RNA analysis using the Agilent 2100 BioAnalyzer

Protocols by Richard

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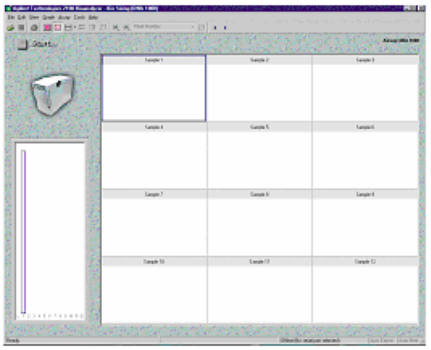
1. Starting the Agilent 2100 Bioanalyzer software

- Switch on the Agilent 2100 Bioanalyzer (back, next to power input) and login to the Hp Vectra computer.
- To start the software, go to your desktop and double-click the icon. 
- The main screen of the software appears. The Agilent 2100 bioanalyzer or chip is represented at the left side of the screen—what is shown depends on the status.



Lid closed, no chip or chip empty

Lid open

Dimmed icon: no communication

Lid closed, chip inserted, RNA or demo selected

2. Essential measurement practices

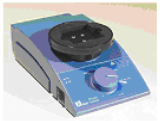
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well leads to bubbles and poor results.
- Keep all reagent and reagent mixes refrigerated at 4ºC when not in use.
- Allow all reagents to equilibrate to room temperature before use.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- Prepared chips must be used within 5 minutes. Reagents might evaporate, leading to poor results.
- Vortex chips at the appropriate time of 1 minute. Inappropriate vortexing leads to poor results.
- Don't touch the Agilent 2100 bioanalyzer during an assay and never place it on vibrating surface.
- Use a new syringe and cleaning chip with each new kit.
- Use RNAse free tips and tubes (use large petridishes for daily usage and pincers for handling).
- Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples. Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.

- Aliquot the amount of ladder you use within a day into a RNase-free microcentrifuge tube and heat at 70ºC for two minutes, after heating store immediately in ice (cool snap) untill usage.
- It is recommended to heat denature all RNA samples before use (70ºC, 2 min).

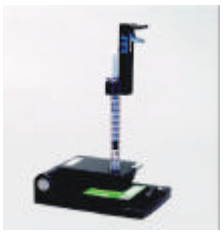
3. Assay equipment set-up

- Before following any of the chip preparation protocols, ensure that the following equipment is set up and ready to use.

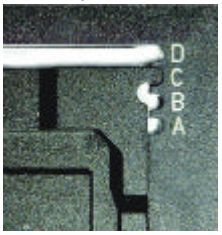
Vortex Mixer: IKA (model MS2-S9)



Chip Priming Station Set Up



Overview



Base Plate: position C

Syringe-clip: topmost position

4. Performing an RNA measurement / analysis

A. Decontaminating the Electrodes

Essential Practices

- Perform the following RNAse decontamination procedure on a daily basis before running any assays.

Procedure

- Slowly fill one of the wells of an electrode cleaner (marked 'RNAse ZAp') with 350 µl RNAseZAP.
- Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.
- Close the lid and leave it closed for about 1 minute.
- Open the lid and remove the electrode cleaner, label the electrode cleaner (if not already done, --> 'RNAse ZAp') 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 350µl RNAse-free water (DEPC-treated milliQ). Electrode cleaner is already marked with 'DEPC-mQ'. If not, label so!
- Place electrode cleaner in the Agilent 2100 bioanalyzer.
- 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.

B. Preparing the Gel-Dye Mix

Essential Practices

- 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 before use.
- 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 (red ●) into the top receptable of a spin filter.
- Place the spin filter in a microcentrifuge and spin at 1,500 x g ± 20% (4000 rpm with Eppendorf microcentifuge) for 10 minutes.
- Place 130µl of the filtered RNA gel matrix into an RNAse free 1.5 ml microfuge tube and add 2µl of RNA dye concentrate (blue ●).
- Cap the tube, vortex thoroughly and visually inspect proper mixing of gel and dye.

