BioAnalyzer RNA analysis

BioAnalyzer RNA analysis (Quick start, Manual)

Preparation

Item	Amount	Location
Nano Chips $^{\alpha}$		VI
RNA Nano Chips	25	RNA bench, Reuse
Electrode Cleaners	2	RNA bench, Reuse
Reagents $^{\beta}$		−80°C (upper door-2-1-4)
(green) RNA 6000 Nano Marker	2 vials	
(blue) RNA 6000 Nano Dye Concentrate	1 vial	
(red) RNA 6000 Nano Gel Matrix	2 vials	
Supplies $^{\gamma}$		−80°C (upper door-2-1-4)
(yellow) RNA 6000 Nano Ladder	1 vial	
Chip Priming Station $^{\delta}$	1 unit	Lab 236 middle bench
Syringe $^{\varepsilon}$	1 unit	Lab 236 middle bench

$^{\alpha}$ **Nano Chips**: Agilent RNA 6000 Nano Kit (5067-1511)

$^{\beta}$ **Reagents**: Agilent RNA 6000 Nano Reagents (5067-1512)

- The marker is transparent color, we already aliquoted it as 75 µL per vial (marked as "M").
- The dye concentrate is blue color, we already aliquoted it as 2 µL per vial (marked as "D")
- The gel matrix is transparent color, put in the same pack with the dye concentrate. We already aliquoted it as 65 µL per vial (marked as "G")

$^{\gamma}$ **Supplies**: Agilent RNA 6000 Nano Ladder (5067-1529)

- The ladder is transparent color, we already aliquoted it as 2 µL per vial

$^{\delta}$ **Chip Priming Station**: Bioanalyzer Parts & Accessories (5065-4401)

$^{\varepsilon}$ **Syringe Kit**: Syringe Kit (G2938-68706)

Procedure

All instruments should be sterilized prior to the experiment

Step 1: Gel-Dye Mix

One set of Gel-Dye Mix is for one chip (12 wells).

1. Allow the **RNA dye concentrate** (🔵) equilibrate to room temperature (**25°C**) for **30 mins**.
2. Vortex the **RNA dye concentrate** (🔵) for **10 secs**, spin down and add **1 µL dye** into a **65 µL gel matrix aliquot**.
3. Vortex solution well. Centrifuge (**13,000 g**, **10 mins**) at 25°C. Use the prepared **Gel-Dye Mix** within one day.

Step 2: Decontaminate the instrument electrode

1. Pipette **350 µL RNaseZap™ solution** into an Electrode Cleaner (we reuse this, marked as "RNaseZap"). Close the machine lid to submerge the electrode probe into the solution for **3 mins**.
2. Pipette **350 µL RNase-free water** into an Electrode Cleaner (we reuse this, marked as "RNase-free water"). Close the machine lid to submerge the electrode probe into the water for **3 mins**.
3. Keep the lid opened for **1 min** to **air-dry** the electrode.

> During this period, switch on the PC and execute the `2100 Expert` software.

Step 3: Load Gel-Dye Mix

1. Put a new RNA Nano Chip on the Chip Priming Station.
2. Pipette **9 µL** of **Gel-Dye Mix** into the well marked Ⓖ (only one well).
3. Make sure the **plunger** is **positioned at 1 mL** and then close the Chip Priming Station. Make sure you hear a click sound to confirm that the latch is securely snapped.
4. Press the plunger until it is held by the clip.
5. Wait for exactly **30 secs** then release clip.
6. Wait for **5 secs**. Slowly **pull back plunger** to **1 mL position**. Open the Chip Priming Station.
7. Pipette **9 µL** of **Gel-Dye Mix** into the wells marked Ⓖ (two wells).
8. Discard the remaining Gel-Dye Mix.

Step 4: Load Marker

1. Pipette **5 µL** of **RNA marker** in all 12 sample wells.

2. Pipette **5 µL** of **RNA marker** in the ladder well marked with a ladder symbol 🪜 (only one well).

- We prepared 75 µL marker per vial. So now we already load in 65 µL into the chip. The remaining 10 µL is, for the empty sample wells, in case you don't have 12 RNA samples. The machine requires all sample wells must have same volume.

Step 5: Load Ladder

1. Pipette **1 µL** of **RNA ladder** in the ladder well marked with a ladder symbol 🪜 (only one well).

Step 6: Load samples RNA

1. Pipette **1 µL** of **sample** in each of the 12 sample wells.

> - substitute with **1 µL Marker** for unused sample wells.

2. Put the chip **horizontally** in the **IKA vortexer** and vortex for **1 min** at **2400 rpm**. Hover your hand over the chip because it might fly out due to an overly strong vortex.

3. Run the chip in the Agilent 2100 Bioanalyzer instrument within 5 mins.

Step 7: On-machine

1. Put the chip into the slot (the shape of the upper right corner of the chip should be matched). Close the machine lid gently to submerge the electrode into the chip.

2. Execute the `2100 Expert` software.

3. Navigate to `Assays` → `RNA` → `Plant RNA Nano.xsy`.

4. Key in your sample name / ID to the list at lower left side.

5. Make sure the `Start Run Checklist` at the lower right side are all checked.

6. Press `Start` and wait for 5 ~ 10 mins for the machine checking.

7. Wait for ≈ 40 mins for the chip to be read.

8. After finishing, discard the chip and clean up the electrode as the procedure in **Step 2: Decontaminate the instrument electrode**. You can skip the RNaseZap step.

9. Check and save all the data with a single PDF report, use `File` → `Print`

> - Quick look at RIN value, `Data` → `Chip summary`
> - Quick look at RNA concentration, `Data` → `Electropherogram`

Done !!!

If you encounter any issues with the protocol, please report them at:
https://github.com/P10911004-NPUST/jklai/issues.