SOP Title: Agilent RNA 6000 Pico 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 RNA is received by the UB Next-Generation Sequencing and expression Analysis Core.

Sample Type: total RNA sample

Equipment Requirements:
- Agilent 2100 Bioanalyzer (Agilent)
- Bioanalyzer Chip Vortex (IKA)
- Vortex (VWR)
- Microcentrifuge (VWR)
- Timer (1 hour)

Reagents & Material Requirements:
- Quant-IT Ribogreen Assay (Invitrogen)
- PMMA Cuvettes (Fischer)
- RNAse- free centrifuge tubes 1.5ml, 200 ul (USA Scientific)
- Pipette Man 2ul, 20ul, 200ul, 1000ul (Rainin)
- Filter Pipette Tips (Rainin)

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

Preparing the Gel
- Pipette 550 µl of RNA 6000 Pico gel matrix (red) into a spin filter.
- Centrifuge at 1500 g for 10 minutes at room temperature.
- Aliquot 65 µl filtered gel into 0.5 ml microfuge tubes. Use filtered gel within 4 weeks.

Preparing the Gel-Dye Mix
- Allow RNA 6000 Pico dye concentrate (blue) to equilibrate to room temperature for 30 minutes.
- Vortex RNA 6000 Pico dye concentrate (blue) for 10 seconds, spin down, and add 1 µl of dye into a 65 µl aliquot of filtered gel.
- Vortex solution well.
- Spin tube at 13000 g for 10 minutes at room temperature. Use prepared Gel-Dye mix within one day.

Loading the Gel-Dye Mix
- Adjust the syringe clip so it is at the highest position.
- Put a new RNA 6000 chip on the chip priming station.
- Pipette 9.0 µl of gel-dye mix in the well marked Ⓖ.
- Make sure that the plunger is positioned at 1 ml and then close the chip priming station.
- Press plunger until it is held by the clip.
- Wait exactly 30 seconds then release clip.
- Wait for 5 seconds. Slowly pull back plunger to 1 ml position.
- Open the chip priming station and pipette 9.0 µl of gel-dye mix in the wells marked [G].
- Discard the remaining gel-dye mix.

Loading the RNA 6000 Pico Conditioning Solution and Markers
- Pipette 9.0 µl of the RNA 6000 Pico Condition Solution (white) in the well marked CS.
- Pipette 5 µl of marker (green) in all 11 sample wells and the ladder well.

Loading the Diluted Ladder and Samples
- Pipette 1 µl of the heat denatured and aliquoted ladder in the well marked ladder.

- In each of the 11 sample wells pipette 1 µl of sample (used wells) or 1 µl of RNA 6000 Pico Marker (green) (unused wells).
- Put the chip horizontally in the adapter and vortex for 1 minute at the indicated setting (2400 rpm).
- Run the chip in the Agilent 2100 bioanalyzer within 5 minutes.