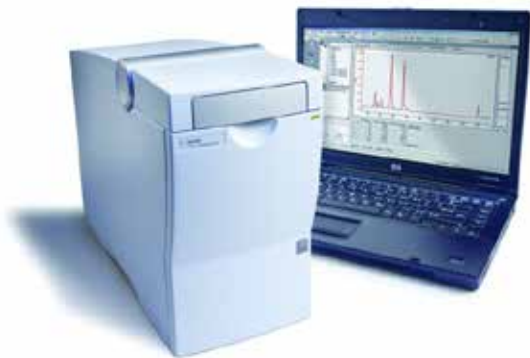


Bioanalyzer Tips & Tricks



Agilent Technologies

Bioanalyzer Tips & Tricks - Outline



System Maintenance



Chip Preparation



Hardware



Software



Assays



Troubleshooting assay runs



Help and Support



Additional Information



System maintenance



Cleaning of the Electrode Cartridge



The following quick and easy steps show how to maintain the Electrode Cartridge and assure proper functionality

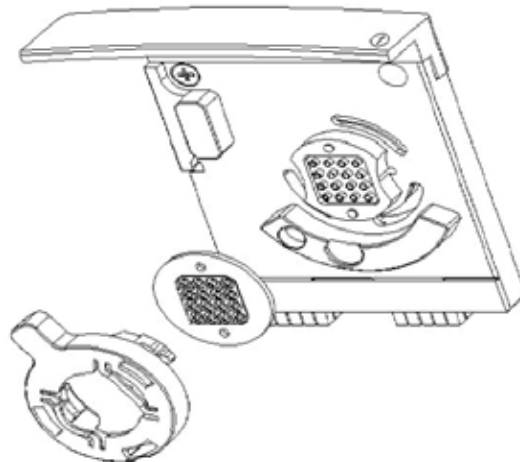
- Remove chip immediately after run is completed.
- Clean the electrode pinset between each run with a dedicated cleaning chip containing **350 µL** of RnaseZAP/water (refer to protocol).
- Empty the cleaning chip in between each run and replace with fresh liquid.
- Perform a thorough cleaning every 3 months or sooner if the electrode is suspected to be dirty.

*****Demonstrate next steps with actual cartridge*****

Cleaning of the Electrode Cartridge



- Turn off the Bioanalyzer instrument, remove the cartridge, remove the electrode pin set and soak for 15 minutes in deionized water.



- Sonicate the pinset in a clean beaker with deionized water and/or use a soft toothbrush.
- RNA assay users: Use half strength RNaseZap as this will be much easier to rinse when finished cleaning. Rinse very well after the cleaning – *residual RNaseZap will negatively impact RNA Pico, Small RNA, and High Sensitivity DNA results.*
- To verify that the pinset is dry, run the short circuit test from the Diagnostics tab of the 2100 Expert Software.

Chip Priming Station

There are specific settings established for each DNA, RNA, and Protein assay. Refer to manual for appropriate positions.

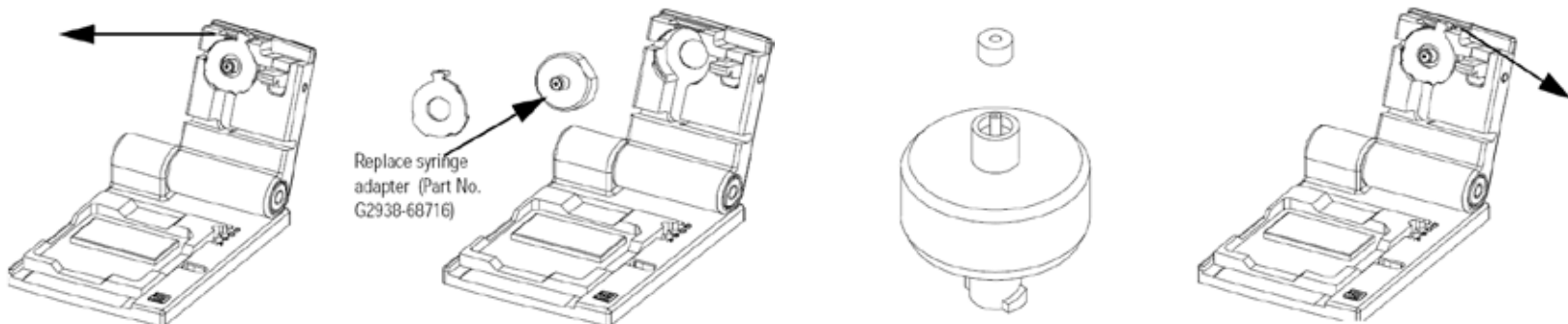


Priming station: steps to proper chip priming

Incomplete priming can cause a run to abort, late migration, and/or leak current issues.



1. Change the syringe with each new kit or after running 25. Sooner if priming station is suspected to be clogged or dirty.
2. Perform a regular maintenance of your priming station (every 3 months at latest). It is recommended to have a spare gasket kit available (p/n G2938-68716).
3. Inspect the white O-ring under the priming station's lid for any dried-out gel. Clean or replace if needed.
4. If the adapter is clogged (check with a backlight), priming will likely be incomplete. Replace the syringe adapter (the part onto which the syringe attaches). The syringe adapter is part of gasket kit (G2938-68716).



Chip preparation

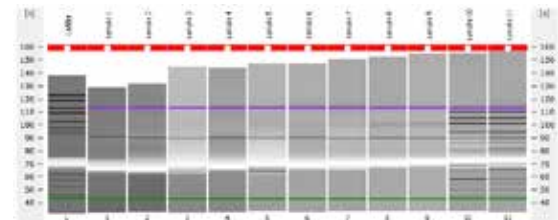
Key essentials of chip and sample preparation.



DNA assays – important points

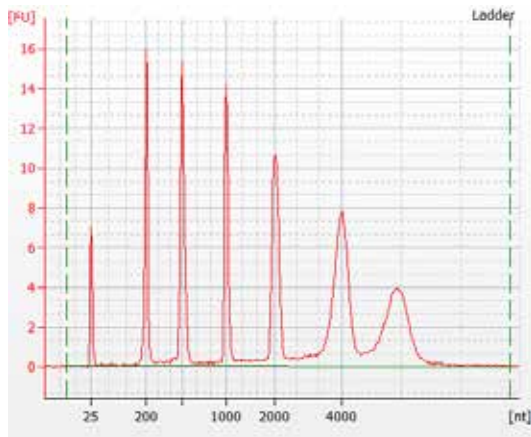


- Make sure the buffer composition matches the specifications of the assay (*applies to all assays*)
- Equilibrate the reagents at room temperature for 30 min (*applies to all assays*)
- Vortex the vials and spin down before use (*applies to all assays*).
- Extraction of the samples uses a wide number of chemicals which can affect the results on the Bioanalyzer. It is best practice to run the samples in TE buffer. **For HS DNA, do not run samples in water.**
- Standard DNA assay chips are interchangeable. For the high-sensitivity DNA assay, the HS DNA Chip is required.
- For HS DNA assay, do not use RNaseZap for cleaning the pinset in between runs. (Residual RNaseZap or SDS on the pins will result in white bands on the gel-like image)

