

A new method for separation and characterization of Small RNA by On-Chip Electrophoresis

Martin Greiner

Agilent Technologies, Waldbronn

2100 Lab-on-a-Chip solutions

The 2100 bioanalyzer

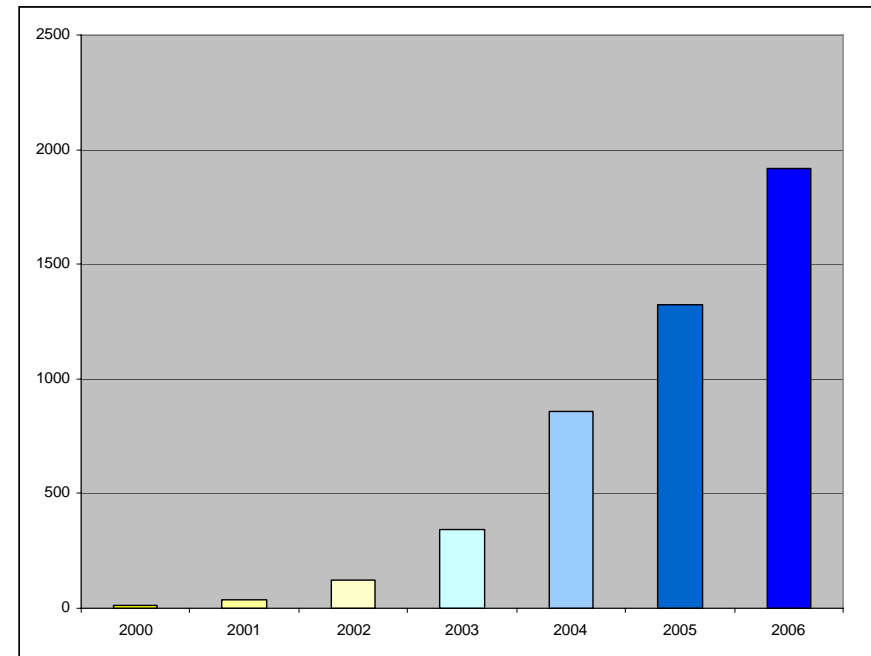
Electrophoretic sizing, quantitation and QC of DNA, RNA, Protein samples on a small Glas Chip as done traditionally on slab gels (Agarose or SDS-PAGE)

First commercially available Lab-on-a-Chip product (since October 1999)