C. Preparing the Ambion RNA 6000 ladder

Essential Practices

- Catalog # 7152, 3 cups with 20 µl RNA
- Concentration: 150 ng/µl.
- The RNA 6000 Ladder is a set of six transcripts with lengths of 0.2, 0.5, 1.0, 2.0, 4.0 and 6.0 kb. The 0.2 kb RNA is at a concentration of 20 ng/µl (A_{260nm}).
- Storage Conditions: Store at –80ºC.
- Storage Buffer: 0.1 mM EDTA in DEPC-treated water.
- Quality Control: 150 ng (1 µl) will generate 6 distinct bands when run on a RNA Labchip™ using the Total RNA or mRNA assay. The ladder remains intact when incubated at 37ºC.
- Slight variations in the signal intensities of the ladder peaks may be seen between different reagent lots (details see product description). These minor variations will not compromise assay performance.
- This product is very sensitive to degradation by nucleases. Wear gloves and use RNase-free plasticware when handling. A degraded ladder can lead to erroneous quantitation. The ladder must be heat denatured before use, see the denaturation instructions below.
- Each of the three tubes contains 20 µl of RNA 6000 Ladder. Since only 1 µl is used per chip, it may be advisable to aliquot the ladder into several RNase-free microfuge tubes. Generally when degradation occurs, the high molecular weight peaks disappear. If the RNA peak pattern changes dramatically from the examples on this data sheet, the ladder may have degraded and a fresh tube of RNA 6000 Ladder should be used.

Procedure (for single day supply)

- Centrifuge the tube for a few seconds to force the contents to the bottom of the tube.
- Aliquot a 1-day supply of the into a fresh PCR microfuge tube and heat the tube at 70ºC in a PCR machine (e.g. BioRad iCyclers) for 2 minutes.
- Place the tube directly on ice for 5 minutes to snap cool the ladder.
- Briefly centrifuge to clear any condensate from the tube's walls and cap.
- Keep the RNA 6000 Ladder on ice throughout the day.

D. Loading the Gel-Dye Mix

Essential Practices

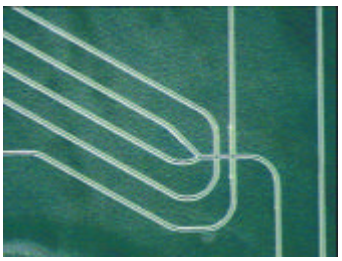
- Insert the tip of the pipette to the bottom of the well when dispensing. This prevents a large air bubble forming under the gel-dye mix.
- Make sure that the Chip Priming Station base plate is in the correct position (C) before loading the gel-dye mix. Make also sure that the adjustable clip is set to the **upper** position. Refer to page 2 of this document for details (picture).

Procedure

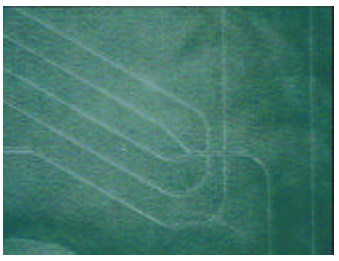
- Take a new RNA chip out of its sealed bag.
- Place the chip on the Chip Priming Station.
- Draw up 9.0µl of the gel-dye mix with a pipette.
- Place the tip of the pipette at the bottom of the well marked G and dispense the gel-dye mix.
- Make sure that the plunger is at 1 ml, then close the Chip Priming Station.
- 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

- Pull back the plunger to the 1 ml position.
- Open the Chip Priming Station.
- Turn over the chip to check for air bubbles.

