GENOMICS UNIT
CABIMER

Bioanalyzer 2100 User Guide

Bioanalyzer Agilent 2100 is designed to determine nucleic acid quality (RNA and DNA) and quantity, and uses a more reliable and accurate technology when compared to other traditional systems. It consists on a microelectrophoretic system based on nanocapillarity. In this way it minimizes the amount of DNA/RNA needed for the assays, as well as the time spent. At present, this equipment has become essential for the determination of DNA and RNA sample quality due to its higher reliability in contrast to classic polyacrilamide and agarose gels. It also minimizes the quantity of sample needed for its analysis (1 µL). Furthermore, in the specific case of RNA samples, the Bioanalyzer software integrates an algorithm called RIN (RNA Integrity Number), which allows the measurement of total eukaryotic RNA sample quality and integrity through a numeric range from 1 to 10, being 1 the value for a completely degraded RNA sample, and 10 the value for an intact one.

This service offers:

- DNA/RNA quantity measurement through spectrophotometric and/or fluorimetric assays.
- Analysis of RNA/DNA sample quality/integrity and size (Bioanalyzer).
- RIN estimation (RNA).
- Results of the nanocapillar electrophoresis at a *.PDF file to visually asses RNA integrity and absence of contaminating genomic DNA.

At present, there exist several possibilities for DNA and RNA analysis using this system, being the following the ones used at this unit:

- For **RNA and mRNA analysis:**
  - **RNA 6000 Nano.** Medium total RNA concentration.
    - Qualitative range: 5-500 ng/µl total RNA, 25-250 ng/µl mRNA.
    - Quantitative range: 25-500 ng/µl total RNA, 25-250 ng/ µl mRNA.
  - **RNA 6000 Pico.** Low total RNA concentration.
    - Qualitative range: 50-5000 pg/µl total RNA, 250-5000 pg/µl mRNA.
    .
- For **DNA and DNA library analysis:**
  - **High Sensitivity DNA.** Low dsDNA concentration.
    - Qualitative range from 5 pg/µl (5-5000 pg/µl).
    - Quantitative range: 5–500 pg/µ.
    - Size range: 50-7000bp.

    NOTE: it is highly important to provide a correct DNA quantification. In the case of genomic DNA, Nanodrop quantification is sufficient; whereas if DNA comes from purification, ChIP or pull-downs, it must be fluorimetrically quantified, or the user should request this measurement to the unit.

For the use of other chips (RNA, DNA and protein) and any other doubt, please contact the genomics staff though these email addresses, Eloisa Andujar, Mónica Pérez or at 954 467 828.

For more information about the kits commercialized by Agilent, visit http://www.chem.agilent.com/en-US/Products/Instruments/lab-on-a chip/ 2100bioanalyzer/ Pages/default.aspx.

- **Sample requirements**

a. RNA isolation using Trizol (Invitrogen cat: 155596-026) and/or purification columns from QIagen RNeasy(Qiagen RNeasy, cat: 74104).

b. RNA quantity:
   - A minimum quantity of 50 ng and maximum concentration of 1000 ng/µl diluted in AT least 2 µl **DNAse free water** (in the case of RNA 6000 Nano, a minimum of 25 ng/µl is required).

DNA quantity:
   - AT LEAST 2 µl at a minimum concentration of 10 pg/µl, for DNA and DNA libraries, diluted in **DNase free water.**

c. All samples must be correctly identified according to the **Application form** attached and correctly filled up. PDF

d. Experiment requirements: if there is not enough RNA/DNA quantity, the analysis will not take place.

- **Sample delivery**

For their quality analysis, DNA/ RNA samples must be column purified and diluted in **DNase free water, inside 1,5mL eppendorf tubes** and kept frozen at dry ice, always with the appropriate application form correctly filled. Samples can be delivered at the next address:

Unidad de Genómica
Centro Andaluz de Biología Molecular y Medicina Regenerativa (Cabimer)
Avda. Américo Vespucio nº24
Parque Tecnológico Cartuja´93
41.092 SEVILLA

Sample reception Schedule: From 9 am to 5 pm.

Sample delivery must be previously informed via phone (954-467828) or e-mail (eloisa.andujar@cabimer.es/monica.perez@cabimer.es).

As soon as samples arrive at the Unit, their correct reception will be notified via mail, as well as the approximate due date for its analysis depending on the waiting list.

Before the analysis, samples will be quantified to ensure there is an adequate amount to proceed. When this does not happen, the user will be notified and advised with some recommendations for an appropriate extraction of the samples, however sample integrity will NOT be measured.

- **Results**

Once the experiment is finished (approximately 2 days after its beginning) the user will be notified via email with the next attachments:

- Results in a *.pdf file including quality analysis (concentration, 18S/28S ratio, RIN), and images (virtual gel image and electropherograms) corresponding to sample migration.

- A guide for RNA sample quality assessment of the results obtained with Bioanalyzer 2100 from Agilent.

➢ **Prices**

Check with the Genomics Unit.

➢ **Additional information**

- Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, Lightfoot S, Menzel W, Granzow M and Ragg T. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Molecular Biology 2006, 7:3 (31 Jan 2006)

- Imbeaud S, Graudens E, Boulanger V, Barlet X, Zaborski P, Eveno E, Mueller O, Schroeder A, and Auffray C. Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces, Nucl. Acids Res. 2005 33: e56.

- Tissue Microarrays Reach New Markets

- RNA Integrity Number (RIN)- Standardization of RNA Quality Control PDF(0.5 MB)

- Bioanalyzer 2100. Agilent Technologies

Analytical specifications	RNA 6000 Nano Kit		RNA 6000 Pico Kit	
	total RNA	mRNA	total RNA	mRNA
Quantitative range	25 - 500 ng/µL	25 - 250 ng/µL	-	-
Qualititative range	5 - 500 ng/µL	5 - 250 ng/µL	50 - 5000 pg/µL in water	250 - 5000 pg/µL in water
Sensitivity (S/N>3)	5 ng/µL in water	25 ng/µL in water	50 pg/µL in water or 200 pg/µL in TE	250 pg/µL in water or 500 pg/µL in TE
Quantitation reproducibility (within a chip)	10% CV	10% CV	20% CV	20% CV
Quantitation accuracy[1]	20%	20%	30%	-
Maximum sample buffer strength	100 mM Tris 0.1 mM EDTA or 125 mM NaCl 15 mM $MgCl_2$	100 mM Tris 0.1 mM EDTA or 125 mM NaCl 15 mM $MgCl_2$	50 mM Tris 0.1 mM EDTA or 50 mM NaCl 15 mM $MgCl_2$	50 mM Tris 0.1 mM EDTA or 50 mM NaCl 15 mM $MgCl_2$
Physical specifications				
Analysis time	30 minutes	30 minutes	30 minutes	30 minutes
Samples per chip	12	12	11	11
Sample volume	1 µL	1 µL	1 µL	1 µL
Kit stability	=4 months at 4°C	=4 months at 4°C	=4 months at 4°C	=4 months at 4°C
Kit size	25 chips 12 samples/chip = 300 samples/kit	25 chips 12 samples/chip = 300 samples/kit	25 chips 11 samples/chip = 275 samples/kit	25 chips 11 samples/chip = 275 samples/kit

[1] Determined analyzing the RNA ladder as sample

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