

# MicroRNA Expression Signature in Human Glioblastoma Multiforme Brain Tumor

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## ABSTRACT

Expression of 180 human miRNAs was examined using recently developed stem-loop primers for reverse transcription (RT) followed by real-time PCR. MicroRNAs can be quantified from as few as single cells or as little as 25 pg total RNA. The  $C_T$  values correlated to the copy number over up to seven orders of magnitude. The TaqMan® miRNA assays discriminated between two miRNAs that differed by as little as a single nucleotide, and between mature miRNAs and their precursors. This method allows accurate and sensitive miRNA expression profiling and uncovers precise changes of miRNA expression. Comparing to normal human brain, the glioblastoma multiforme (GBM) tumors have a distinct expression signature of miRNAs. Nearly half of miRNAs showed the reduced expression by > 2-folds. In contrast, only 13% miRNAs had increased expression (>2-folds) in GBM. Expression of miR-10a and miR-10b etc. located within class I HOX and miR-129, miR-139, and miR-153 etc. within class II HOX gene clusters is either elevated or reduced (>10-fold), suggesting that these miRNAs may be involved in brain cancers.

## INTRODUCTION

MicroRNAs are endogenous RNAs of ~22 nucleotides that play important regulatory roles (1). More than 750 miRNAs have been identified across species. Their expression levels vary greatly among species and tissues (2). Low abundant miRNAs have been difficult to detect using current technologies. Here, we present a new real-time quantitation method termed looped-primer RT-PCR for accurate and sensitive detection of miRNAs as well as expression profiling for human brain tumors.

## MATERIALS & METHODS

**miRNA targets:** 180 human miRNAs.

**Tissue RNA samples:** Four normal human brain and two brain tumor (GBM) samples.

**Cells:** Heat-treated OP9 cells were directly used for quantification.

**RT-PCR:** The assay includes two steps, RT and PCR (Figure 1). RT reactions containing RNA samples, looped-primers, 1X buffer, reverse transcriptase, and RNase inhibitor were incubated for 30 min each, at 16°C and at 42°C. Real-time PCR was performed on an AB 7900HT Sequence Detection System.

**Data analysis:** The copy number per cell was estimated based on the standard curve of synthetic lin-4 miRNA. Agglomerative hierarchical clustering between normal human brain and tumor tissues was performed by using CLUSTER program (3). The fold-change was calculated against Ambion's brain RNA.

## RESULTS

Fig. 1. Assay scheme

**Step 1. Stem-loop RT:** Stem-loop RT primers are annealed to miRNA targets and extended in the presence of reverse transcriptase.

**Step 2. Real-time PCR:** miRNA-specific forward primer, TaqMan® probe, and reverse primer are used for PCR. Quantitation of miRNAs is estimated based on measured  $C_T$  values.

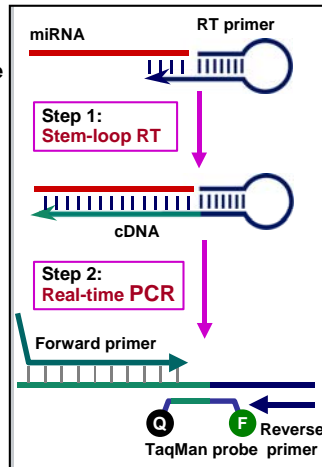
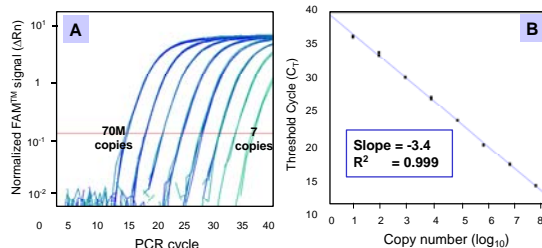


Figure 2. Quantitation of synthetic lin-4 miRNA



(A) Amplification plot of synthetic lin-4 miRNA over 7-logs. (B) Standard curve for lin-4 miRNA.  $C_T$  values were plotted against copy number.

