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Development of low cost high throughput miRNA screening

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INTRODUCTION

Micro-RNAs (miRNAs) form a class of 17-24 base single-strand non-coding RNAs which regulate gene expression post-transcriptionally in eukaryotes and viruses. Around 700 miRNAs have been identified in many different organisms. They are known to inhibit translation by fixing onto the 3' UTR of mRNAs. Recent data have suggested that miRNAs regulate 1-10 % of mRNAs and that some miRNAs are involved in numerous processes such as HIV infection, oncogene Ras regulation and leukaemia.

We have developed a low cost high throughput method to quantify the expression of these miRNAs via classic total RNA extraction without interference from genomic DNA. More than 150 human and more than 100 mouse miRNAs have been validated using as little as 100 ng total RNA per miRNA. This method provides accurate sensitive miRNA expression profiles which can be used to identify specific miRNA signatures associated with tissues, biological processes or diseases.

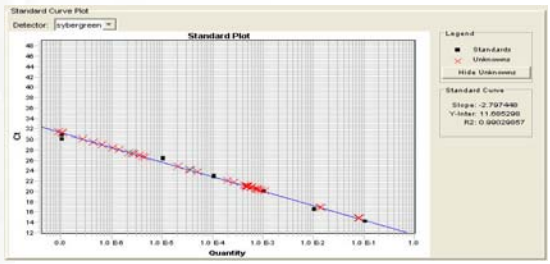
MATERIALS

Tissue RNA samples:
More than 12 different frozen human tissue samples were cut up and the total RNA extracted by a classic phenol/ chloroform method. In addition, five different human RNA samples purchased from Sigma were studied.

RESULTS

Fig.1 Quantification of 2 synthetic miRNAs

A



B

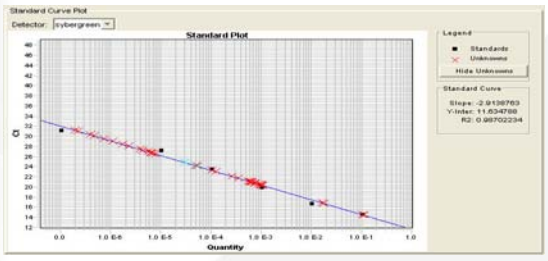


Fig. 1A and Fig. B show the efficiency of PCR on two independent miRNAs (mi18 and mir196b). The PCR sensitivity permits amplification to 1 copy of the miRNA

Table 1. Human miRNA expression quantification in five different tissues

NAME	LIVER	HEART	BRAIN	LUNG	KIDNEY
mir10b	0	2	4	93	502
mir374	0	6	20	2	9
mir7-3	9	4	212	43	6
mir301	9	27	93	196	49
mir7-1	13	9	43	9	20
mir205	24	0	2	924	43
mir214	48	196	4299	445	196
mir122a	430	114	0	0	2
mir219-2	987	457	92612	430	438
mir32	924	438	2016	1961	924
mir29b-2	1961	430	4299	1987	1976
mir221	4299	924	924	42987	9258
mir18	43004	2015	4350	19608	4299

The copy numbers per cell is estimated from a standard curve of mir18 and synthetic mir 196b.

We have designed specific primers for pre-miRNA sequences to amplify all the different precursors of an miRNA, thus giving better accuracy.

All the PCRs were performed in duplicate on genomic DNA and cDNA. It was checked that they gave no dimeric structures. All products were sequenced and showed perfect specificity.

DNAVision carries out assays on samples to quantify the expression of target miRNAs.

METHODOLOGY

Cells /Tissue

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Total RNA → Microarray analyses

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miRNA quantification

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Quality control according to norm ISO 17025, using Bioanalyser 2100

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Statistical analyses and identification of miRNA signature and detailed confidential report