Agilent Bioanalyser High Sensitivity DNA

(5-500pg/µL, 50-7000bp)

1. Allow High Sensitivity DNA reagents need to equilibrate to room temperature for **30 minutes** before use.

2. DO NOT decontaminate the electrodes. Please ask a member of Genomics Staff to change the electrodes, then wash the electrodes:
- Take electrode cleaner labelled water and fill one of the wells with 350 µL of RNase free dH2O.water.
- Place electrode cleaner in Bioanalyser, close lid and leave it for **1 minute**.
- 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 gel-dye mix:

a. Vortex the blue-capped High Sensitivity DNA dye concentrate for 10 seconds and spin down.

b. Pipette 15 µl of the High Sensitivity DNA dye into an unused red-capped High Sensitivity DNA gel matrix vial. Replace the dye concentrate into the box (in the dark again).

c. Cap the tube and vortex for 10 seconds. Visually inspect proper mixing of gel and dye.

d. Transfer the gel-dye mix to the top receptacle of a spin filter.

e. Centrifuge for **10 minutes** at room temperature at 2240g (RCF).

f. Discard the filter and aliquot 40 µl filtered gel-dye mix into labeled 1.5 ml **amber** tubes. Label with date. Use the gel-dye mix within 6 weeks. Protect from light at all time and store in the fridge.

4. Concentration of DNA should be between 5-500pg/ul.

Double-stranded DNA is used for analysis and samples are therefore not denatured.

5. 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 **lowest** position.
Place HS DNA Chip in the Chip Priming Station

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

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

8. Wait for **1 minute exactly** and then release plunger with the clip release mechanism. Allow the plunger to move back to the 1ml position, aid if necessary.

9. Open the chip priming station.

10. Pipette 9.0 µl gel-dye mix into the other three wells marked **G**.

11. Pipette 5µl of green-capped HS DNA Marker into the well marked  and into each of the 11 sample wells.
N.B. Do not leave any wells empty.

12. Pipette 1 µl of yellow-capped ladder into the bottom right hand corner well marked with  .

13. Pipette 1 µl of each to sample into its assigned well. If running fewer than 11 samples, an extra 1 µl of marker should be added in place of the sample.

14. 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.

15. 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.

16. Close the lid. If there are problems, check the chip is inserted properly. Do not force the lid as this will damage the electrodes.

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

18. From the "Assay" icon select 'Double-stranded DNA' then 'High Sensitivity DNA'.

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

20. Click on the 'Start' icon.

21. 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.

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