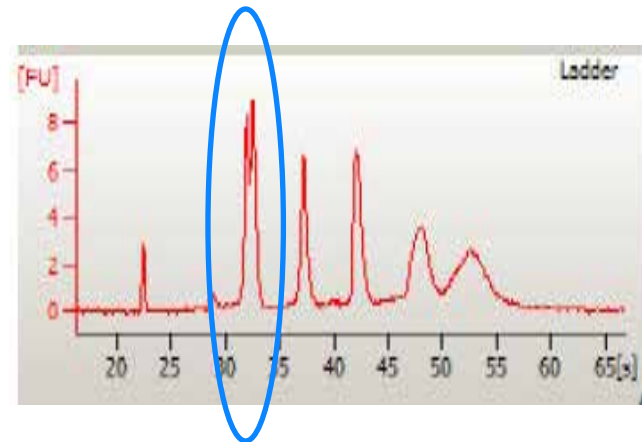


RNA assays - important points

- Wear gloves when handling RNA samples and reagents.
- Use RNase-free microfuge tubes, tips and water.
- Heat-denature RNA samples and Ladder at 70°C for 2 min and keep them on ice to reduce formation of secondary structure. This is especially important for the ladder as this is used for quantification.



Ladder Properly denatured

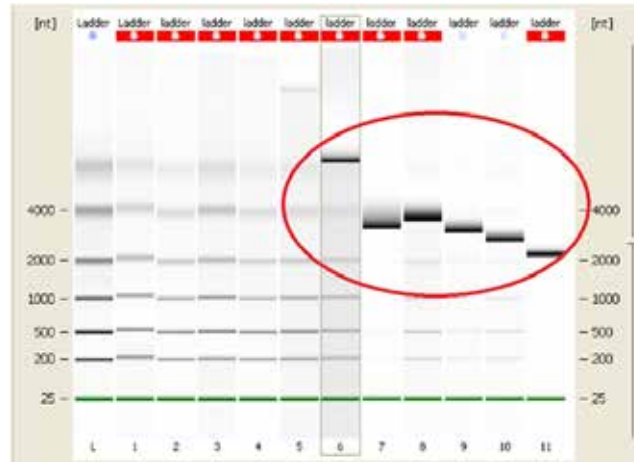


Ladder *not* properly denatured

RNA assays – important points



- For RNA Pico and Small RNA assays, do not use RNaseZap for cleaning in between runs. Residual RNaseZap will show up as an overwhelming strong peak in the electropherogram.



- Small RNA gel is very viscous, therefore when preparing the gel /dye mix for this assay, it is important that add the dye first and then the gel is added on top.
- Every assay (RNA Nano, RNA Pico, Small RNA) uses its own chip.

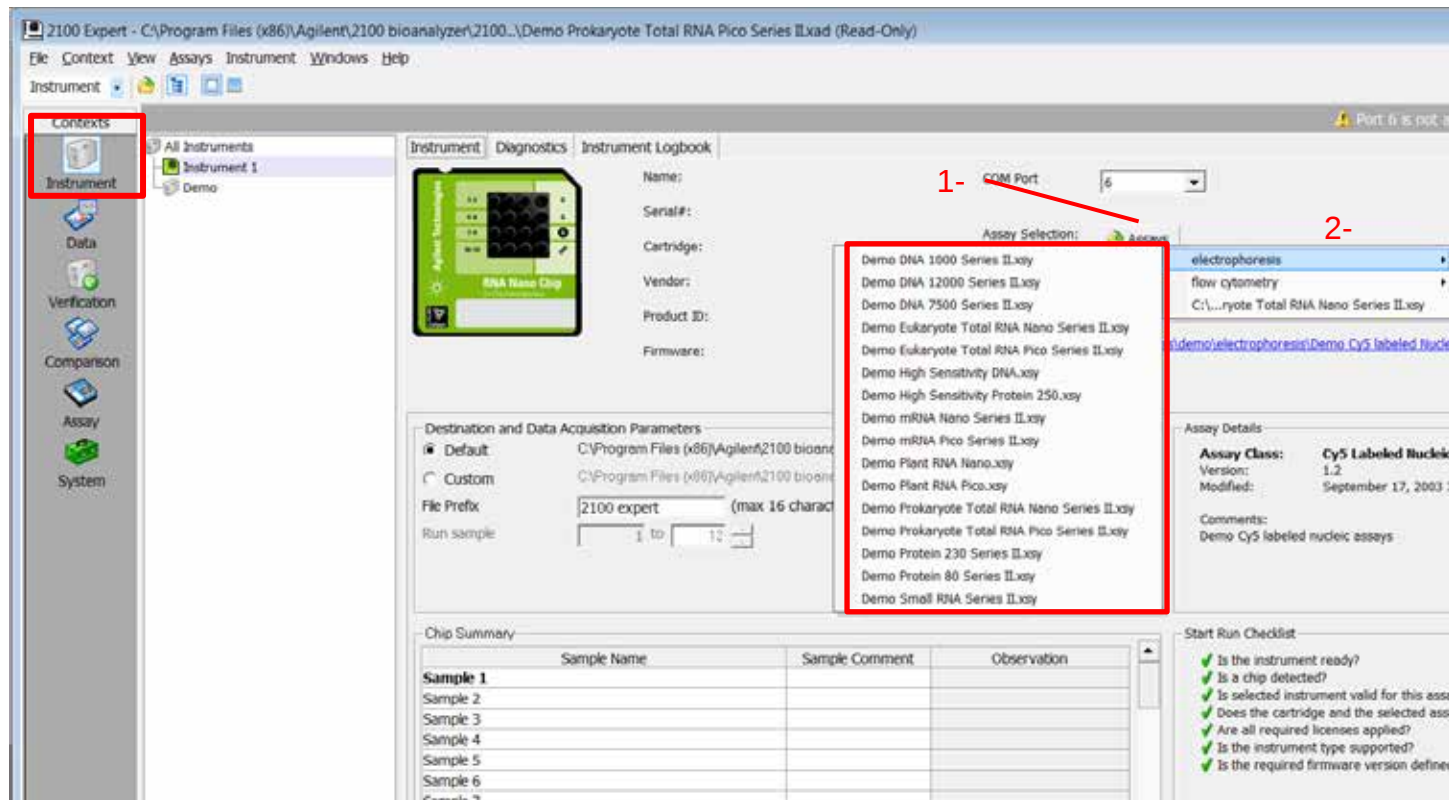
Protein assays - important points



- Do not vortex Protein Chips. Due to detergents, this will cause liquid spillage on the chip and leak currents.
- Use 0.5 μ L tubes for the denaturation. Using larger tubes may lead to poor results.
- Protein analysis under reducing conditions requires a 1 M DTT solution. If you want to analyze the proteins under non-reducing conditions, replace the amount of DTT required with MilliQ water.
- Protocol for standard Protein assays are different from High Sensitivity Protein assay. It is recommended that you check and follow the protocol prior to chip preparation.
- The High Sensitivity Protein gel matrix comes pre-filtered. It is ready to use after thawing.