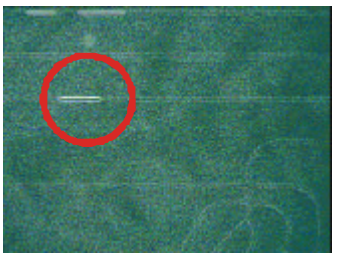
You will commonly see small bubbles in the wells after pipetting but the vortexing procedure (done after loading samples) will typically remove these bubbles. If you see bubbles in the channels of the chip, repeat steps 6 and 10. This is a fairly rare occurrence but can happen if a good seal is not formed when the chip is pressurized the first time.



Empty chip



Loaded chip: no air bubbles



Loaded chip: with air bubbles

- Pipette 9.0 µl of the gel-dye mix in each of the wells marked G.

E. Loading the RNA 6000 Nano Marker **(not Ambion RNA 6000 ladder !!)**

Essential Practices

- All 12 wells must be filled with sample buffer, even when running less then 12 samples.

Procedure

- Draw up 5µl of the RNA 6000 Nano Marker (green ●).
- Place the pipette tip all the way to the bottom of the well marked with the ladder symbol . Dispense the buffer into the well.
- Dispense 5µl of the RNA 6000 Nano Marker (green ●) into each of the 12 sample wells.

F. Loading the ladder

- Draw up 1 µl of the RNA 6000 ladder into a pipette.
- Place the pipette tip all the way to the bottom of the well marked with the ladder symbol . Dispense the buffer into the well.

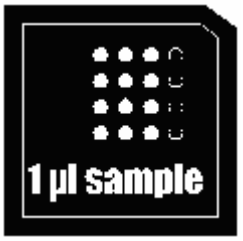
G. Loading the samples

Essential Practices

- Do not leave any wells empty or the chip will not run properly. Add an additional 1µl of RNA 6000 Nano Marker (green ●) to the 5µl of sample buffer in the unused sample well(s).
- For determination of RNA concentration, total RNA in sample must be between 25- 500 ng/µl. If concentration of your particular sample is above this range dilute with RNAse-free water.

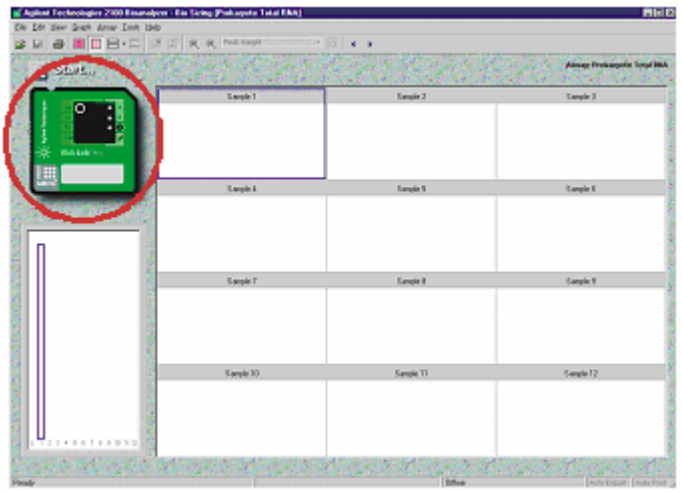
Procedure

- Pipette 1µl 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 2100 bioanalyzer and start the run within five minutes.



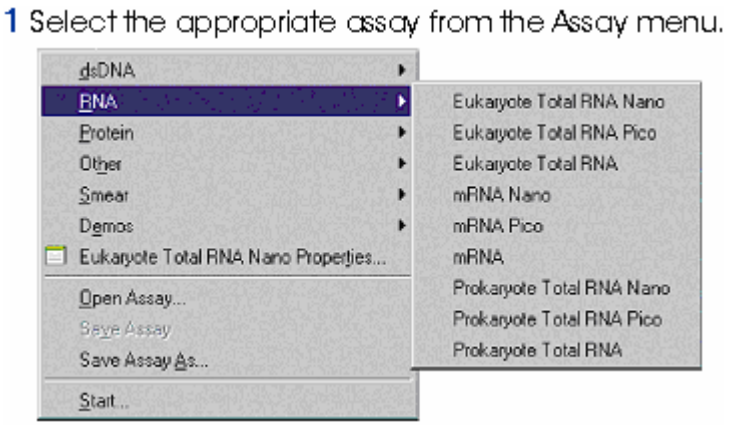
H. Inserting a Chip in the Agilent 2100 Bioanalyzer

