

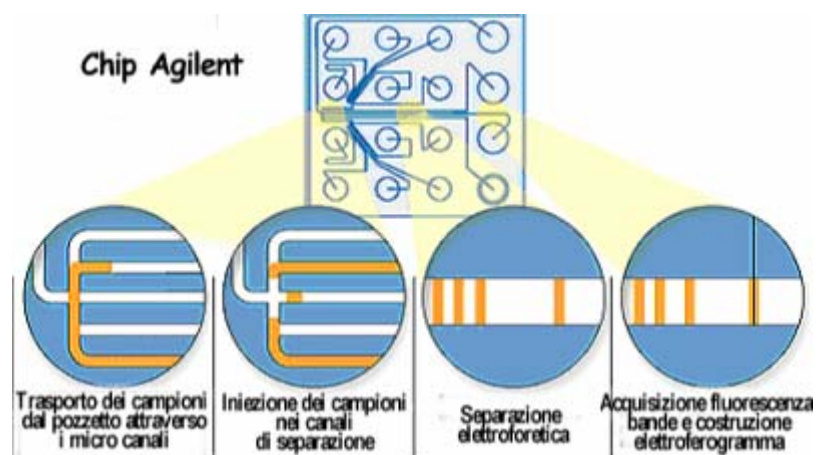
The Molecular Biology Service has recently acquired a new equipment for the qualitative and quantitative analysis of the nucleic acids, the Agilent Bioanalyzer 2100, and have implemented such technology in the services offered. Such technology is mainly used for the qualitative analysis (QC) of RNA samples to be used in sensitive applications (qPCR, microarray, next gen seq, libraries generation, etc)

Qualitative analysis with the Agilent BioAnalyzer

cDNA, total RNA Totale, mRNA and small RNA

The service consists in the analysis of the samples of cDNA and RNA (total RNA, mRNA or RNA shorter than 200 nucleotidic (microRNA, siRNA, scRNA, snoRNA, piRNA, 5S rRNAs, 5.8S rRNAs) with the Agilent Bioanalyzer 2100 that allows to qualitatively and quantitatively characterize the samples through a capillary electrophoresis. According to the samples quantity it is possible to use the NANO chip, the PICO chip or, in the case of small RNA the Small RNA chip. The Agilent Bioanalyzer 2100, in the case of the RNAs chip, calculate a quality value **R.I.N.** (RNA Integrity Number) that allows to attribute a qualitative index to the examined sample. Such parameter goes from 10, RNA of good quality, to zero that points out RNA instead completely degraded. The Small RNA chip, is able to calculate the percentage of enrichment of the 20-40 nt long fragment correspondents to the population of the miRNAs. Every chip contains a series of micro-channels tightly interconnected: the nucleic acids fragments are separated according to their molecular weight as in a normal agarose gel electrophoresis.

The micro-channels of every chip are filled with a matrix and a fluorochrome: 1. The samples migrate through the micro channels starting from the loading well; 2. Samples are inserted in the separation channel; 3. sample is separated through a electrophoretic run; 4. At the end of the run the samples are read in base to their fluorescence and such information is translated in a typical image of a gel and in electropherogram



The following tables allow to identify the right the product to satisfies the needs of the user.

RNA

Analytical Specifications	RNA 6000 Nano Total RNA Assay	RNA 6000 Nano mRNA Assay	RNA 6000 Pico Total RNA Assay	RNA 6000 Pico mRNA Assay	Small RNA Assay
Quantitative range	25–500 ng/μL	25–250 ng/μL	–	–	50–2000 pg/μL of purified miRNA in water
Qualitative range	5–500 ng/μL	25–250 ng/μL	50–5000 pg/μL in water	250–5000 pg/μL in water	50–2000 pg/μL of purified miRNA in water
Sizing range	–	–	–	–	6–150 nt

DNA

Analytical Specifications	DNA 1000 Assay	DNA 7500 Assay	DNA 12000 Assay	High Sensitivity DNA Assay
Sizing range	25–1000 bp	100–7500 bp	100–12000 bp	50–7000 bp
Sizing resolution	25–100 bp: 5 bp 100–500 bp: 5 % 500–1000 bp: 10 %	100–1000 bp: 5 % 1000–7500 bp: 15 %	100–1000 bp: 5 % 1000–12000 bp: 10 %	50–600 bp: ±10 % 600–7000 bp: ±20 %

Terms and organization of the service

To access the Service is necessary:

1. To book on the register at the SBM.
2. To furnish the necessary material to the SBM for the analysis (Kit and Chip) that at the moment the Service doesn't furnish waiting to arrange with the user a more functional organization.
3. To deliver the samples, according to the following instructions, to the SBM personnel.