Select the correct assay (Eukaryote, Prokaryote, mRNA, Plant) before running a chip.

The results cannot be converted to a different assay type.



Starting a chip run

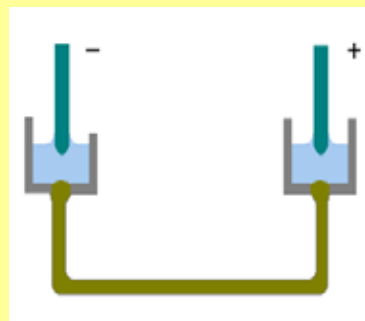
It is recommended to run the chip within 5 min after preparation



Troubleshooting: My chip does not start

At the beginning of each run there is a brief conductivity check to assure that all wells are completely filled and that there are no leak currents on the chip due to spilled liquid.

How it should look like !



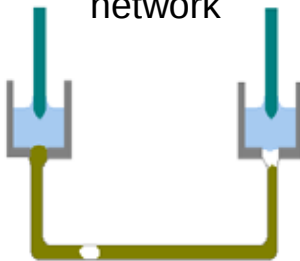
However, there can be multiple things happening to prevent this check from completing successfully:

Bubble at channel entrance or no connection



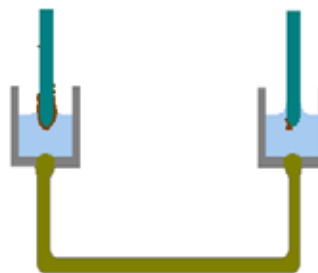
Pipetting sample

Bubble in channel network



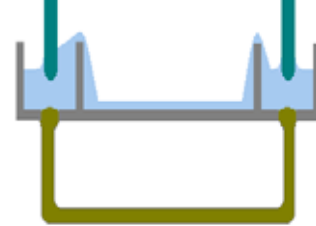
Pipetting gel; cold gel; priming

Dirty electrodes



No regular maintenance of electrode cartridge

Spillage across the chip



Vortexer; vortexer settings

Troubleshooting: Run aborted

Hint: Look at the Log Book of the actual data file.

2100 Expert - E:\basic training files\2100 expert_Protein 80_DE34903170_2009-10-29_11-52-42.xad

File Context View Log Book Windows Help

Data

Contexts

Files

Demo Eukaryote Total RNA Nano Series

Instrument

Data

Verification

Comparison

Assay

System

Assay Properties Chip Summary Gel Electropherogram Result Flagging **Log Book** Recorder

Run aborted on port 1

Instrument error occurred on port 1, Unusual high or low voltage or current was detected during the start phase of the on-chip analysis. Wells marked with (+) or (-) have been causing problems. The top left well equals sample 1 on the microfluidic chip:

{ } { } { } { }

{ } { } { } { }

(-) { } { } { } { }

Please check carefully all points below and perform corrective actions prior to any new chip preparation or analysis.

- Chip was primed according to instructions.
- All gel-wells are filled with appropriate volume.
- Sample and Ladder wells are filled with appropriate volume.
- No liquid spills occurred during chip handling or vortexing.
- Electrode pins are clean and not bent.
- Instrument lid is closed firmly.

Please refer to the Kit guide and the Help section of the 2100 Expert for assistance on chip preparation and troubleshooting. Consider maintenance operations like "Cleaning the pin set" succeeded by the "Short circuit test".

Run status: bioana 80_DE34903170_2009-10-29_11-52-42.xad

Product Name : Vendor : Serial : Firmware : Cartridge :

Number	Source	Category	Sub Category	Oct
1550	Instrument	Run		oct

If the error occurs on one or several channels:

- **Check volumes of respective wells**
 - Visually or by pipetting the liquid out
 - add further liquid (gel/marker), but attention! quantitation will be wrong
- **Check if vortexing spills liquid out, reduce speed or**
- **Check if electrode pins are ok, bend, broken or coated.**
- **Press on the lid of the BioA to get the pins in the liquid.**
- **If available - consider a different cartridge**

Hardware

The 2100 Bioanalyzer
instrument – Tips & Tricks for
best performance



Hardware

If the instrument hardware has a problem it might go into a not ready state. There are a few steps to try:

- Close the software, turn off the 2100 Bioanalyzer instrument and turn it back on, wait for the green LED and restart the software.



Status Indicator

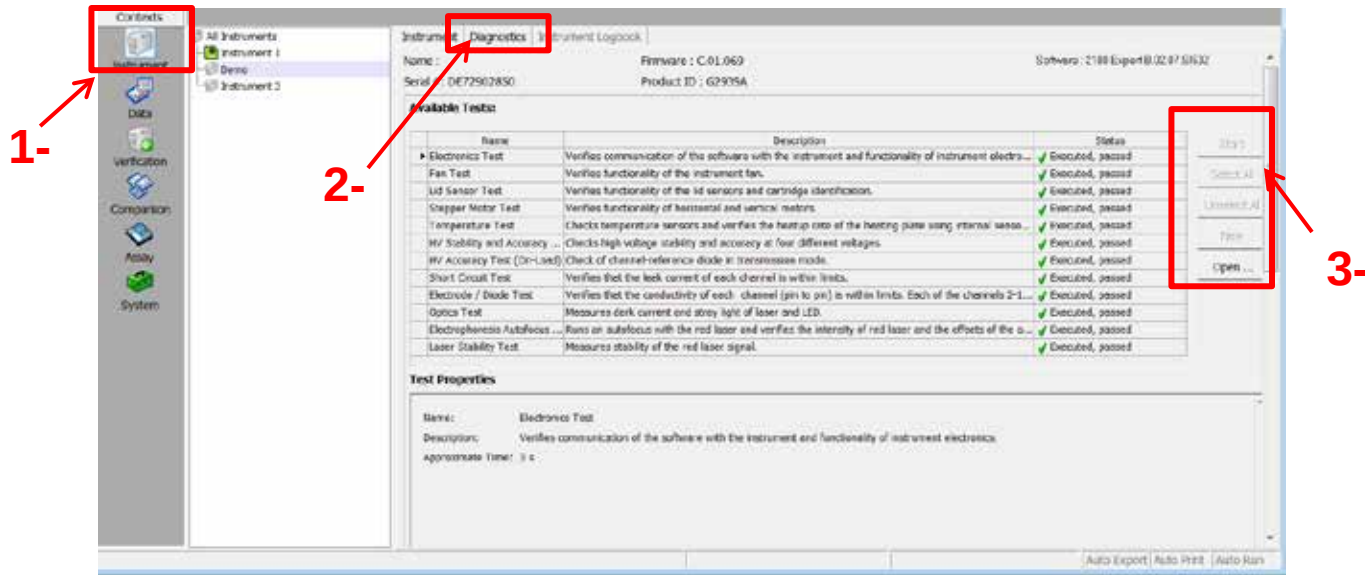
- Green—Ready
- Green, flashing—Busy
- Orange—Self-test
- Red—Error

- If status indicator remains red, please contact Agilent Tech Support.
- If status indicator is green, but there is suspicion whether it is not functioning as expected, you may run Diagnostic tests.

