Standard Operating Procedure for Agilent Bioanalyzer
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Internal Medicine IV
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Purpose
This protocol describes the required procedure for analyzing RNA using the Agilent RNA 6000 Pico Kit and Bioanalyzer

Scope
This procedural format is utilized by the contributing centres to the project `Functional Significance of Changes in Genome Wide Gene Expression Profiles Associated with Renal Aging´. Adherence to the protocol will standardize the analysis of RNA extracted from the human kidney biopsy samples collected for this project.

Materials
RNA Pico Chip
Agilent RNA 6000 Pico Reagents Part I & Part II
Chip Priming Station & syringe kit
Heating block set to 70°C
IKA vortexer
Electrode cleaner chips
RNase-free water
RNase ZAP
RNase-free microfuge tubes and pipette tips

Procedure
The following procedure is adapted from the Agilent RNA 6000 Pico Assay Protocol, April 2007.
The dye is light-sensitive, take care to minimize exposure of dye and dye mixtures to light.

Preparing the Gel and Gel-Dye mix
- Pipette 550 ul of RNA 6000 Pico Matrix gel (red lid) into a spin filter and centrifuge at room temperature for 10 minutes at 1500 xg. Aliquot 65 ul filtered gel into 0.5 ul RNase-free microfuge tubes and store for up to 4 weeks at 4°C.
- Allow the RNA 6000 Pico dye concentrate (blue lid) to equilibrate to room temperature for 30 minutes, vortex for 10 seconds and zip spin. Add 1 ul of dye to 65 ul aliquot of filtered gel. Vortex mixture thoroughly and centrifuge for 10 minutes at 13000 xg.
- Use the gel-dye mix within one day.

Preparing the Sample
- Use 1 ul of RNA sample diluted with RNase-free water to a concentration on approximately 1 ng / ul.
- Denature RNA samples at 70°C for 2 minutes and store on ice until ready to load onto the chip.
- The RNA ladder also needs to be heat denatured (2 min at 70°C), this can be done once and the ladder stored in aliquots at -80°C ready to use.

Preparing the Pico Chip
- Place a new Pico chip on the chip priming station and pipette 9 ul of gel-dye mix into the well marked ● containing the letter G. Set the syringe plunger to the 1ml mark and close the chip priming station.

- Press the plunger until it is held by the clip, wait for exactly 30 seconds then release the clip.
- After 5 seconds slowly return plunger to 1 ml position and open the chip priming station.
- Pipette 9 ul of gel-dye mix into the 2 wells marked G and discard the remaining gel-dye mix.

Loading the Pico Chip

- Add 9 ul of the RNA 6000 Pico Conditioning Solution (white lid) to the well marked CS.
- Add 5 ul of RNA 6000 Pico marker (green lid) to all 11 sample wells and the well marked with a ladder.
- Add 1 ul of denatured ladder well and 1 ul of denatured sample into the appropriate wells (ensure the pipette tip is reaching the bottom of the well when dispensing samples). Into any unused sample wells add 1 ul of RNA 6000 Pico Marker.
- Place the loaded chip into the adapter on the IKA vortexer and vortex for 1 minute at 2400 rpm. Run the chip in the Agilent Bioanalyzer within 5 minutes.

Operating the Bioanalyzer

- Turn on the Bioanalyzer and click on the 2100 expert icon on the attached computer.
- Click on Assays button to reveal the menu and select Eukaryote Total RNA Pico
- Place the loaded Pico chip into the Bioanalyzer and close the lid, a tick should appear in the start run checklist next to 'Is a chip detected?'.
- Click on Start button, the run takes around 25 minutes.
- Save the results as a PDF file using the option in the print menu.
- Discard the used Pico chip. Clean the electrodes after a run by pipetting 350ul Rnase-free H_2O into the Electrode cleaner chip, place chip in Bioanalyzer and close lid. Wait for 30 seconds, remove chip and wait for 30 seconds for the electrode pins to dry before closing the lid.