Assessing the quality of RNA using the Agilent 2100 Bioanalyzer

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1.0 Title
Assessing the Quality of RNA Using the Agilent 2100 Bioanalyzer

2.0 Purpose
To prepare and run Agilent Bioanalyzer 2100 in order to assess the quality of RNA before processing it for Gene Expression Profiling

3.0 Definitions and Abbreviations
RNA Ribonucleic acid
DNA Deoxyribonucleic acid
PBS Phosphate Buffered saline

4.0 Equipment / Reagents

Equipment

<table>
<thead>
<tr>
<th>Name</th>
<th>Vendor</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Agilent 2100 Bioanalyzer</td>
<td>Agilent Technologies Inc.</td>
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<tr>
<td>4.2 Chip priming station</td>
<td>Agilent Technologies Inc.</td>
<td>5065-4401</td>
</tr>
<tr>
<td>4.3 IKA Vortex</td>
<td>IKA</td>
<td>Model MS2-S8 / MS2-S9</td>
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<tr>
<td>4.4 Pipettes</td>
<td>Can be chosen by the Lab</td>
<td></td>
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<tr>
<td>4.5 0.5 ml and 1.5 ml Tubes</td>
<td>Can be chosen by the Lab</td>
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<td>4.6 Micro-centrifuge</td>
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<td>4.7 Heating Block</td>
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<tr>
<td>4.8 16-pin Electrode Cartridge</td>
<td>Agilent Technologies, Inc.</td>
<td>5065-4413</td>
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Reagents

<table>
<thead>
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<th>Name</th>
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<tr>
<td>4.9 RNA 6000 Nano LabChip Kit</td>
<td>Agilent Technologies, Inc.</td>
<td>5065-4476</td>
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</tbody>
</table>
4.10 RNA 6000 Nano reagents and Supplies  Agilent Technologies, Inc.  5065-4475
4.11 RNA 6000 ladder  Ambion, Inc.  7152
4.12 RNaseZAP®  Ambion, Inc.  9780
4.13 RNase-free water

5.0 Procedures

Note: This protocol should serve as a reference ONLY. For more details please refer to the Reagent Kit Guide “RNA 6000 Nano Assay” (Agilent Technologies Inc., part No. G2941-90126)

5.1 Setting up all necessary equipment

5.1.1 Setting up the chip priming station

5.1.1.1 Replace the Syringe with each new Reagent Kit!

5.1.1.2 Before use, make sure that the base-plate is adjusted properly.
5.1.3 Before use, make sure that the Syringe Clip is properly adjusted.

5.1.2 Setting up Bioanalyzer

5.1.2.1 Open the lid of the instrument and make sure that the electrode cartridge is in place.

5.1.2.2 Adjust the chip selector position to “1”.

5.1.3 Setting up the Software

5.1.3.1 Double-click on the icon that opens the software for the Agilent 2100 Bioanalyzer.

5.2 Preparation of the Agilent 2100 Bioanalyzer

Note: the following decontamination procedure should be performed daily before running any assays.
5.2.1 Slowly fill a well of an electrode cleaning cartridge with 350 μl of RNA-Zap or RNAsAway.

5.2.2 Place the electrode cleaner into the Agilent Bioanalyzer 2100, close the lid and leave the cartridge in the machine for 1 minute.

5.2.3 After 1 minute, remove the electrode cleaner from the Bioanalyzer and store it for future use. This cartridge can be re-used for all the chips from the same kit.

5.2.4 Slowly fill a well of an electrode cleaning cartridge with 350 μl with Nuclease-free water.

5.2.5 Place electrode cleaner into the Bioanalyzer and leave it there for 10 seconds. After 10 seconds, remove the chip.

5.2.6 Keep the lid of the Bioanalyzer open for another 10 seconds and let it stay close for some time. This will allow the water to evaporate from the capillaries.

5.2.7 Between Bioanalyzer chips clean electrodes ONLY with the Nuclease free water cartridge. This will prevent any RNAsese build up.

At the end of the Bioanalyzer session repeat steps 5.2.1 to 5.2.6.

5.3 Preparation of RNA 6000 Nano gel matrix

Note:
- Keep all reagents and reagents mixes at 4°C when not in use
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use
- Use loaded chips within 5 minutes

5.3.1 Place 550 μl of RNA 6000 Nano gel matrix on top of spin filter.

5.3.2 Spin at 1,500 g for 10 minutes.

5.3.3 Aliquot gel matrix into 65 μl samples.

5.3.4 Vortex RNA 6000 Nano Dye concentrate (blue cap).
5.3.5 Add 1 μl of Nano Dye concentrate to a 65 μl filtered gel matrix.

5.3.6 Cap the tube and vortex thoroughly.

5.3.7 Spin tube for 10 minutes at 13,000 g at RT. Use prepared mix within one day.

5.4 Sample preparation
5.4.1 Denature samples at 70˚ C for 2 minutes. After 2 minutes spin shortly and then place on ice.

5.5 Preparing and running the Bioanalyzer chip
5.5.1 Place chip on the Chip Priming Station.

5.5.2 Load 9 μl of gel dye mix into the well marked and pressure it with syringe to spread into all of the capillaries and wait for exactly 30 seconds before releasing the clip. Add 9 μl of the gel to the other “Gel” wells (wells without a dot).

5.5.3 Please observe proper loading techniques.
5.5.4 Pipette 5.0 μl of the RNA 6000 Nano Marker (green top) into the well marked as “Ladder” and 5.0 μl into each of 12 sample wells. For each unused well add 6 μl of the Nano Marker (green top).

5.5.5 Load 1 μl of Ladder into “Ladder” well.

5.5.6 Load 1 μl of sample into each sample well.

5.5.7 Vortex the chip for 1 minute at 2400 rpm.

5.5.8 Load the chip into an Agilent Bioanalyzer 2100 within 5 minutes.

5.5.9 Run Agilent Bioanalyzer 2100.

6.0 Cleaning of the equipment

For cleaning instructions, please follow the steps listed in Section 5.2 (Preparation of the Agilent Bioanalyzer 2100).

6.1 Perform cleaning of the Agilent Bioanalyzer 2100 daily before and after any runs.

6.2 For equipment that is used on daily basis, please perform a more thorough cleaning.

6.2.1 Once a month use the toothbrush to clean the capillary’s area inside of the machine.
6.2.2 Please refer to the 2100 Maintenance and Troubleshooting Guide provided with Agilent Bioanalyzer 2100 for further instructions on cleaning procedures.
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