Hardware Diagnostic tests

A set of self Hardware Diagnostic Tests can be found within the Software (Instrument context > Diagnostics)

- Performing the hardware diagnostics requires dedicated test chips for electrophoresis (G2938-68300) and flow cytometry (G2938-68200) tests.
- The chips come with an expiration date and should not be used after that. Using expired test chips may lead to a false positive or false negative test result.



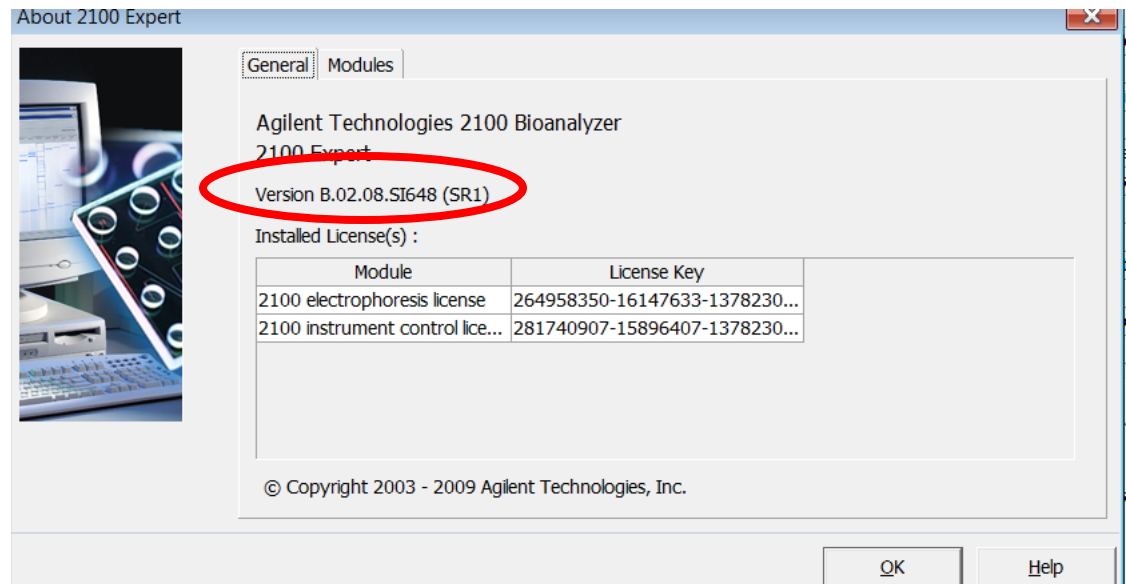
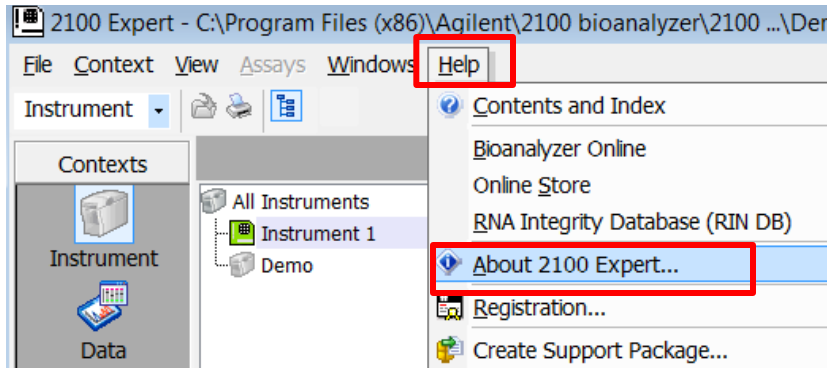
PC and software



Few important points to consider...

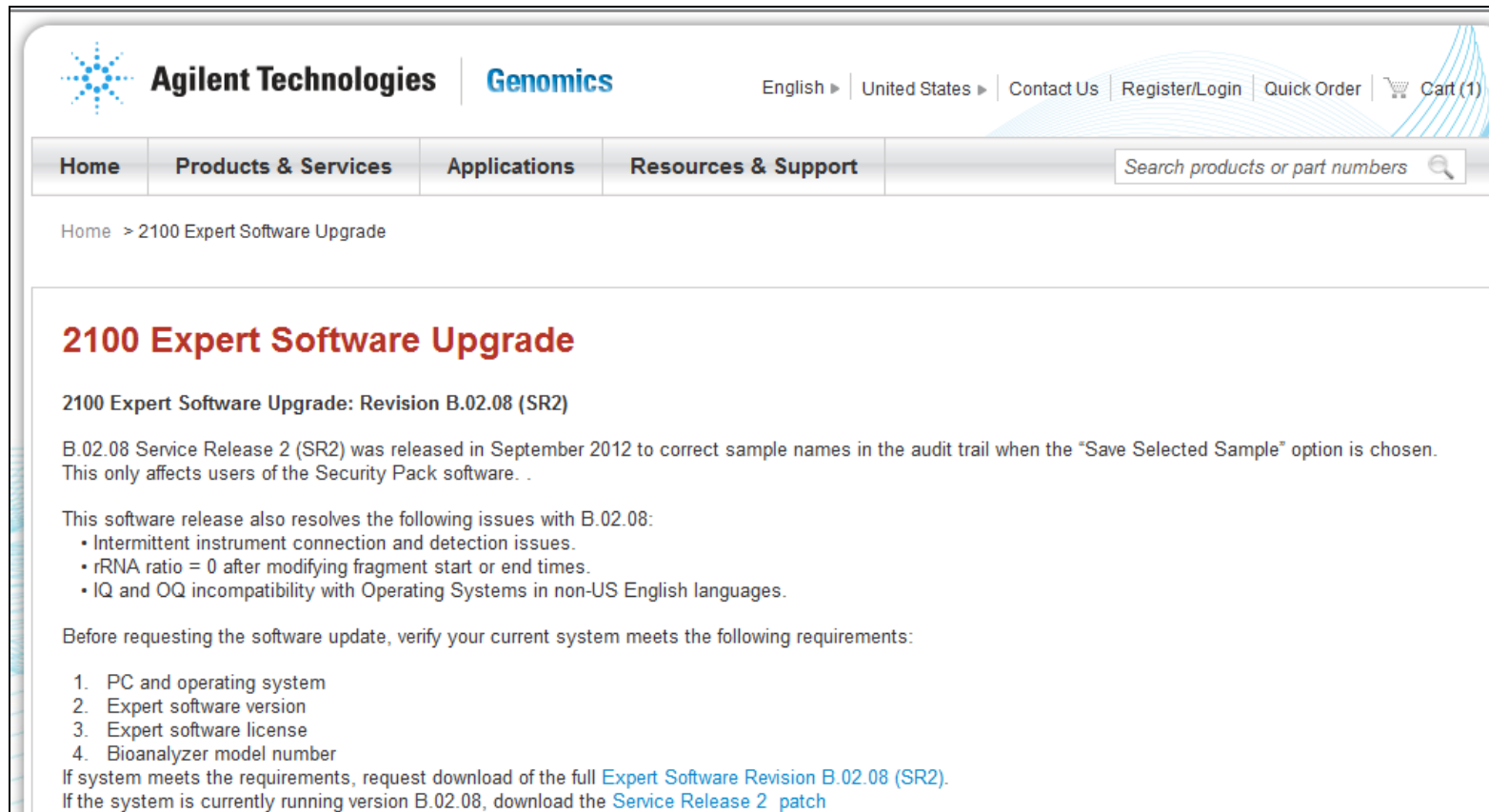
- The latest revision of the 2100 Expert software requires 1024 MB RAM. Especially, if you have other applications in use, you may drain off too much RAM to operate reliably. Restart the PC from time to time to free up enough RAM to restore functionality.
- If the free HD space is below 20 GB, the 2100 Expert software can run very slowly. It is recommended to archive old data and defragment your PC to free up disk space.
- If the PC is networked, regularly use a utility like CleanSweep, CleanUp or the Windows accessory to get rid of temp files, cookies etc.
- Keep the software updated with the current Software version available