Digital Analysis of up to 12 samples in 30 minutes

DNA, RNA, Proteins and Cells

Number of BioA publications/year
March 2007: > 5000 citations



2100 bioanalyzer workflow for RNA analysis

1. Load sample



2. Run analysis

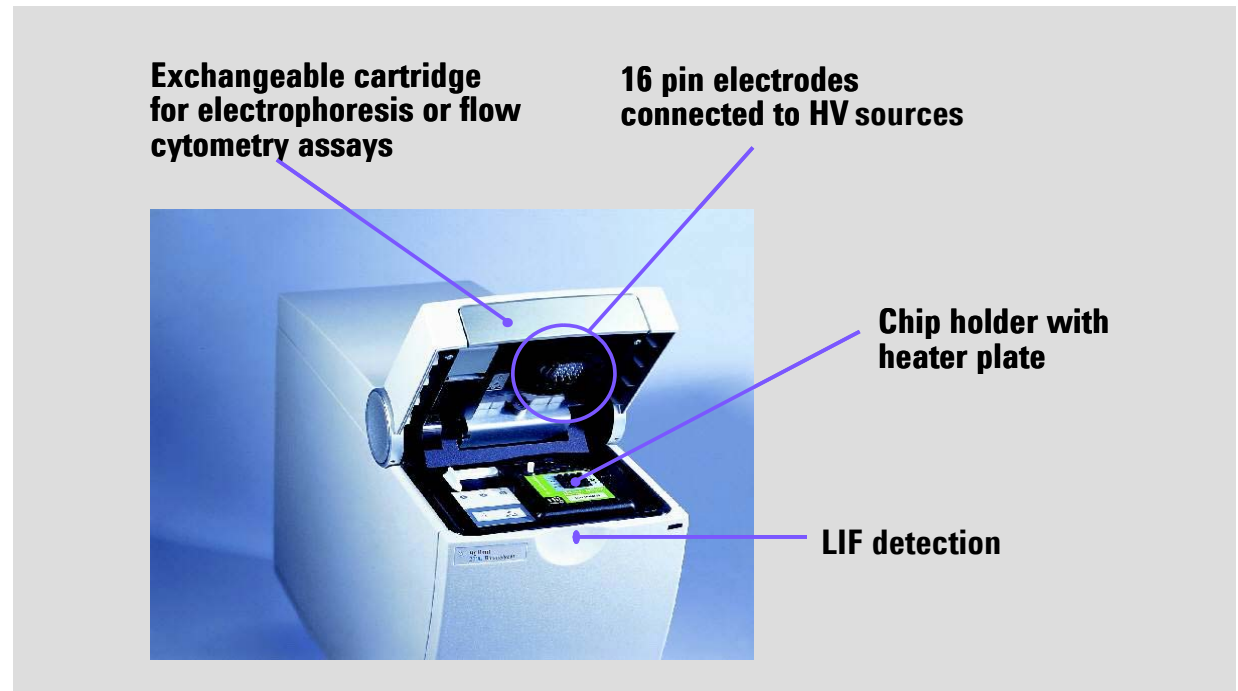


3. Analyze data



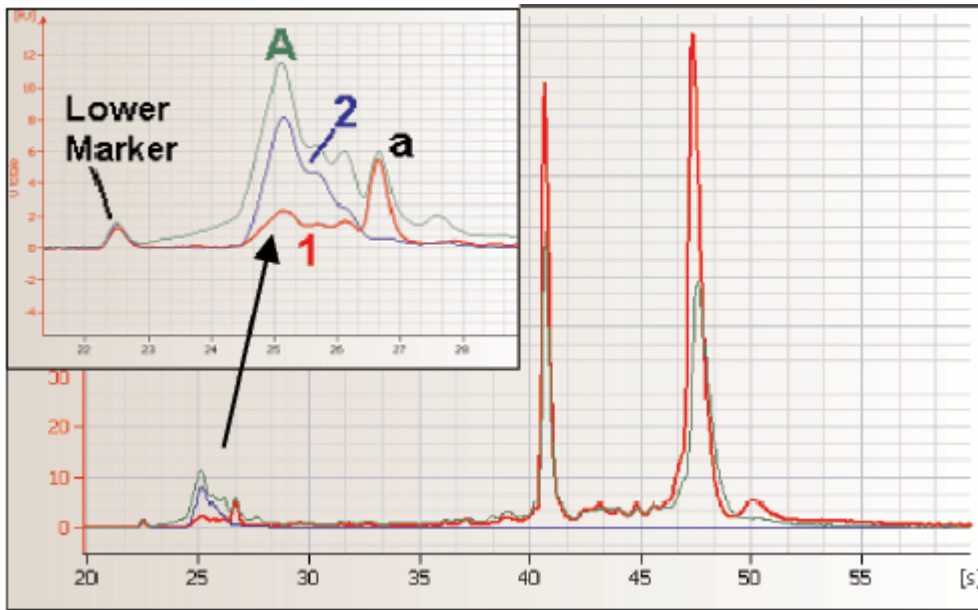
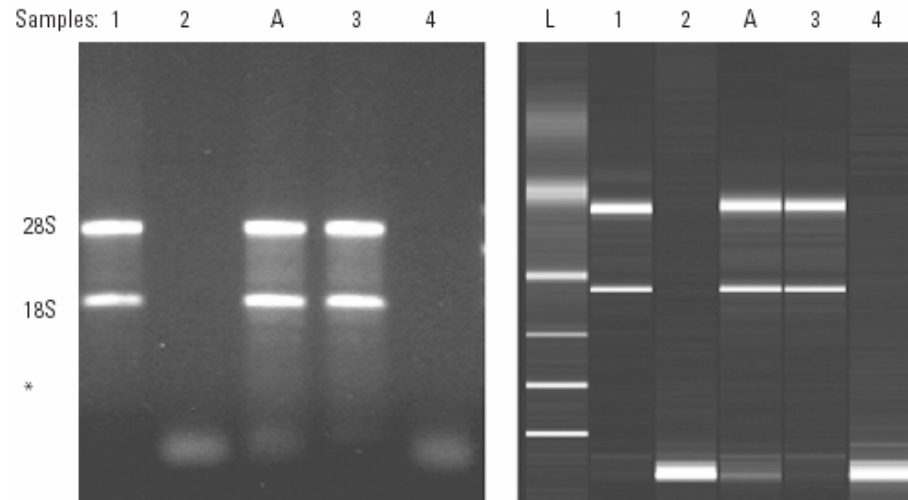
Experimental setup for Small RNA Assay:

- 1 Priming and pipetting of Chip (5 minutes)
- 2 Run up to 11 samples (in 30 minutes)
- 3 Analyze data qualitative and quantitative



Analysis of Small RNA (using RNA 6000 Assay)

