EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

# EIGC.006_Quality Control using the Agilent Bioanalyzer

## Standard Operating Procedure Staff Review Page:

**I have read and understand the procedure listed above.**

| Employee name | Date SOP review complete |
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# Standard Operating Procedure Approval Page:

Date Implemented: 1 May 2005

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| Updated: | Ashima Amin<br>**Name** | 15 June 2020<br>**Date** |
| Supersedes: | Ashima Amin<br>**Name** | 14 April 2016<br>**Date** |

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

**Changes Made:** Review, Renumbered SOP. Previously EIGC.007

***Changes to previous procedures:***
N/A

***Agilent Bioanalyzer Usage, Troubleshooting, and Maintenance***

***Note: No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.***

## Instrument Principles

The Agilent 2100 Bioanalyzer is a microfluidics-based platform for sizing, quantification and quality control of DNA, RNA, proteins and cells.

The Agilent Bioanalyzer (Nano and Pico) uses chip-based electrophoresis to examine the 18S and 28S ribosomal RNA peaks to determine the amount of degradation in the RNA sample.

The Bioanalyzer DNA chips are ideal for automated sizing and quantification of PCR products, restriction digests or fragmented DNA samples.

## Specimen Types

RNA and DNA

# Agilent RNA 6000 Nano

## Purpose

Quantitative and qualitative analysis of RNA samples concentrated 25-250 ng/µl.

## Quality Control

An RNA ladder is run as a control to determine if the fluorescence is appropriate, and if the gel resolution is adequate.

## Safety

Powder-free gloves should be worn.

| **Agilent RNA 6000 Nano Reagents Kit _Agilent (Cat# 5067-1511)** | |
|---|---|
| RNA 6000 Nano Chip | Room Temperature |
| RNA 6000 Nano Gel Mix | 4°C |
| RNA 6000 Nano Marker | 4°C |
| RNA 6000 Nano Dye Concentrate | 4°C |
| RNA 6000 Nano Ladder | -80°C |

## Preparing the RNA Ladder

1. Spin the ladder down and pipette into an RNase-free vial.
2. Heat denature the ladder for 2 min at 70 °C.
3. Immediately cool the vial on ice.
4. Prepare aliquots in recommended 0.5 mL RNase-free vials with the required amount for typical daily use. (3 µL)
5. Store aliquots at -80 °C. After initial heat denaturation, the frozen aliquots should not require repeated heat denaturation.
6. Before use, thaw ladder aliquots on ice (avoid extensive warming).

## Preparing the Gel

1. Pipette 550 µL of RNA gel matrix (red ●) into a spin filter.
2. Centrifuge at 1500g ± 20% for 10 min at room temperature. Use filtered gel within 4 weeks. Store at 4 °C. Write date and initials on filtered gel.

## Preparing the Gel-Dye Mix


1. Allow all reagents (except ladder) to equilibrate to room temperature for 30 min.
2. Vortex RNA dye concentrate (blue ●) for 10 s, spin down and mix 1 µL of dye with 65 µL of filtered gel.
3. Vortex solution well. Spin tube at 13000g for 10 min at room temperature. Use prepared gel-dye mix within one day.

## Loading the Gel-Dye Mix

1. Make sure that the chip priming station base is positioned at C (check user manual for assistance).
2. Release the lever of the syringe clip and slide it up to the top position.
3. Put a new RNA chip on the chip priming station.
4. Pipette 9 µL of gel-dye mix in the well marked G.
5. Make sure that the plunger is positioned at 1 mL and then close the chip priming station.
6. Press plunger until it is held by the clip.
7. Wait for exactly 30 s then release clip.
8. Wait for 5 s. Slowly pull back plunger to 1 mL position.
9. Open the chip priming station and pipette 9 µL of gel-dye mix in the wells marked G.

## Loading the Marker

1. Pipette 5 µL of RNA marker (green ●) in the well marked and in each sample well to be used.
2. Pipette 6 µL of RNA marker (green ●) in each unused sample well.

## Loading the Ladder and Samples

1. Pipette 1 µL of prepared ladder in well marked .
2. Pipette 1 µL of sample in the sample wells.
3. Put the chip horizontally in the IKA vortexer and vortex for 1 min at 2400 rpm.

