Agilent Bioanalyser RNA 6000 Nano Assay

(5-500 ng/µL)

1. Allow RNA Nano reagents need to equilibrate to room temperature for **30 minutes** before use.

2. Decontaminate the electrodes:
- Take electrode cleaner labelled RNaseZAP and fill one of the wells with 350 µL of RNaseZAP.
- Place electrode cleaner in Bioanalyser, close lid and leave it for **1 minute**.
- Take electrode cleaner labelled water and fill one of the wells with 350µL of RNase free dH2O.
- Place electrode cleaner in Bioanalyser, close lid and leave it for **10 seconds**.
- Open lid and remove electrode cleaner, and leave Bioanalyser lid open for **10 seconds** to allow the water on the electrodes to evaporate.
- Close the Bioanalyser lid.

3. If necessary prepare filtered gel matrix:

a. Pipette 550µl of RNA gel matrix (red●) into top of spin filter provided.
b. Spin at 1500g for **10 minutes** (DO NOT spin using rpm setting)
c. Remove spin filter. Aliquot 65µl filtered gel into **amber** microcentrifuge tubes (one aliquot can be used for 2 chips on the same day)
d. Label with date. Store at 4°C in the white plastic box and use within **one month**.

4. Prepare gel-dye mix:

a. Vortex RNA dye concentrate (blue●) for **10 seconds** and spin down briefly.
b. To 65µl filtered gel aliquot add 1µl of the RNA dye concentrate
c. Vortex thoroughly. Check that proper mixing of gel and dye has occurred.
d. Spin tube for **10 minutes** at room temperature at 13 000g.
e. Keep at 4° C and protected from light until needed. Use within one day and discard unused gel-dye mix.

5. Concentration of RNA should be between 25-500ng/µl (optimum 250ng/µl). If necessary dilute samples with RNase-free water.

6. Denature RNA samples and an aliquot (1.2µl) of RNA 6000 Ladder (stored in the -80°C freezer, bottom shelf box J02) by incubating at 70°C for 2 minutes. If you use the last aliquot, please let Genomics Lab staff know.

7. Place denatured samples and ladder on ice.

8. Check that the Chip Priming Station O ring is not dirty. If it is, contact a member of the Genomics Lab, who will clean and if necessary replace it. The Chip Priming Station must be set at base plate **position C** and the syringe clip at the **topmost** position.
Place RNA 6000 Nano chip in the Chip Priming Station.

9. Pipette 9µl of gel-dye mix into bottom of well marked .

10. Ensure syringe plunger is at 1ml. Close the Chip Priming Station and push down plunger until it is held by the syringe clip.

11. Wait for **30 seconds** exactly and then release plunger with the clip release mechanism. Allow the plunger to move back to the 1ml position. Aid if necessary.

12. Remove the chip from the Chip Priming Station and check for bubbles. Bubbles will appear as straight lines in the capillaries. Steps 11-12 can be repeated if any bubbles are present. If bubbles are still present check seals of Chip Priming Station

13. Pipette 9µl of gel-dye mix into the 2 remaining wells marked **G**.

14. Pipette 5µl of RNA 6000 Nano Marker (green) into the 13 remaining empty wells (including that marked).

15. Pipette 1µl of denatured RNA 6000 Ladder into the bottom right hand corner well marked .

16. Pipette 1µl of each sample into its assigned well. If running fewer than 12 samples, an extra 1µl of the RNA 6000 Nano Marker should be added in place of the sample.

17. Place the chip in adapter of vortex mixer. Check that chip is secure and vortex speed is that of the 'set' point. Vortex the chip for **1 minute** exactly. DO NOT vortex for longer as this will lead to poor results.

18. To run the chip, place in Bioanalyser. It fits one way and must not be forced. Once prepared chips must be used within **5 minutes** because reagents can evaporate.

19. Close the lid. If there are problems, check the chip is inserted properly. DO NOT force the lid as this will damage the electrodes.

20. If the chip has been detected it will appear in the top left hand corner of the software screen.

21. From the "Assay" icon select 'RNA', then for total RNA select 'Eukaryote Total RNA Nano Series II' and for cRNA select 'Eukaryotic mRNA Nano Series II'.

22. Name your samples in the chart (optional) and select the number of samples you are running in the Data Acquisition Parameters box.

24. Click on the 'Start' icon.

25. The assay run takes approximately 30 minutes. DO NOT touch the Bioanalyser during an assay run. Movements of the Bioanalyser during a run may result in erroneous results.

26. Once finished remove the chip and perform the electrode decontamination protocol (like at step 2) before running further chips or shutting down. Empty electrode cleaners by flicking out and then gently tapping upside down onto a paper towel.