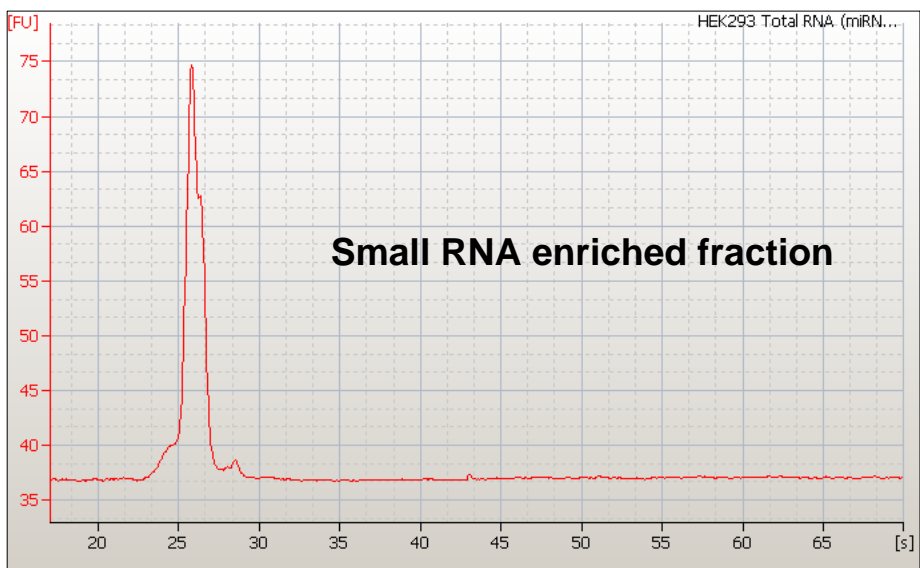
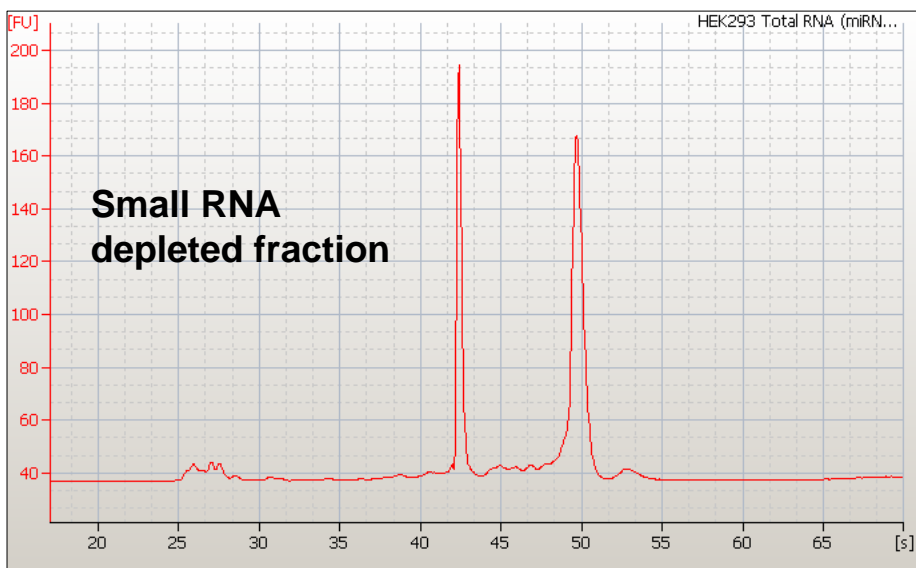
Small RNA
fraction: < 200 nts
e.g. miRNA,
siRNA, snRNA,
tRNA, 5S RNA



Total RNA assay
allows some
discrimination of
different profiles

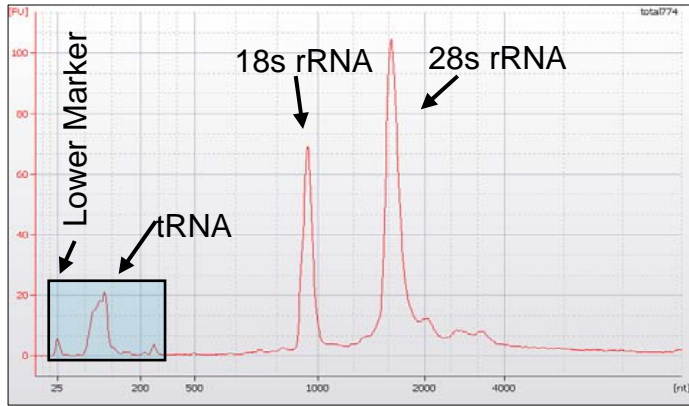
HEK 293 Cells: direct miRNA extraction

using the Ambion „mirVana“ Kit and RNA 6000nano



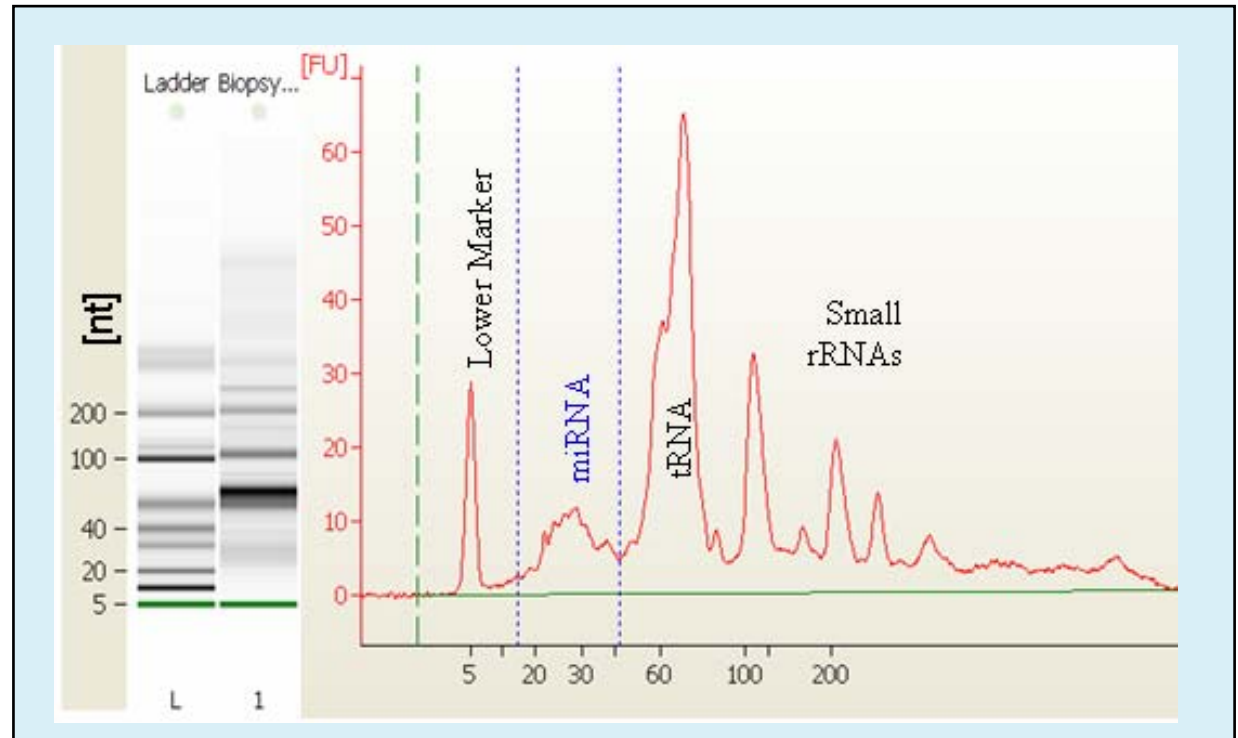
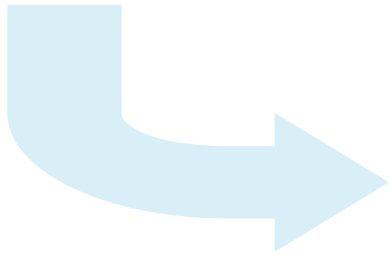
Existing RNA
6000 nano kit
(no Lower Marker used)

