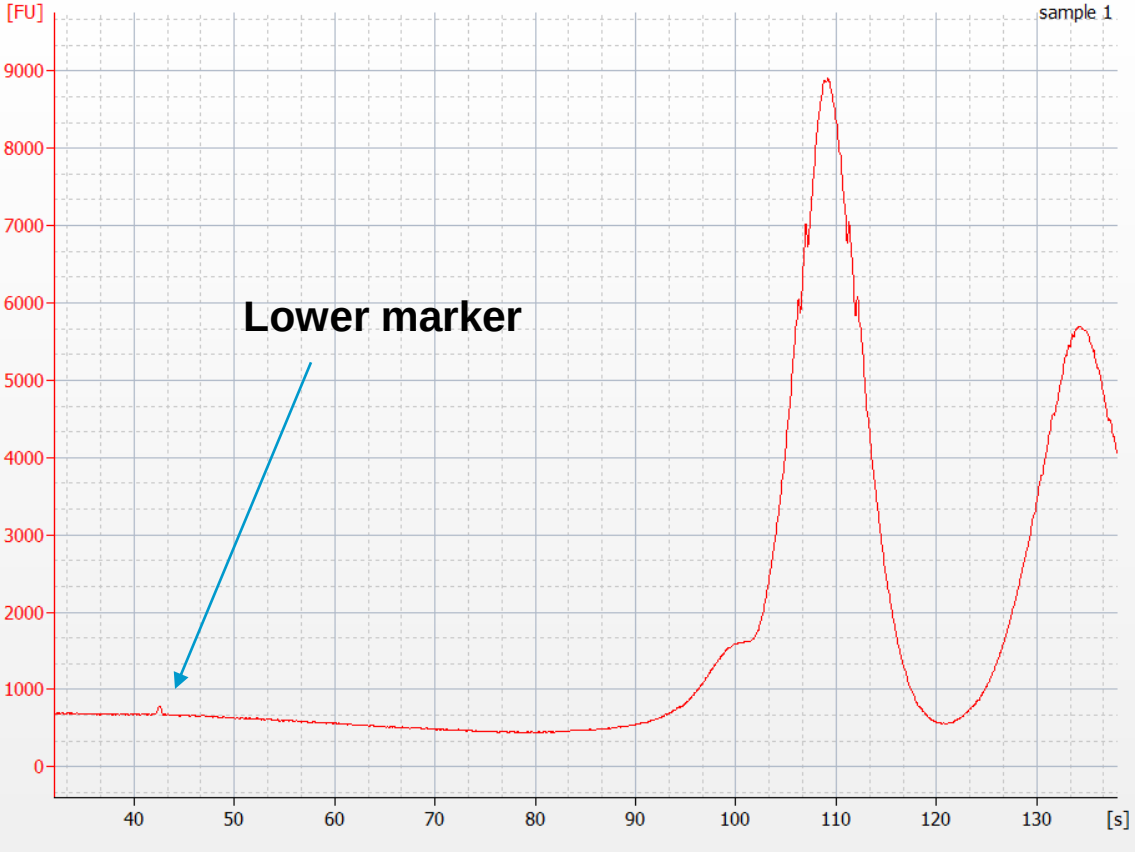
- No sizing/quantitation information, red flags, error message: “optical signal too high“ -

Issue:

Overloaded chip

Solution:

Adjust sample concentration by dilution



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No detectable peaks, distorted baseline

Hints:

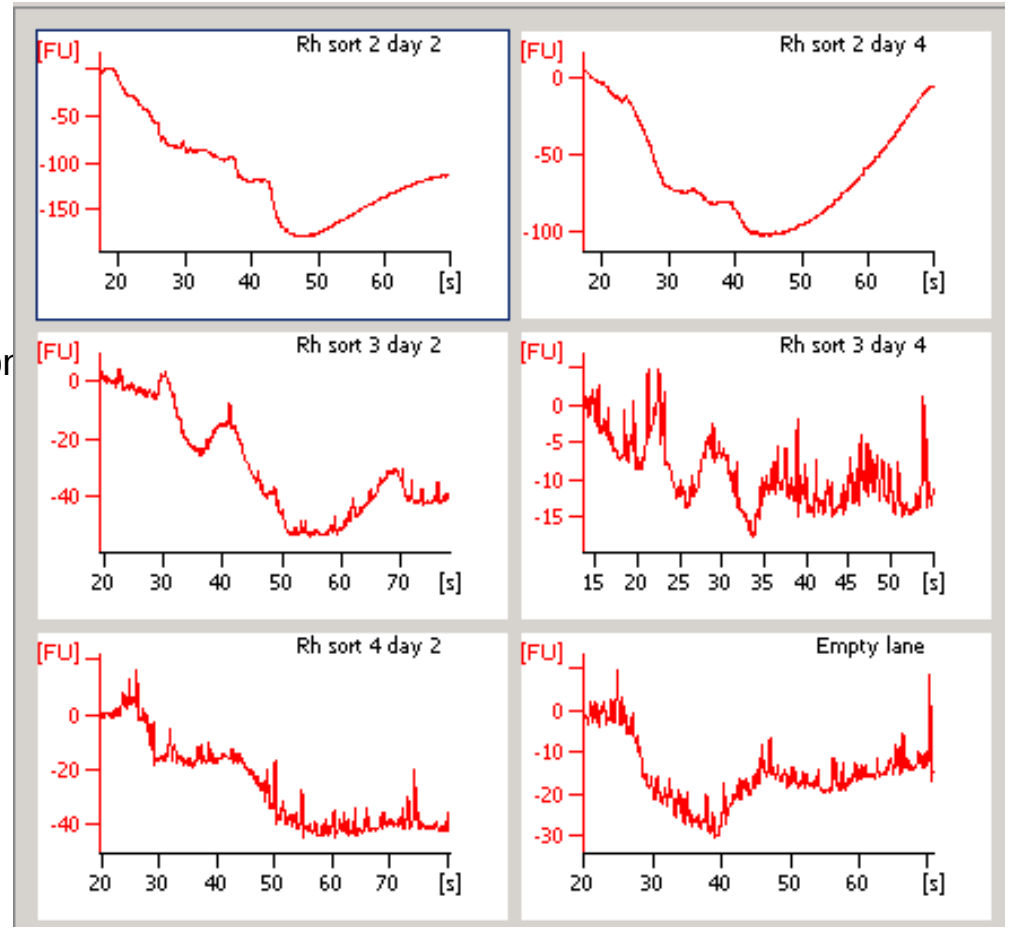
- Check for liquid spillage on chip surface
- Check chip priming station settings
- How much water was added to the cleaning chip
- When was maintanance for chip priming station last performed
- When was maintenance for pin set last performed

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



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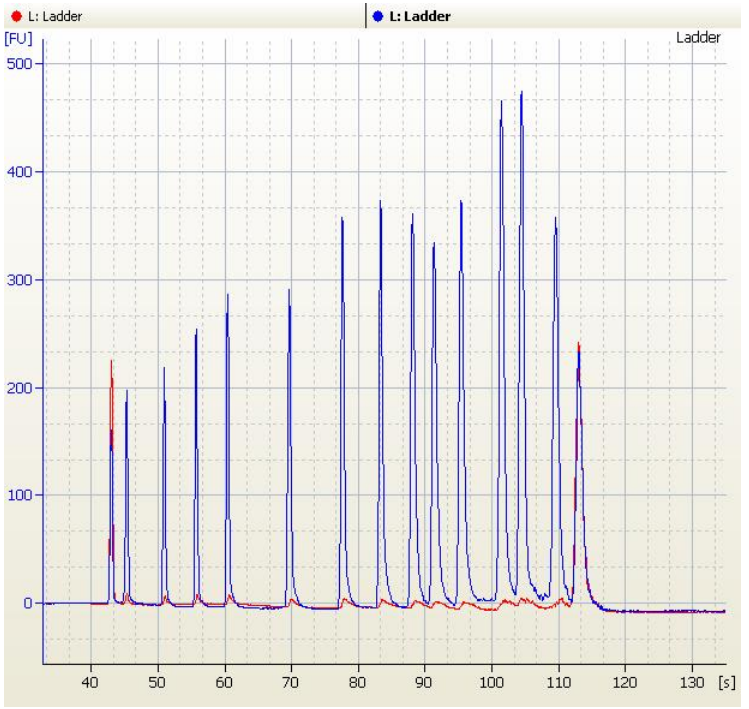
Low signal intensity (FU) with the HS DNA Ladder

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)

