

Bioanalyzer Tips & Tricks



Agilent Technologies

Bioanalyzer Tips & Tricks - Outline



System Maintenance



Chip Preparation



Hardware



Software



Assays



Troubleshooting assay runs



Help and Support

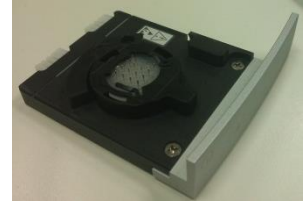


Additional Information

System maintenance



Cleaning of the Electrode Cartridge

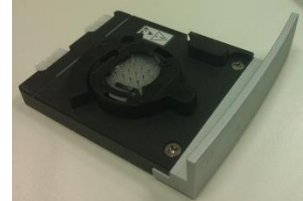


The following quick and easy steps show how to maintain the Electrode Cartridge and assure proper functionality

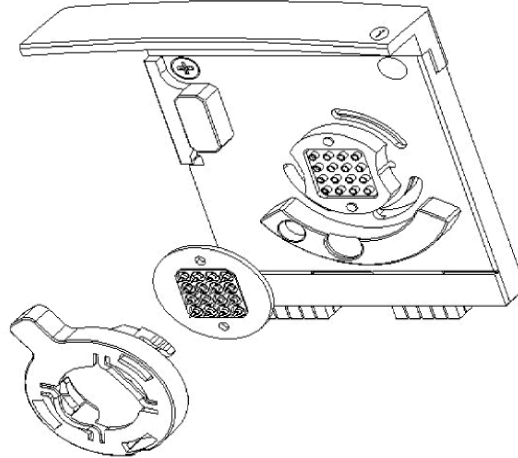
- Remove chip immediately after run is completed.
- Clean the electrode pinset between each run with a dedicated cleaning chip containing **350 μL** of RnaseZAP/water (refer to protocol).
- Empty the cleaning chip in between each run and replace with fresh liquid.
- Perform a thorough cleaning every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

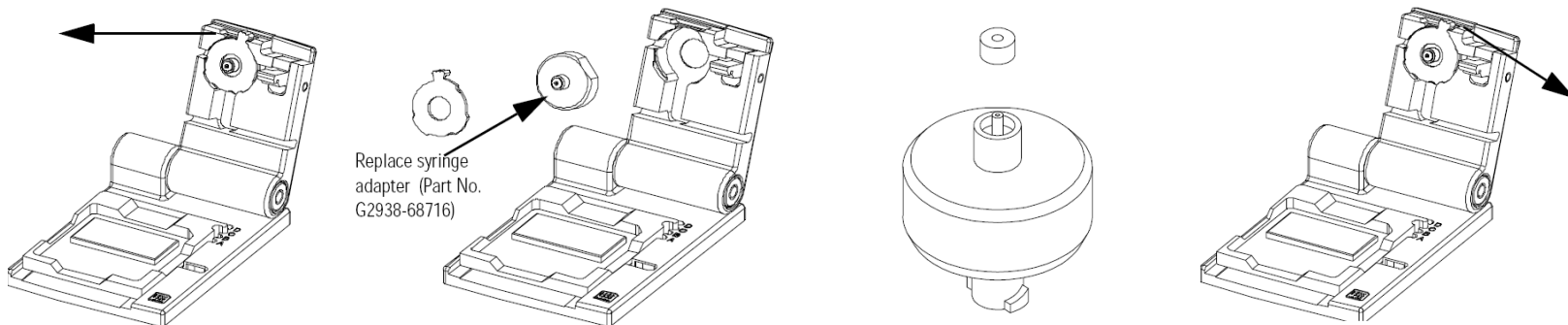


Priming station: steps to proper chip priming



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

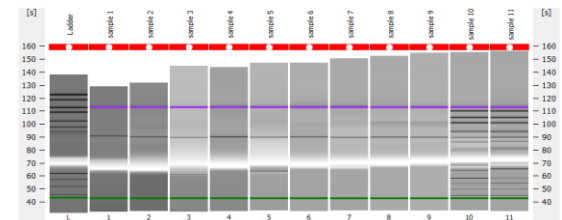
Key essentials of chip and sample preparation.