4. Run the chip in the Agilent 2100 Bioanalyzer instrument within 5 min.

### Using the Expert Software

1. Double click on Expert to start the software.
2. In instrument context, select the correct assay/method - RNA Nano total (eukaryotic, prokaryotic, or plant) or mRNA.
3. Select the number of samples (1-12). Enter sample IDs.
4. Press Start once the chip is loaded.
5. Wait until the run is complete (30 min).
6. Save the data as a chip data file and as a PDF file in the PPMS Projects folder.

### Wash the Electrodes

1. Fill an electrode cleaner with 350 µL RNAse-free $H_2O$.
2. Place electrode cleaner in the Agilent bioanalyzer.
3. Close the lid for 1 minute.
4. Open the lid, remove the electrode cleaner, and leave the lid open for 1 minute.

### Record Keeping

A data folder is produced with the corresponding date of the run and stored indefinitely on the Bioanalyzer computer.

### Analysis/Interpretation

Concentration is displayed in ng/µl. The quality of RNA is determined based on an electropherogram and RIN (RNA integrity number). The RIN of 8-10 is considered good, while 6.5-8 is marginal. All samples with the RIN number below 6.5 are considered of poor quality.

### Benefits/Limitations

The quality and quantity of RNA are required for proper execution of all downstream experiments. One of the major limitations of this procedure is that 1 µL of sample needs to be loaded in the wells of the chip. If the sample is very dilute, the bands are not properly detected.

### Troubleshooting

See user's manual.

# Agilent RNA 6000 Pico

## Purpose
Quantitative and qualitative analysis of RNA samples concentrated 250-5000 pg/µL.

## Quality Control
An RNA ladder is run as a control to determine if the fluorescence is appropriate, and if the gel resolution is adequate.

## Safety
Powder-free gloves should be worn.

| **Agilent RNA 6000 Pico Reagents Kit _ Agilent (Cat# 5067-1513)** | |
|---|---|
| RNA 6000 Pico Chip | Room Temperature |
| RNA 6000 Pico Gel Mix | 4°C |
| RNA 6000 Pico Marker | 4°C |
| RNA Pico Dye Concentrate | 4°C |
| RNA 6000 Pico Conditioning Solution | 4°C |
| RNA 6000 Pico Ladder | -80°C |

## Preparing the RNA Ladder

1. Spin the ladder down and pipette in an RNase-free vial.
2. Heat denature the ladder for 2 min at 70 °C.
3. Immediately cool the vial on ice.
4. Prepare aliquots in recommended 0.5 mL RNase-free vials with the required amount for typical daily use. (5 µL)
5. Store aliquots at -80 °C. After initial heat denaturation, the frozen aliquots should not require repeated heat denaturation.
6. Before use, thaw ladder aliquots on ice (avoid extensive warming).

## Preparing the Gel

1. Pipette 550 µL of RNA gel matrix (red ●) into a spin filter.
2. Centrifuge at 1500g ± 20 % for 10 min at room temperature. Use filtered gel within 4 weeks. Label filtered gel tubes with date and initials. Store at 4 °C.

## Preparing the Gel-Dye Mix


1. Allow all reagents (except ladder) to equilibrate to room temperature for 30 min.
2. Vortex RNA dye concentrate (blue ●) for 10 s, spin down and mix 1 µL of dye with 65 µL of filtered gel.
3. Vortex solution well. Spin tube at 13000g for 10 min at room temperature. Use prepared gel-dye mix within one day.

## Loading the Gel-Dye Mix

1. Make sure that the chip priming station base is positioned at C (check user manual for assistance).
2. Release the lever of the syringe clip and slide it up to the top position.
3. Put a new RNA chip on the chip priming station.
4. Pipette 9 µL of gel-dye mix in the well marked G.
5. Make sure that the plunger is positioned at 1 mL and then close the chip priming station.
6. Press plunger until it is held by the clip.
7. Wait for exactly 30 s then release clip.
8. Wait for 5 s. Slowly pull back plunger to 1 mL position.
9. Open the chip priming station and pipette 9 µL of gel-dye mix in the wells marked G.

## Loading the Conditioning Solution and Marker

1. Pipette 9 µL of the RNA conditioning solution (white ○) into the well marked CS.
2. Pipette 5 µL of RNA marker (green ●) in the well marked and in each sample well to be used.
3. Pipette 6 µL of RNA marker (green ●) in each unused sample well.

