Non Controlled Version

**Note: Controlled Versions of this document are subjected to change without notice*

Operation and Maintenance of the Agilent 2100 Bioanalyzer for DNA samples

I. Purpose

To provide specific guidelines for operating and maintaining the Agilent 2100 Bioanalyzer. This is achieved using reagents and instructions provided by Agilent to determine the quality and quantity of DNA samples

II. Scope

All procedures are applicable to the BCGSC Library Construction, Sequencing and Library TechD Groups.

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a QA associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Group Leader to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Assurance Management to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
Agilent DNA 7500 and DNA 12000 Assay kit Quick Start Guide	G2938-90025
Agilent DNA 1000 Assay Quick Start Guide	G2938-90013
Agilent High Sensitivity DNA Kit Guide	G2938-90321

VI. Related Documents

Document Title	Document Number
N/A	N/A

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the material safety data sheet (MSDS) for additional information.

VIII. Materials and Equipment

Name	Supplier	Number: #	Model or Catalogue #	
Small Autoclave waste bags 10"X15"	Fisher Scientific	01-826-4		✓
Ice bucket – Blue	Fisher	11-676-36		✓
wet ice	In house	N/A		N/A
Gilson P2 pipetman	Mandel	GF-44801		✓
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P1000 pipetman	Mandel	GF-23602		✓
Diamond Filter Tips 10ul	Mandel	GF-F171203		✓
Diamond Filter Tips 1000ul	Mandel	GF-F171703		✓
Galaxy mini-centrifuge	VWR	37000-700		✓
VX-100 Vortex Mixer	Rose Scientific	S-0100		✓
Large Kimwipes	Fisher	06-666-117		✓
Black ink permanent marker pen	VWR	52877-310		✓
Eppendorf BenchTop Refrigerated Centrifuge 5810R	Eppendorf	5810 R		✓
Agilent DNA 1000 Kit	Agilent	5067-1504		✓
Agilent DNA 1000 Reagents	Agilent	5067-1505		✓
Agilent DNA 7500 Kit	Agilent	5067-1506		✓
Agilent DNA 7500 Reagents	Agilent	5067-1507		✓
Agilent DNA 12000 Kit	Agilent	5067-1508		✓
Agilent DNA 12000 Reagents	Agilent	6067-1509		✓
Agilent High Sensitivity DNA Kit	Agilent	5067-4626		
Chip Priming Station	Agilent	5065-4401		✓
IKA Vortex Mixer	Agilent	MS2-S8	✓	
Agilent 2100 Bioanalyzer	Agilent			✓
Medicom Safetouch Nitrile gloves- large	Ultident	1137-D	✓	
Heat Block ISOTEMP 125D	Fisher		✓	
UltraPure DNase/RNase Free water	Gibco	10977-015		✓
1.5ml Eppendorf Tubes	Ambion	12400		

IX. Procedure

Note: **All Chip priming, running of the Agilent 2100 Bioanalyzer is to be completed on the 6th Floor. (Figure 1)**

Figure 1: Agilent 2100 Bioanalyzer System

1. Agilent 2100 Bioanalyzer System Determine Which Assay Is Needed Based On Sample Type and bp Fragment size

1.1. DNA samples can be analyzed on Agilent DNA 1000, DNA 7500, DNA 12000 or Agilent High Sensitivity DNA chips.

1.2. With the expected DNA sample sizing range, select the appropriate assay based on the following table:

Table 1: Determining the Agilent Chip to Use

Specification	Agilent DNA 1000	Agilent DNA 7500	Agilent DNA 12000	Agilent High Sensitivity
Sizing range	25-1000bp	100-7500bp	100-12000bp	50-7000bp
Quantitative range	0.1-50ng/µl	0.5-50ng/µl	0.5-50ng/µl	5-500pg/µl

2. Replacing the Chip Priming Station Syringe

2.1. Put on a clean pair of gloves

2.2. Unscrew the old syringes from the lid of the Chip Priming Station (see Figure 2)

2.3. Remove the plastic cap of the new syringe and insert it into the clip

2.4. Slide it into the hole of the luer lock adapter and screw it tightly to the Chip Priming Station

Figure 2. Chip Priming Station

3. Setting Up and Running the Chip

3.1. Put on a clean pair of gloves.

3.2. Remove the reagents for the desired kit from the 4°C fridge (and allow them to equilibrate to room temperature for 30 minutes before use. Put reagents in pre-made box that shelters dye and gel-dye mix from light.

3.3. Arrange samples by approximate lowest to highest concentration. Arrange in the order as they will be loaded onto the chip to prevent mix-up.

