Agilent Bioanalyzer 2100 protocol

Things to do first and to make sure

1. All reagent and reagent mix (exclude RNA ladder) should be kept in 4C. Take reagents out of the fridge to warm at room temperature for at least 30 minutes.
2. Protect dye and gel dye mix from light.
3. Turn on heating block and set to 70°C
4. Always wear glove when touching the equipment to avoid contamination of RNase
5. Start the software of Expert 2100 before loading the chip
6. insert pipette tip to the bottom of the well to avoid bubble forming under the gel-dye mix
7. Do not touch or even think about opening the Bioanalyzer while it is running.

Preparation of Gel matrix

1. Add 400 ul of gel matrix to a spin filter
2. Spin for 10 min at 4000 rpm (1500g+-20%)
3. Gel Matrix can be stored at 4C and should be used within one month.

Clean the electrodes

1. Fill two electrode cleaners, one with 350ul RNase free water and one with 350ul RNase Zap.
2. Place electrode cleaner with RNase Zap in the machine for 1 minute- remove- place the electrode cleaner with water in the machine for 10 seconds- remove- leave machine open for 10 seconds for water to dry

Preparation of Gel-dye matrix

1. Vortex dye for 10 seconds and spin down
2. Add 2 ul of dye to a 120 ul tube of gel matrix
3. Vortex thoroughly for 10 seconds and visually inspect proper mixing of gel and dye.
4. Centrifuge at maximum speed (13000g or 14000rpm for eppendorf microcentrifuge) for 10 minutes

Note:

- Protect dye and gel-dye mix from light.
- Always re-spin the gel-dye mix for ten minutes before use.

Loading chip with the gel matrix and RNA 6000 Nano Marker:

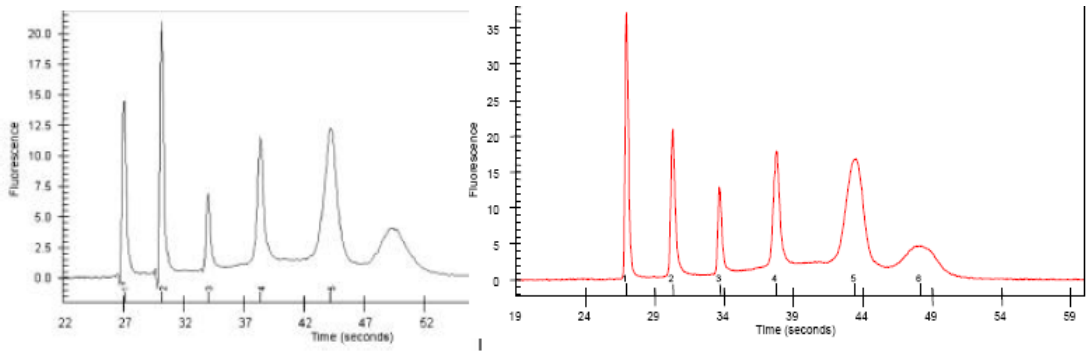
1. place new chip on priming station and load 9 ul of gel-dye matrix into the well marked G (fourth column, third row)
2. set the timer for 30 sec and make sure that the plunger is at the 1 ml mark. close the chip priming station; press the plunger until it is held by the syringe clip; wait 30 seconds
3. Release the plunger with clip release mechanism; wait 5 seconds (*it should release to about 0.8 ml if sealed very well) and slowly pull the plunger back up to 1 ml; gently open the chip priming station
4. Pipette 9 ul of the gel dye matrix in the two additional wells marked G (fourth column, first and second rows)
5. Pipette 5 ul of marker (green cap) into the well marked ladder and each of the 12 RNA wells. 6ul Marker should be loaded into each unused sample otherwise the chip will not run properly.

   Note:
   - loaded chip must be used within 5 mins, reagents might evaporate, leading to poor result
   - for each new kits, use new syringe and electrode cleaner

Loading the RNA sample and the RNA 6000 ladder:

1. Incubate the RNA sample and RNA ladder in the 70° C heating block for 2 minutes and put on ice for 5 minutes. Briefly centrifuge to clear any condensate from the tube's walls and cap.
2. Load 1 ul of ladder into the well marked "ladder" and load 1 ul of RNA into each of the 12 wells (or as many wells as there are samples for)
3. Place the Chip in the adapted vortexer and mix for 1 minute at 2400rpm. If there is liquid spill at the top of the chip, carefully remove it with a tissue. Use hand to press the chip otherwise it would be thrown out from the vortexer.

   Note:
   - Prior to use the RNA ladder, centrifuge the tube for a few seconds to force the contents to the bottom of the tube.
   - There might be slight variation in the signal intensities of ladder peaks between different reagent lot. But the RNA might degrade if it apparently different from the figure below.



Typical signal intensity variations seen between two different lots of RNA 6000 Ladder

Running the chip

1. Place the loaded Chip in the Bioanalyzer
2. In the Instrument context, select Assay→ RNA→ the appropriate Assay
3. Press "Start" and enter the sample names.
4. Files are automatically saved at the end of the run and the End of Run message appears when the chip run is finished

Note: Chip should be run within 5 min after loading the sample

Clean the electrodes

1. Removing the Chip from Bioanalyzer right after the run is finished.
2. Repeat the steps in **Clean the electrodes**