DNA assays – important points

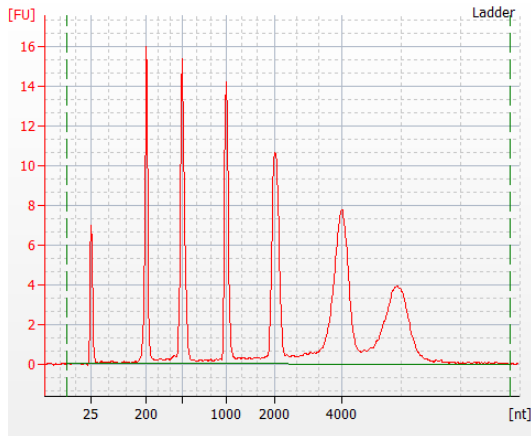


- Make sure the buffer composition matches the specifications of the assay (*applies to all assays*)
- Equilibrate the reagents at room temperature for 30 min (*applies to all assays*)
- Vortex the vials and spin down before use (*applies to all assays*).
- Extraction of the samples uses a wide number 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 assays 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)

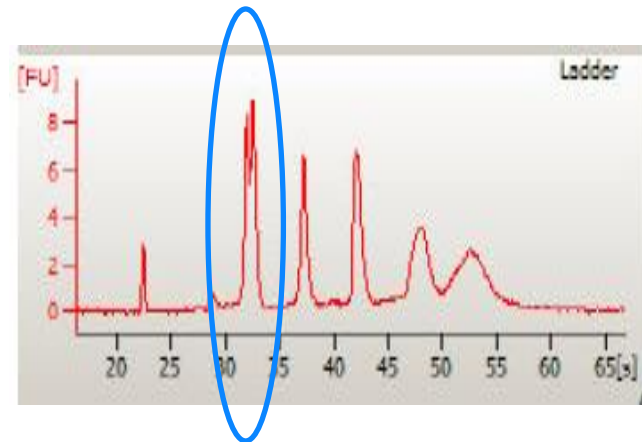


RNA assays - important points

- 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

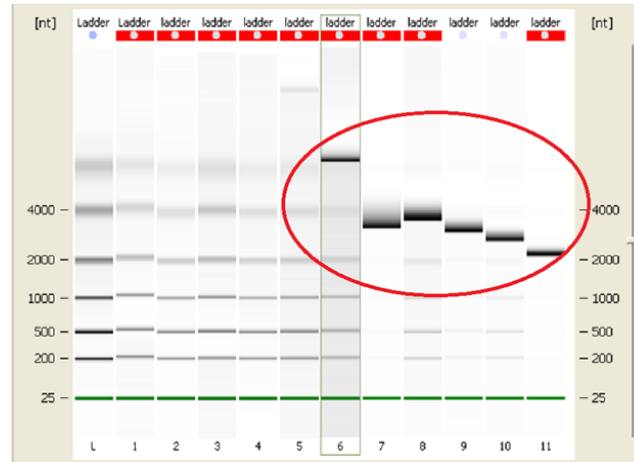


Ladder *NOT* properly denatured

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.

The screenshot shows the Agilent 2100 Expert software interface. The 'Contexts' sidebar on the left has 'Instrument' highlighted. The main window displays the 'Instrument' tab for an 'RNA Nano Chip'. A dropdown menu for 'Assay Selection' is open, showing a list of assays. A red box highlights the list of assays, and a red arrow points to the 'COM Port' dropdown menu. The 'Assay Selection' dropdown is currently set to 'electrophoresis'. The 'Assay Details' section on the right shows 'Assay Class: Cy5 Labeled Nucleic' and 'Version: 1.2'. The 'Start Run Checklist' on the bottom right shows several green checkmarks indicating that the instrument is ready for use.