Where to look for the Software version on your PC??



2100 Expert Software Upgrade

<http://www.genomics.agilent.com/article.jsp?pageId=2354>



The screenshot shows the Agilent Technologies Genomics website. The header includes the Agilent logo, the word "Genomics", and navigation links for English, United States, Contact Us, Register/Login, Quick Order, and a shopping cart with 1 item. A main navigation bar contains links for Home, Products & Services, Applications, and Resources & Support, along with a search bar. The breadcrumb trail reads "Home > 2100 Expert Software Upgrade". The article title "2100 Expert Software Upgrade" is displayed in red. The subheading is "2100 Expert Software Upgrade: Revision B.02.08 (SR2)". The text states that B.02.08 Service Release 2 (SR2) was released in September 2012 to correct sample names in the audit trail when the "Save Selected Sample" option is chosen. It also lists issues resolved by this release: intermittent instrument connection and detection issues, rRNA ratio = 0 after modifying fragment start or end times, and IQ and OQ incompatibility with Operating Systems in non-US English languages. A section titled "Before requesting the software update, verify your current system meets the following requirements:" lists four items: PC and operating system, Expert software version, Expert software license, and Bioanalyzer model number. It concludes with instructions to download the full Expert Software Revision B.02.08 (SR2) if the system meets the requirements, or the Service Release 2 patch if the system is currently running version B.02.08.

2100 Expert Software Upgrade

2100 Expert Software Upgrade: Revision B.02.08 (SR2)

B.02.08 Service Release 2 (SR2) was released in September 2012 to correct sample names in the audit trail when the "Save Selected Sample" option is chosen. This only affects users of the Security Pack software. .

This software release also resolves the following issues with B.02.08:

- Intermittent instrument connection and detection issues.
- rRNA ratio = 0 after modifying fragment start or end times.
- IQ and OQ incompatibility with Operating Systems in non-US English languages.

Before requesting the software update, verify your current system meets the following requirements:

1. PC and operating system
2. Expert software version
3. Expert software license
4. Bioanalyzer model number

If system meets the requirements, request download of the full [Expert Software Revision B.02.08 \(SR2\)](#).
If the system is currently running version B.02.08, download the [Service Release 2 patch](#)

Make sure the PC meets the specifications required and listed on the Agilent webpage

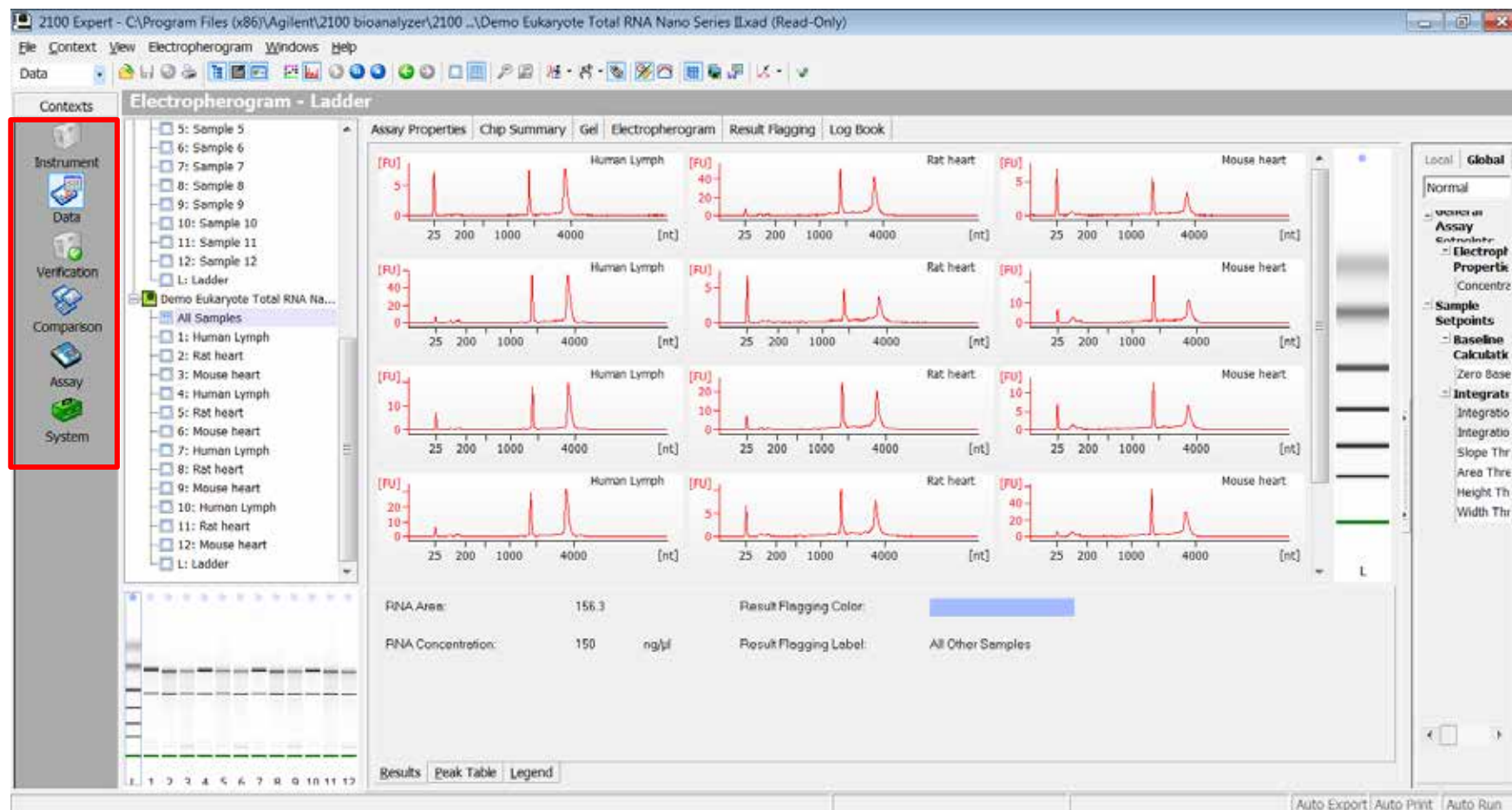
Software

The 2100 Expert software offers great functionality.