The analysis will be performed by the SBM staff and the results sent via email, within the four following days to the receipt of the samples in pdf format. The SBM staff is available for explanations in the interpretation of the data.

Preparation of the samples

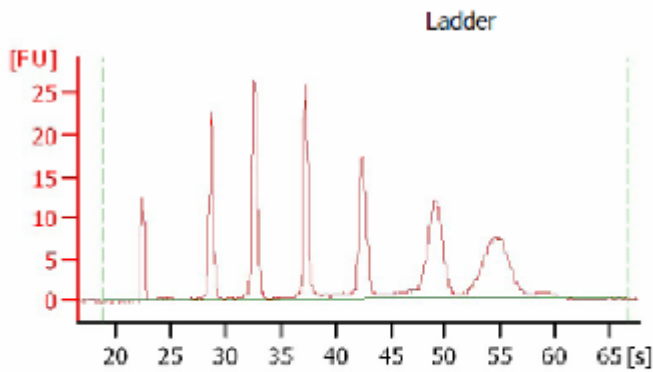
The samples to be analyzed must be delivered in 1.5ml Eppendorf, diluted in RNase free water. In the following table, according to the different chip, are reassumed the recommended quantities to get a good result both in quantitative and qualitative terms

Tipo di RNA	NANO Chip	PICO Chip	Small RNA Chip
RNA Totale	100 – 200 ng/μl	400 – 800 pg/μl	30 – 100 ng/μl
mRNA	50 – 100 ng/μl	700 – 1000 pg/μl	///
Small RNA < 150 nt	///	///	5 – 20 ng/μl
Oligonucleotidi	///	///	500 – 1.500 pg/μl

Every samples must be delivered in a minimum volume of 3.0 ul to allow the SBM staff to repeat the analysis in the case of technical problems. On every single test-tube it has to be clearly indicated the name of the samples.

Result interpretation

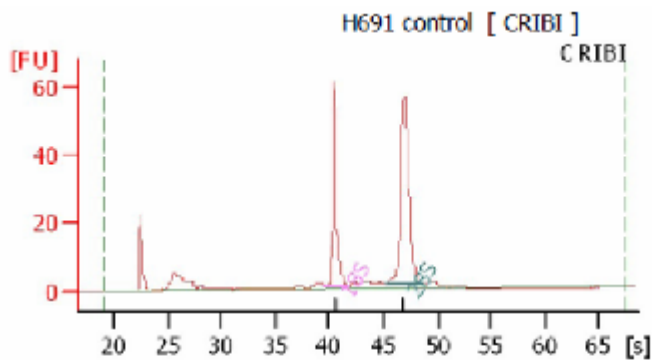
The Agilent Bioanalyzer 2100 for every single sampler furnish, as result, an electropherogram and the virtual image of a typical agarose gel. To help the user in the interpretation of the gotten profiles we list a series of peculiar electropherogram for the total RNA, for the mRNA and for the small RNA



RNA 6000 ladder

Overall Results for Ladder

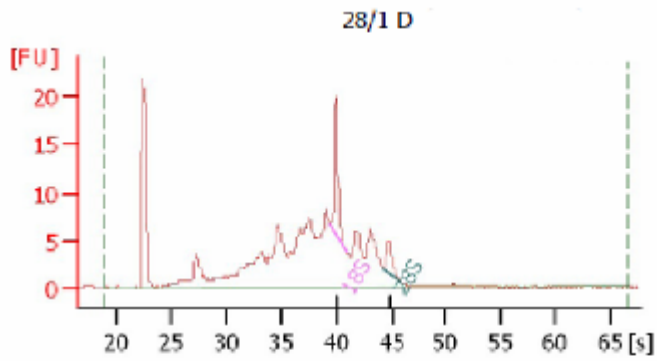
RNA Area: 245.1
 RNA Concentration: 150 ng/ μ l
 Result Flagging Color:
 Result Flagging Label: All Other Samples