1- COM Port

2-

Assay Selection:

- Demo DNA 1000 Series II.xsy
- Demo DNA 12000 Series II.xsy
- Demo DNA 7500 Series II.xsy
- Demo Eukaryote Total RNA Nano Series II.xsy
- Demo Eukaryote Total RNA Pico Series II.xsy
- Demo High Sensitivity DNA.xsy
- Demo High Sensitivity Protein 250.xsy
- Demo mRNA Nano Series II.xsy
- Demo mRNA Pico Series II.xsy
- Demo Plant RNA Nano.xsy
- Demo Plant RNA Pico.xsy
- Demo Prokaryote Total RNA Nano Series II.xsy
- Demo Prokaryote Total RNA Pico Series II.xsy
- Demo Protein 230 Series II.xsy
- Demo Protein 80 Series II.xsy
- Demo Small RNA Series II.xsy

electrophoresis

flow cytometry

C:\...ryote Total RNA Nano Series II.xsy

Assay Details

Assay Class: **Cy5 Labeled Nucleic**

Version: 1.2

Modified: September 17, 2003 3

Comments:

Demo Cy5 labeled nucleic assays

Start Run Checklist

- ✓ Is the instrument ready?
- ✓ Is a chip detected?
- ✓ Is selected instrument valid for this assay?
- ✓ Does the cartridge and the selected assay match?
- ✓ Are all required licenses applied?
- ✓ Is the instrument type supported?
- ✓ Is the required firmware version defined?

Sample Name	Sample Comment	Observation
Sample 1		
Sample 2		
Sample 3		
Sample 4		
Sample 5		
Sample 6		
Sample 7		

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.



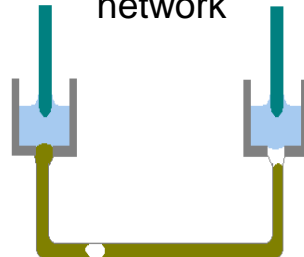
However, there can be multiple things happening to prevent this check from completing successfully:

Bubble at channel entrance or no connection



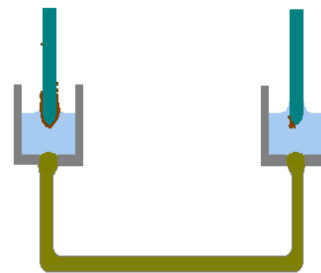
Pipetting sample

Bubble in channel network



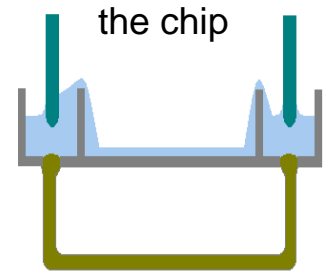
Pipetting gel; cold gel; priming

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.

The screenshot shows the 2100 Expert software interface. The title bar reads "2100 Expert - E:\basic training files\2100 expert_Protein 80_DE34903170_2009-10-29_11-52-42.xad". The menu bar includes File, Context, View, Log Book, Windows, and Help. The toolbar contains icons for Data, File operations, and Analysis. The left sidebar shows a tree view of contexts: Demo Eukaryote Total RNA Nano Series, Instrument, Data, Verification, Comparison, Assay, and System. The main window displays the Log Book tab, showing a table with columns: Description, Number, Source, Category, Sub Category, and Date. The first row is highlighted in blue and contains the following text:

Description	Number	Source	Category	Sub Category	Date
Run aborted on port 1	1550	Instrument	Run		oct

The description for this entry reads: "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:"

```
{ } { } { } { }
{ } { } { } { }
{ } { } { } { }
(-) { } { } { }
```

Below this entry, there is a warning icon and text: "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'."

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.

The screenshot shows the software interface with the following elements:

- 1-** A red arrow points to the 'Contexts' menu in the left sidebar, which is highlighted with a red box.
- 2-** A red arrow points to the 'Diagnostics' tab in the top navigation bar, which is highlighted with a red box.
- 3-** A red arrow points to the 'Start' button in the 'Available Tests' table, which is highlighted with a red box.