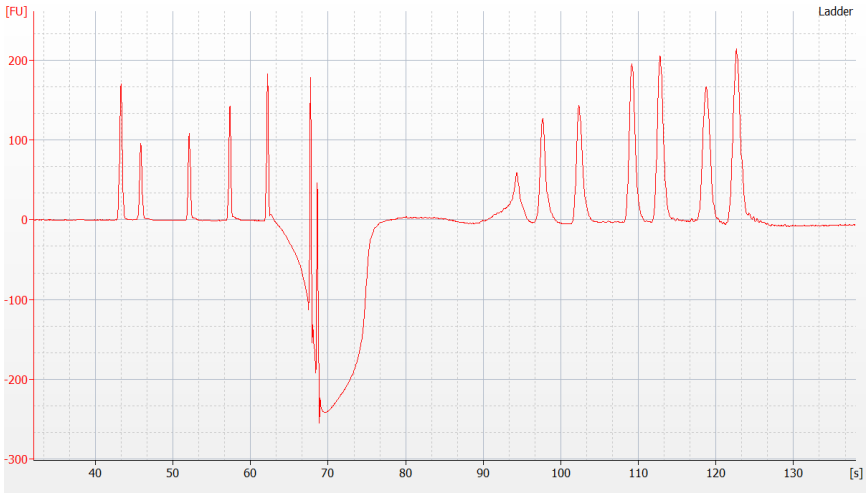
Broad dips in all/some samples and/or ladder

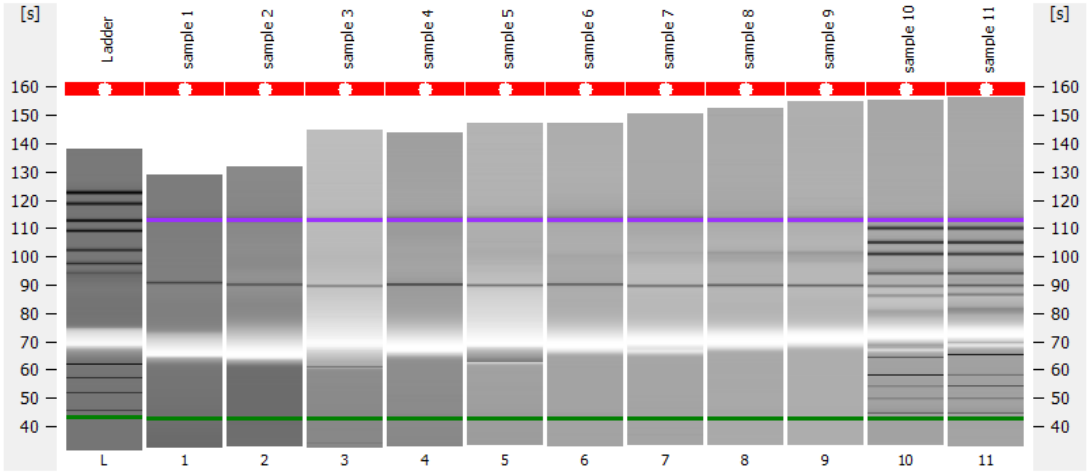
Explanation:

Residual RNaseZap or SDS on the electrodes

Solution:

Additional washes with cleaning chip with H_2O (2x)





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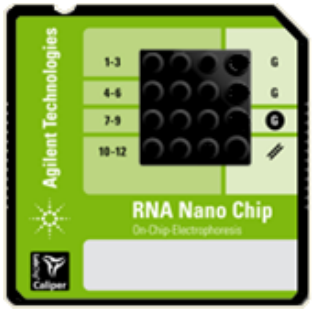
RNA Chip Troubleshooting



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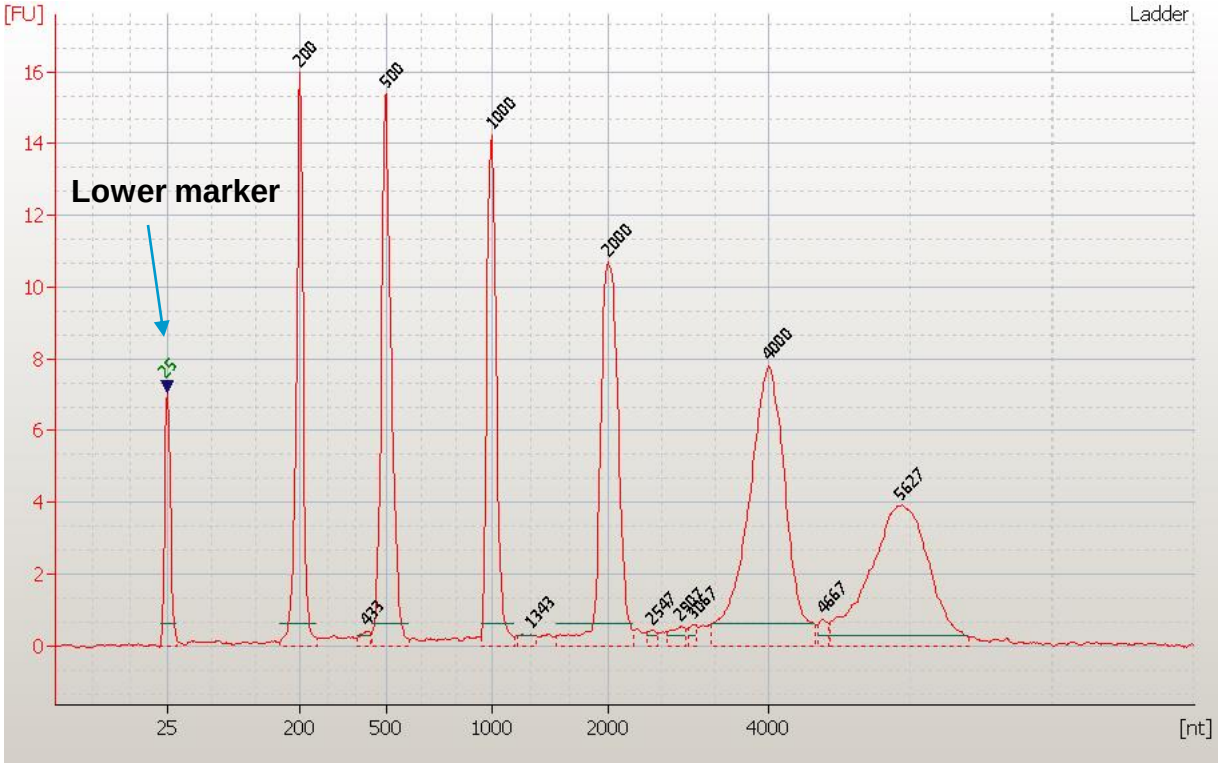
Data Analysis: RNA Ladders

RNA Ladder is used for sizing *and* quantitation:

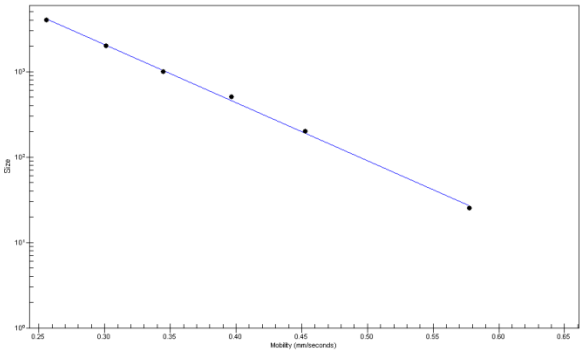
6 ladder peaks + Lower Marker

Ladder concentration: **RNA Nano: 150 ng/µl** **Small RNA ladder: 1000pg/µl**

RNA Pico: 1000 pg/µl



Chip Summary >Standard Curve:



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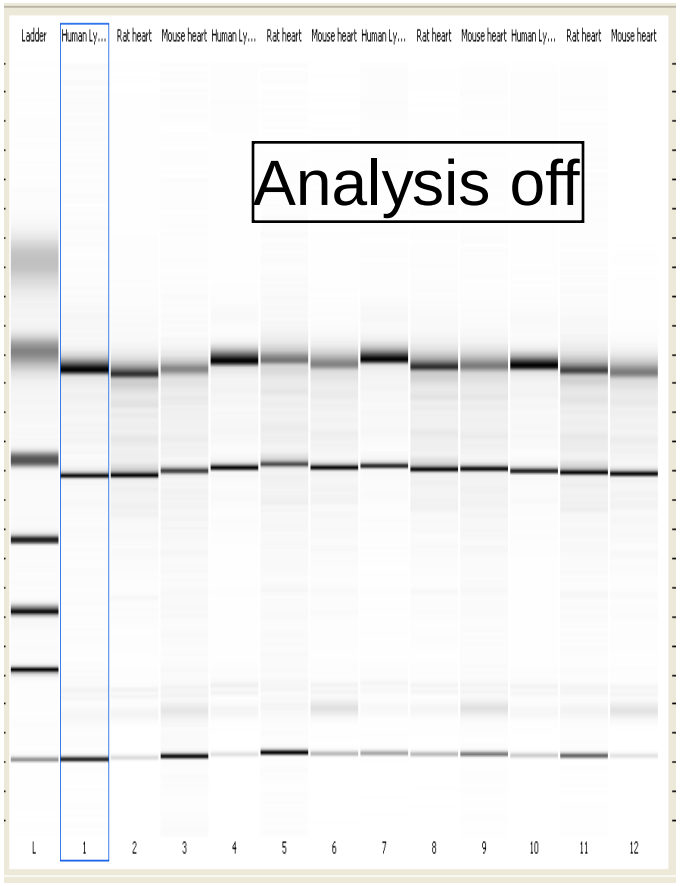
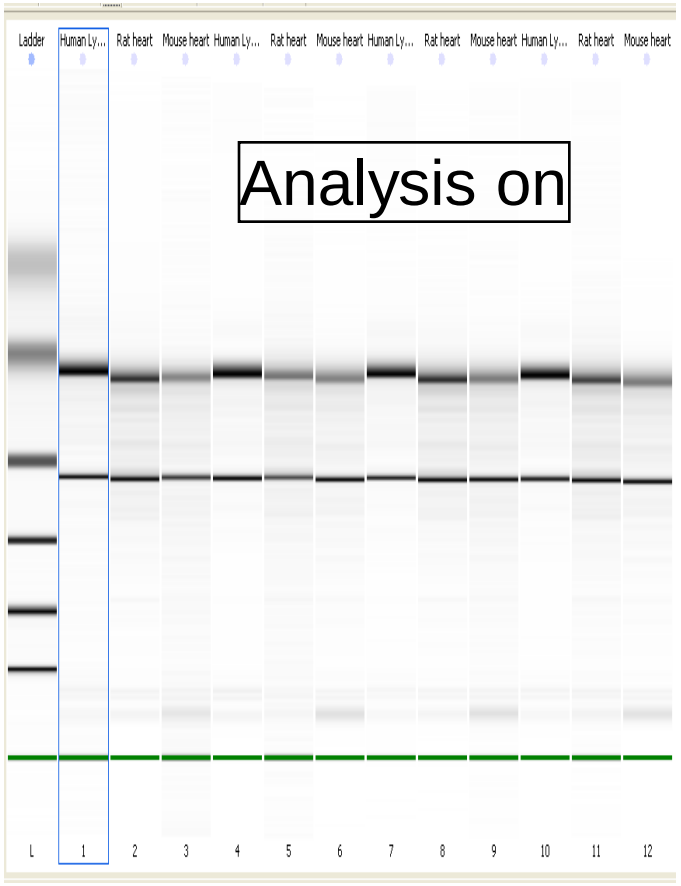
Use of Lower Marker for Alignment





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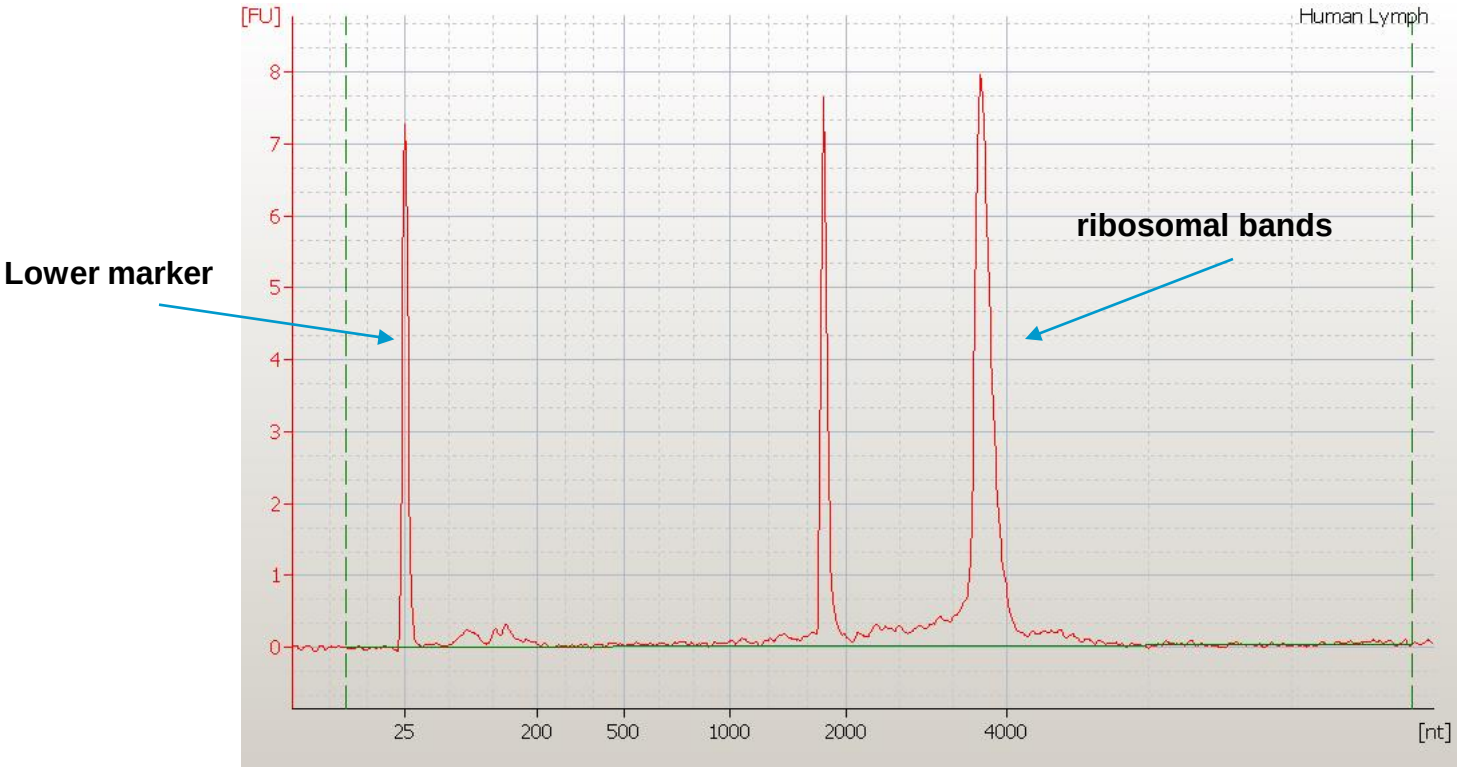
Alignment

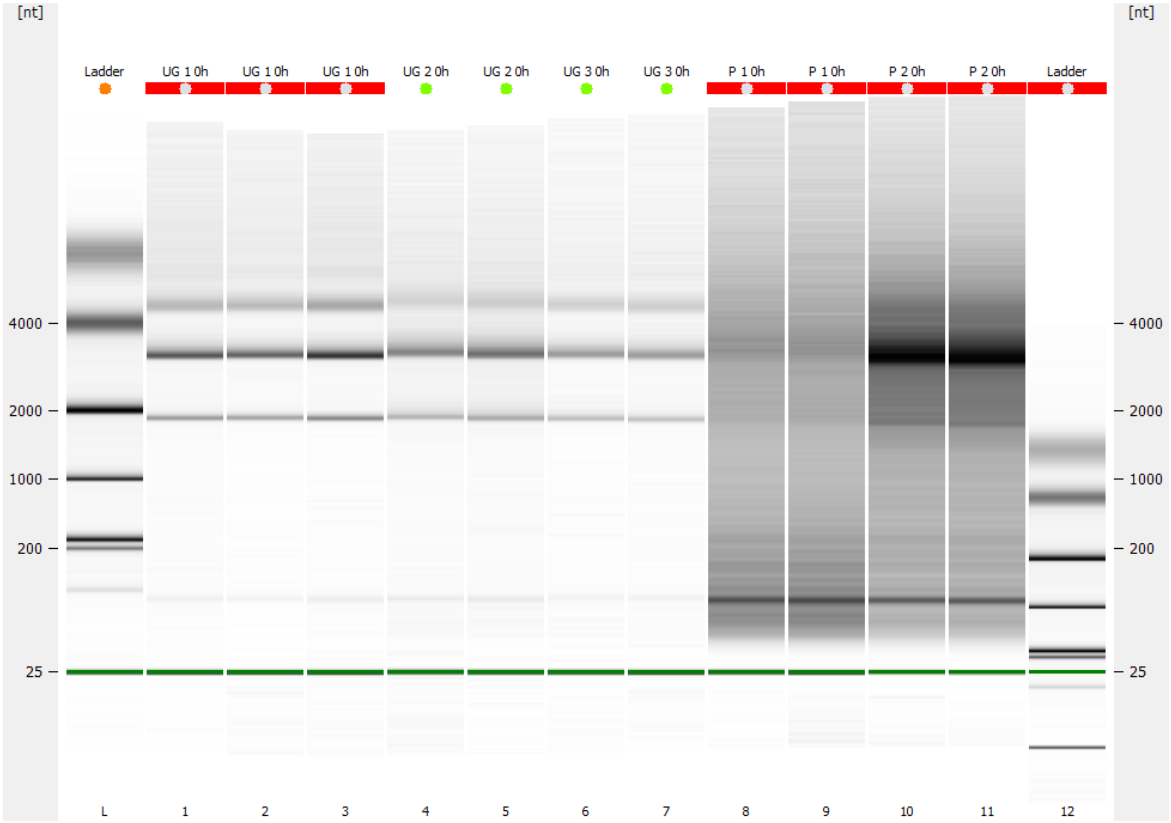
- A DNA Fragment (25 nt) is used as Lower Marker in RNA Nano/Pico Assay
- The 2100 Expert SW automatically detects the Lower Marker
- Lower Marker is used for the alignment



Why is RIN N/A?