## Loading the Ladder and Samples

1. Pipette 1 µL of prepared ladder in well marked .
2. Pipette 1 µL of sample in the sample wells.

3. Put the chip horizontally in the IKA vortexer and vortex for 1 min at 2400rpm.
4. Run the chip in the Agilent 2100 Bioanalyzer instrument within 5 min.

### Using the Expert Software

1. Double click on Expert to start the software.
2. In instrument context, select the correct assay/method - RNA Pico total (eukaryotic, prokaryotic, or plant) or mRNA.
3. Select the number of samples (1-11) and enter sample IDs.
4. Press Start once the chip is loaded.
5. Wait until the run is complete (30 min).
6. Save the data as a chip data file and as a PDF file in the PPMS Projects folder.

### Wash the Electrodes

1. Fill an electrode cleaner with 350 µL RNAse-free $H_2O$.
2. Place electrode cleaner in the Agilent bioanalyzer.
3. Close the lid for 1 minute.
4. Open the lid, remove the electrode cleaner, and leave the lid open for 1 minute.

### Record Keeping

A data folder is produced with the corresponding date of the run and stored indefinitely on the Bioanalyzer computer.

### Analysis/Interpretation

Concentration is displayed in ng/µl. The quality of RNA is determined based on an electropherogram and RIN (RNA integrity number). The RIN of 8-10 is considered good, while 6.5-8 is marginal. All samples with the RIN number below 6.5 are considered of poor quality.

### Benefits/Limitations

The quality and quantity of RNA are required for proper execution of all downstream experiments. One of the major limitations of this procedure is that 1 µL of sample needs to be loaded in the wells of the chip. If the sample is very dilute, the bands are not properly detected.

### Troubleshooting

See user's manual.

# Agilent DNA 1000

## Purpose

Quantitative and qualitative analysis of PCR samples or NGS libraries concentrated 0.5-50 ng/µL with fragment sizes 25-1000 bp.

## Quality Control

A DNA ladder is run as a control to determine if the fluorescence is appropriate, and if the gel resolution is adequate.

## Safety

Powder-free gloves should be worn.

| **Agilent DNA 1000 Reagents Kit _ Agilent (Cat 5067-1504)** | |
|---|---|
| DNA 1000 Chip | Room Temperature |
| DNA 1000 Ladder | 4°C |
| DNA 1000 Marker | 4°C |
| DNA Dye Concentrate | 4°C |
| DNA Gel Matrix | 4°C |

## Preparing the Gel-Dye Mix

1. Allow all reagents to equilibrate to room temperature for 30 min.
2. Add 25 µL of DNA dye concentrate (blue 🔵) to a High Sensitivity DNA gel matrix vial (red 🔴).
3. Vortex solution well and spin down. Transfer to spin filter.
4. Centrifuge at 2240 g ± 20 % for 15 min. Protect solution from light. Label gel-dye with date and initials. Store at 4 °C.
5. Use prepared gel-dye mix within 6 weeks of preparation.

## Loading the Gel-Dye Mix

1. Make sure that the chip priming station base is positioned at C (check user manual for assistance).
2. Release the lever of the syringe clip and slide it down to the bottom position.

3. Put a new High Sensitivity DNA chip on the chip priming station.
4. Pipette 9 µL of gel-dye mix in the well marked G.
5. Make sure that the plunger is positioned at 1 mL and then close the chip priming station.
6. Press plunger until it is held by the clip.
7. Wait for exactly 1 min then release clip.
8. Wait for 5 s. Slowly pull back plunger to 1 mL position.
9. Open the chip priming station and pipette 9 µL of gel-dye mix in the wells marked G.

### Loading the Marker

1. Pipette 5 µL of marker (green ●) in the well marked and in each sample well to be used.
2. Pipette 6 µL of marker (green ●) in each unused sample well.

### Loading the Ladder and Samples

1. Pipette 1 µL of High Sensitivity DNA ladder (yellow ●) in well marked .
2. Pipette 1 µL of sample in the sample wells.
3. Put the chip horizontally in the adapter and vortex for 1 min at the indicated setting (2400 rpm).
4. Run the chip in the Agilent 2100 Bioanalyzer instrument within 5 min.

### Using the Expert Software

1. Double click on Expert to start the software.
2. In instrument context, select the correct assay/method – High Sensitivity DNA.
3. Select the number of samples (1-12) and enter sample IDs.
4. Press Start once the chip is loaded.
5. Wait until the run is complete (35 min).
6. Save the data as a chip data file and as a PDF file in the PPMS Projects folder.