Name	Description	Status
▶ Electronics Test	Verifies communication of the software with the instrument and functionality of instrument electro...	✓ Executed, passed
Fan Test	Verifies functionality of the instrument fan.	✓ Executed, passed
Lid Sensor Test	Verifies functionality of the lid sensors and cartridge identification.	✓ Executed, passed
Stepper Motor Test	Verifies functionality of horizontal and vertical motors.	✓ Executed, passed
Temperature Test	Checks temperature sensors and verifies the heatup rate of the heating plate using internal senso...	✓ Executed, passed
HV Stability and Accuracy ...	Checks high voltage stability and accuracy at four different voltages.	✓ Executed, passed
HV Accuracy Test (On-Load)	Check of channel-reference diode in transmission mode.	✓ Executed, passed
Short Circuit Test	Verifies that the leak current of each channel is within limits.	✓ Executed, passed
Electrode / Diode Test	Verifies that the conductivity of each channel (pin to pin) is within limits. Each of the channels 2-1...	✓ Executed, passed
Optics Test	Measures dark current and stray light of laser and LED.	✓ Executed, passed
Electrophoresis Autofocus ...	Runs an autofocus with the red laser and verifies the intensity of red laser and the offsets of the o...	✓ Executed, passed
Laser Stability Test	Measures stability of the red laser signal.	✓ Executed, passed

Test Properties

Name: Electronics Test
Description: Verifies communication of the software with the instrument and functionality of instrument electronics.
Approximate Time: 3 s

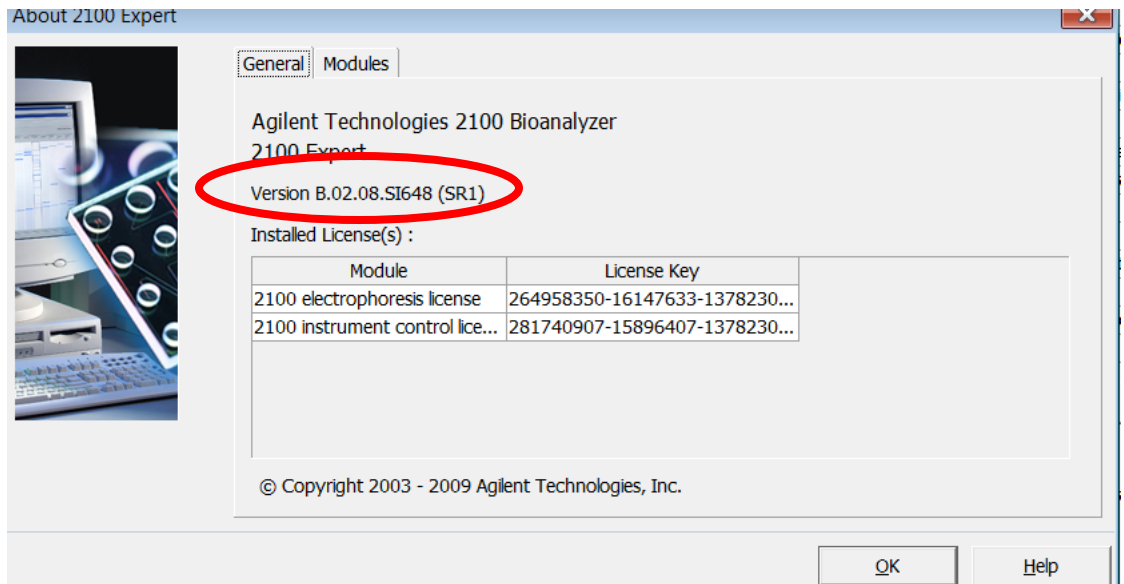
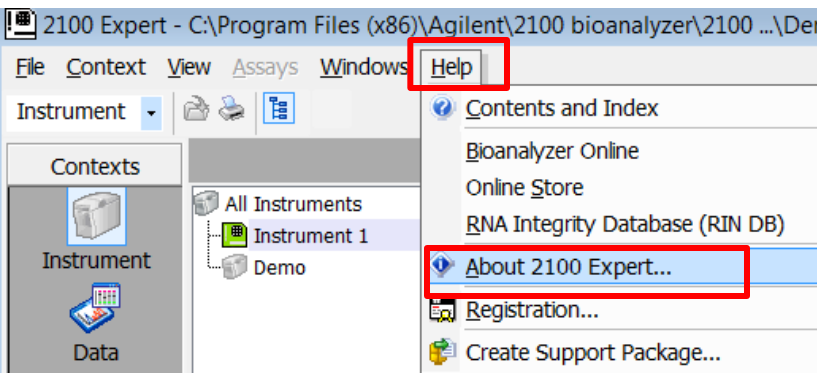
PC and software



Few important points to consider...

