

# TaqMan<sup>®</sup> MicroRNA Assays And Their Applications For Stem Cell Research

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# Content

- Introduction
- TaqMan<sup>®</sup> miRNA assays
  - *How they work*
  - *How they performed*
- Applications
  - *MicroRNA expression profiles classify **ES**, differentiated cells and tissues*
  - *MicroRNA expression profiling in a **single** cell*

# miRNAs: Genomics

- ~22 nt, single-stranded, endogenous, non-coding RNAs
- Highly conserved among related species
- Found in animals and plants
- Estimated >250 miRNA genes per species
- Wide range of expression: Few to 50,000 copies per cell
- Mature miRNAs can regulate multiple genes
- Target mRNAs for translational repression or cleavage

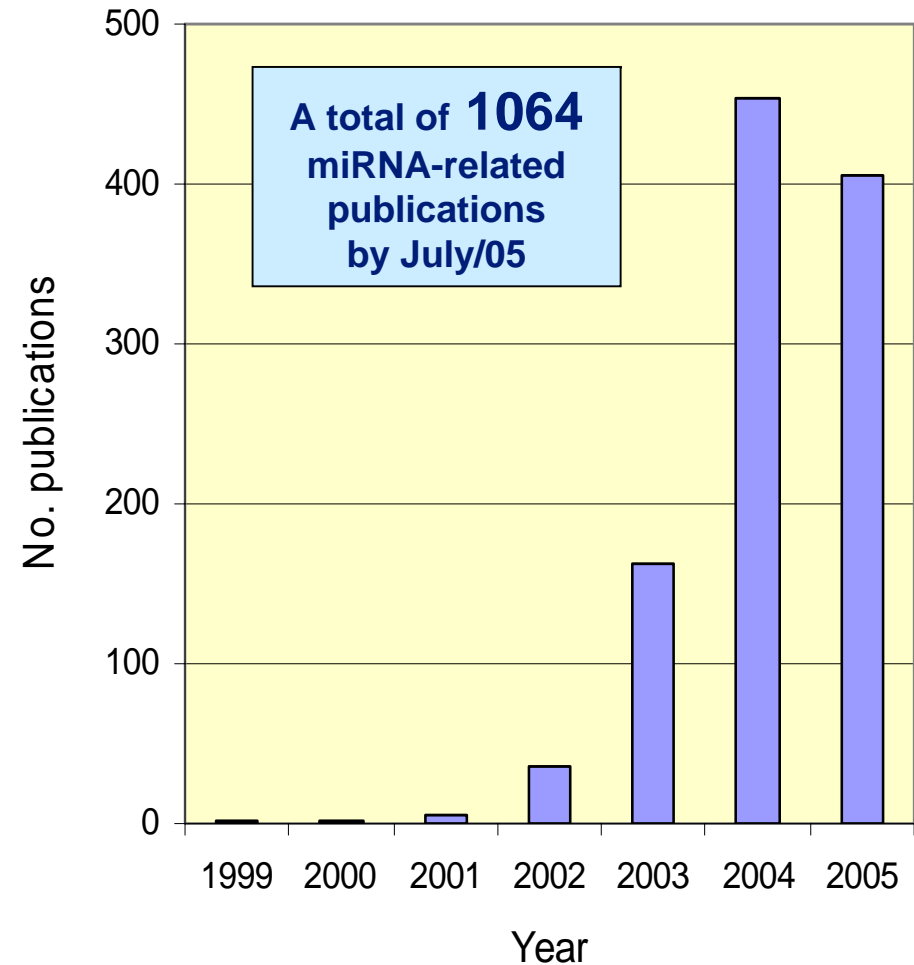
**cel-lin-4, 1st miRNA discovered by Ambros et al. (1993) :  
5'-pUCCCUGAGACCUCAAGUGUGA-OH-3'**