### Wash the Electrodes

1. Fill an electrode cleaner with 350 µL RNAse-free $H_2O$.
2. Place electrode cleaner in the Agilent bioanalyzer.
3. Close the lid for 1 min.
4. Open the lid, remove the electrode cleaner, and leave the lid open for 1 min.
5. Wait 15 min before running another chip.

### Record Keeping

A data folder is produced with the corresponding date of the run and stored indefinitely on the Bioanalyzer computer.

### Analysis/Interpretation

The quality of DNA is determined based on an electropherogram. The software also calculates the DNA concentration based on the ladder (displayed in ng/µL).

### Troubleshooting

See user's manual.

# Agilent High Sensitivity DNA

## Purpose

Quantitative and qualitative analysis of PCR samples concentrated 5-500 pg/µL or NGS libraries concentrated 100 pg/µL to 10 ng/µL with fragment sizes 50-7000 bp.

## Quality Control

A DNA ladder is run as a control to determine if the fluorescence is appropriate, and if the gel resolution is adequate.

## Safety

Powder-free gloves should be worn.

| **Agilent High Sensitivity DNA Reagents Kit _ Agilent (Cat# 5067-4626)** | |
|---|---|
| High Sensitivity DNA Chip | Room Temperature |
| High Sensitivity DNA Ladder | 4°C |
| High Sensitivity DNA Marker | 4°C |
| High Sensitivity DNA Dye Concentrate | 4°C |
| High Sensitivity DNA Gel Matrix | 4°C |

## Preparing the Gel-Dye Mix

1. Allow all reagents to equilibrate to room temperature for 30 min.
2. Add 15 µL of High Sensitivity DNA dye concentrate (blue 🔵) to a High Sensitivity DNA gel matrix vial (red 🔴 ).
3. Vortex solution well and spin down. Transfer to spin filter.
4. Centrifuge at 2240 g ± 20 % for 15 min. Protect solution from light. Label gel-dye with date and initials. Store at 4 °C.
5. Use prepared gel-dye mix within 6 weeks of preparation.

## Loading the Gel-Dye Mix

1. Make sure that the chip priming station base is positioned at C (check user manual for assistance).
2. Release the lever of the syringe clip and slide it down to the bottom position.
3. Put a new High Sensitivity DNA chip on the chip priming station.
4. Pipette 9 µL of gel-dye mix in the well marked G.
5. Make sure that the plunger is positioned at 1 mL and then close the chip priming station.
6. Press plunger until it is held by the clip.
7. Wait for exactly 1 min then release clip.
8. Wait for 5 s. Slowly pull back plunger to 1 mL position.
9. Open the chip priming station and pipette 9 µL of gel-dye mix in the wells marked G.

## Loading the Marker

1. Pipette 5 µL of marker (green ●) in the well marked and in each sample well to be used.
2. Pipette 6 µL of marker (green ●) in each unused sample well.

## Loading the Ladder and Samples

1. Pipette 1 µL of High Sensitivity DNA ladder (yellow ●) in well marked .
2. Pipette 1 µL of sample in the sample wells.
3. Put the chip horizontally in the adapter and vortex for 1 min at the indicated setting (2400 rpm).
4. Run the chip in the Agilent 2100 Bioanalyzer instrument within 5 min.

## Using the Expert Software

1. Double click on Expert to start the software.
2. In instrument context, select the correct assay/method – High Sensitivity DNA.
3. Select the number of samples (1-11) and enter sample IDs..
4. Press Start once the chip is loaded.
5. Wait until the run is complete (45 min).
6. Save the data as a chip data file and as a PDF file in the PPMS Projects folder.

## Wash the Electrodes

1. Fill an electrode cleaner with 350 µL RNAse-free $H_2O$.
2. Place electrode cleaner in the Agilent bioanalyzer.
3. Close the lid for 1 min.

4. Open the lid, remove the electrode cleaner, and leave the lid open for 1 min.
5. Wait 15 min before running another chip.

*Don't use chips from different assays, just the ones specific for each assay.

### Record Keeping

A data folder is produced with the corresponding date of the run and stored indefinitely on the Bioanalyzer computer.

### Analysis/Interpretation

The quality of DNA is determined based on an electropherogram. The software also calculates the DNA concentration based on the ladder (displayed in ng/μL).

### Troubleshooting

See user's manual.