A new microfluidic Assay for the Analysis of small RNAs

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Introduction

RNA (ribonucleic acid) is the central mediator of gene expression in living cells. Its main function is the transport of the sequence information from the DNA (deoxyribonucleic acid) in the nucleus into the cytoplasm as messenger RNA (mRNA) where it serves as template for the synthesis of proteins. This flow of genetic information from DNA via mRNA to protein has been termed the central dogma of molecular biology. In addition RNA is acting as adaptor for amino acids (tRNA), as structural component of the the ribosomes (rRNA) and can exhibit catalytic activity (Ribozymes).

Recently, a novel class of RNA molecules was discovered, the so-called microRNAs (miRNAs). These short (18-23 nucleotide) molecules have been identified as sequence-specific regulators of many cellular processes such as apoptosis (programmed cell death), proliferation (cell division) and differentiation (cell specialization). Within a cell miRNAs bind to specific mRNAs and modulate their translation (protein expression) by forming double-stranded RNA molecules. In a process called “RNA interference” double-stranded RNA activates a biochemical machinery in the cell which degrades those mRNA molecules that carry a genetic code identical to that of the double-stranded RNA (Andrew Z. Fire and Craig C. Mello received the 2006 Nobel Prize in Medicine for the discovery of this mechanism).

Meanwhile hundreds of different miRNA sequences have been discovered in the genomes of animals and plants, but they are only at the beginning of being classified by their functional roles. Researchers are typically quantifying the content of individual miRNAs (within complex mixtures) in biological samples using different methods. Currently one of the major drawbacks in miRNA research is the lack of adequate analytical methods for the analysis of small RNA samples before proceeding to the following downstream experiments. Here we present a very sensitive microfluidic high resolution assay for the analysis of such short RNA molecules on the Agilent 2100 Bioanalyzer.

The Bioanalyzer is an instrument platform that is designed to run different applications on microfluidic chips. Assays are available to customers for the analysis of RNA, DNA, proteins and cells. Since it’s introduction 7 years ago, the RNA 6000 Nano assay has evolved into the industry standard method for the assessment of total RNA integrity.

The novel high resolution RNA assay utilizes a novel sieving matrix formulation and a dye system for both single and double stranded nucleic acids (DNA and RNA). It measures integrity, size and concentration and is tuned for small single-stranded nucleic acid species. It is capable of analyzing synthetic oligo-nucleotides, purified small RNA samples as well as total RNA samples. The sample size range displayed is 10 to 150 nucleotides.

The 2100 Bioanalyzer

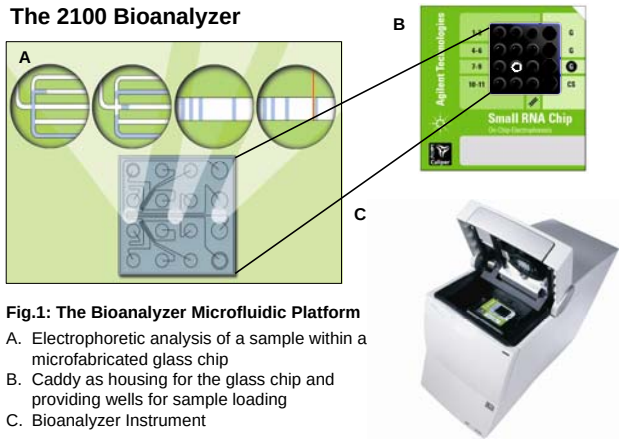

Fig.1: The Bioanalyzer Microfluidic Platform

A. Electrophoretic analysis of a sample within a microfabricated glass chip
B. Caddy as housing for the glass chip and providing wells for sample loading
C. Bioanalyzer Instrument

The Role of miRNA

The central dogma of molecular biology describes the process how genetic information is transcribed from DNA to RNA and then translated from RNA into proteins. The main transmitter molecule is the messenger RNA (mRNA). It transports the genetic information from the nucleus to the ribosomes, where proteins are synthesized according to the mRNA sequence. Many factors are known to influence and regulate this process. The recently discovered microRNAs represent a novel kind of regulation mechanism for the abundance of messenger RNA molecules in a cell. They bind to specific target mRNAs and (a) inhibit their binding to the ribosomes and (b) initiate their cleavage by enzymes in a process called “RNA interference”. Both effects lead to a “shut off” of the signal, thus inhibiting the synthesis of the protein product of the corresponding gene.