Software overview

Icons and tabs available to make software user-friendly

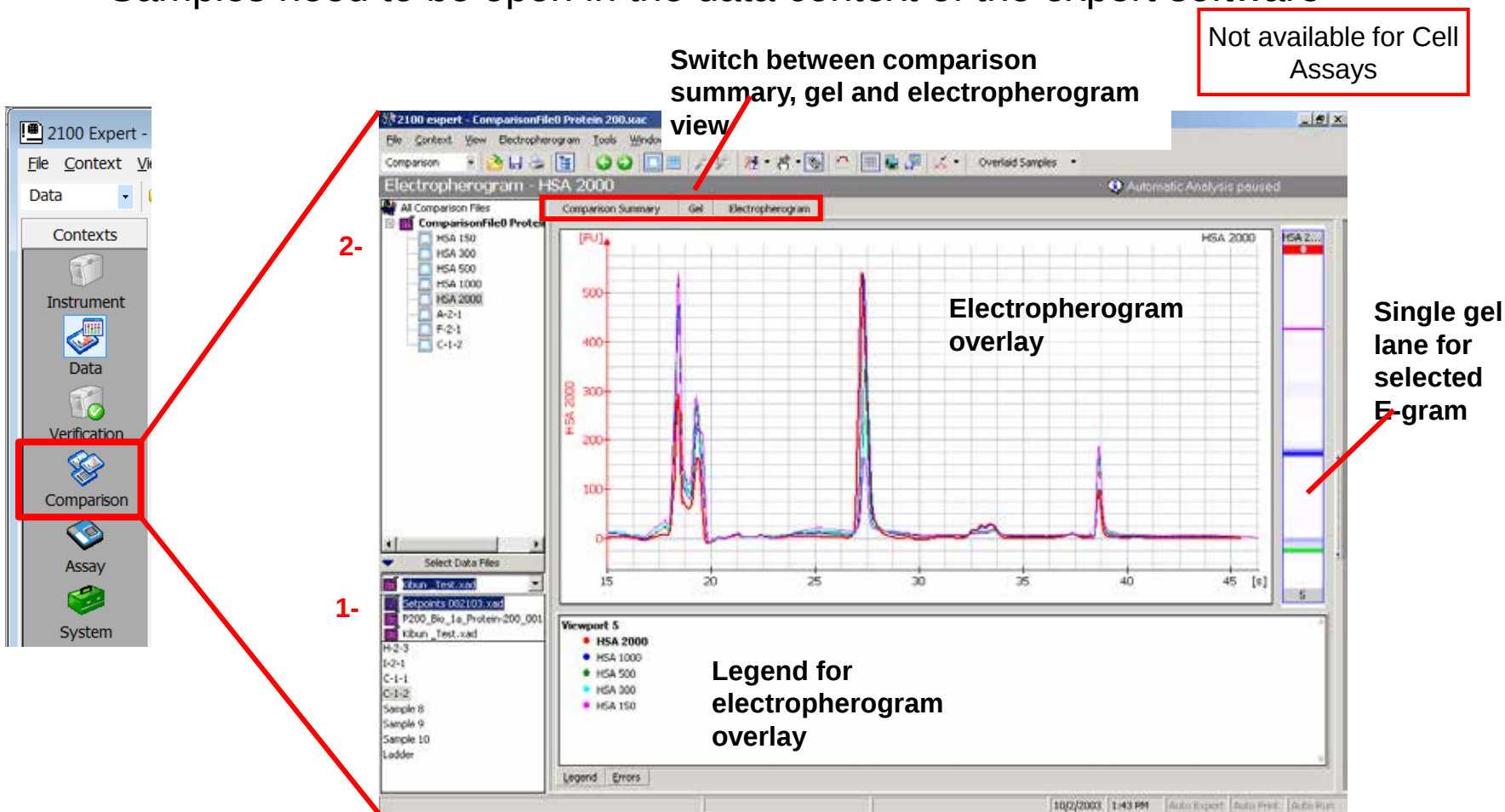


2100 Expert Software

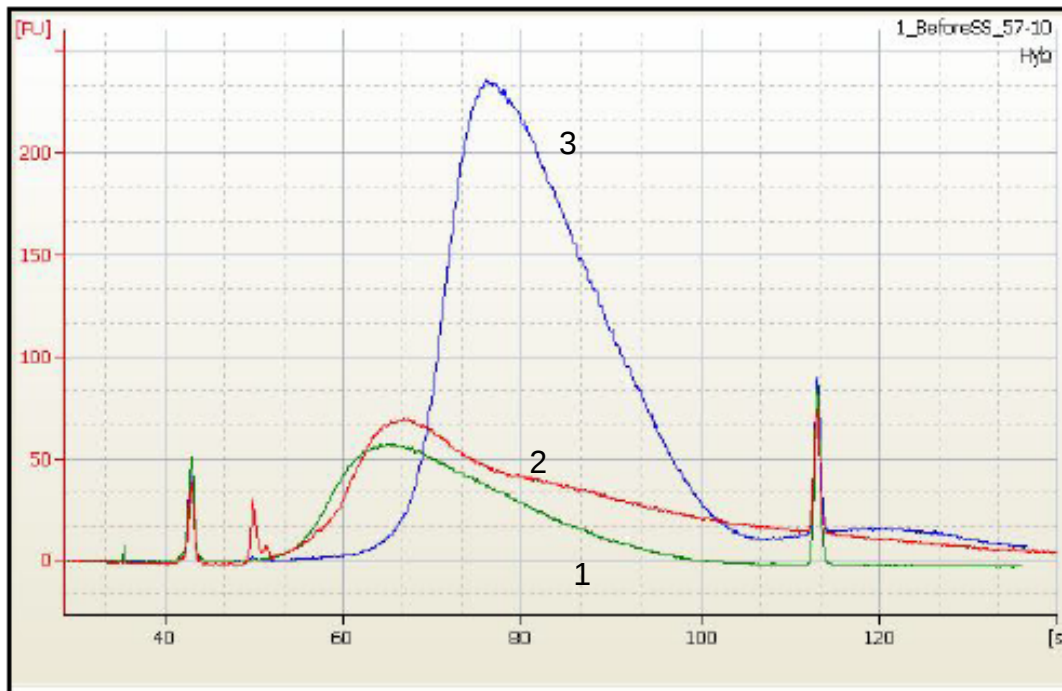


Comparison context

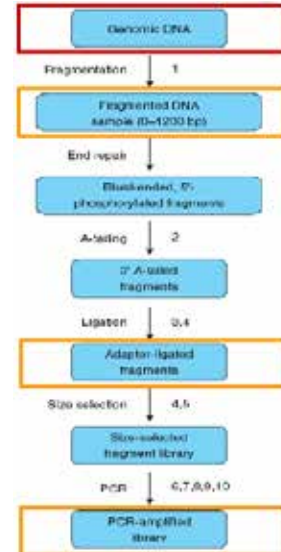
- Easy comparison of multiples chip run from the same assay class
- Samples need to be open in the data context of the expert software



