Title: DNA analysis using Agilent High Sensitivity DNA Kit for 2100 Bioanalyzer Systems		
Date last updated: 7th July 2024	Total Pages: 7	Written by: Jeanie Wu

DNA analysis using Agilent High Sensitivity DNA Kit for 2100 Bioanalyzer Systems

1. PURPOSE

To analyse DNA with sample sizes ranging from 50bp to 7000bp after NGS library preparation.

2. MATERIALS & EQUIPMENTS

Samples

- Dengue or Zika prepared NGS library

Reagents

- Agilent High Sensitivity DNA Kit, includes reagents and chips (Agilent, 5067-4626)

Consumables

- 0.2 ml PCR tubes
- Nuclease-free water

Equipments

- Agilent 2100 Bioanalyzer Systems (Bioanalyzer, chip priming station and IKA vortex mixer)
- Centrifuge (> 13000g)

3. PROCEDURE

Essential Measurement Practices

- Handle and store all reagents according to the instructions on the label of the individual box
- 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
- Keep all reagents and reagent mixes refrigerated at 2 – 8°C when not in use
- Allow all reagents and samples to equilibrate to room temperature for 30 min before use

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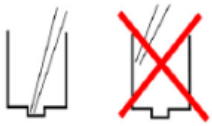
- Protect dye and dye mixtures from light
- Always insert pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results



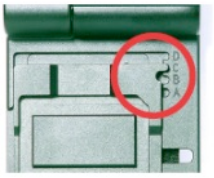
- Use a new syringe and electrode cleaners with each new kit
- Use loaded chips within 5 min after preparation. Reagents might evaporate, leading to poor results
- Do not touch the 2100 Bioanalyzer instrument during analysis and never place it on a vibrating surface

Step 1: Sample Preparation

a. For high sensitivity chip, prepare samples in the concentration range of 100pg/ul – 10ng/ul

Step 2: Setting up Chip Priming Station

a. Replace the syringe by unscrewing old syringe from the lid of chip priming station
b. Release old syringe from the clip and discard
c. Remove plastic cap of the new syringe and insert into the clip
d. Slide it into the hole of the luer lock adapter and screw it tightly to chip priming station
e. Adjust the base plate by pulling the latch and opening the chip priming station
f. Using a screwdriver, open the screw at the underside of the base plate
g. Lift the base plate and insert it again in position C. Retighten the screw
h. Adjust the syringe clip by releasing the lever of the clip and sliding it down to the lowest position



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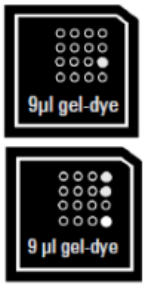
Step 3: Preparing Gel-Dye Mix

a. Allow High Sensitivity DNA dye concentrate (blue 🔵) and High Sensitivity DNA gel matrix (red 🔴) to equilibrate to room temperature for 30 min
b. Add 15 ul of High Sensitivity DNA dye concentrate (blue 🔵) to a High Sensitivity DNA gel matrix vial (red 🔴)
c. Vortex solution well and spin down. Transfer to spin filter
d. Centrifuge at 2240 g ± 20% for 15 min.
e. Protect solution from light
f. Store at 2 – 8°C. Use prepared gel-dye mix within 6 weeks of preparation

Step 4: Loading the Gel-Dye Mix



a. Allow the gel-dye mix to equilibrate to room temperature for 30 min before use
b. Put a new High Sensitivity DNA chip on the chip priming station
c. Pipette 9 ul of gel-dye mix in the well marked G
d. Make sure the plunger is positioned at 1 ml and then close the chip priming station
e. Press plunger until it is held by the clip
f. Wait for exactly 60 s then release clip
g. Wait for 5 s, then slowly pull back the plunger to the 1 ml position
h. Open the chip priming station and pipette 9 ul of gel-dye mix in the wells marked G

Step 5: Loading the Marker

a. Pipette 5 ul of marker (green 🟢) in all sample and ladder wells. Do not leave any wells empty

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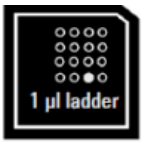
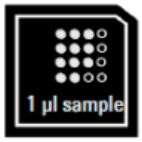
Step 6: Loading the Ladder and Samples

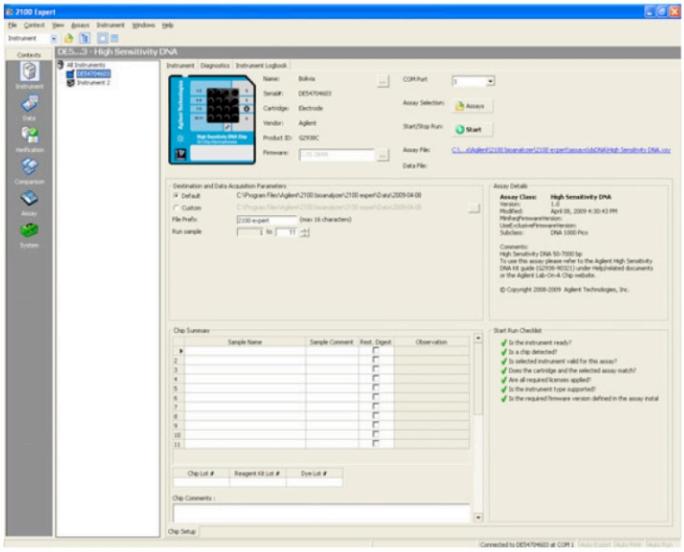
a. Pipette 1 ul of High Sensitivity DNA ladder (yellow) in the well marked
b. In each of the 11 sample wells, pipette 1 ul of sample (used wells) or 1 ul of marker (unused wells)
c. Put the chip horizontally in the adaptor and vortex for 1 min at indicated setting (2400 rpm)
d. Run the chip in the 2100 Bioanalyzer instrument within 5 min





