**Agilent RNA Pico 6000** (04 April 2023; Scott Herke)

**Cleaning the Electrodes before Running Assays**

1. Unless RNAse contamination is expected, skip to Step 5, with 5’ incubations.

*If RNAse contamination is suspected, do Steps 2-4 before running any RNA Pico assays.*

1. Slowly fill **RNAseZAP electrode cleaner** with 340 µl of fresh RNaseZAP.
2. Place electrode cleaner in Bioanalyzer, close lid, and incubate (5 min). \_\_\_|
3. Remove electrode cleaner, flick out RNAseZAP, and store cleaner chip.

>> Repeat Steps 5-7, ≥4 times:

1. Slowly fill the **RNA-WATER electrode cleaner** with 340 µl of fresh RNase-free water.
2. Place electrode cleaner in Bioanalyzer, close lid, & incubate.
   1. Normal procedure: \_\_\_ 5 minute incubations.
   2. RNAseZAP procedure: \_\_\_ 10 minute incubations. \_\_\_|\_\_\_| \_\_\_| \_\_\_|
3. Remove electrode cleaner, flick out water.

>> After the 3-4X repetitions, store cleaner chip.

1. Leave lid open (5 min) to allow water to evaporate from electrodes.

**Basic start-up procedures:**

1. Before working with the chip, clean work areas with 80% EtOH and then with RNAseZAP.
2. Start software before you load the chip; verify that a connection is live.
3. Adjust syringe clip: Release clip lever and slide it up to the **TOP** position.
4. Inspect white seal of chip priming station to ensure no old gel is plugging the hole; clean if needed.
5. Allow all reagents to equilibrate to room temperature for 30 minutes before use.
6. Protect dye and dye mixtures from light.
7. Keep samples on ice.
8. RNA ladder: remove heat-denatured ladder aliquot from -70°C freezer, and thaw on ice... do NOT allow ladder to warm extensively.
9. Heat-denature (70°C, 2’) RNA samples & snap-chill, unless samples might have ‘[hidden breaks](https://genomics.lsu.edu/genomics-sacks-main.php#Agilent-Pico-2)’... in which case, it might be best to skip the denaturation step.
10. Always vortex dye concentrate (10 s) before preparing the gel- dye mix and spin down afterwards.
11. Set the timer to 30 s *(not 1 minute, as for the DNA-HS assay)* and vortexer to 2000.

**LADDER PREPARATION**: Area under ladder is used to quantitate samples... dilute correctly!

To avoid RNase contamination and repetitive freeze/thaw cycles, RNA ladder must be aliquoted in RNase-free vials.

➔ Some plastics can bind RNA. This can affect RNA ladder concentration which affects the ladder identification and the quantitation of the sample. Use Eppendorf Safe-Lock PCR clean or Eppendorf DNA LoBind 0.5ml Microcentrifuge tubes for RNA ladder aliquoting.

After reagent kit arrival, prepare a work area by cleaning with 80% EtOH and then RNAseZAP, and then...

1. Spin ladder down and transfer to 0.2-ml Axygen DNA LoBind PCR tube.
2. Heat denature the ladder for 2 min (70°C) in thermal cycler.
3. Immediately cool the vial on ice.
4. Following the heat-denaturation, add 90 µL of RNase- free water and mix thoroughly.
5. Prepare aliquots in 0.5 mL RNase- free vials (30) with 3.2 ul (each) for typical daily use.
6. Store aliquots at -70°C.
7. Before use, thaw ladder aliquots and keep on ice (avoid extensive warming upon thawing process).

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**Preparing gel:** (Note: If the previously prepared gel is >1 month old, repeat the filtering step.)

1. Add 550 ul gel to spin filter and centrifuge (10’, @ 1200-1800 rcf).
2. Aliquot 65 µl filtered gel into a 0.5 ml RNase- free microcentrifuge tube.
3. Store remainder in the 1.5-ml tube at 4°C.

**Adding dye concentrate to gel:**

1. Vortex RNA 6000 Pico dye concentrate (blue) for 10 seconds and spin down.
2. Add 1 µl of RNA 6000 Pico dye concentrate (blue) to a 65 µl aliquot of filtered gel.
   * Return dye concentrate to 4 °C (dark).
3. Cap tube, vortex thoroughly and visually inspect proper mixing of gel and dye.
4. Spin tube (10’ @ RT, 13000 rcf).
5. Use prepared gel-dye mix within 1 day (discard if not used).

**Preparing samples and Ladder:**

1. To minimize 2o-structure, heat-denature (70°C, 2’) & snap-chill samples before loading on chip.

*(Note: If samples might have ‘*[*hidden breaks*](https://genomics.lsu.edu/genomics-sacks-main.php#Agilent-Pico-2)*’, it might be best to skip the denaturation step.)*

1. Thaw ladder aliquot and keep on ice (avoid extensive warming upon thawing process).
2. Pipette 1 µl of the diluted RNA 6000 Pico ladder into a 0.2-ml tube.
3. Pipette 1 µl of each sample (or water) into 11 tubes (0.2-ml).
4. Centrifuge 12 tubes; add 5 ul RNA 6000 Pico marker (green) to each tube; briefly vortex; touch-spin.

**Loading the chip:**

1. Adjust syringe clip: Release clip lever and slide it up to the **TOP** position.
2. Inspect white seal of chip priming station to ensure no old gel is plugging the hole.
3. Remove a new RNA chip from bag and place it on the chip priming station.
4. Pipette 10.0 µl of gel-dye mix at bottom of ‘loading’ well.
   1. *When pipetting gel-dye mix, do NOT draw up particles that may sit at the bottom of the gel-dye mix vial.*
   2. *To prevent forming a large air bubble under the gel-dye mix, insert tip of pipette to bottom of chip well when dispensing. (Placing pipette at edge of well may lead to poor results.)*
5. Set timer (30 s), make sure that plunger is positioned at 1 ml and then close chip priming station.
6. Press syringe plunger down until it is held by the clip.
7. Wait for exactly 30 seconds and then release plunger with the clip release mechanism.
8. Visually inspect that the plunger moves back at least to the 0.3 ml mark almost immediately.
9. Wait for 5-10 seconds, then slowly pull back plunger to 1 ml position.
10. Open chip priming station.
11. Pipette 10.0 µl of gel-dye mix into the other two “G” wells.
12. Pipette 10 µl of RNA 6000 Pico conditioning solution (white) into the “CS” well.
13. Pipette samples/ladder into the 12 wells. *(Note: Do not leave any wells empty or the chip will not run properly.*

**Final chip preparation**

1. Place chip in IKA vortex mixer (ensuring it is flat and securely held); vortex (60 s, 2000 rpm).
2. Centrifuge chip (30 s, 100 rcf) in a Plate Centrifuge (using a [cardboard](https://genomics.lsu.edu/images/genomics/agilent-bioanalyzer-chip-holder-cardboard.jpg) or [3D-printed](https://genomics.lsu.edu/images/genomics/agilent-bioanalyzer-chip-holder-3d-printed.jpg) ‘chip holder’; see also [webpage](https://genomics.lsu.edu/genomics-sacks-main.php#Agilent-2)).
3. Place chip in Bioanalyzer, close lid, and start the run (within 5’ of chip loading).
4. After the run is finished, remove chip and clean [2X] electrodes (steps 5-7 of Cleaning procedure).

Do not leave chip on Bioanalyzer for >1 hour, or electrodes may need vigorous cleaning to remove dried contaminates.