Total RNA good quality

Overall Results for sample 1 : H691 control

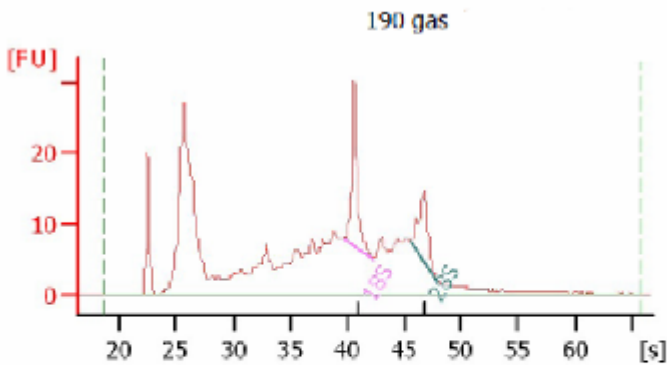
RNA Area: 240.5
 RNA Concentration: 104 ng/ μ l
 rRNA Ratio [28s / 18s]: 1.9
 RNA Integrity Number (RIN): 9.8 (8.02.05)
 Result Flagging Color:
 Result Flagging Label: RIN: 9.80



Total RNA partially degraded

Overall Results for sample 4 : 28/1 D

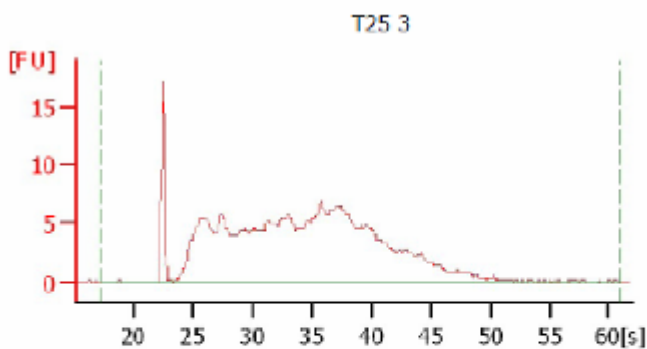
RNA Area: 206.1
 RNA Concentration: 89 ng/ul
 rRNA Ratio [28s / 18s]: 0.3
 RNA Integrity Number (RIN): 5.1 (B.02.05)
 Result Flagging Color:
 Result Flagging Label: RIN: 5.10



Total RNA degraded

Overall Results for sample 7 : 190 gas

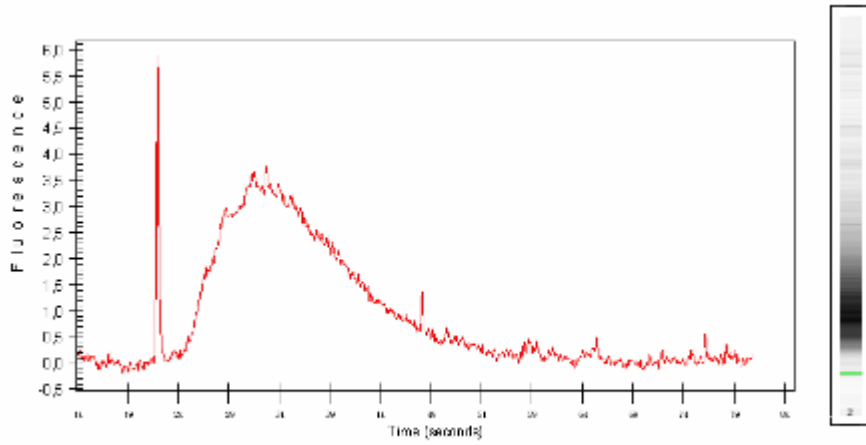
RNA Area: 488.6
 RNA Concentration: 236 ng/ul
 rRNA Ratio [28s / 18s]: 0.6
 RNA Integrity Number (RIN): 5.4 (B.02.05)
 Result Flagging Color:
 Result Flagging Label: RIN: 5.40



