

# TaqMan<sup>®</sup>-based miRNA profiles classify mouse ES and differentiated cells

Caifu Chen, Dana A. Ridzon, Yu Liang, Linda Wong, Karl J. Guegler, Advanced Research & Technology, Applied Biosystems, 850 Lincoln Centre Dr., Foster City, CA 94404, USA; William M. Strauss, Department of Molecular, Cellular, & Developmental Biology, University of Colorado, Boulder, CO 80309, USA

## ABSTRACT

TaqMan<sup>®</sup> miRNA assays have been developed using stem-loop primers for reverse transcription (RT) followed by real-time PCR. A total of 254 mouse and human miRNA assays were examined with thirteen different mouse embryonic stem cell (ES) lines and their differentiated embryoid bodies (EBs) and six mouse tissues. MicroRNA expression profiles can classify the ES cells, differentiated EBs and adult tissues. We have identified a number of ES-specific and differentiation-related miRNA candidates that could be used as molecular markers to determine ES cell identity and to monitor the differentiation. There exists a highly conserved miRNA expression signature in 13 mouse ES lines. This signature, when compared to all cellular lineages, includes three components of miRNA expression: an unchanging per-developmental microRNA expression pattern, increasingly complex somatically acquired microRNA expression pattern, and stem cell specific microRNA expression pattern. Only ¼ miRNA genes are highly expressed in ES cells, and during development an increasingly elaborate miRNA signature is expressed. The stem cell specific expression of a small set of microRNAs is lost in an apparently coordinate fashion during development and does not reappear in any somatic lineage. Based on the elucidation of this regulated miRNA molecular signature, it seems likely that there is a significant role for miRNA action in the early embryo.

## INTRODUCTION

MicroRNAs are endogenous RNAs of ~22 nucleotides that play important regulatory roles in animals & plants (1). We propose that miRNAs are likely important regulators for stem cell self-renewal. Our hypothesis is based on the observation that distinct sets of miRNAs are specifically expressed in pluripotent ES cells but not in differentiated EBs (2), and that EBs express miRNAs not found in ES cells. To test this hypothesis we have conducted genome-wide expression of 252 miRNAs in mouse ES cells, EBs, and adult tissues. This survey of miRNA expression utilized TaqMan miRNA assays to profile miRNA expression signatures or fingerprints. miRNA expression signatures in single cells will be useful in determining tissue/cell identity and monitoring the ES differentiation.

## MATERIALS AND METHODS

**miRNA genes:** 254 mouse and/or human miRNAs.

**RNA samples:** Six from mouse tissues, 13 from mouse ES lines, and 5 from embryoid bodies at day 6 or 9.

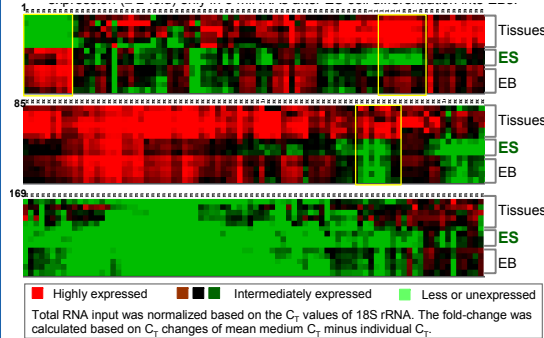
**Stem cell differentiation:** Aggregated ES cells were cultured under feeder- and serum-free condition (Kanno et al. 2004). Adherent (Ad) and non-adherent (NA) cells were harvested at 6 and 9 days.

**RT-PCR:** The assay includes two steps, RT and PCR (Figure 1). RT reactions 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.