New Small RNA Assay versus existing RNA Assay



RNA 6000 Assay

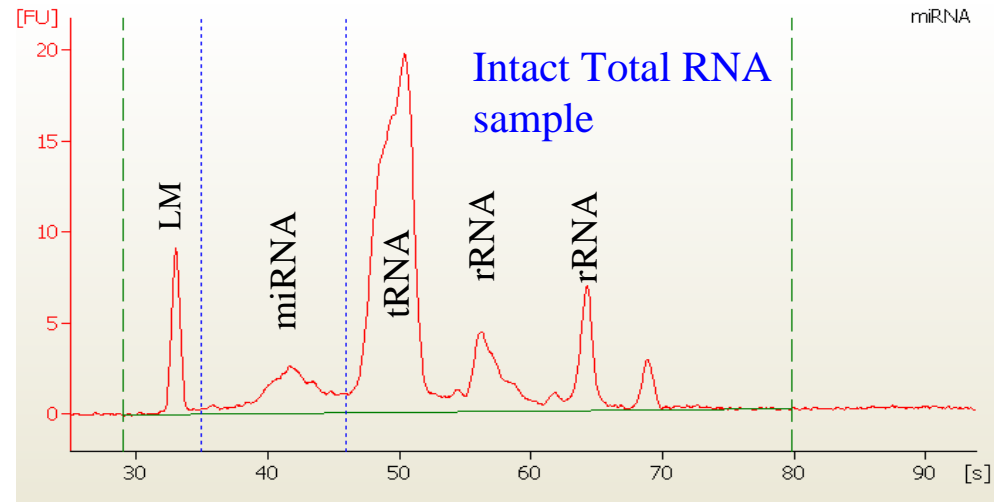
New Small RNA Assay



The new Assay as a tool for:

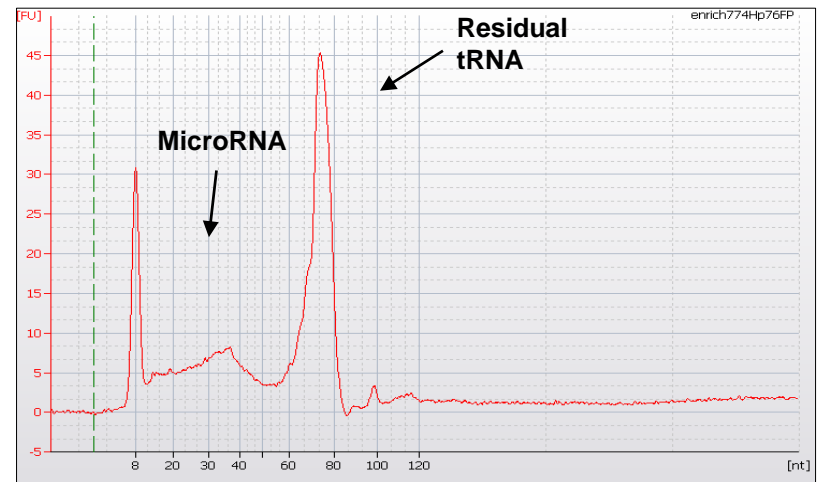
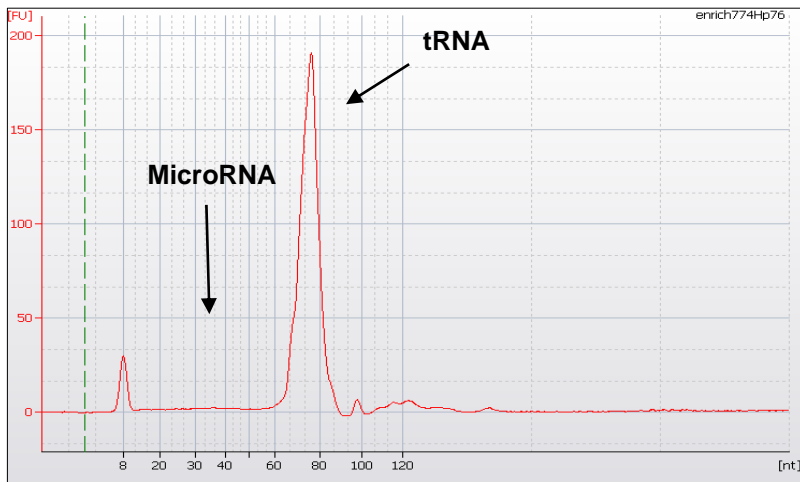
Verification, comparison and optimization for the small RNA region:

- High sensitivity to detect low abundant fragments
- High resolution for ss oligos, miRNA, pre-, t-, 5S-RNA's
- compatible with Total RNA samples or purified small RNAs.
- Semi-quantitative for single stranded RNA.
- Denaturing
- Analysis up to 150nt



Monitoring purification of microRNA samples

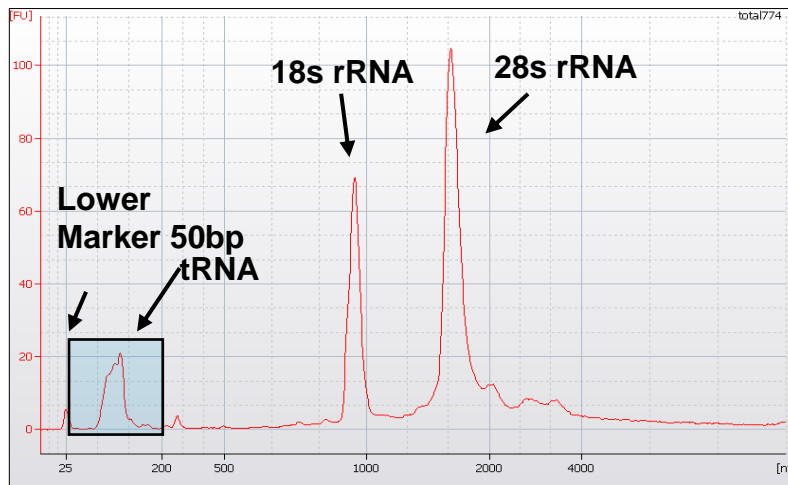
totalRNA pre-purified with a commercial smallRNA kit (cut-off 200nt) further enriched using preparative polyacrylamide gel (cut-off 40nt)



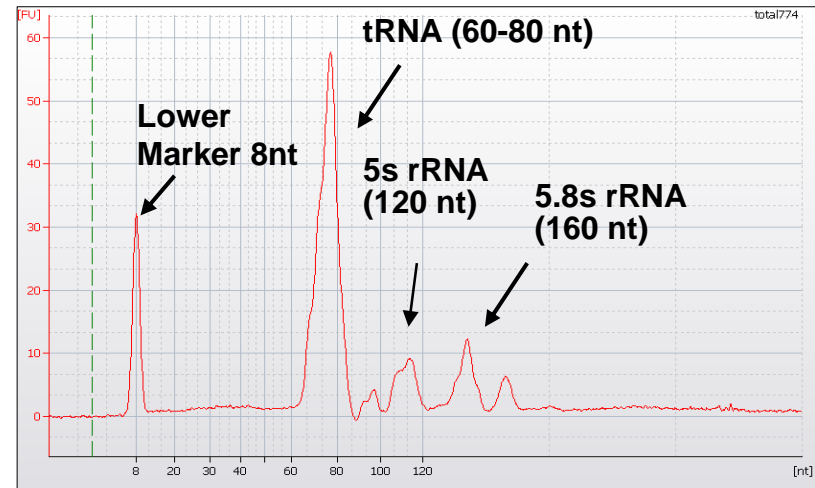
miRNA to tRNA ratio was increased by a factor of ~20

Small RNA region in Trizol+Glycogen isolated total RNA

Two complimentary assays, one to evaluate total RNA integrity and the second to characterize the small RNA fraction



RNA 6000 pico



Small RNA Assay

Resolution test using DNA Oligonucleotides

Analysis of DNA Oligonucleotides

Based on this analysis the resolution of the assay can be estimated at approx 3-4 nt for samples from 10 to 40 nt and 4-6 nt in for samples from 50 to 120 nt. RNA oligos typically show peaks twice as broad