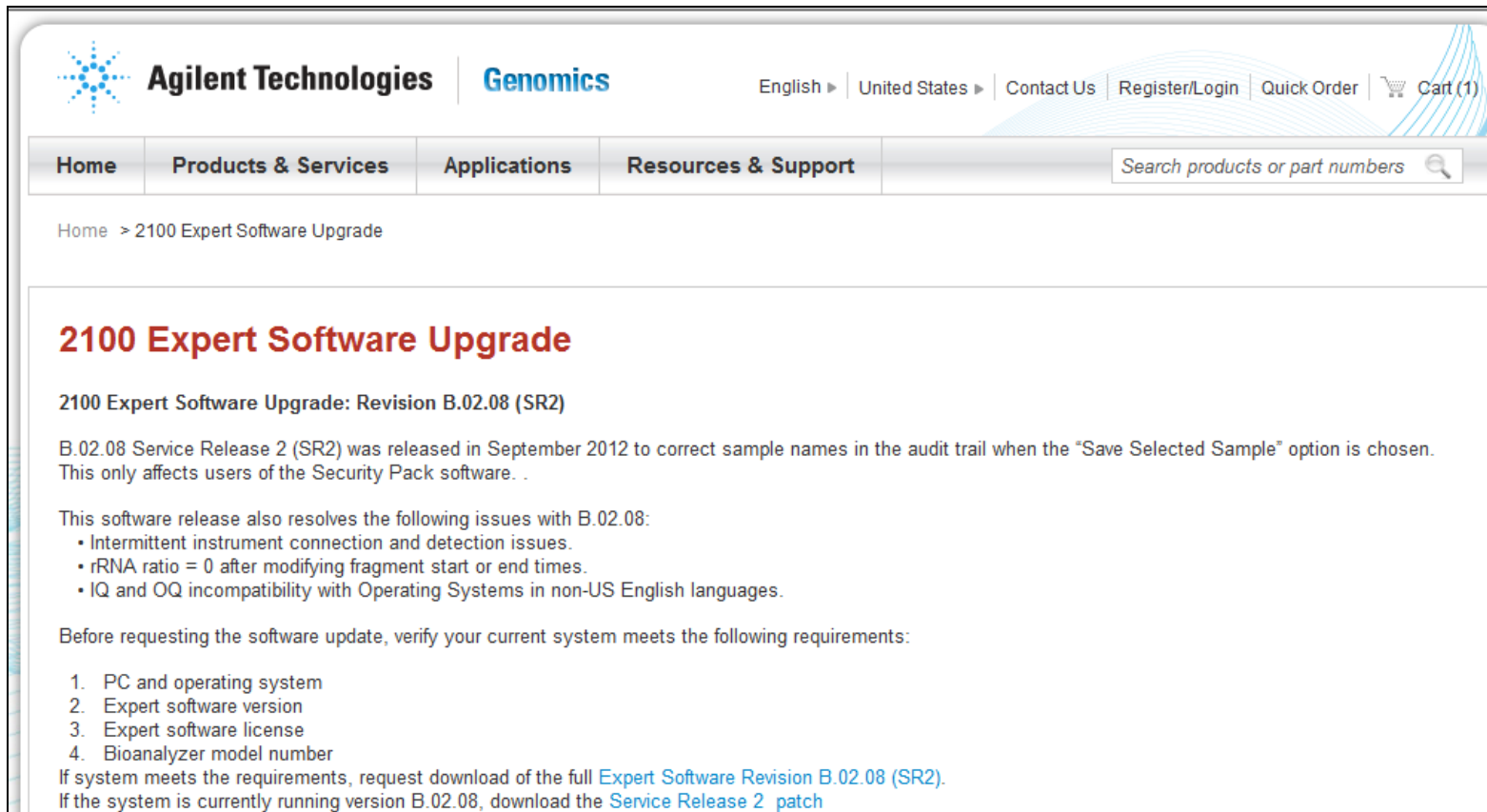
- The latest revision of the 2100 Expert software requires 1024 MB RAM. Especially, if you have other applications in use, you may drain off too much RAM to operate reliably. Restart the PC from time to time to free up enough RAM to restore functionality.
- If the free HD space is below 20 GB, the 2100 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?pagelId=2354>