Hints:

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



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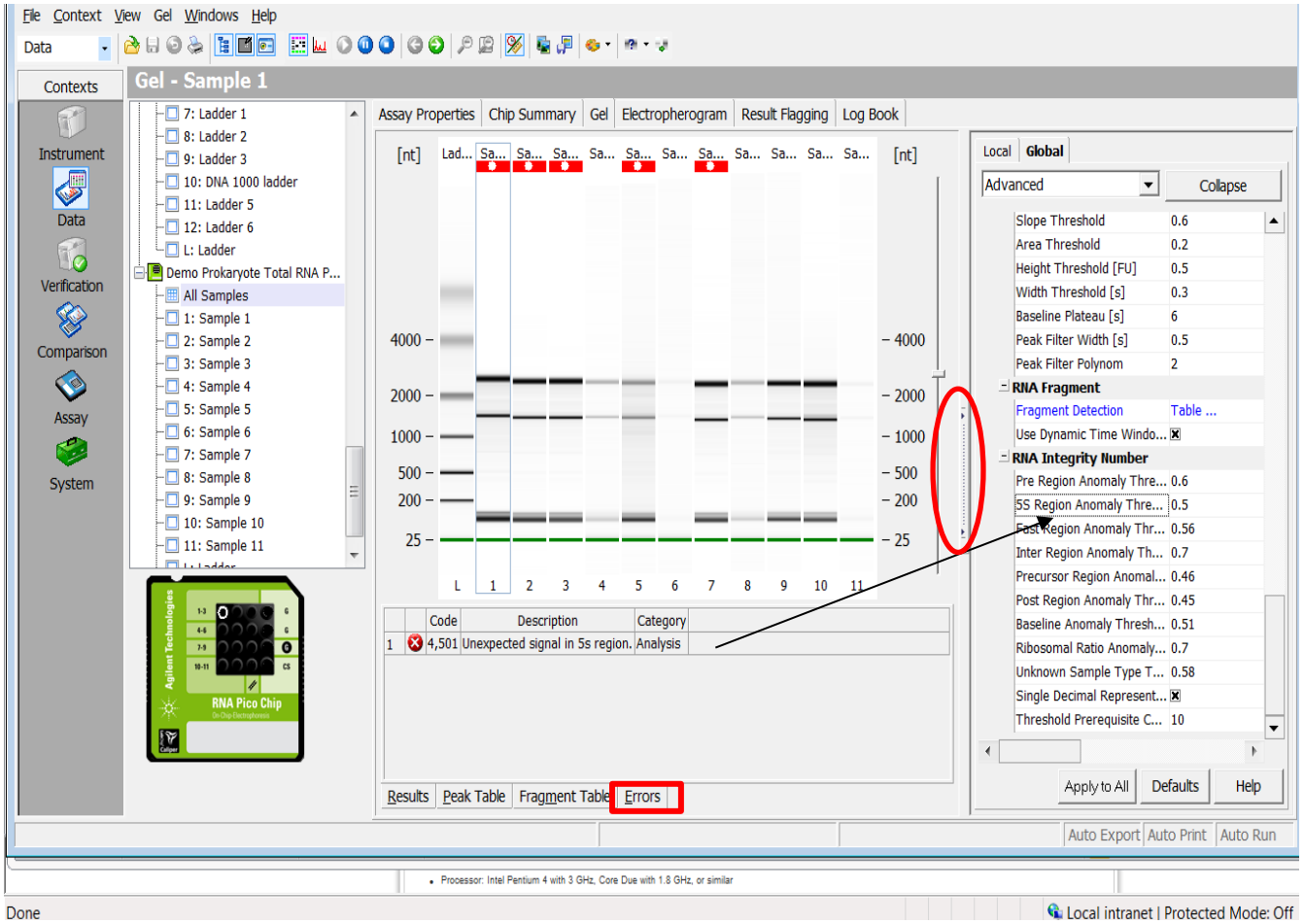
Why is RIN N/A?

Solutions:

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



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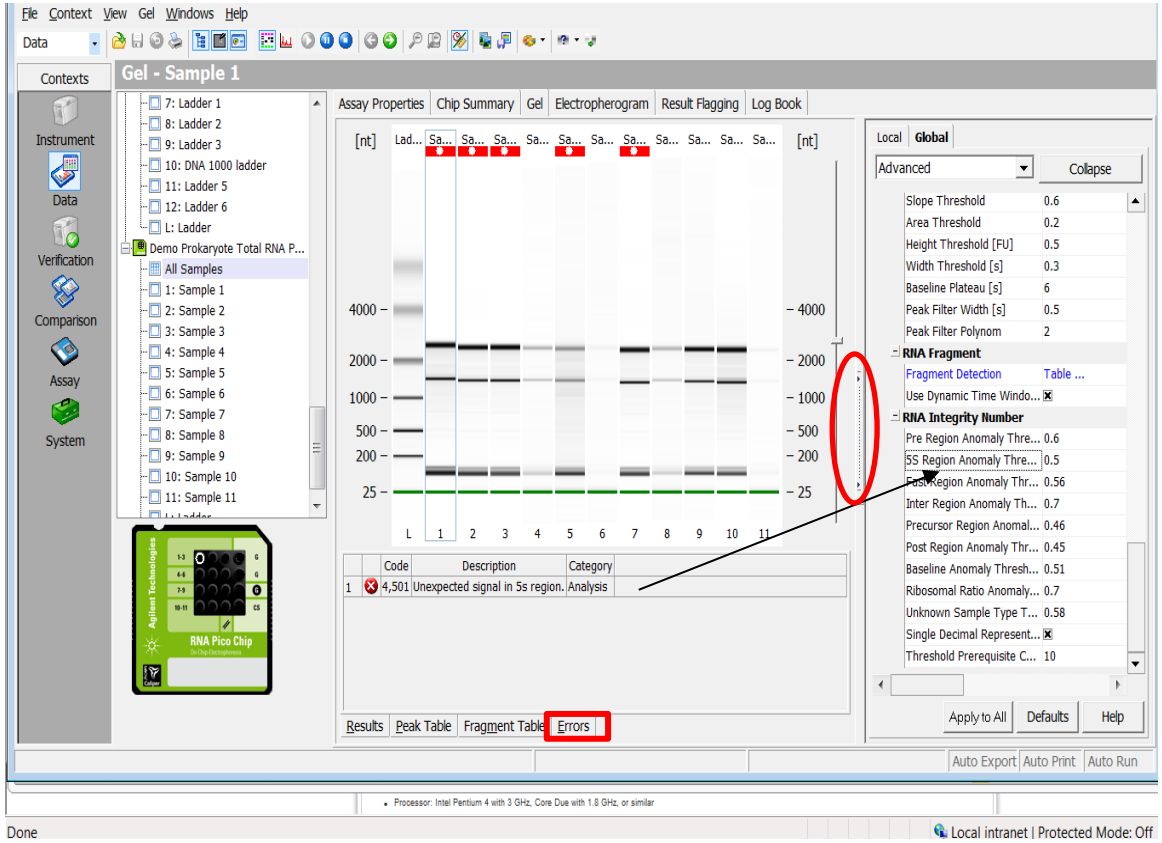
2100 Expert Software