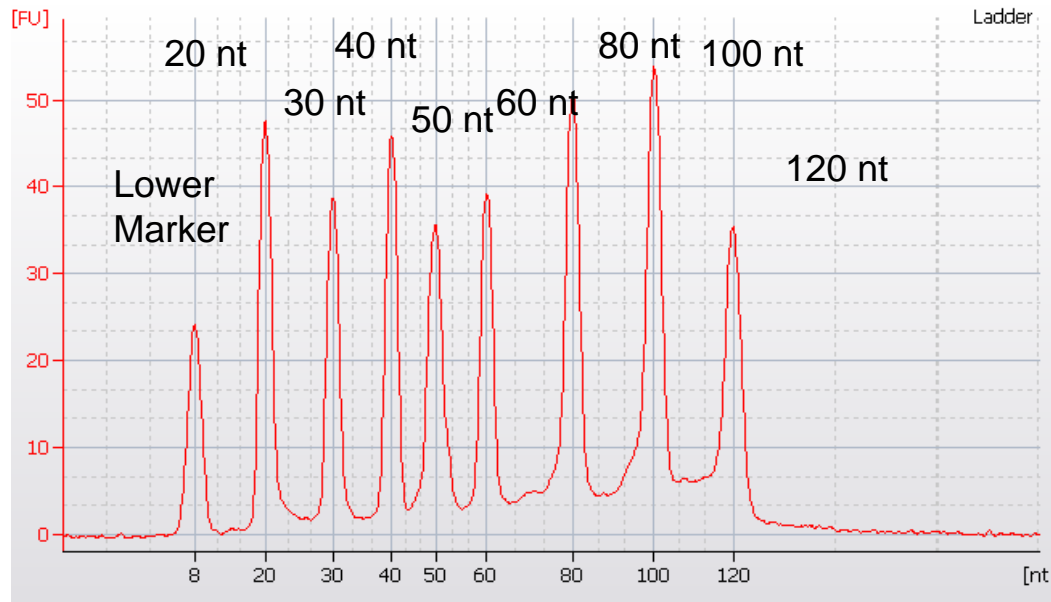
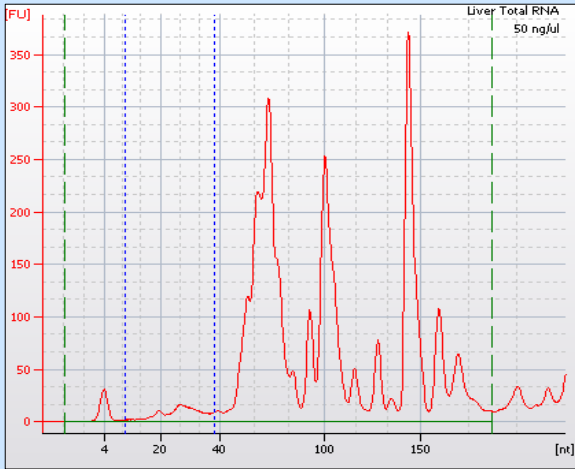
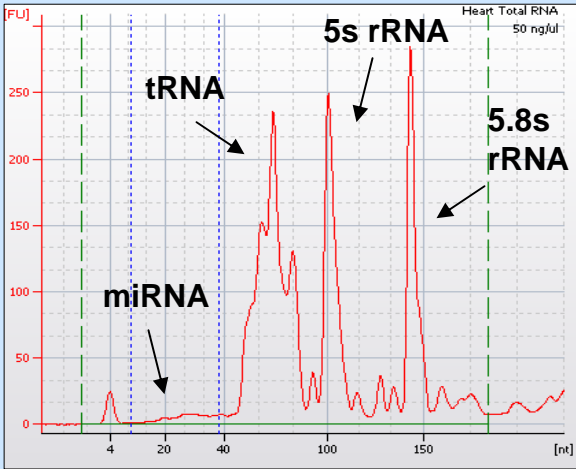
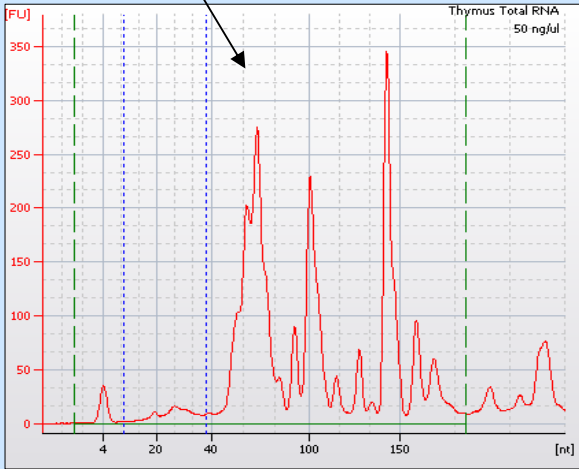
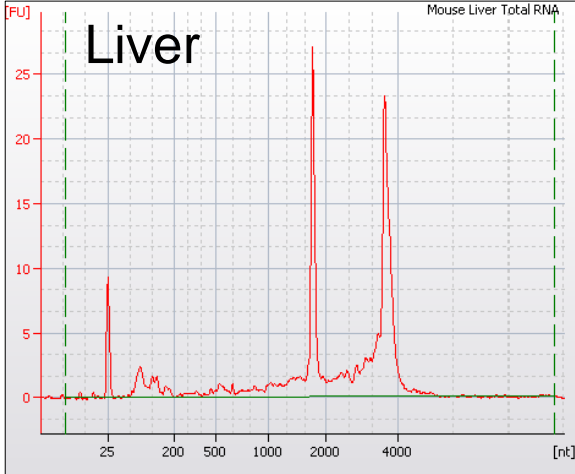
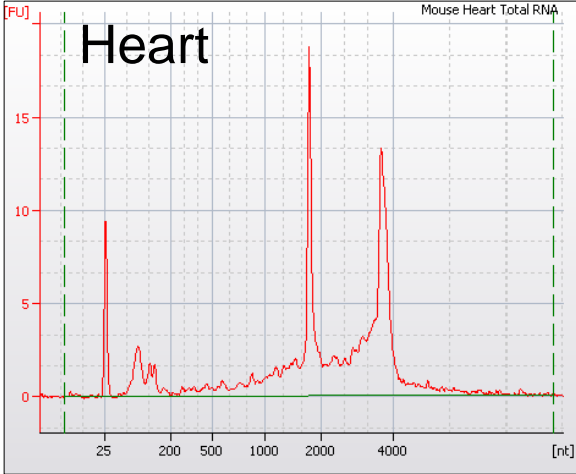
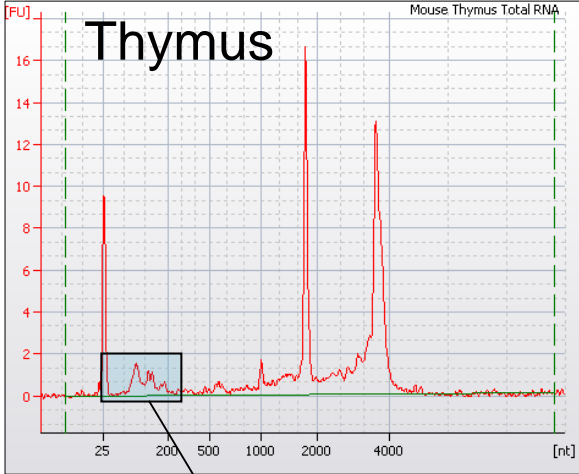