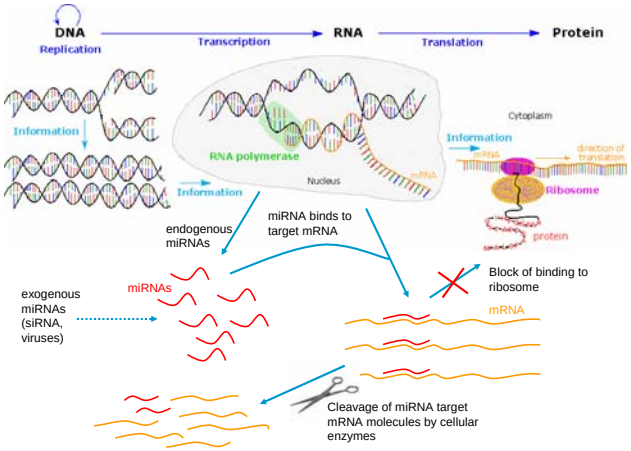

Fig. 2: The role of miRNA in messenger RNA turnover

Analysis of DNA Oligonucleotides

A low-concentrated mixture of eight synthetic DNA oligonucleotides (20-120 nt) was analyzed using the novel assay. All individual components of the mixture are well separated and can be detected with high sensitivity. The resolution of short single-stranded DNA molecules in the assay can be estimated to approx. 3-4 nt for samples from 10 to 40 nt and 4-6 nt for samples from 50 to 120 nt.

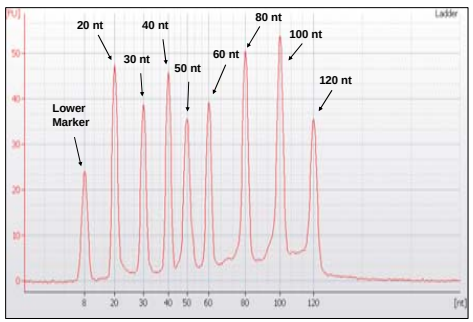

Fig. 3: Separation of a mixture containing different synthetic DNA oligonucleotides (approx. 500 pg/µl of each component)

Characterization of miRNA Content in Total RNA Samples

Total RNA was analyzed using the commercially available RNA 6000 Nano assay (A) and the novel assay (B). The blue box in panel A represents the complete size range that is displayed in the Small RNA assay with high resolution in panel B. In the RNA 6000 Nano assay most components that are contained in total RNA samples are visualized (the major abundant species are the 18s and 28s ribosomal RNA).

In contrast to the RNA Nano assay the peaks of the tRNA (approximately 75 nt), 5s rRNA (100-120 nt) , 5.8s rRNA (130-160 nt) are well resolved in the novel assay. Furthermore the very low-abundant miRNA fraction is clearly detected and its concentration is calculated by the software. This represents the first analytical method to characterize and quantify the total miRNA content in biological samples.

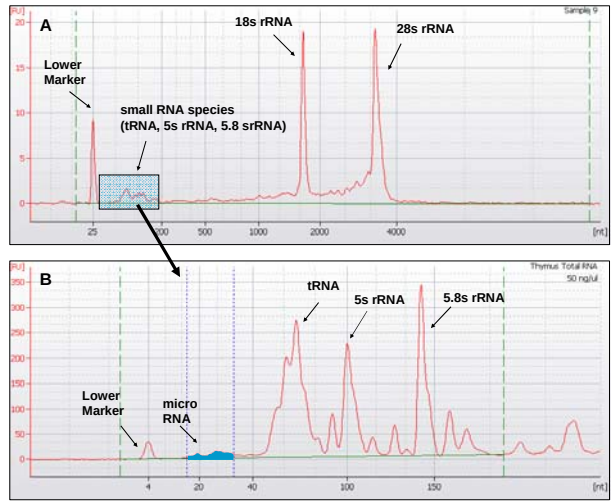

Fig. 4: Analysis of 50 ng/µl of Mouse Thymus total RNA

A. RNA 6000 Nano assay
B. Small RNA assay

Summary

- A novel, very sensitive microfluidic assay for small RNA molecules and DNA Oligonucleotides is presented
- The Assay delivers information about size, concentration and integrity of the sample
- Characterization of the miRNA fraction in complex total RNA is possible even without purification
- RNA Sample Size Range: 10-150 nucleotides
- Sample Concentration Range: 100 pg/µl – 50 ng/µl
- Direct Applications: Analyzing small RNA integrity, Monitoring of miRNA purification, DNA Oligonucleotide QC, monitoring of smallRNA labeling, etc.
- Applications to be investigated: Measurement of siRNA purity and concentration, high resolution dsDNA analysis, microsatellite analysis