3.4. Ensure that the Chip Priming Station is set up correctly for type of chip being run. The base plate should be in position "C".

3.5. Check that the correct electrode cartridge head is being used. If the electrode cartridge head needs to be changed then it is important that the 2100 Bioanalyzer is off.

3.6. Clean the electrode before (and after) as follows:

3.6.1. Slowly fill one of the wells of the electrode cleaner chip with 350µL of fresh RNase-free water.

3.6.2. Never fill too much fluid in the electrode cleaner. This could cause liquid spill which may cause leak currents between electrodes.

3.6.3. Open the lid and place electrode cleaner chip in the Agilent 2100 Bioanalyzer.
3.6.4. Close the lid and leave it closed for 1 minute.

3.6.5. Open the lid and remove the electrode cleaner chip. Leave the lid open for 10 seconds to allow the water on the electrodes to evaporate.

3.6.6. Make sure there is a date on the cleaner chips and keep for the end of protocol cleaning. Following the end of protocol cleaning, tap out the liquid of each cleaner chip and leave upside down on a paper towel adjacent to the Bioanalyzer

3.7. Ensure the syringe clip is secured in the **very bottom position for DNA1000 assay and High Sensitivity assay**, and in the **very top position for DNA7500 and DNA12000 assay**.

4. Preparing the Gel Dye Mix

4.1. Allow DNA dye concentrate and DNA gel matrix to equilibrate to room temperature 30 minutes. Vortex and spin down.

4.2. Add DNA dye concentrate to a DNA gel matrix vial.

	DNA 1000 Assay	**DNA 7500 Assay**	**DNA 12000 Assay**	**HS DNA Assay**
DNA Dye Concentrate	25µL	25µL	25µL	15µL
DNA Gel Matrix	500µL	500µL	500µL	300µL

4.3. Vortex the solution well and spin down briefly. Transfer to spin filter.

4.4. Centrifuge for 10 minutes at room temperature.

	DNA 1000 Assay	**DNA 7500 Assay**	**DNA 12000 Assay**	**HS DNA Assay**
g	2250	1500	1500	2250
rpm	4895	4000	4000	4895

4.5. Remove the filter and label the tube as “Filtered Gel Matrix” with the date, your initials and the expiry date.

4.6. Use the filtered gel within 4 weeks. Discard afterwards.

5. Agilent 2100 Expert Software

5.1. Turn on the Agilent 2100 Bioanalyzer by flipping the switch at the rear of the instrument. A green light will come on.

5.1.1. Login to the computer supporting the Agilent 2100 Bioanalyzer and double click on the "2100 Expert" icon.

5.1.2. The screen of the software appears in the *Instrument* context.

5.1.3. Click on the *Assay Selection* icon and choose the correct assay to be run. Enter the number of samples to be run in the appropriate box.

5.1.4. Under "Destination" click on the circle adjacent to "Custom" and save the file under *R:Library Core\QC\Agilent\DNA.* Clinical Sequencing users must save to the appropriate Clinical folder on the S:\ drive as directed by the clinical SOP. Identify the chip with the next assay# by entering this new *assay#* in the file prefix section (refer to whiteboard to naming scheme: **DNA#** (Assay).) Note: This last step is not necessary for Clinical Sequencing users.

5.1.5. Change the Assay number on the whiteboard to the Assay number currently being run. Note: This step is not necessary for Clinical Sequencing users.

6. Chip Priming DNA 1000 Assay

6.1. Set a timer for the appropriate priming time.

6.2. Remove a new DNA chip from its sealed bag.

6.3. When pipetting the gel dye mix, be careful not to draw up any particles that may sit at the bottom of the tube. Pipette 9.0µL of the gel dye mix into the bottom of the well marked G (with black circle). Take care not to introduce bubbles when dispensing the gel dye mix. If bubbles are present, very gently tap the chip on the bench top or remove the gel dye mix from the well and repipette 9.0µL. The chip will not run properly if air gaps are pushed into the capillaries.