Fig. 3. Quantification of miRNAs from single cells

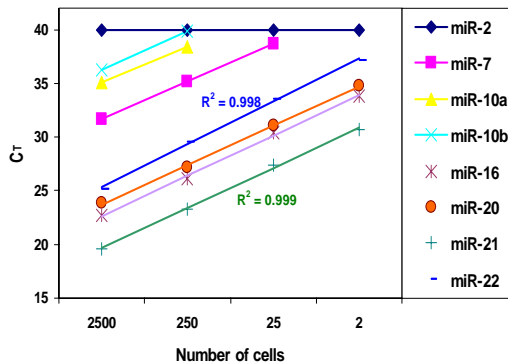
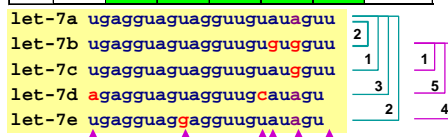


Fig. 4. Single base discrimination of miRNA assays

miRNA assay	Synthetic miRNA target					Relative detection (%)
	let-7a	let-7b	let-7c	let-7d	let-7e	
let-7a	100	0.3	3.7	0.0	0.0	2
let-7b	0.0	100	0.3	0.0	0.0	
let-7c	0.0	2.5	100	0.1	0.0	
let-7d	0.1	0.0	0.0	100	0.0	
let-7e	0.0	0.0	0.0	0.0	100	

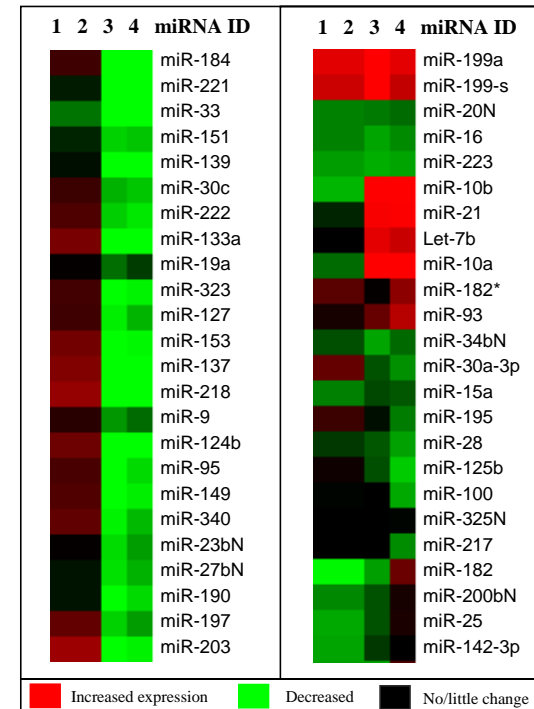


\* Relative detection (%) calculated based on  $C_T$  difference between perfectly matched and mismatched assays

Table 1. Expression changes of miRNAs in GBM tumor

Fold change	miRNA representatives	No.	%
Down 10X	miR-218, miR-124a, miR-124b, miR-137, miR-184, miR-129, miR-33, miR-139, miR-128b, miR-128a, miR-330, miR-133a, miR-203, miR-153, miR-326, miR-105, miR-338, miR-133b, miR-132, miR-154*, miR-29bN	21	12
	miR-7N, miR-323, miR-219, miR-328, miR-149, miR-122a, miR-321, miR-107, miR-190, miR-29cN, miR-95, miR-154, miR-221, miR-299, miR-31, miR-370, miR-331, miR-342, miR-340 etc.	66	37
Up 10X	miR-10b, miR-10a, miR-96	3	2
Up 2-10X	miR-182, miR-199b, miR-21, miR-124, miR-199a, miR-199-s, miR-199a*, miR-106b, miR-15b, miR-188, miR-148a, miR-104, miR-224, miR-368, miR-23a, miR-210N, miR-183, miR-25, miR200cN, miR-373, miR-17-5p	21	11
	miR-143, miR-186, miR-337, miR-30a-3p, miR-355, miR-324-3p etc.	69	38

Figure 5. Heat map displaying miRNA expression in normal brain (1-2) and glioblastoma brain tumors (3-4)



## REFERENCES

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- Eisen et al. 1998. PNAS 95:14863-14868

## ACKNOWLEDGEMENTS

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## NOTES

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