The screenshot shows the Agilent Technologies Genomics website. The header includes the Agilent logo, the text "Agilent Technologies" and "Genomics", and navigation links for "English", "United States", "Contact Us", "Register/Login", "Quick Order", and "Cart (1)". A search bar is located in the top right corner. The main navigation menu includes "Home", "Products & Services", "Applications", and "Resources & Support". The breadcrumb trail reads "Home > 2100 Expert Software Upgrade". The article title is "2100 Expert Software Upgrade". The sub-heading is "2100 Expert Software Upgrade: Revision B.02.08 (SR2)". The main text states: "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. .". It then 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: "1. PC and operating system", "2. Expert software version", "3. Expert software license", and "4. Bioanalyzer model number". It concludes with: "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

The screenshot displays the 2100 Expert software interface. The main window is titled "Electropherogram - Ladder" and contains a 4x3 grid of electropherograms. The columns are labeled "Human Lymph", "Rat heart", and "Mouse heart". The rows represent different samples. Each plot shows fluorescence intensity (FU) on the y-axis and time (nt) on the x-axis. The x-axis has major ticks at 25, 200, 1000, and 4000. The y-axis scales vary by plot, ranging from 0-5 to 0-40. A sidebar on the left contains a list of contexts and a set of icons. The icons are: Instrument (red box), Data, Verification, Comparison, Assay, and System. The bottom of the interface shows a summary table with the following data:

RNA Area:	156.3	Result Flagging Color:	[Blue bar]
RNA Concentration:	150 ng/ul	Result Flagging Label:	All Other Samples

At the bottom right, there are buttons for "Auto Export", "Auto Print", and "Auto Run".

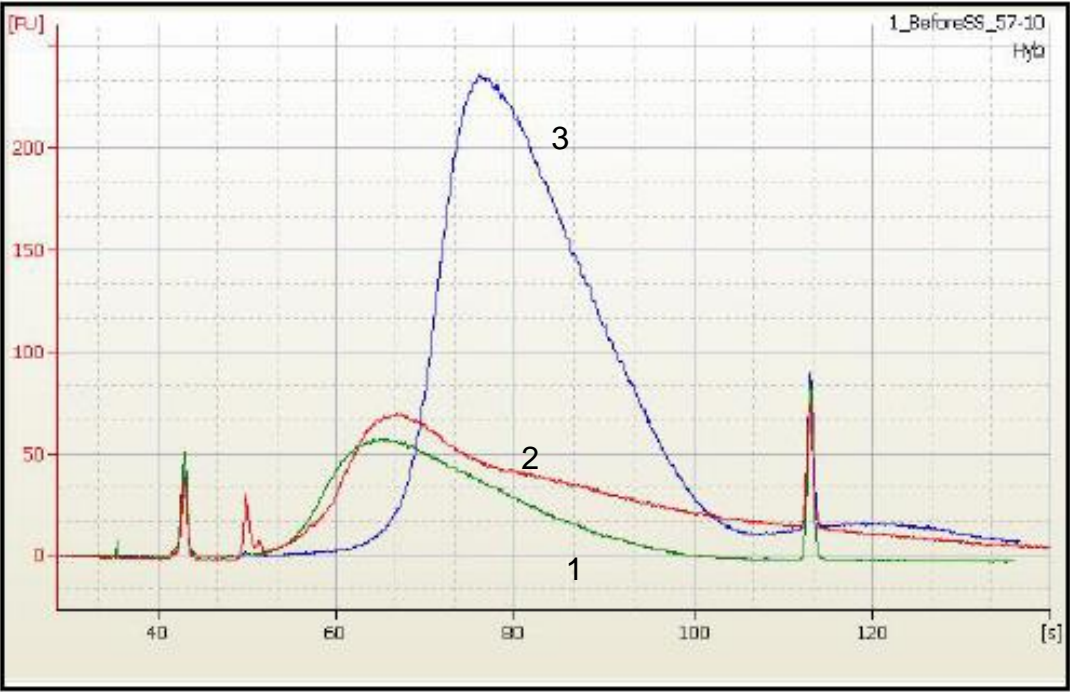
2100 Expert Software

Comparison context

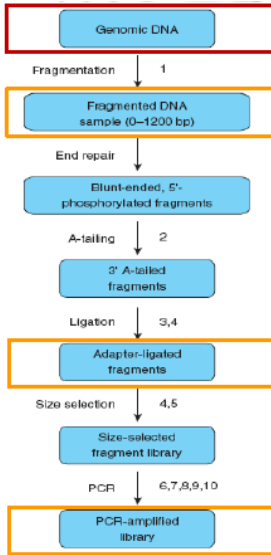
- Easy comparison of multiples chip run from the same assay class
- Samples need to be open in the data context of the expert software