Step 7: Inserting a Chip in the 2100 Bioanalyzer Instrument

a. Open lid of 2100 Bioanalyzer instrument
b. Place chip carefully into the receptacle. The chip fits only one way
c. Carefully close the lid. The electrodes in the cartridge fit into the wells of the chip
d. The 2100 Expert software screen shows that you have inserted a chip and upon closing the lid, a chip icon is displayed at the top left of the **Instrument** context



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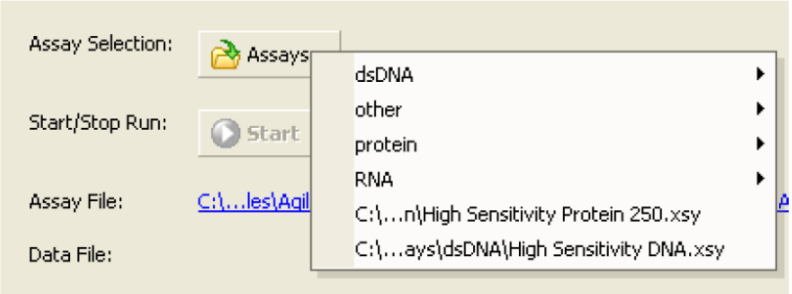
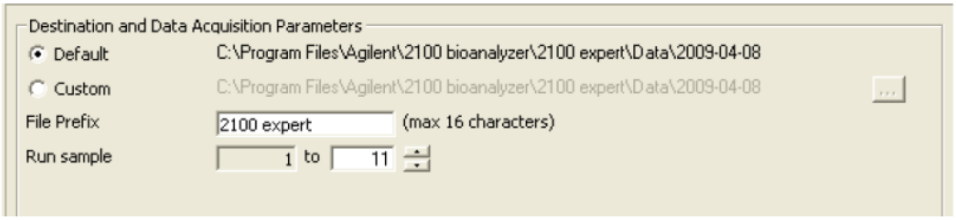
Step 8: Starting the Chip Run

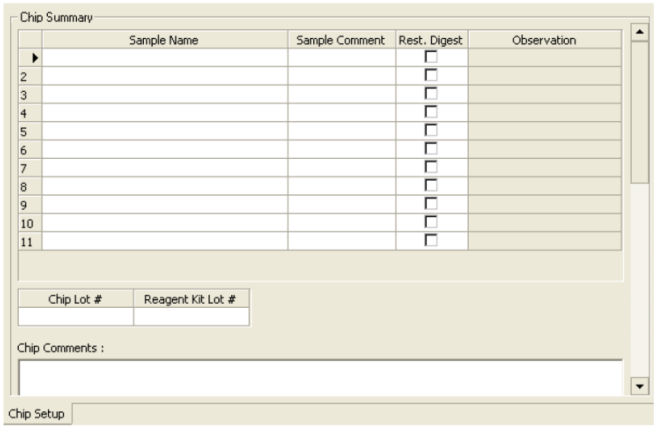
a. In the **Instrument** context, select the appropriate assay from the "Assay" menu



b. Data will be saved automatically to a file with a name using the prefix you have just entered. You can also customize the file storage location and the number of samples that will be analyzed.



c. Complete sample name table by entering sample information like sample names and comments



d. Click the **Start** button in upper right of window to start the chip run. The incoming raw signals are displayed in the **Instrument** context.

e. After the chip run is finished, remove the chip immediately and dispose it according to good laboratory practices.

Step 9: Cleaning Electrodes after a High Sensitivity DNA Chip Run

a. Slowly fill one of the wells of electrode cleaner with 350 ul deionized analysi-grade water
b. Open lid and place electrode cleaner in the 2100 Bioanalyer instrument
c. Close lid and leave it closed for about 10 – 30 s
d. Open lid and remove electrode cleaner
e. Wait another 30 s to allow water on the electrodes to evaporate before closing lid

<u>High Sensitivity DNA ladder Well Results</u>

To check results of your run, select the Gel or Electropherogram tab in the **Data** context. The electropherogram of the ladder well should resemble those shown below:

Major features of a successful ladder run are:

- 15 peaks for High Sensitivity DNA ladder
- All peaks are well resolved
- Flat baseline
- Correct identification of both markers

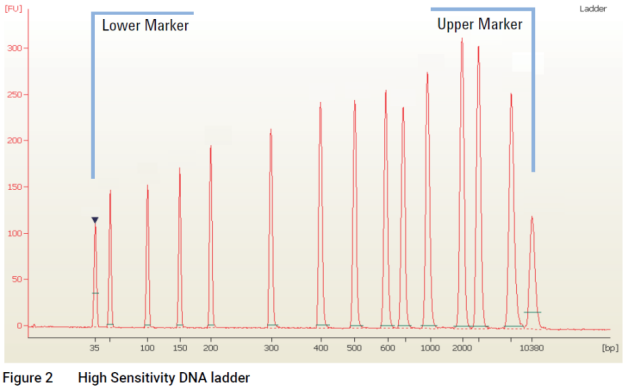


Figure 2 High Sensitivity DNA ladder

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<u>High Sensitivity DNA Sample Well Results</u>

To review the results of a specific sample, select the sample name in the tree view and highlight the *Results* sub-tab.

Typical Nextera libraries show a broad size distribution of ~ 250 – 1000 bp, as shown below.

Figure 1 Example Bioanalyzer Trace