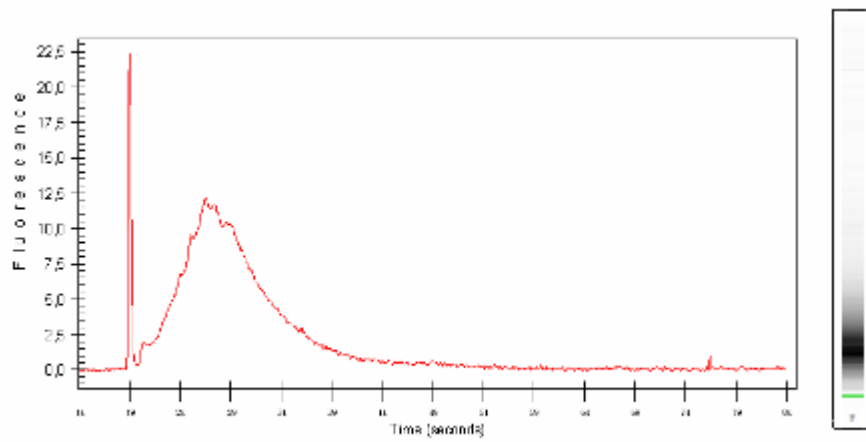
Total RNA entirely degraded

Overall Results for sample 3 : T25 3

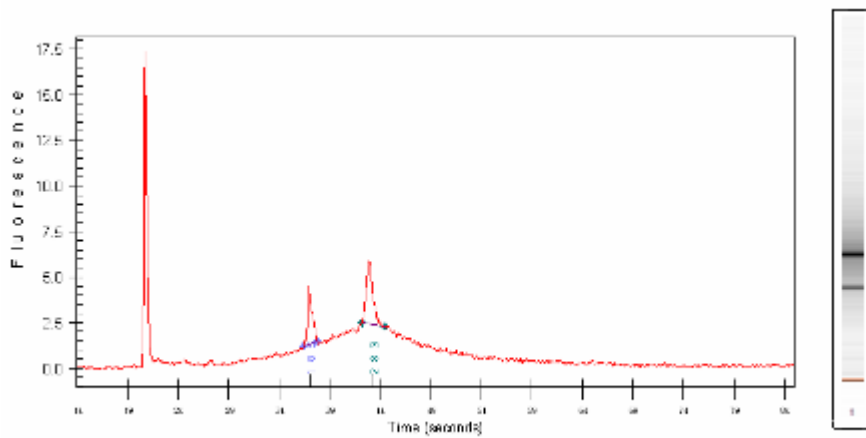
RNA Area: 284.4
 RNA Concentration: 186 ng/ul
 rRNA Ratio [28s / 18s]: 0.0
 RNA Integrity Number (RIN): 2.3 (B.02.06)
 Result Flagging Color:
 Result Flagging Label: RIN: 2.30



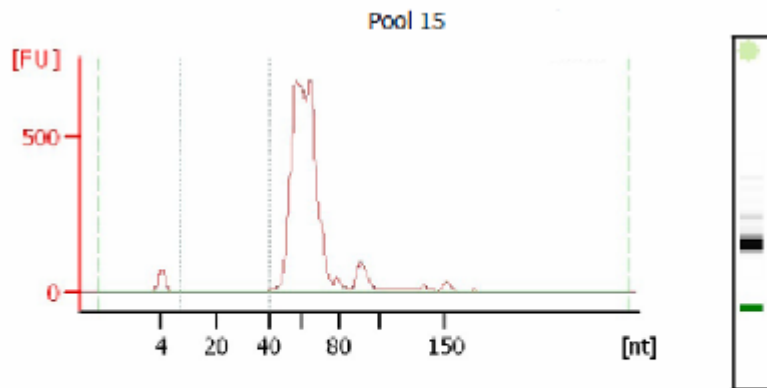
mRNA good quality



mRNA sintetico



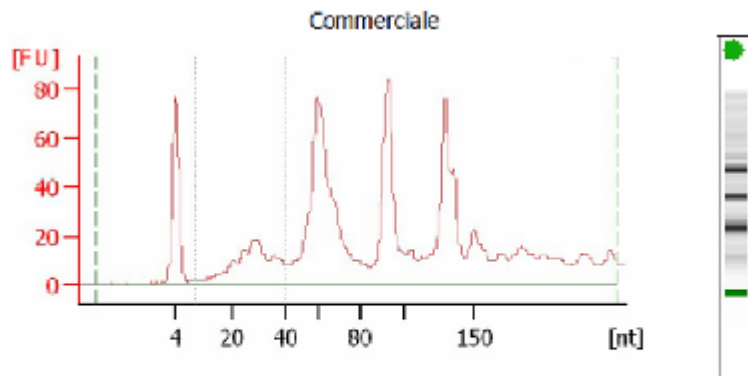
mRNA with ribosomal
RNA contamination



Evaluation of RNA < 150 nt with Small RNA chip (% miRNA = 3%)

Overall Results for sample 2 : Pool 15

Small RNA Concentration [pg/ μ l]: 12.350,3
 miRNA Concentration [pg/ μ l]: 312,9
 miRNA / Small RNA Ratio [%]: 3
 Result Flagging Color:
 Result Flagging Label: 3 % miRNA; Concentration: 312.90 pg/ μ l



Evaluation of RNA < 150 nt with Small RNA chip (% miRNA = 37%)

Overall Results for sample 1 : Commerciale

Small RNA Concentration [pg/ μ l]: 3,397.0
 miRNA Concentration [pg/ μ l]: 1,250.0
 miRNA / Small RNA Ratio [%]: 37
 Result Flagging Color:
 Result Flagging Label: 37 % miRNA; Concentration: 1250 pg/ μ l