SOP Title: Agilent DNA 7500 Quality Control	Version 1.1, Page 1
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Staff Able to Perform Procedure: Research Technician and higher

Principle of the Method:

This protocol describes the initial quality and quantification control steps when total DNA is received by the UB Next-Generation Sequencing and expression Analysis Core.

Sample Type: total DNA sample

Equipment Requirements:

- o Agilent 2100 Bioanalyzer (Agilent)
- o Bioanalyzer Chip Vortex (IKA)
- o Vortex (VWR)
- o Microcentrifuge (VWR)
- o Timer (1 hour)

Reagents & Material Requirements:

- o Quant-IT Picogreen Assay (Invitrogen)
- o PMMA Cuvettes (Fischer)
- o RNAse- free centrifuge tubes 1.5ml, 200 ul (USA Scientific)
- o Pipette Man 2ul, 20ul, 200ul, 1000ul (Rainin)
- o Filter Pipette Tips (Rainin)

Sample

o Wearing gloves, place total DNA samples at -80°C in labeled 5x5 Cryo freezer box.

Preparing the Gel-Dye Mix

- o Allow DNA dye concentrate (blue) and DNA gel matrix (red) to equilibrate to room temperature for 30 minutes.
- o Vortex DNA dye concentrate (blue) and add 25 μl of the dye to a DNA gel matrix vial (red).
- o Vortex solution well and spin down. Transfer to spin filter.
- o Centrifuge at 1500 g for 10 minutes. Protect solution from light. Store at 4°C.

Loading the Gel-Dye Mix

- o Adjust the syringe clip so it is at the top position.
- o Allow the gel-dye mix equilibrate to room temperature for 30 minutes before use.
- o Put a new DNA chip on the chip priming station.
- o Pipette 9.0 μ l of gel-dye mix in the well marked \hat{G} .
- o Make sure that the plunger is positioned at 1 ml and then close the chip priming station.
- o Press plunger until it is held by the clip.
- o Wait exactly 30 seconds then release clip.
- o Wait for 5 seconds. Slowly pull back plunger to 1 ml position.
- o Open the chip priming station and pipette 9.0 μ l of gel-dye mix in the wells marked G

Loading the Markers

o Pipette 5 µl of marker (green) in all 12 sample and ladder wells. Do not leave any wells empty.

Loading the Ladder and the Samples

- o Pipette 1 μl of DNA ladder (yellow) in the well marked ladder.
- o In each of the 12 sample wells pipette 1 μ l of sample (used wells) or 1 μ l of de-ionized water (unused wells).
- O Put the chip horizontally in the adapter and vortex for 1 minute at the indicated setting (2400 rpm).
- o Run the chip in the Agilent 2100 bioanalyzer within 5 minutes.