6.4. Ensure the lever clip is in the correct position. Raise the syringe plunger to the 1mL mark.

	DNA 1000 Assay	DNA 7500 Assay	DNA 12000 Assay	HS DNA Assay
Clip Postition	bottom	top	top	bottom
Priming Time	1 min	30 sec	30 sec	1 min

6.5. Place the chip onto the Chip Priming station. Lock the Chip Priming station.

6.6. Press the plunger until it is held by the clip. Start the timer.

6.7. When the priming time has finished, release the clip. Allow the plunger to rise by itself.

6.8. Wait for 5 seconds before slowly pulling the plunger back to the 1mL mark.

6.9. Remove the chip from the Chip Priming station.

7. Loading the Remaing Gel Dye Mix and DNA Marker

7.1. Pipette 9.0µL of gel dye mix into the remaining wells marked G.

7.2. Pipette 5.0 µL of DNA Marker into every sample well and the ladder well.

8. Loading the Ladder and samples

8.1. Pipette 1.0µL of DNA ladder into the ladder well.

8.2. Pipette 1.0µL of sample into each of the sample wells.

8.3. Pipette 1.0µL of DEPC-water or DNA Marker into each unused sample well.

8.4. Put the chip in the Agilent Vortex Mixer adapter and secure with labeling tape if necessary. Set speed at **2200 rpm** and vortex chip for one minute. ***A prepared chip must be loaded within 5 minutes.***

8.5. Open the lid of the Bioanalyzer and carefully place the chip into the receptacle. The chip will only fit one way.

8.6. Carefully close the lid. The electrodes fit into the well of the chip. The 2100 Expert software should display the appropriate assay chip on the screen.

8.7. Ensure that the appropriate assay has been selected.

8.8. Ensure the file will save to the appropriate directory. If unsure, check with a supervisor. Enter the ID Naming Scheme in File Prefix box - Add the EQU Number of the Agilent Bioanalyzer that is being used to analyze the sample after the DNA Chip # (DNA#XXX_EquXXX)

8.9. Check on the white board to see what the next available DNA Chip # is and enter it into the File Prefix in the following format: DNA#272_Equ2902. Note: This step is not necessary for Clinical Sequencing users.

8.10. Enter the sample information, including the Library ID, PCR cycles, Initials and any other information such as dilution if applicable, in the chip summary table. Note: This step is not necessary for Clinical Sequencing users.

8.11. Click the Start button in the upper right window. Ensure that the instrument is running properly after downloading the script prompt (ie. No error message is displayed).

8.12. If auto export is not set up, please export your agilent run to \\isaac\labinstruments\Bioanalyzer_Run\Exports

9. Shutdown and Cleaning

9.1. Immediately after the chip is finished, remove the chip from the receptacle of the Bioanalyzer and dispose of it in the waste container adjacent the instrument.

9.2. Refer to Steps 3.6.3 to 3.6.6 for the appropriate cleaning procedure.

9.3. Close the software and Log off of the computer. Turn off the monitor as well. Switch off the Agilent 2100 BioAnalyzer.

Appendix A

1. Creating Bioanalyzer Runs for your Samples

1.1. Under the Lib_Construction tab, scan in your samples and the Agilent equ# that you used .

1.2. A list will be generated of all the samples that you have scanned in.

1.3. In the "Sample Positions" table, fill in the position each sample loaded on the Agilent chip. Even though it is a 4x4 well chip, only the sample wells will be 'numbered'. So in row 1 of the chip, you have positions 1-3. Row 2 has positons 4-6 and so on. For the DNA assays, there is a maximum of 12 positions except for the HS DNA assay there is a maximum of 11 positions.

1.4. Enter in the Chip ID. If you accidentally enter in a Chip ID that was previously used for this assay, your set of samples will be stuck in "pending". The Chip ID must be entered the same format as the assay was saved. Eg DNA#1234_Equ2902.

1.5. One run is created for each sample you scanned in.

1.6. To monitor the progress and to view the run results, select the "Bioanalyzer Runs summaries" under the Summaries tab.

1.7. Your samples will show up under "Pending Runs".

1.8. Once the Runs have been validated by LIMS, your samples will show up in the "Waiting Analysis Runs" section.

1.9. Select the samples you want to analyze and select "Add Analysis Data".

1.10. Multiple attribute fields will show up for you to fill out. Depending on which stage your QC was done on your samples, you only need to fill in the fields that are applicable to your samples at that time. The value for DNA_concentration_ng_uL attribute will be from the Qubit results. Once you have finished filling out the information, select "Submit Analysis Data".

1.11. If the volumes are tracked and all relevant attribute information was entered in the previous step, then LIMS will calculate the molarity of your samples for you.

1.12. If you want to edit any of the attributes, you can click on the "Add/Edit Analysis Data" button. For example, if the molarity was not calculated for you because the volumes of your samples were not updated, you can edit the volume in LIMS, then go to the Bioanalyzer homepage and select the Run you want to edit under "Analyzed Runs".

1.13. You would need to enter the attribute values again and "Submit Analysis Data".

1.14. The run will be updated with the new values that you have entered and any calculations required will be updated as well.