Bartel, 2004

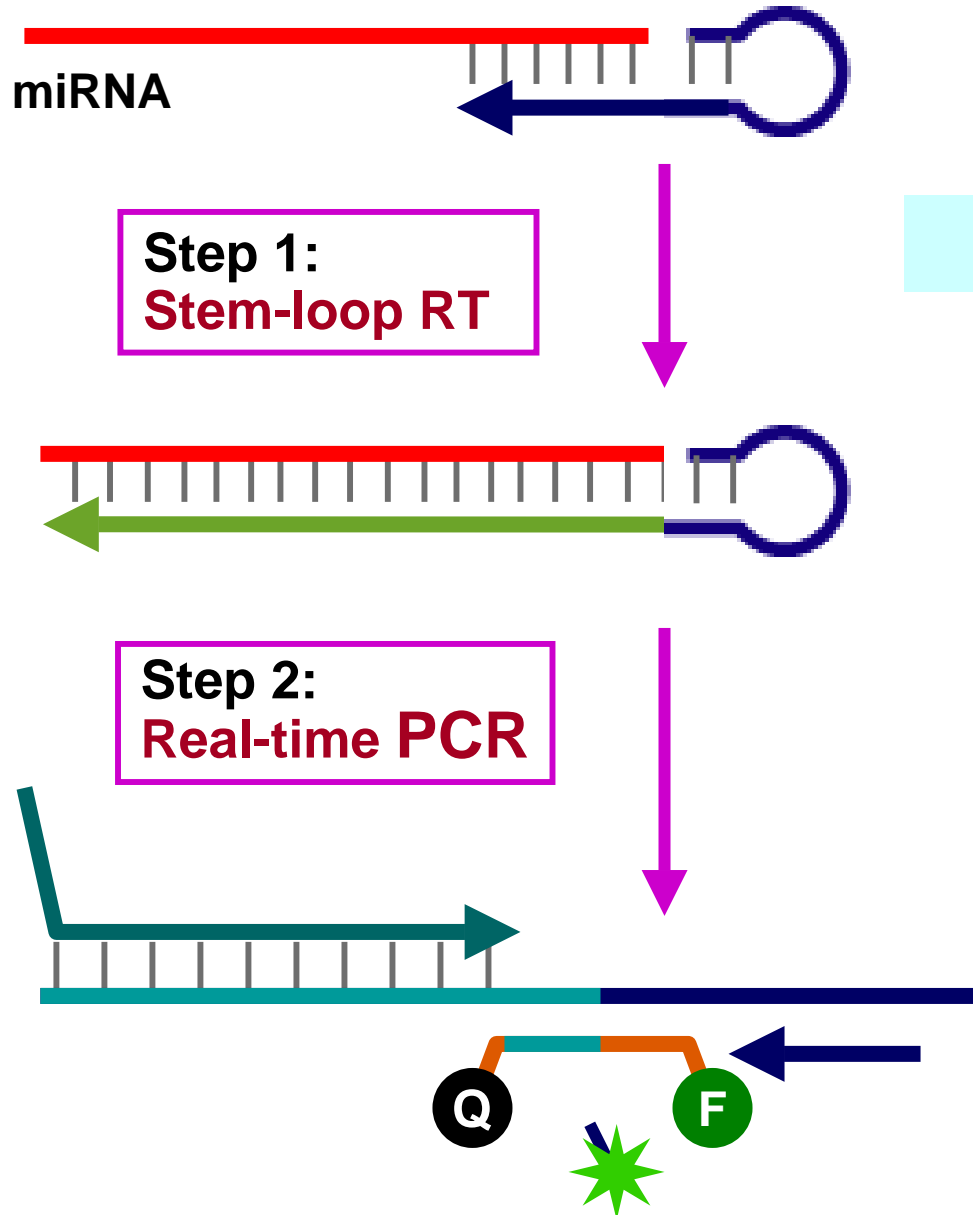
# Micro RNAs: Macro Significance

- **A new class of post-transcriptional regulatory elements**
- **ES cell division is regulated by miRNA pathway (Hatfield, 2005)**
- **Some miRNAs control development**
- **miRNAs are associated with disease processes**
- **miRNAs may be used as biomarkers**
- **miRNAs may have clinical research applications**



# Current miRNA Detection Methods

- **Cloning & sequencing**
  - Cloning/sequencing (Elbashir et al., 2001)
  - MPSS (Lynx, 2004)
  
- **Hybridization**
  - Northern (Lagos-Quintana et al., 2001)
  - Ribonuclease protection-based PAGE (Ambion, 2004)
  - Microarrays (Liu et al., 2004)
  - Bead-based detection (Lu et al. 2005)
  