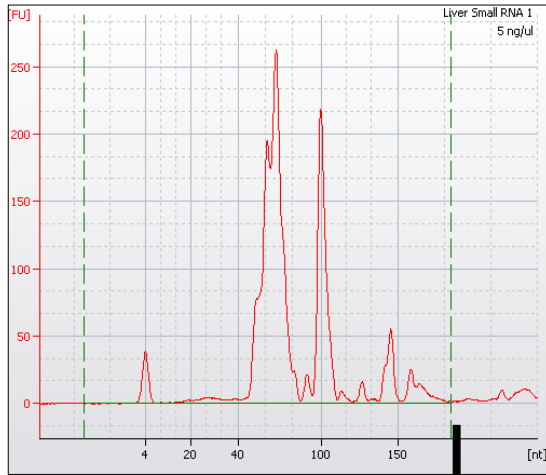
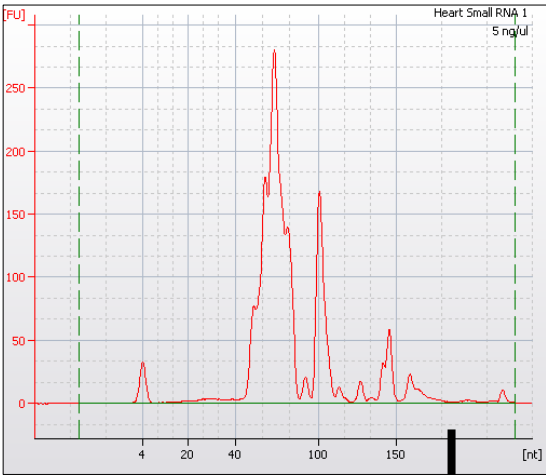
Fig 1: Separation of a mixture containing different synthetic DNA oligonucleotides (20, 30, 40, 50, 60, 80, 100, 120 nt)

Analysis of different total RNA Samples by RNA Nano and Small RNA Assay



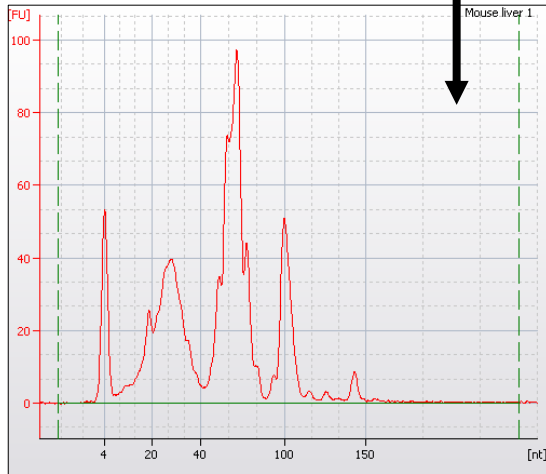
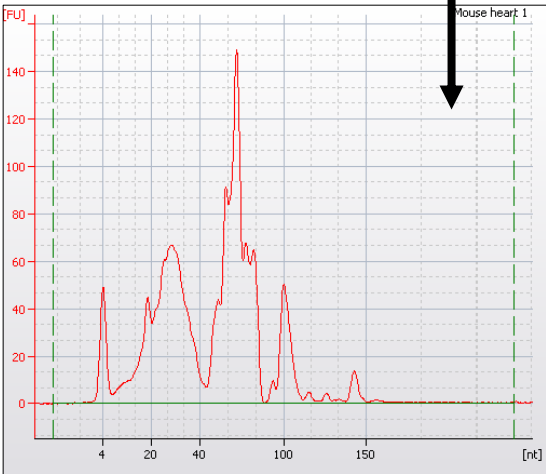
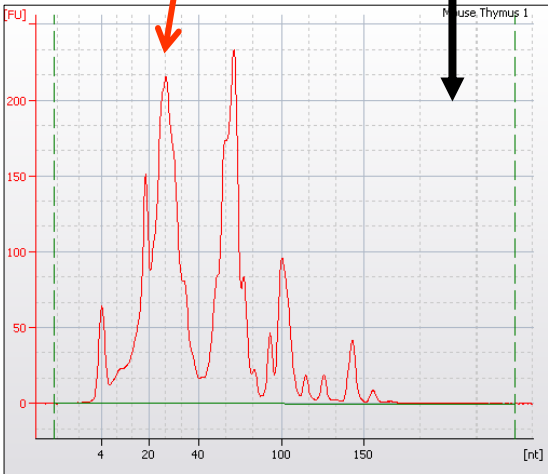
FlashPAGE Purification of miRNA