Setpoint Explorer

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

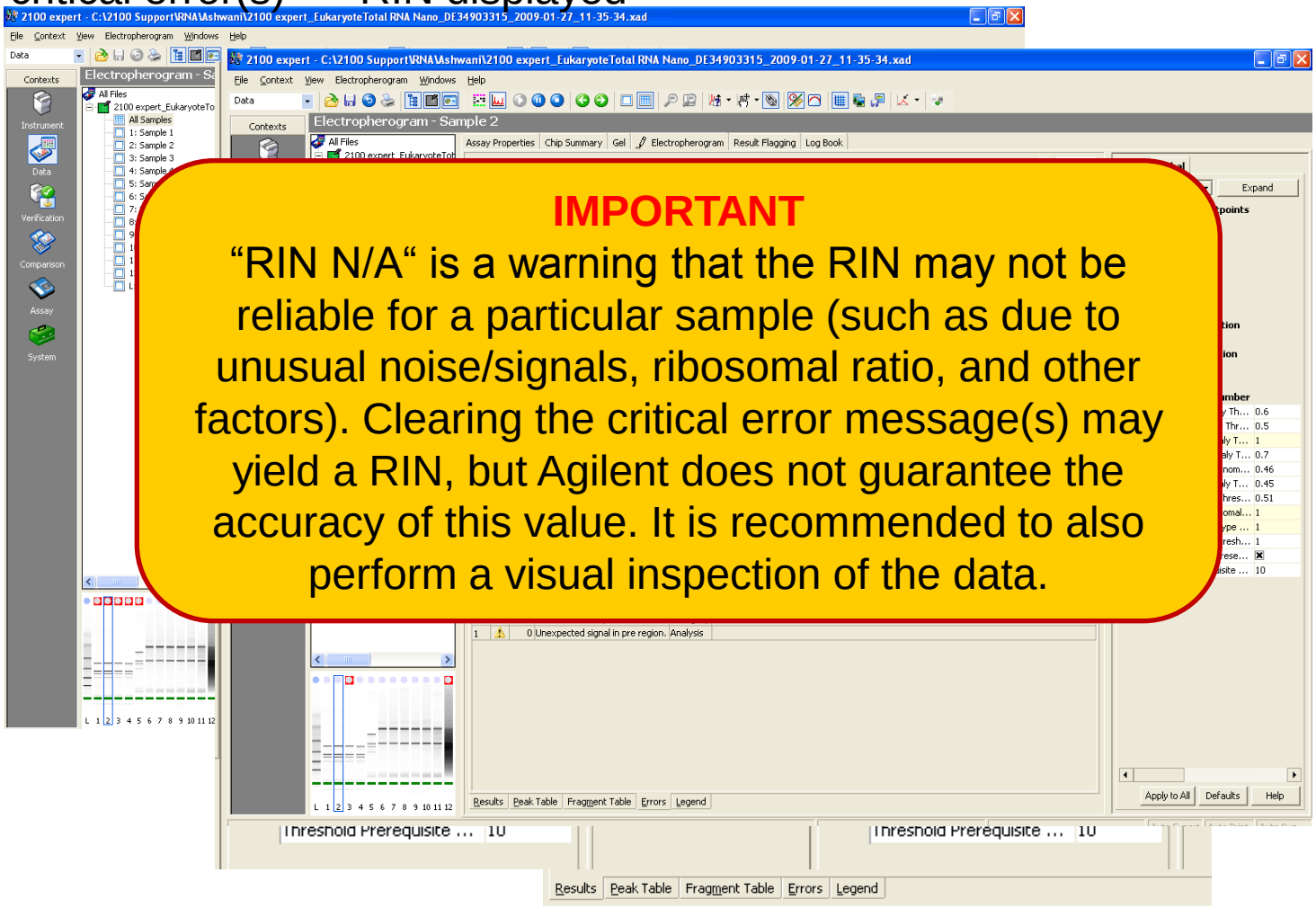


Relax 5S Region Anomaly Threshold to 1 (most relaxed) will clear the critical error

2100 Expert Software

Setpoint Explorer

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



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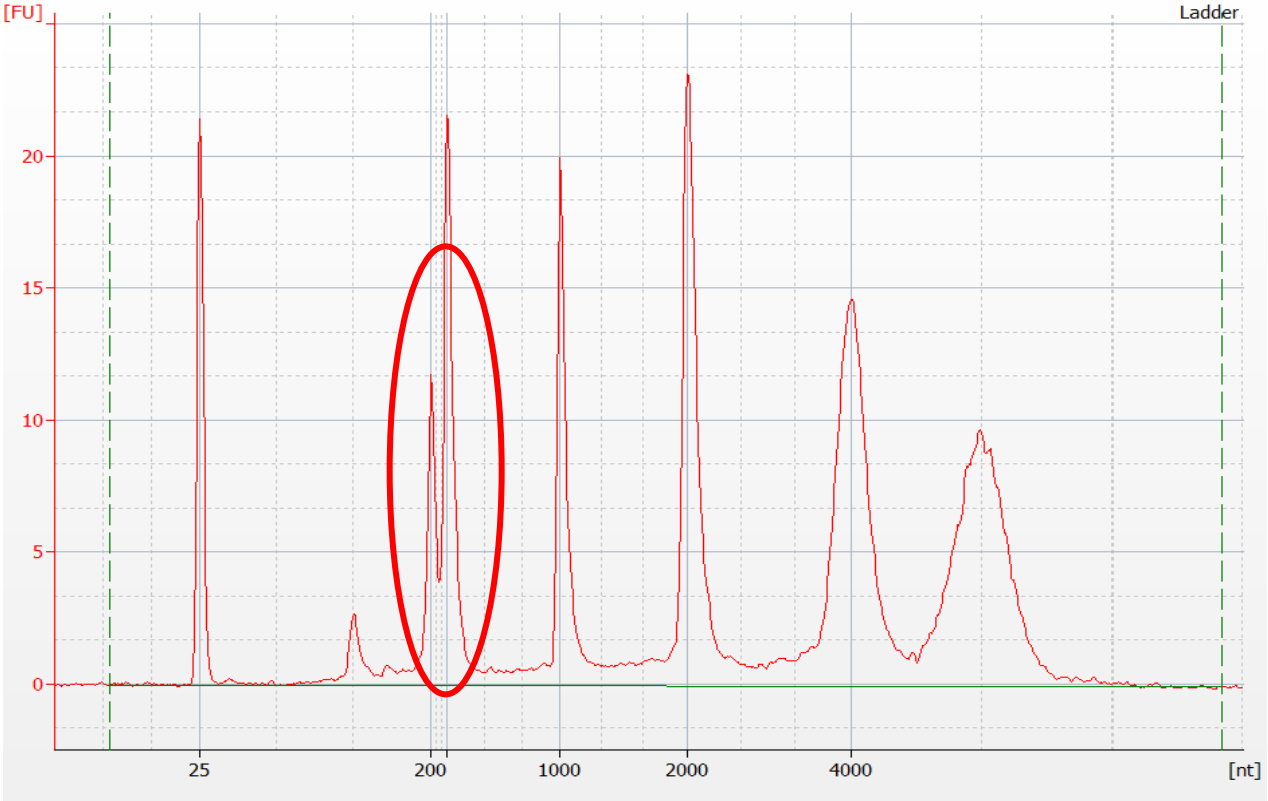
Troubleshooting: additional ladder fragments

Solutions:

- Heat denature samples and ladder at 72°C for 2 min and immediately place on ice

- Aliquot the ladder after heat denaturation



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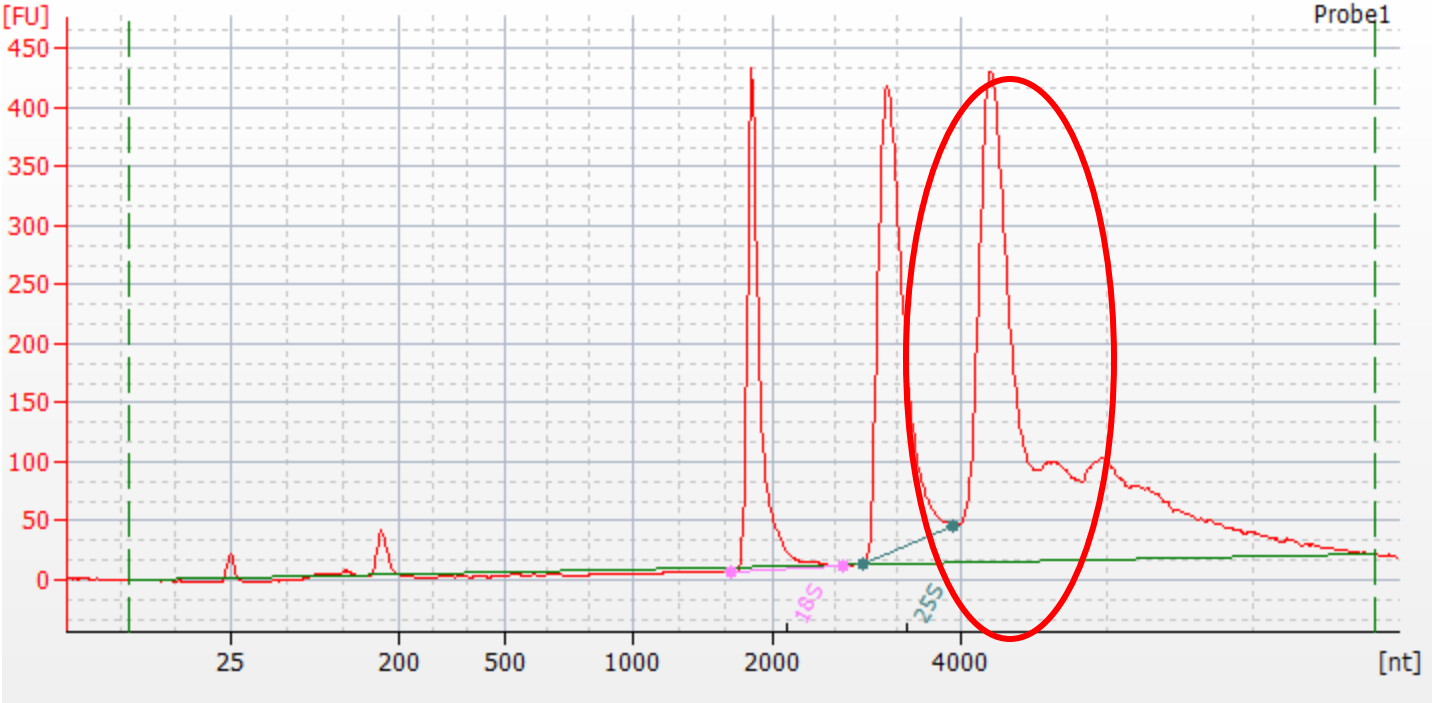
Troubleshooting: additional RNA sample peak

