

Appendix 7.2 – Agilent BioAnalyzer Operation

Date: \_\_\_\_\_

Experiment ID: 7.2-~~yyyymmdd~~

Technician: \_\_\_\_\_

Project: \_\_\_\_\_

Client Signature: \_\_\_\_\_

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1. Prepare RNA Ladder

1.1 NanoChip – Pipette 1ul aliquots and store at -70C as described in 5.1. Enter aliquots into the reagent logbook. \_\_\_\_\_

1.2 PicoChip – Dilute ladder by adding 90ul nuclease-free water. Pipette 1ul aliquots and store at -70C as described in 5.1. Enter aliquots into the reagent logbook. \_\_\_\_\_

2. Prepare the BioAnalyzer for Operation

2.1 Pipette 350ul RnaseZAP to the cleaning chip. \_\_\_\_\_

2.2 Place on the instrument and close the lid for one minute.

2.3 Remove the cleaning chip and rap sharply on paper towels to remove the RnaseZAP.

2.4 Pipette 350ul nuclease-free water to the cleaning chip. \_\_\_\_\_

2.5 Place on the instrument and close the lid for 30 seconds.

2.6 Remove the cleaning chip and leave the door open for 30 seconds.

3. Prepare the BioAnalyzer Chip. \_\_\_\_\_

Allow reagents to equilibrate to room temperature.

3.1 Add 550ul gel matrix to spin column.

3.2 Centrifuge at 1500 rcf for 10 minutes.

3.3 Transfer the flowthrough to 65ul aliquots. Aliquots can be stored at 4C for one month. Enter the aliquots into the reagent logbook. \_\_\_\_\_

3.4 Add 1ul dye to 65ul gel matrix.

3.5 Vortex and briefly centrifuge.

- 3.6 Place new chip on the priming station.**
- 3.7 Pipette 9ul gel-dye mix into the bottom of the well marked with a circled G.**
- 3.8 Set the plunger at 1ml. Close the priming station lid.**
- 3.9 Depress the plunger until it is held by the clip for 30 seconds.**
- 3.10 Release the clip. Allow the plunger to rise until it stops. Manually pull the plunger to 1ml.**
- 3.11 Remove the chip from the priming station.**
- 3.12 Pipette 9ul gel-dye mix into the two other wells marked G. Discard remaining gel-dye mix.**
- 3.13 (PicoChip only) Add 9ul conditioning solution to the well marked CS.**
- 3.14 Add 5ul RNA marker to all sample wells being used and the ladder well.  
Add 6ul RNA marker to any unused sample well.**

#### **4. Prepare RNA Samples**

- 4.1 Denature all samples and ladder at 70C for ten minutes.**
- 4.2 Pipette 1ul RNA sample and ladder into appropriate wells.**
- 4.3 Place the chip on the vortexer and run for 1 minute at 2400 rpm.**

#### **5. Run Chip on BioAnalyzer**

- 5.1 Turn on the instrument and open the software (2100 Expert).**
- 5.2 Load the chip onto the instrument.**
- 5.3 Select the appropriate assay and designate how many samples to run.**
- 5.4 Click <Start>. The instrument will take several minutes to begin.**
- 5.5 Enter sample ids into the table on the data tab.**
- 5.6 Name the data file after the experiment ID. The instrument auto-saves to a destination folder on the hard drive.**