The screenshot displays the 2100 Expert software interface. On the left, a sidebar contains a 'Contexts' menu with options: Instrument, Data, Verification, Comparison (highlighted with a red box), Assay, and System. A red arrow labeled '1-' points from the 'Comparison' option to the main window. The main window title is '2100 expert - ComparisonFile0 Protein 200.xac'. It features a menu bar (File, Context, View, Electropherogram, Tools, Window) and a toolbar. Below the toolbar, there are tabs for 'Comparison Summary', 'Gel', and 'Electropherogram', with 'Electropherogram' selected. A red box highlights these tabs, with a red arrow labeled '2-' pointing to it and the text 'Switch between comparison summary, gel and electropherogram view'. The main display area shows an 'Electropherogram overlay' with a y-axis labeled '[FU]' (0 to 500) and an x-axis labeled '[s]' (15 to 45). Multiple colored lines represent different samples. A legend at the bottom left, titled 'Legend for electropherogram overlay', lists: HSA 2000 (red), HSA 1000 (blue), HSA 500 (green), HSA 300 (cyan), and HSA 150 (magenta). On the right side of the plot, a vertical gel image is shown, with a red arrow pointing to it and the text 'Single gel lane for selected E-gram'. A red box at the top right contains the text 'Not available for Cell Assays'. The status bar at the bottom shows '10/2/2003 1:43 PM' and options for 'Auto Export', 'Auto Print', and 'Auto Run'.

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



- Sheared
- Adaptor-ligated
- PCR-amplified



Save Selected Samples

The screenshot shows the Agilent 2100 Expert software interface. The File menu is open, and 'Save Selected Sample...' is highlighted. A red arrow labeled '1' points to the File menu, and another red arrow labeled '2' points to 'Save Selected Sample...'. The main window displays a gel electrophoresis image with a ladder and a table of peak data.

Peak #	Size [bp]	Conc. [pg/μl]	Molarity [pmol/l]	Observations
1	35	125.00	5,411.3	Lower Marker
2	50	150.00	4,545.5	Ladder Peak
3	100	150.00	2,272.7	Ladder Peak
4	150	150.00	1,515.2	Ladder Peak
5	200	150.00	1,136.4	Ladder Peak
6	300	150.00	757.6	Ladder Peak
7	400	150.00	568.2	Ladder Peak

Save Selected Samples

The screenshot shows the Agilent 2100 Expert software interface. The main window displays an electropherogram titled "Electropherogram - Ladder" with a y-axis labeled "[FU]" ranging from 400 to 500. A "Save Selected Samples ..." dialog box is open in the foreground, showing a table of samples. The table has columns for ID, Sample Name, Comment, Category, and Selected. The "Ladder" sample (ID 12) is selected, indicated by a checked checkbox in the "Selected" column. Red arrows point to the "Apply" button and the "Selected" checkbox for the "Ladder" sample.

ID	Sample Name	Comment	Category	Selected
1	GAI library 1:20		Sample	<input type="checkbox"/>
2	DNA ladder (1:200)		Sample	<input type="checkbox"/>
3	FLX library 1700 pg/μl (1:10)		Sample	<input type="checkbox"/>
4	DNA library (1:30)		Sample	<input type="checkbox"/>
5	DNA ladder II (1:200)		Sample	<input type="checkbox"/>
6	DNA library (1:30)		Sample	<input type="checkbox"/>
7	GAI library (1:800)		Sample	<input type="checkbox"/>
8	ChIPseq library (1:2)		Sample	<input type="checkbox"/>
9	5 pg/μl ladder		Sample	<input type="checkbox"/>
10	GAI library (1:800)		Sample	<input type="checkbox"/>
11	Fragmented DNA		Sample	<input type="checkbox"/>
12	Ladder		Ladder	<input checked="" type="checkbox"/>

