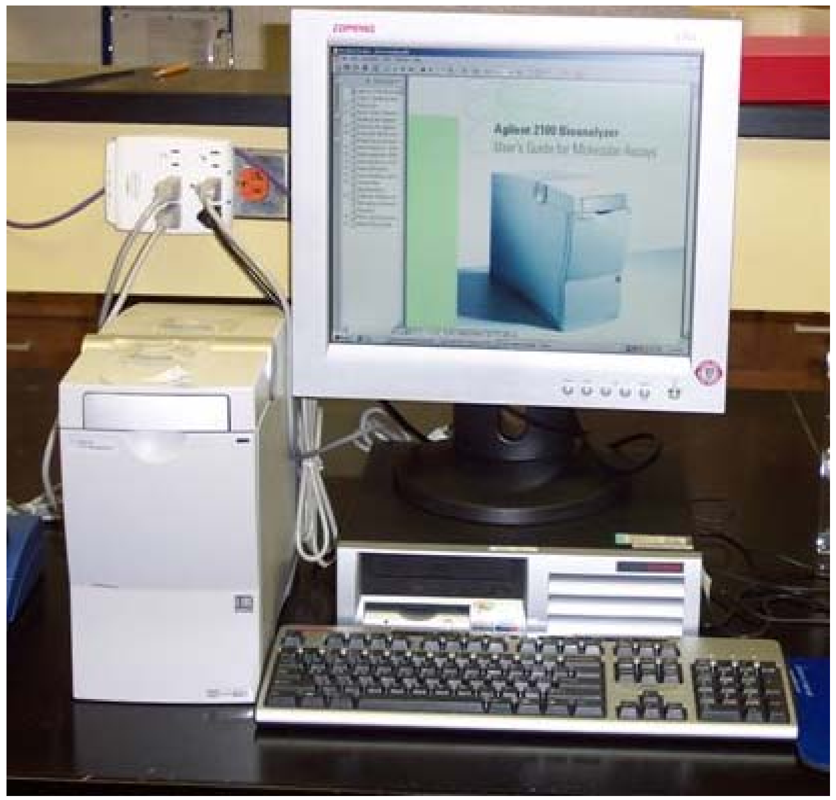
Agilent 2100 Bioanalyser:



This lab-on-a-chip system can analyze up to 12 DNA, RNA or protein samples in a matter of minutes. Also, because of the very high sensitivity, extremely small sample sizes are sufficient. We routinely use the Bioanalyser to perform a QC on our total RNA prior to gene micro-array experiments.

The following manual guides are given by Agilent Technologies:
Biosizing – User Guide
Biosizing - Maintenance and troubleshooting guide

RNA Isolation:

Refer:
* **ISOLATION OF TOTAL RNA FROM ANIMAL TISSUES USING RNeasy MINI KIT**

* **ISOLATION OF TOTAL RNA FROM ANIMAL TISSUES USING RNeasy MICRO KIT**

The Process: (Strictly for Nano Assay)

- Decontaminate the electrodes
- Prepare the Gel-Dye Mix
- Load the Gel-Dye Mix
- Load the RNA 6000 Nano Marker
- Load the Ladder
- Load the samples
- Insert the chip in the Agilent 2100 Bioanalyser
- Run the RNA Assay
- Check Your RNA results

- Clean up after an RNA Assay

Decontaminating the electrodes:

- Slowly fill one of the wells of an electrode cleaner with 350 µl RNaseZAP.
- Open the lid and place electrode cleaner in the Agilent 2100 Bioanalyser.
- Close the lid and leave it closed for about 1 minute.

- Open the lid and remove the RNase ZAP chip (cleaning chip) and keep for future use. You can reuse the electrode cleaner for all the chips in the kit. Remove the RNaseZAP at the end of the day.
- Slowly fill one of the wells of another electrode cleaner with 350 µl RNase-free water.
- Place electrode cleaner in the Agilent 2100 Bioanalyser.
- Close the lid and leave it closed for about 10 seconds.
- Open the lid and remove the electrode cleaner.
- Wait another 10 seconds for the water on the electrodes to evaporate.