- Open the lid of the Agilent 2100 bioanalyzer.
- Place the chip into the receptacle. The chip fits only one way. Do not use force.
- Carefully close the lid. The electrodes located in the cartridge fit into the wells of the chip.
- Do not force the lid closed or electrodes may be damaged. If the lid will not shut completely, check to be sure the chip and cartridge are inserted properly and then try to close the lid again.
- The Agilent 2100 bioanalyzer software screen shows that you have inserted a chip and closed the lid by displaying the chip icon at the left of the screen:



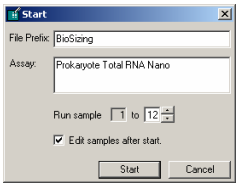
I. Running the RNA assay

- Select the appropriate assay from the Assay menu.

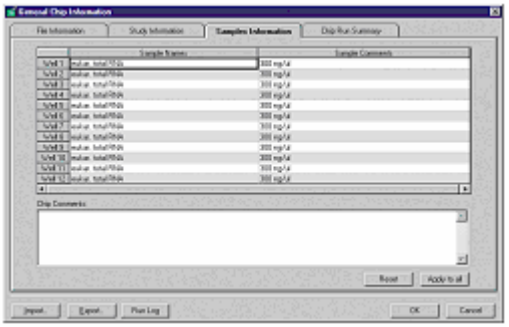


- Position the mouse cursor over the word Start above the icon of the chip (the word becomes active instead of dimmed). Click the Start button to open the Start dialog box.

- 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. Data will be saved automatically to a file with a name using the prefix you have just entered. Then click t he start button of the dialog to begin the assay.



- If appropriate, complete the sample name table and press OK.



If the error message 'Voltages out of range' occurs, there is not enough liquid in the wells. Prepare another chip and ma ke sure to dispense all the liquid from the pipette into the wells.

After the run begins, the **Start** button on the Agilent 2100 bioanalyzer software screen changes to **Stop**:

If you should need to end the run for any reason, click the Stop button. A dialog box will appear, asking if you're sure you want to end the run in progress. Clicking Yes will stop the run. The file information at the top of the screen will show a red error circle and a third line of file information will show Status: Aborted by the user.

Information collected up to the stop point is saved.

- To view results for individual wells as data is acquired or after the run is finished, click a well in the chip, a single well displayed on the large12-well display, or a lane in the gel. Data regarding that well appears in a result table at the bottom of the display.
- When the assay is complete, remove the chip from the receptacle of the Agilent 2100 bioanalyzer and dispose of it in the 'Biological Waste bins'

J. Cleaning up after an RNA Assay

When the assay is complete, **immediately** remove the used chip from the Agilent 2100 bioanalyzer and dispose of it in the 'Biological Waste bins'. Then perform the following procedure to ensure that the electrodes are clean, i.e. no residues are left over from the previous assay.

Essential Practices

- Empty and refill the electrode cleaner at regular intervals, for example, every five assays.
- The electrode cleaner can be used for 25 assays.
- Use a new cleaning chip with each new kit.