Pre-purified small RNA (0-150 nt)



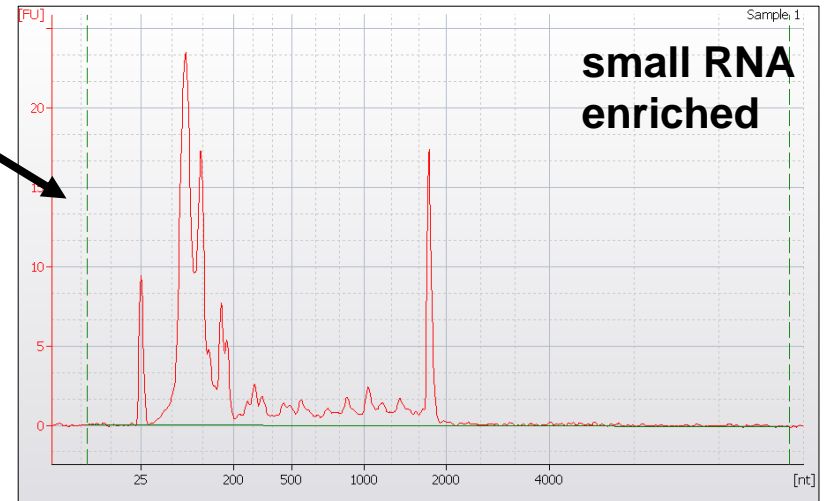
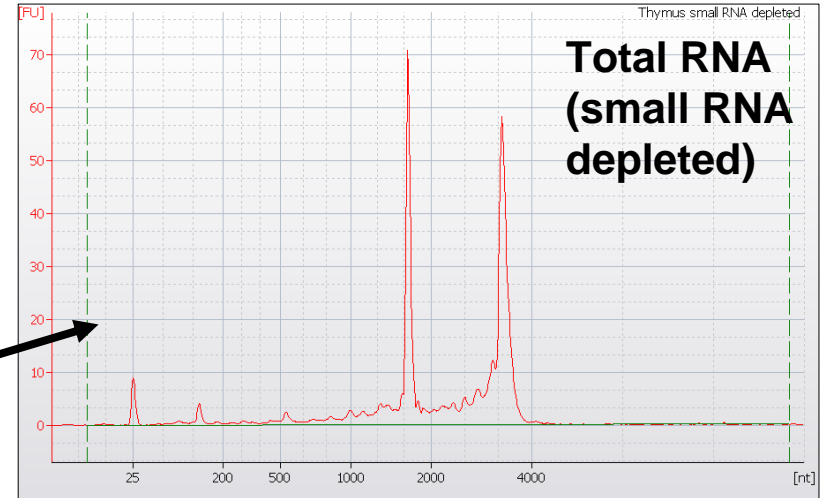
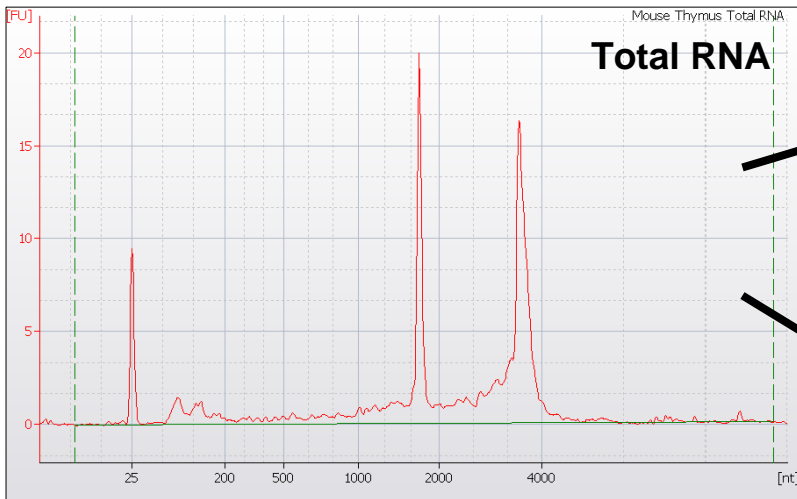
miRNA Peak

“Flash PAGE” purified miRNA



Small RNA purification

Analysis of the individual fractions using the RNA Nano assay



Summary

- Highly sensitive Assay (~ 10 ng total RNA, 100 pg miRNA)
- Separation range 10 -150 nt for ss- and ds-Nucleotides
- Verification tool for RNA extraction quality and optimization
- Semi quantitative based on RNA ladder
- Quick and reproducible (11 samples in 30 minutes)
- Only 1 μ l sample volume required

For more information about 2100 bioanalyzer solutions please visit our webpage at: <http://www.agilent.com/chem/labonachip>