All samples and ladder must be heated in a water-bath at 70°C for 2 minutes and place directly on ice to minimize secondary structure.

Note: Bioanalyzer may need a stronger cleaning if erratic operation is noted (after 2-3 day period of disuse). Please see the Bioanalyser Administrator (Joel Sanneman, Dr.Marcus Laboratory) or contact him at 532 – 4535 or jsanneman@vet.ksu.edu.

Perform all of the following preparatory steps in the PCR workstation.

Preparing the Gel-Dye Mix:

- Store the gel-dye mix at 4°C when not in use for more than 2 hours.
- Use the gel-dye mix within one week of preparation.
- Use the filtered gel within one month of preparation.
- Protect the gel-dye mix from light-- the dye will degrade when exposed to light and this reduces signal intensity.
- Allow the gel-dye mix to equilibrate to room temperature (> 30 min) before use. ****VERY IMPORTANT**
- Protect the gel-dye mix from light while bringing it to room temperature.

Procedure (sufficient for 4 chips):

- Place 400 µl of RNA gel matrix into the top receptacle of a spin filter

- Place the spin filter in a micro centrifuge and spin at 1500 g ± 20% for 10 minutes.
- Place 130 µl of the filtered RNA gel matrix into an RNase-free 1.5 ml micro centrifuge tube and add 2 µl of RNA dye concentrate.
- Cap the tube, vortex thoroughly and visually inspect proper mixing of gel and dye.
- Spin Gel/Dye mixture in centrifuge for 30 seconds to remove any micro bubbles that could be present in the tube.

Loading the Gel-Dye Mix:

Note: Insert the tip of the pipette to the bottom of the well when dispensing. This prevents large air bubbles forming under the gel-dye mix.

- Take a new RNA chip out of its sealed bag.
- Place the chip on the Chip Priming Station.
- Draw up 9.0 µl of the gel-dye mix with a pipette.
- Pipette 9.0 µl of the gel-dye mix in the chip well marked with this symbol.
- Make sure that the plunger of the Priming Station is at 1 ml and the base plate is at position C.
- Then close the Chip Priming Station until the latch is secure.
- Press the plunger until it is held by the syringe clip.
- Wait for exactly 30 seconds and then release the plunger with the clip release mechanism.
- Note: The plunger should automatically return to 6-8 ml if a proper seal was achieved.
- Pull back the plunger to the 1 ml position to release all pressure. Open the Chip Priming Station.
- Turn over the chip to check for air bubbles. Do not touch underside of the chip.
- Pipette 9.0 µl of the gel-dye mix in both of the wells marked with this symbol .

Loading the RNA 6000 Nano Marker:

- Draw up 5 µl of the RNA 6000Nano Marker
- Pipette into well marked with this symbol .
- Dispense 5 µl of the RNA 6000 Nano Marker into each of the 12 sample wells.

Loading the Ladder:

- Draw up 1 µl of the RNA 6000 ladder into a pipette.
- Pipette in the well marked with this symbol .
- Dispense the ladder into the well.

Loading the samples:

Note: Do not leave any wells empty or the chip will not run properly. Add an additional 1 µl of RNA 6000 Nano Marker to the 5 µl of sample buffer in the unused sample well.

- Pipette 1 µl of each sample into each of the 12 sample wells.
- Place the chip in the adapter of the vortex mixer. Vortex for 1 minute at the IKA vortexer set-point.
- Place the chip in the Agilent Bioanalyzer and start the run within five minutes.

Inserting a Chip in the Agilent 2100 Bioanalyzer:

- Open the lid of the Agilent Bioanalyzer.
- Place the chip into the receptacle. The chip is keyed to only fit one way. Do not force it.
- Carefully close the lid. The electrodes located in the cartridge fit into the wells of the chip.
- The Agilent 2100 Bioanalyser software screen shows that you have inserted a chip and closed the lid by displaying the chip icon at the left of the screen.