Procedure

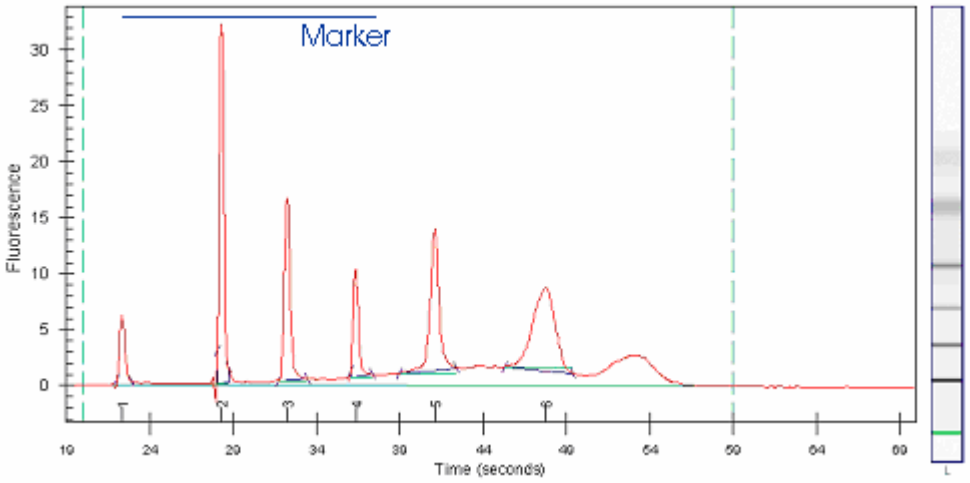
- Slowly fill one of the wells of the electrode cleaner with 350µl RNAse-free water (DEPC-treated milliQ).
- Never fill too much water in the electrode cleaner. This could cause liquid spill or contamination of the electrodes.

- Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.
- 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.

5. Checking your RNA Results

RNA Ladder Well Results

To check the results of your run, go to View > Single Wells and select the ladder well (left-most in the gel view window). The electropherogram of the ladder well window should resemble the one shown here:



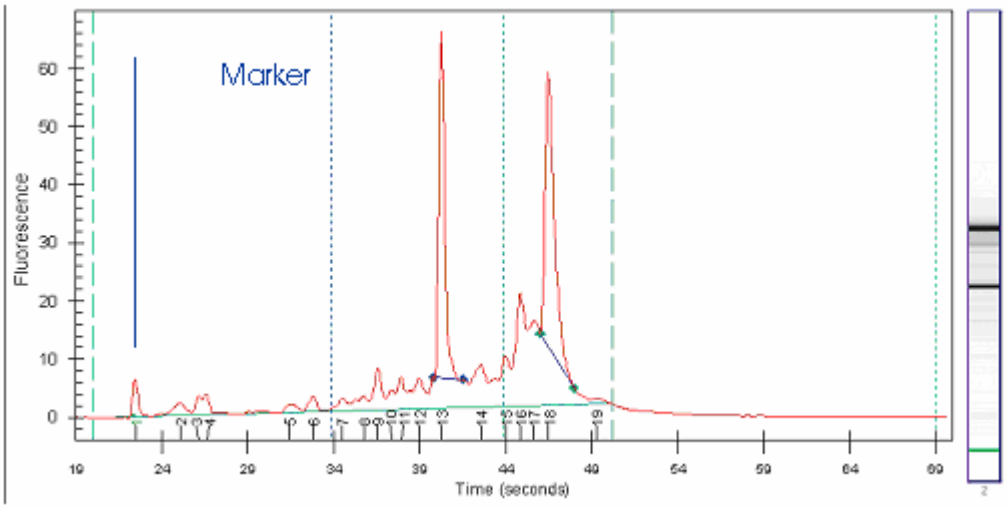
Major features of a successful ladder run are:

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

The software might not detect the last peak, depending on the Peak Find Settings. This will not affect the results although the separation might look different than expected.

RNA Sample Well Results (adjust later to graphs from *L. lactis* and *B. subtilis* RNA)

If you are not viewing your results in single well mode, go to View > Single Wells and select one of the sample wells. The electropherogram of the sample well window for total RNA (eukaryotic) should resemble the one shown here:



Major features for a successful total RNA run are:

- 2 ribosomal peaks (with successful sample preparation)
- 1 marker peak