Solution:

- DNAse treatment

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



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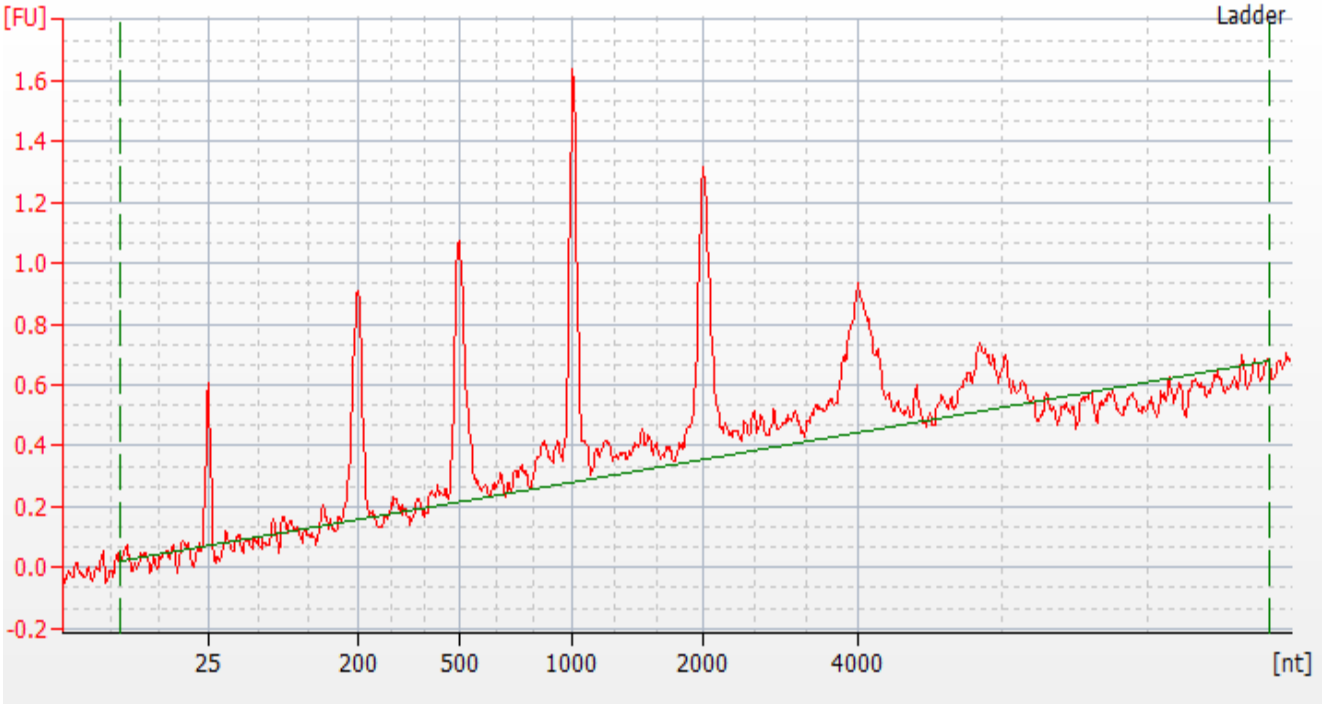
Troubleshooting: low signal intensity

Solutions:

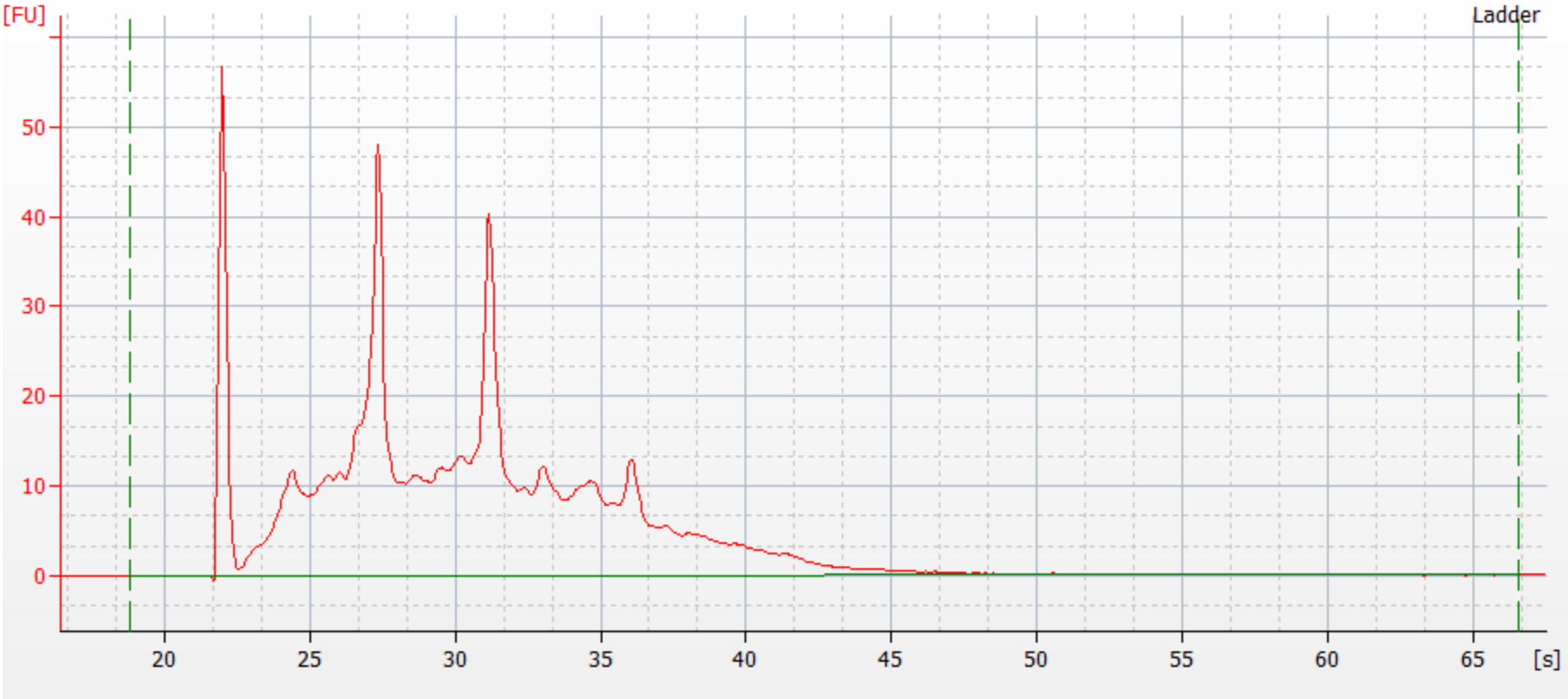
- Prepare a fresh gel-dye mix; protect dye and gel-dye mix from light
- Check kit (both chips and reagents) is within expiration
- Run full set of hardware diagnostic tests



Troubleshooting: degraded RNA Ladder

Improper handling, preparation, and/or storage

RNA Ladders are available separately



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Protein Chip Troubleshooting

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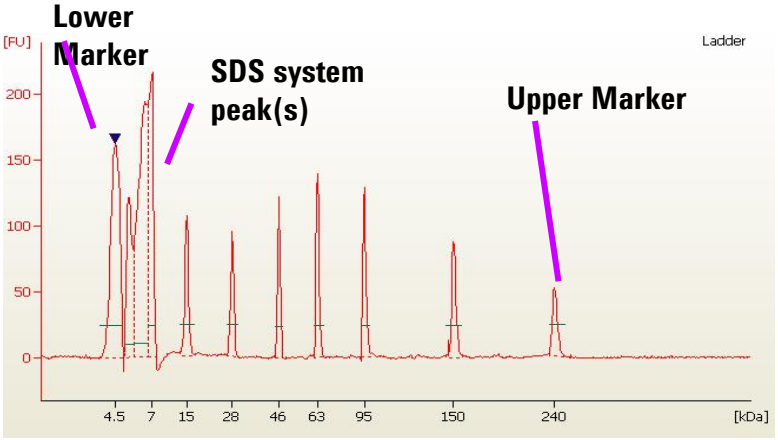
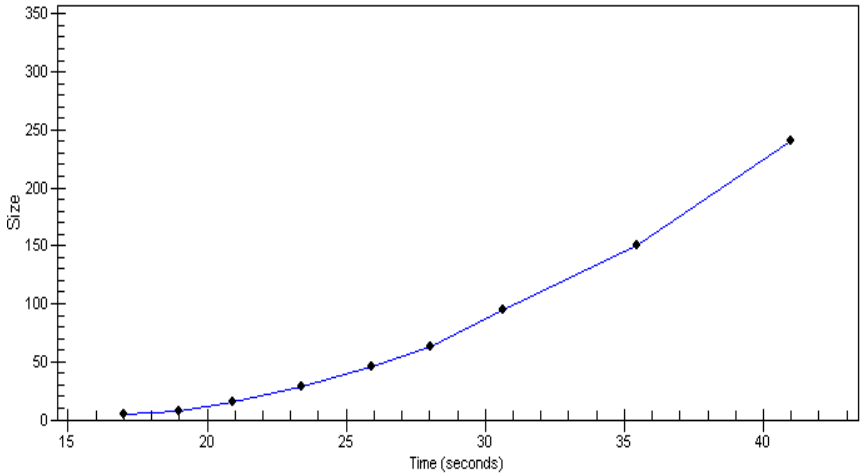
Data Analysis: Protein Ladder