Running the RNA Assay:

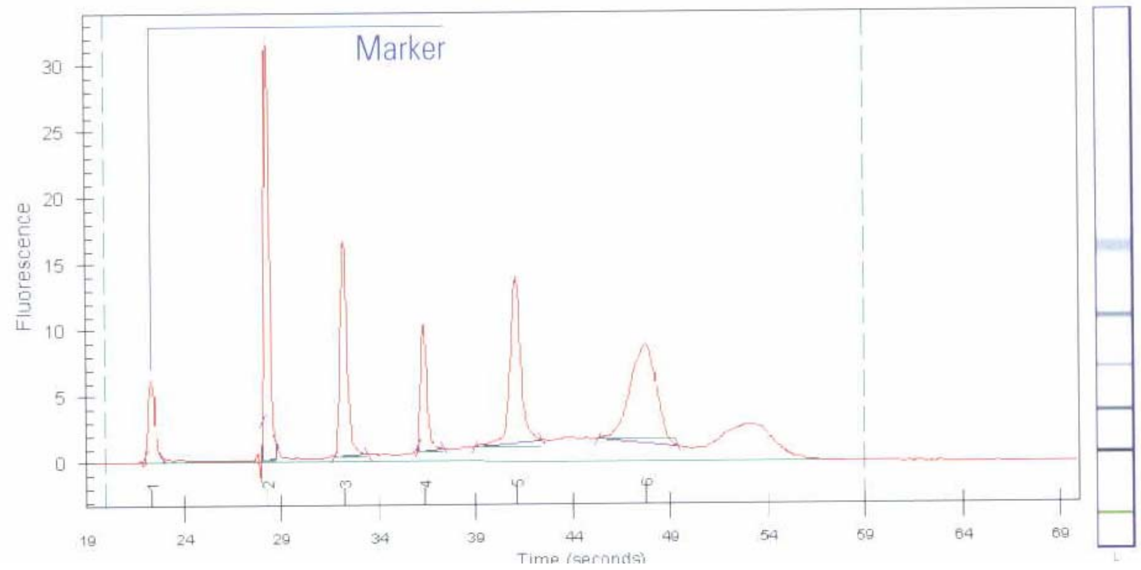
- Select the appropriate assay from the Assay menu.
- Position the mouse cursor over the word Start...above the icon of the chip. Click the Start button to open the Start dialog box.
- The software is set up to allow sample description editing after the run has been initiated. When the Start dialog box appears, the name of the loaded assay is listed as the current assay. You can enter a new file prefix at this time [Your Initials]. Data will be saved automatically to a file located in C:\Program Files\ Agilent 2100 BioAnalyzer\ Biosizing\ Data with a name using the prefix you have just entered. Then click the start button of the dialog to begin the assay. The whole file name can be set by the user.
- If appropriate, complete the sample name table and press OK.
- After the run begins, the Start button on the Agilent 2100 Bioanalyser software screen changes to Stop.
- To view results for individual wells as data is acquired or after the run is finished, click a well in the chip diagram, a single well displayed on the large 12 well display, or a lane in the gel. Data regarding the chosen well appears in a result table at the bottom of the display.
- When the assay is complete, immediately remove the chip from the receptacle of the Agilent 2100 Bioanalyser and dispose of it to prevent electrode contamination.

Checking your RNA Results:

RNA Ladder Well Results:

Major features of a successful ladder run are:

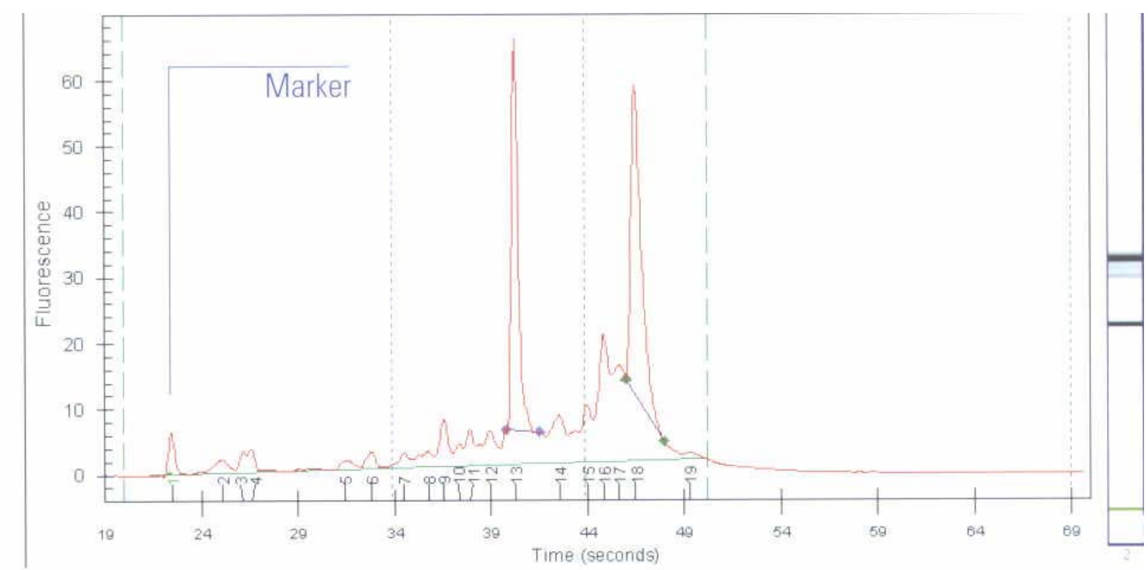
- 6 RNA peaks
- 1 Marker peak
- all 7 peaks are well resolved



RNA Sample Well Results:

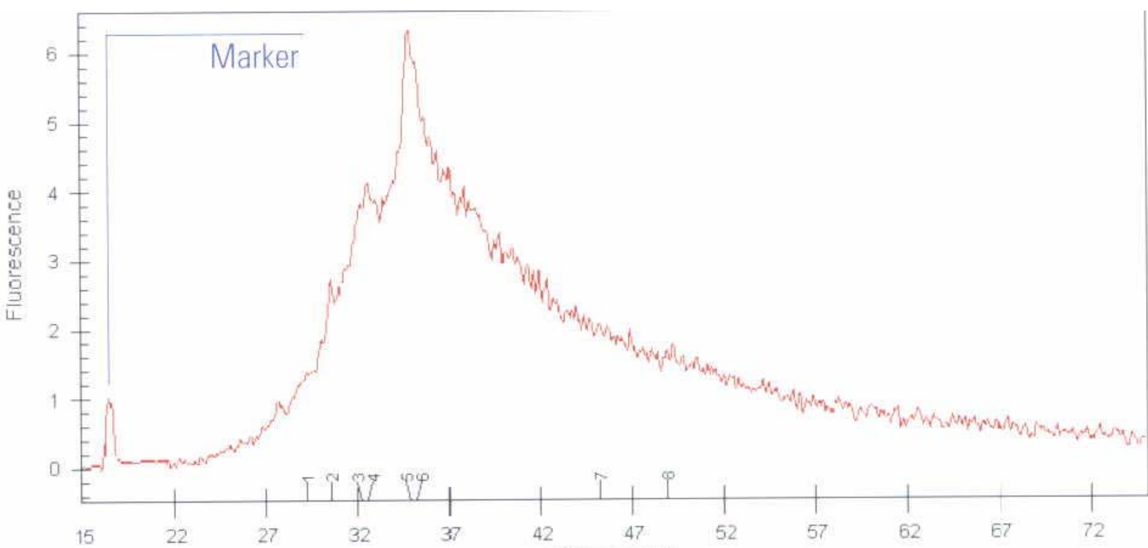
Major features for a successful total RNA run are:

- 2 ribosomal peaks
- 1 marker peak



Major features for a successful mRNA run are:

- broad hump
- contamination with ribosomal RNA shown as 2 overlaid peaks
- 1 marker peak



Cleaning up after an RNA Assay:

When the assay is complete, immediately remove the used chip from Agilent 2100 Bioanalyser and dispose of it.

Refer to Decontamination of electrodes.