

Bioanalyzer : DNA High Sensitivity

DNA High Sensitivity Kit

- Qualitative range: 5 -500 pg (ie for amplicons) or 2-3 ng (ie for libraries or sheared DNA)
- Sizing range: 50 – 7000 bp

Agilent DNA High Sensitivity Assay Protocol

1. Turn ON:
 - Centrifuge
 - Instrument
 - Launch software 2100 Expert
2. Decontaminate the electrodes:
 - Add 350ul to DEPC H2O cleaning chip (1 of 2) and cleaning chip (2 of 2). Place cleaning chip (1 of 2) in instrument for ~10 sec, remove cleaning chip and repeat with cleaning chip (2 of 2).
3. Chip:
 - 11 wells for samples; 1ul sample/well
 - 4 wells for gel; 9ul gel-dye/well
 - 1 well for standard ladder used for sizing; 1ul ladder
4. Chip Priming Station settings:
 - Position C
 - Adjustable clip set to the last position
 - Plunger at 1ml
5. Remove the DNA HS chip from the sealed bag.
6. Place the chip in the priming station.
7. Always insert the pipette tip to the bottom of the well when dispensing the liquid.
8. Pipette and dispense 9ul gel-dye mixture at the bottom of the circled G well.
9. Close the priming station. Latch will click when it is closed properly.
10. Set timer for 1 minute.
11. Press syringe plunger down until it is held by the clip. Start timer for 1 min. After 1 min, release the clip of the plunger.
12. Wait 5 seconds then slowly pull the plunger back to the 1 ml position.
13. Open the chip priming station.
14. Pipette 9 ul of the gel-dye mix into each of the remaining G wells.
15. Pipette 5 ul of marker into the well marked with the ladder symbol and into each of the sample wells.
 - Note: use 6 ul of marker in wells without samples.
16. Pipette 1 ul of ladder into the well marked with the ladder symbol.
17. Pipette 1 ul of sample into the sample wells.
18. Set timer to 1 minute.
19. Vortex chip at 2400 rpm for 1 min (clean any liquid spills before vortexing)
20. Run chip within 5 minutes as reagents might evaporate, leading to poor results.
21. Carefully place the chip into the receptacle on the Bioanalyzer. Carefully close the lid.
22. Chip icon will show up on the software.
23. Select the appropriate assay from the *Assay Menu*.
24. Click the *Start* button. The ladder will show up first (after ~7min) and it will take ~45 minutes to run a full chip.
25. To enter the sample names, select the *Data File* link.
26. To review the raw signal trace, return to the *Instrument* context.

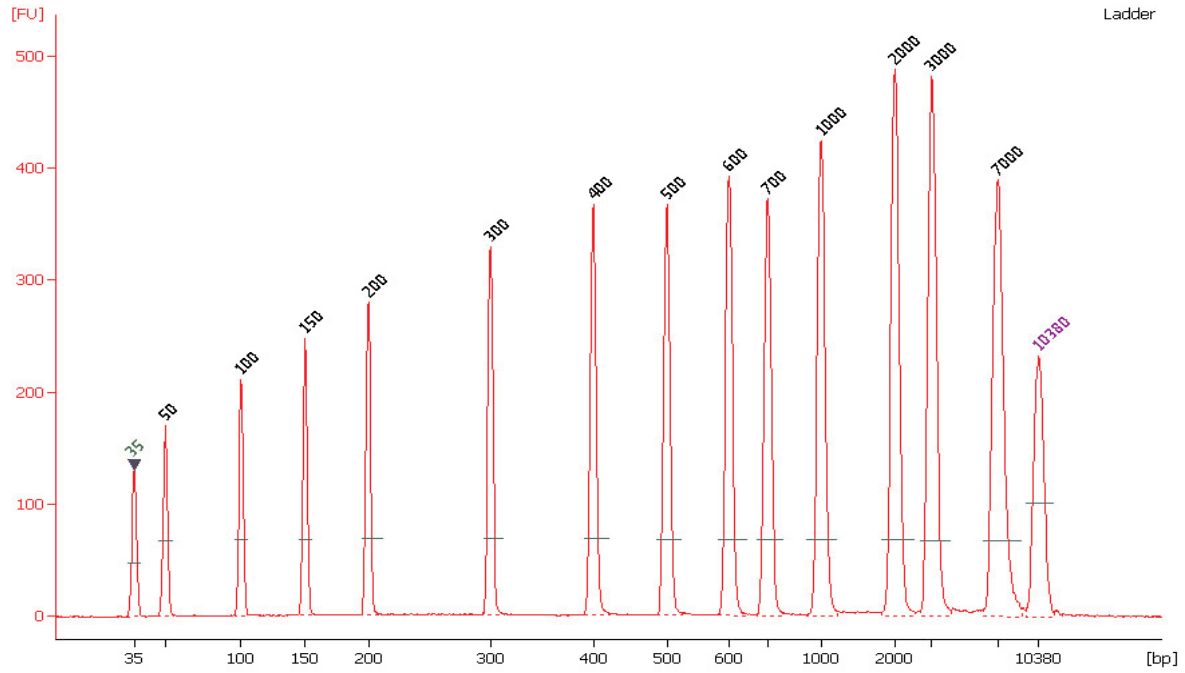
Run Complete:

1. Immediately remove the chip from the receptacle of the Bioanalyzer and dispose of it to prevent residues on the electrodes.
2. Return the DEPC cleaning chips to the instrument for ~10 sec, repeat with second cleaning chip, then remove it when done and leave the lid open for another 10sec to allow the electrodes to dry.

3. To save run as a PDF file: select *Print* then name accordingly. Ask a core member to upload your file into your folder in your Genome Center Account.
4. Turn off instrument.

DNA Analysis:

High Sensitivity Ladder



- 15 peaks for High Sensitivity Ladder (including markers)
- Lower marker: right after 40 sec; late migration seen when ladder starts around 50 sec, leaving the upper marker unseen.