Check if all Protein Ladder fragments have been identified correctly:

Protein 230 Ladder Electropherogram



Standard Curve (Chip Summary tab)

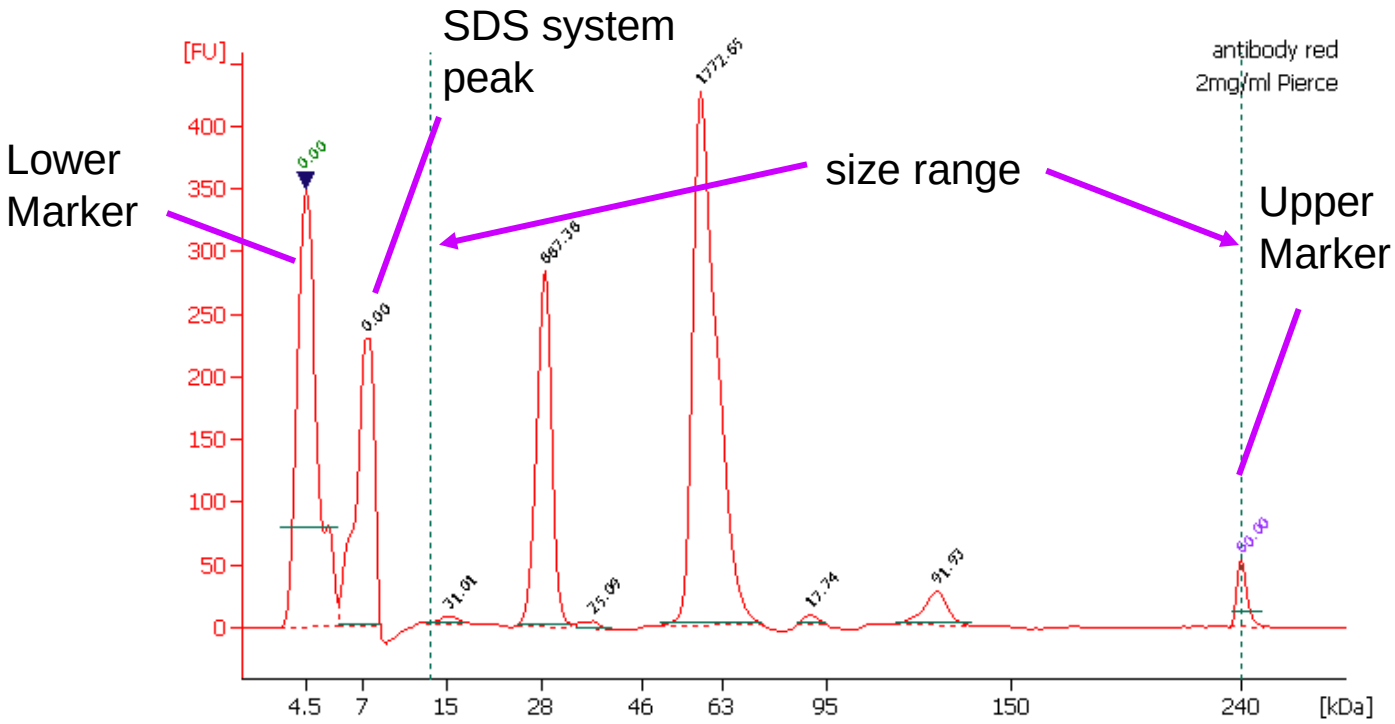


- **The standard curve is generated with a point to point fit using the migration times and sizes of the ladder peaks**

- **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



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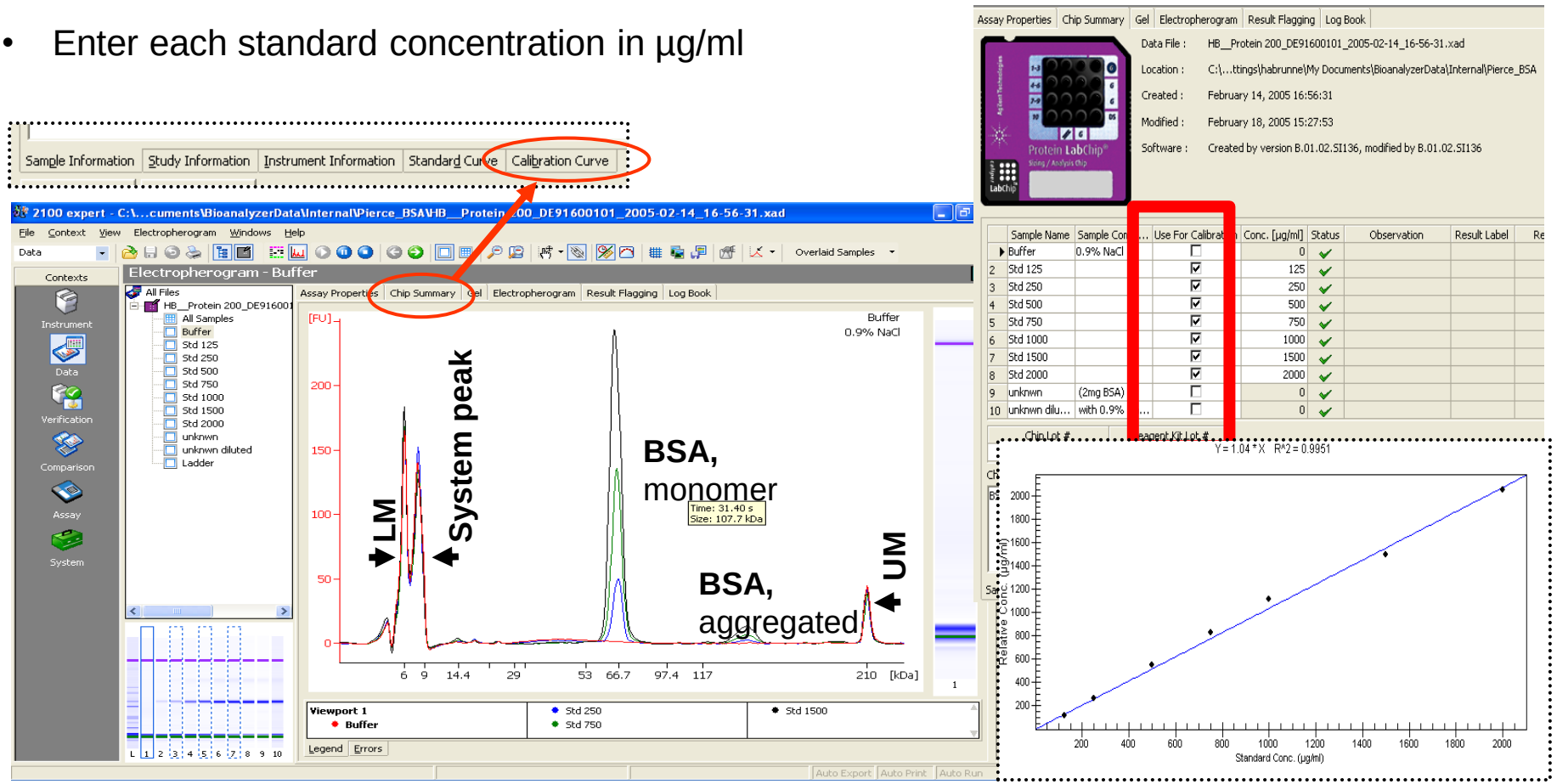
Absolute Quantification Using the Calibration Curve