Library prep: Using the comparison context to assess successful library preparation



- Sheared
- Adaptor-ligated
- PCR-amplified



Save Selected Samples

1

2

The screenshot shows the Agilent 2100 Expert software interface. The 'File' menu is open, and 'Save Selected Sample...' is highlighted. The main window displays a DNA ladder electropherogram with peaks labeled by size (bp) and concentration (pg/μl). A table at the bottom lists the ladder data.

Size [bp]	Conc. [pg/μl]	Molarity [pmol/l]	Observations
35	125.00	5,411.3	Lower Marker
50	150.00	4,545.5	Ladder Peak
100	150.00	2,272.7	Ladder Peak
150	150.00	1,515.2	Ladder Peak
200	150.00	1,136.4	Ladder Peak
300	150.00	757.6	Ladder Peak
400	150.00	568.2	Ladder Peak



Save Selected Samples

The screenshot shows the '2100 Expert' software interface. A 'Save Selected Samples' dialog box is open, displaying a list of 12 samples. The 'Ladder' sample is selected, indicated by a checkmark in the 'Selected' column. Red arrows point to the 'Apply' button and the 'Ladder' sample.

ID	Sample Name	Comment	Category	Selected
1	GAII library 1:20		Sample	☐
2	DNA ladder (1:200)		Sample	☐
3	FLX library 1700 pg/ul (1:10)		Sample	☐
4	DNA library (1:30)		Sample	☐
5	DNA ladder II (1:200)		Sample	☐
6	DNA library (1:30)		Sample	☐
7	GAII library (1:800)		Sample	☐
8	ChIPseq library (1:2)		Sample	☐
9	5 pg/ul ladder		Sample	☐
10	GAII library (1:800)		Sample	☐
11	Fragmented DNA		Sample	☐
12	Ladder		Ladder	☒

3-Select the samples you want to save

4-Click Apply and save



2100 Expert Software

Setpoint explorer – allows to change default **settings** in order to modify the data evaluation for sample analysis:

- Change setpoints locally (selected electropherogram) or globally (all electropherograms)
- Accessible parameters

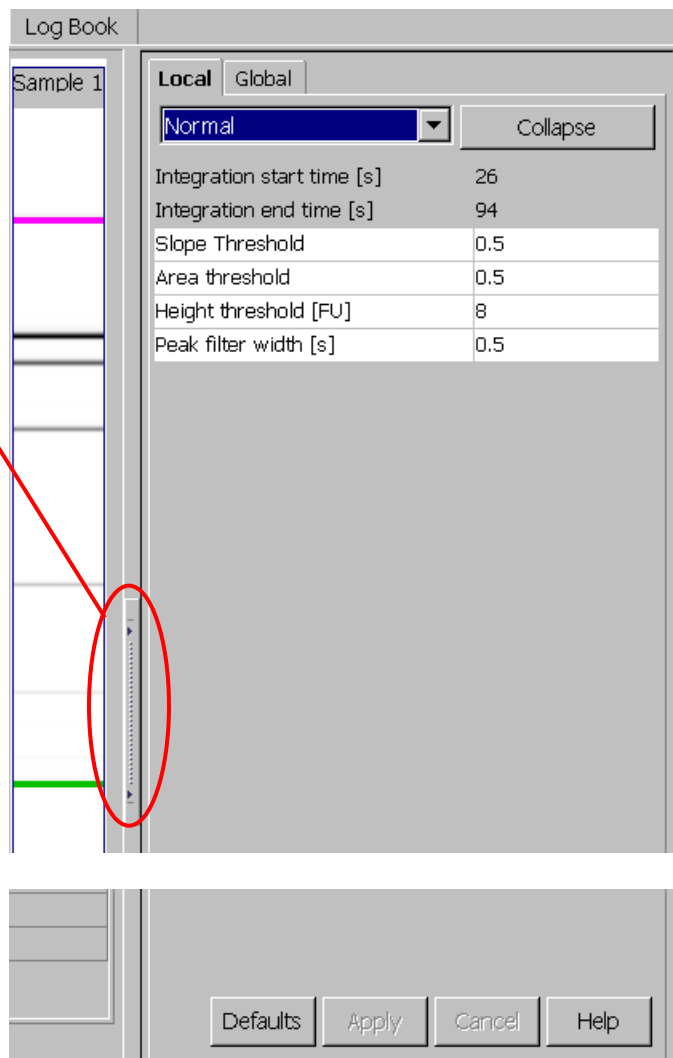
Normal mode

Slope threshold
Area threshold
Height threshold
Peak filter width

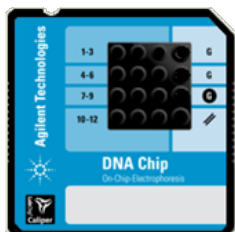
Advanced mode

Smear Analysis (RNA)
Calibrate all (Protein)

Open and collapse setpoint explorer



When setpoint explorer feature is needed?