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

Log Book

Sample 1

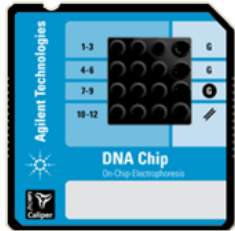
Local Global

Normal Collapse

Integration start time [s]	26
Integration end time [s]	94
Slope Threshold	0.5
Area threshold	0.5
Height threshold [FU]	8
Peak filter width [s]	0.5

Defaults Apply Cancel Help

When setpoint explorer feature is needed?



Analyze the run data and need to modify default parameters

- Peak height for individual samples
- Enabling smear analysis
- Align to upper and/or lower marker
- Adding/deleting ribosomal fragments (for RNA assays only)

2100 Expert Software

Setpoint explorer-

The screenshot displays the Agilent 2100 Expert software interface. The main window shows a gel electrophoresis image with lanes labeled Ladder, Sample 1, Sample 2, Sample 3, Sample 4, Sample 5, Sample 6, Sample 7, Sample 8, Sample 9, and Sample 10. The y-axis represents size in nucleotides [nt], ranging from 25 to 4000. A red arrow points from the 'Setpoint explorer-' text to the 'RNA Integrity Number' section in the setpoint explorer panel on the right.

The setpoint explorer panel shows the following parameters:

- Slope Threshold: 0.6
- Area Threshold: 0.2
- Height Threshold [FU]: 0.5
- Width Threshold [s]: 0.3
- Baseline Plateau [s]: 6
- Peak Filter Width [s]: 0.5
- Peak Filter Polynom: 2
- RNA Fragment**
 - Fragment Detection: Table ...
 - Use Dynamic Time Windo...
- RNA Integrity Number**
 - Pre Region Anomaly Thre...: 0.6
 - 5S Region Anomaly Thre...: 0.5
 - Fast Region Anomaly Thr...: 0.56
 - Inter Region Anomaly Th...: 0.7
 - Precursor Region Anomal...: 0.46
 - Post Region Anomaly Thr...: 0.45
 - Baseline Anomaly Thresh...: 0.51
 - Ribosomal Ratio Anomaly...: 0.7
 - Unknown Sample Type T...: 0.58
 - Single Decimal Represent...
 - Threshold Prerequisite C...: 10

2100 Expert Software

Setpoint explorer-

Changes in the setting will be recorded in the results

The screenshot displays the 2100 Expert software interface. The main window shows a gel electrophoresis result for 'Gel - Sample 1'. The gel image shows lanes for a Ladder and 11 samples. The y-axis represents nucleotide length [nt] from 25 to 4000. Below the gel, the 'RNA Integrity Number (RIN)' is displayed as 8.5 (B.02.08, Anomaly Threshold(s) manually adapted). The 'Setpoint Explorer' panel on the right shows various parameters, with the '5S Region Anomaly Thre...' set to 0.9. A red arrow points from the 'Setpoint explorer-' text to the '5S Region Anomaly Thre...' setting. A red oval highlights the RIN value and its associated text.

Parameter	Value
Integration End Time [s]	69
Slope Threshold	0.6
Area Threshold	0.2
Height Threshold [FU]	0.5
Width Threshold [s]	0.3
Baseline Plateau [s]	6
Peak Filter Width [s]	0.5
Peak Filter Polynom	2
RNA Fragment	
Fragment Detection	Table ...
Use Dynamic Time Windo...	<input checked="" type="checkbox"/>
RNA Integrity Number	
Pre Region Anomaly Thre...	0.6
5S Region Anomaly Thre...	0.9
Fast Region Anomaly Thr...	0.56
Inter Region Anomaly Th...	0.7
Precursor Region Anomal...	0.46
Post Region Anomaly Thr...	0.45
Baseline Anomaly Thresh...	0.51
Ribosomal Ratio Anomaly...	0.7
Unknown Sample Type T...	0.58
Single Decimal Represent...	<input checked="" type="checkbox"/>
Threshold Prerequisite C...	10

Questions?