- 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 µg/ml



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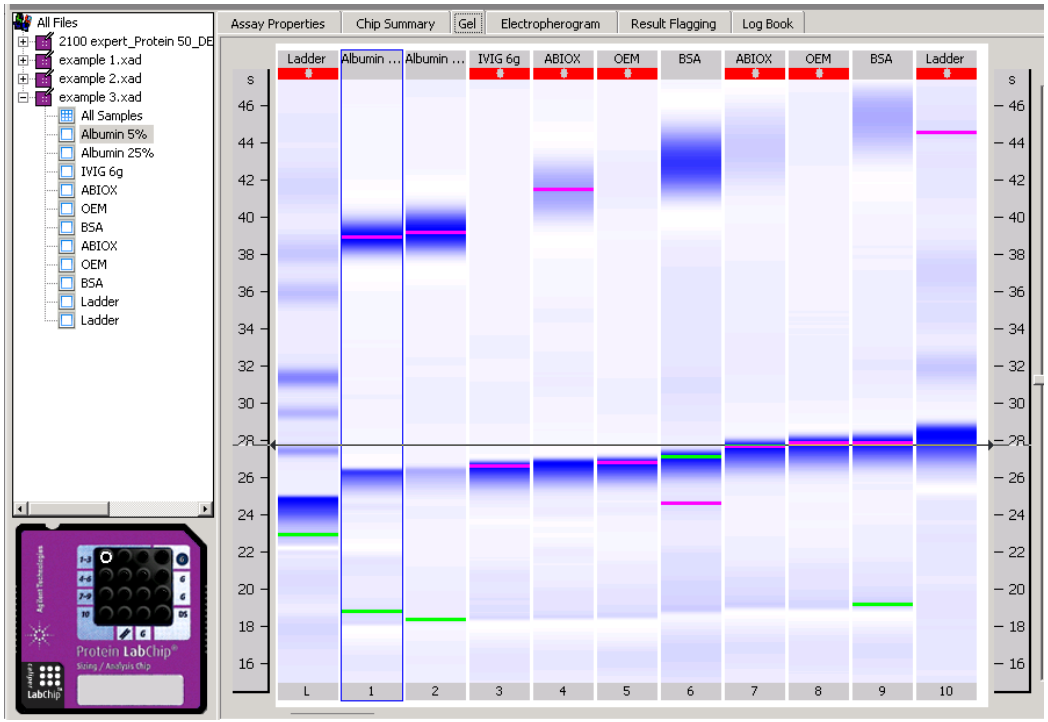
Troubleshooting: clogged priming station

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:

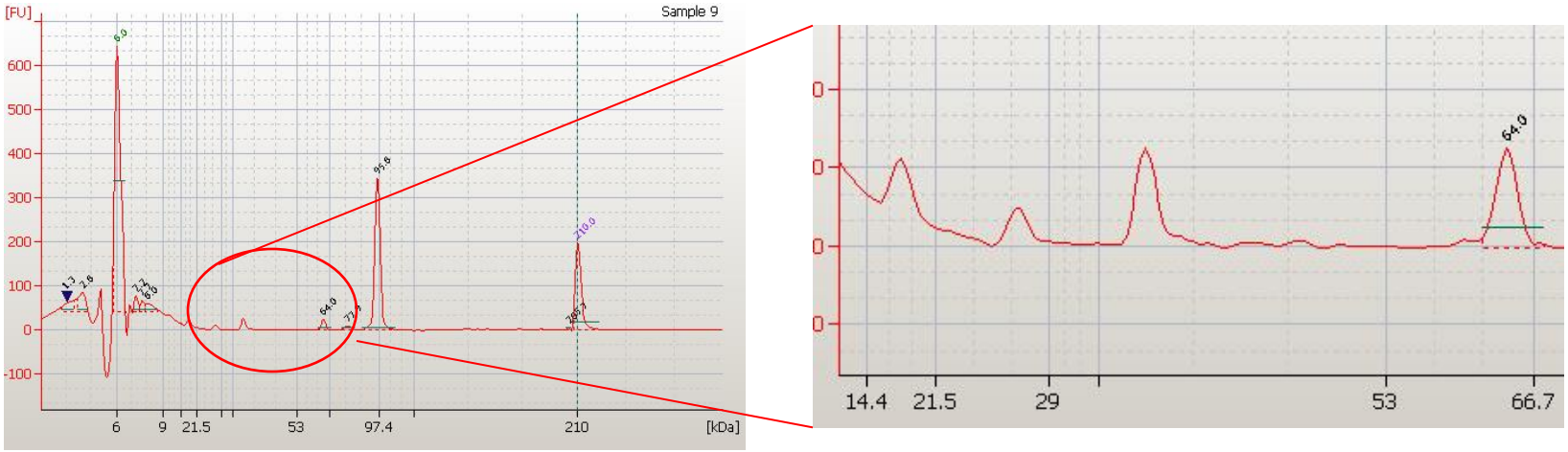
Quick view if residual gel is visible on the adapter/gasket;

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:



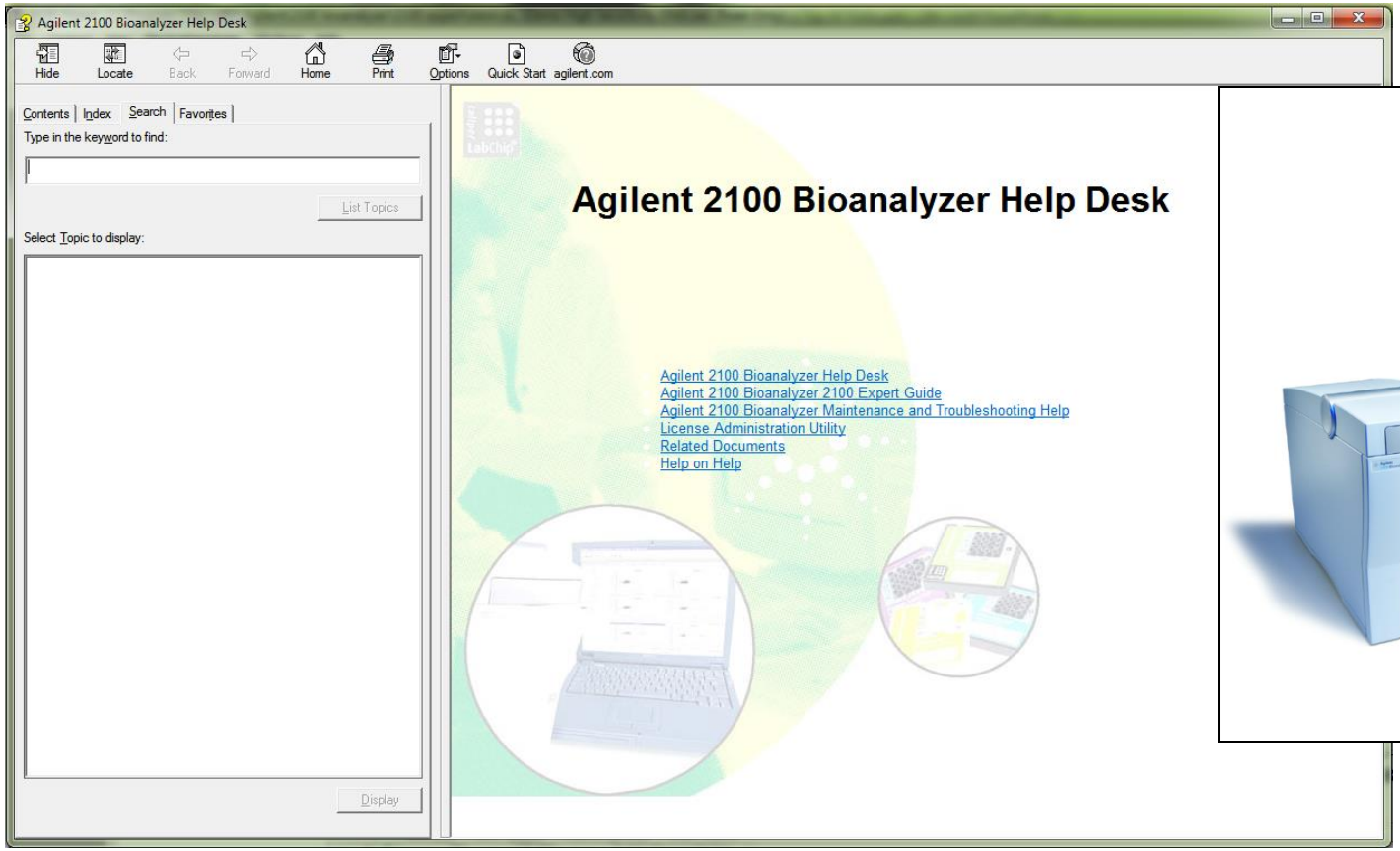
Solutions:

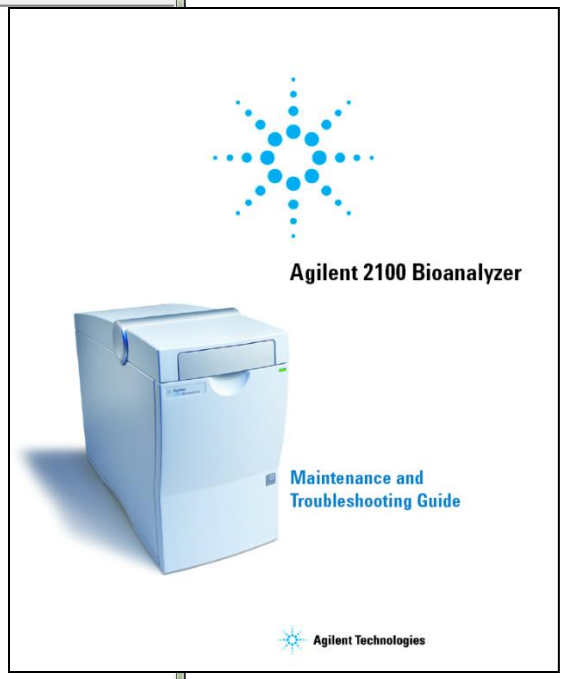
- Use Setpoint Explorer to modify integration limits e.g. height threshold

- 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





Need help?

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

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Need to get in contact with us?

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

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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\

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Questions?

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