Analyze the run data and need to modify default parameters

Ø Peak height for individual samples

Ø Enabling smear analysis



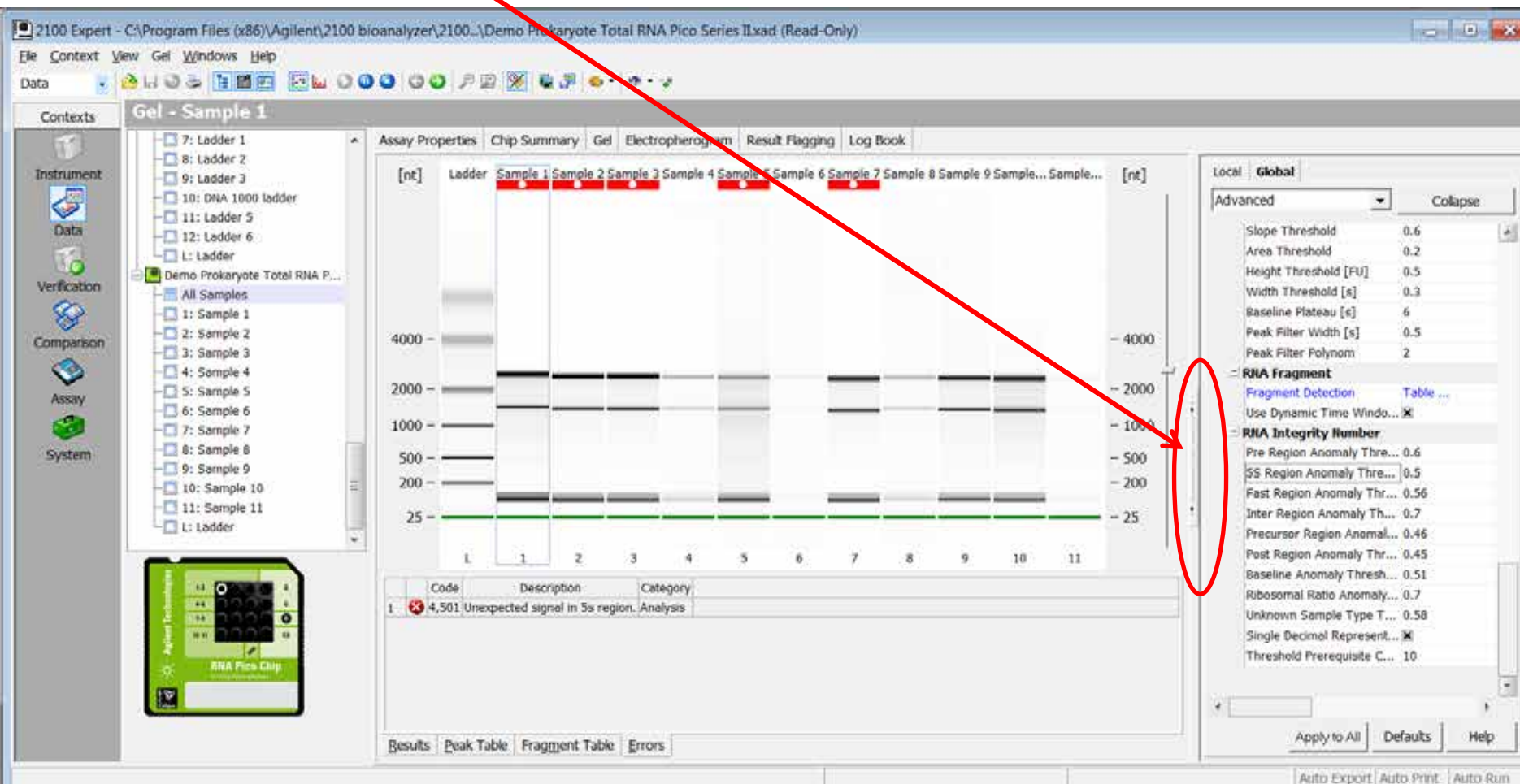
Ø Align to upper and/or lower marker

Ø Adding/deleting ribosomal fragments (for RNA assays only)



2100 Expert Software

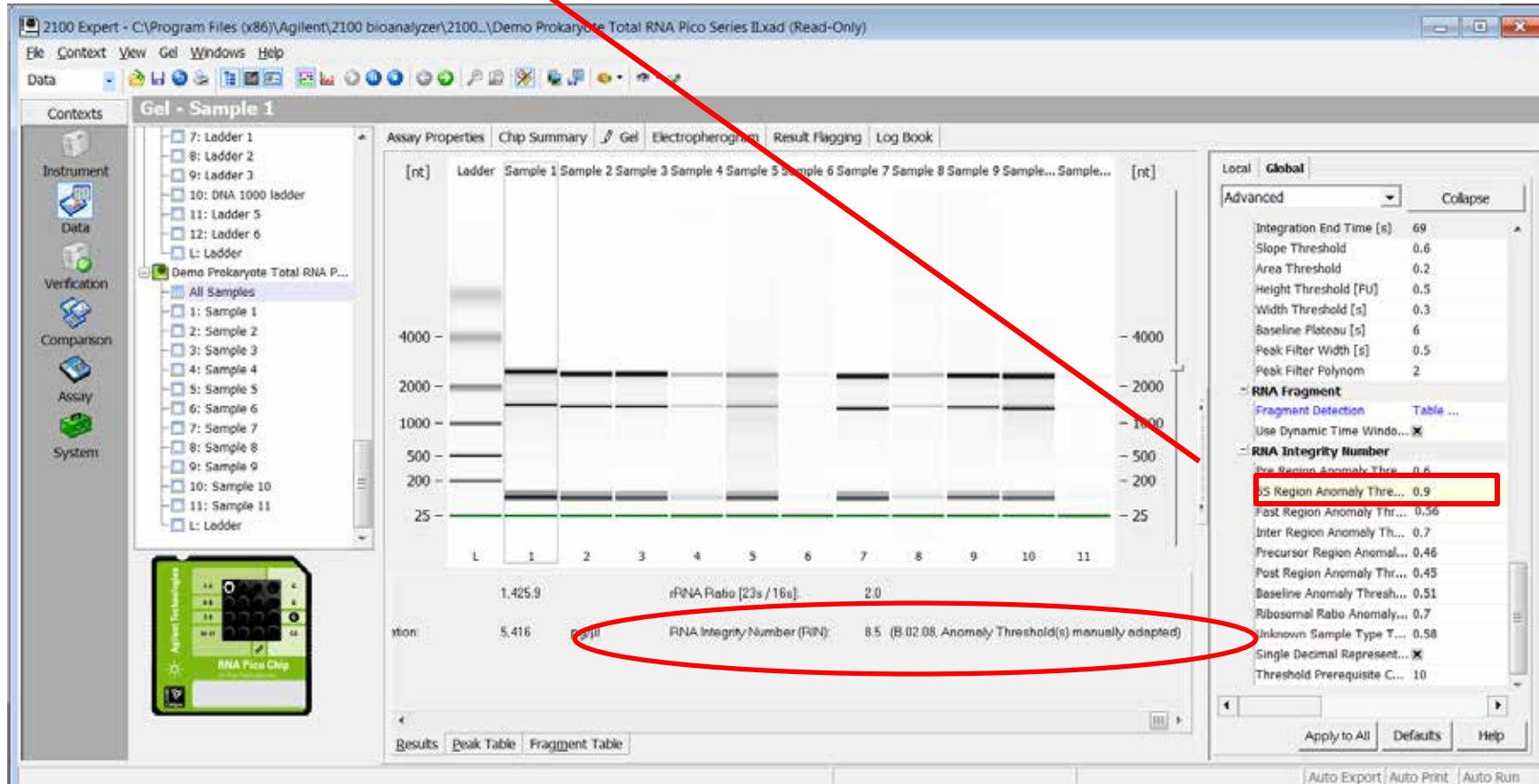
Setpoint explorer-



2100 Expert Software

Setpoint explorer-

Changes in the setting will be recorded in the results



Questions?