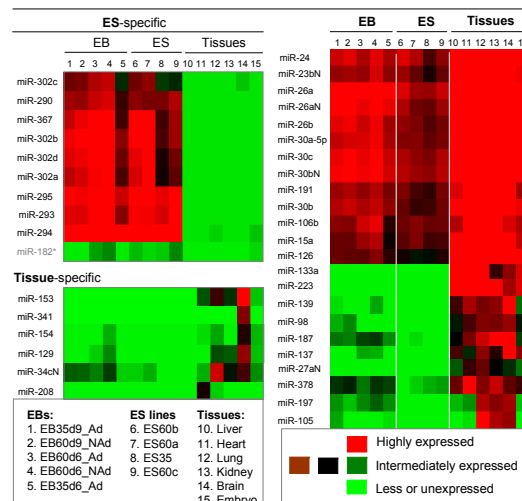
**Data analysis:** Total RNA input was normalized based on  $C_T$  values of 18S rRNA assay. All  $C_T$  values with 35 or higher are adjusted to 35. The fold-change was calculated based on  $C_T$  changes of mean medium  $C_T$  minus individual  $C_T$  of a miRNA. Agglomerative hierarchical clustering was performed using CLUSTER program (3).

## RESULTS

**Figure 1. TaqMan<sup>®</sup>-based miRNA profiles classify ES cells, differentiated embryoid bodies (EB) and tissues (embryo, brain etc.)**



**Figure 2. Mouse ES cell-, EB- or tissue-specific MicroRNAs**

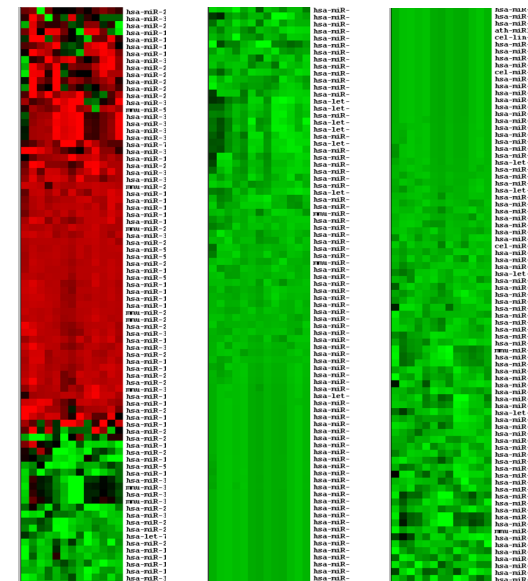


**Table 1. miRNA expression in mouse ES and differentiated cells**

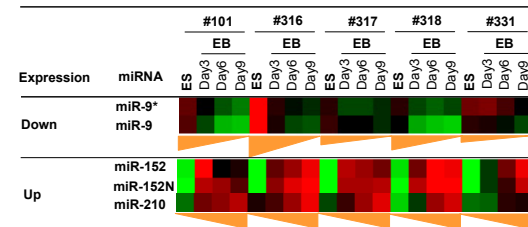
Expressed miRNAs*	Mouse tissues							
	ES	EB	Brain	Embryo	Heart	Kidney	Liver	Lung
No.	119	149	206	178	175	189	147	201
%	47	59	82	71	69	75	58	80

\* miRNAs are defined as "Expressed" if their  $C_T$  values are less than 35 (an estimate of >1.5 copies per cell). A total of 252 miRNAs are used to calculate % of expressed ones in a tissue or cell type

**Figure 3. Highly conserved miRNA expression profile in 13 mouse ES cell lines. Most miRNAs in ES cells are expressed at a low level. However, ¼ (61) miRNAs are highly expressed in ES. MicroRNA expression signature, together with ES-specific mRNA marker Oct3/4 confirmed the stemness of these ES lines. However, the variability of expression levels in some miRNAs, not mRNA genes suggests that miRNAs could be a better indicator to monitor spontaneous ES differentiation during cell culture.**



**Figure 4. MicroRNA expression changes of five ES cell lines upon differentiation**



## REFERENCES

- Bartel, D. 2004. Cell 116: 281-297
- Houbaviy et al. 2003. Developmental Cell 5:351
- Eisen et al. 1999. PNAS 95:14863-14868

## NOTES

For Research Use Only. Not for use in diagnostic procedures. The PCR process and 5' nuclease process are covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd. Applied Biosystems, AB and FAM are trademarks of Applied Biosystems, Inc. or its subsidiaries in the US and/or certain other countries. TaqMan is a trademark of Roche Molecular Systems, Inc. © 2005 Applied Biosystems. All rights reserved.