- **Real-time quantitation**
  - Invader assays (Allawi et al., 2004)
  - Real-time PCR (Chen et al. 2005)

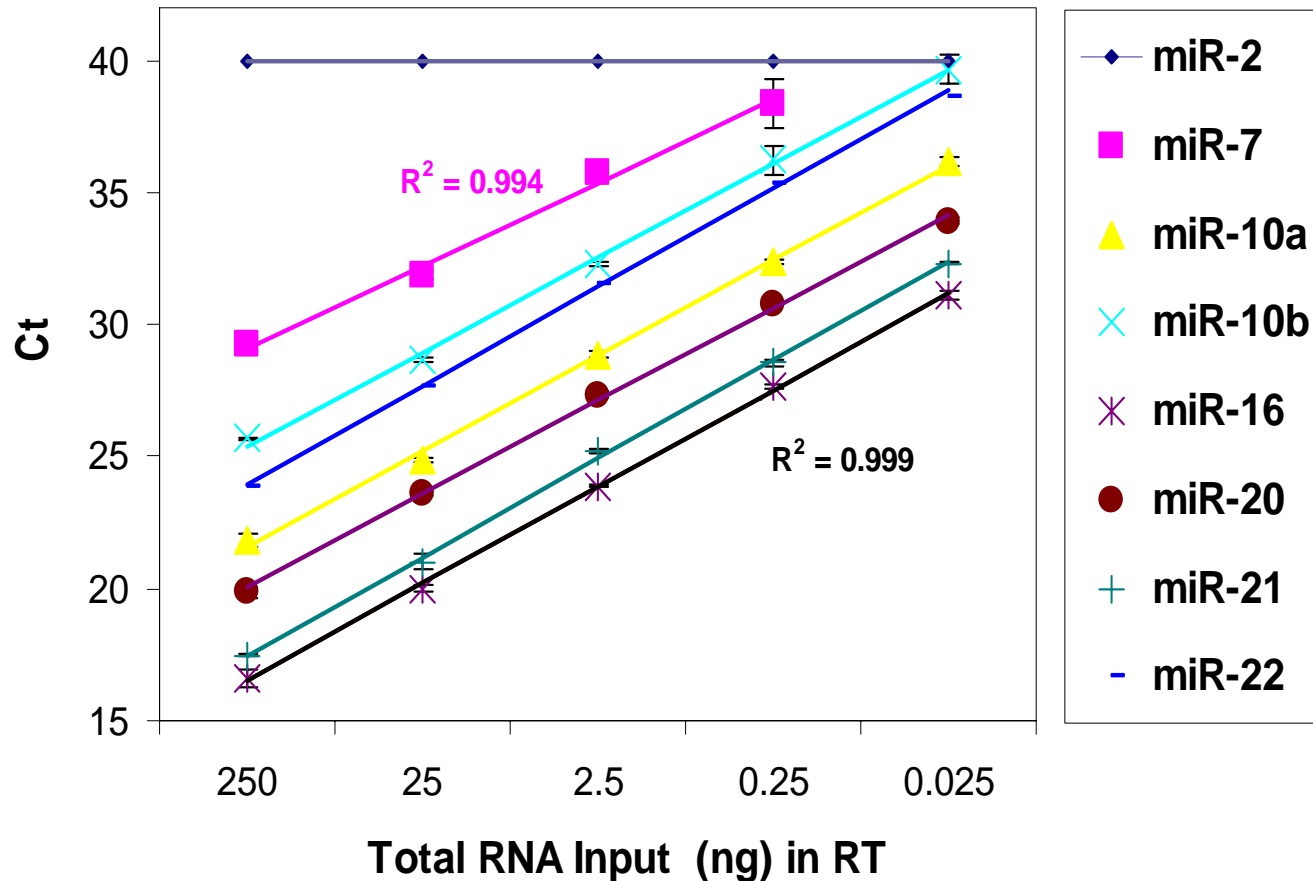


TaqMan<sup>®</sup> MicroRNA Assays

# miRNA Quantitation – Total RNA

Detection sensitivity: **25 pg** total RNA

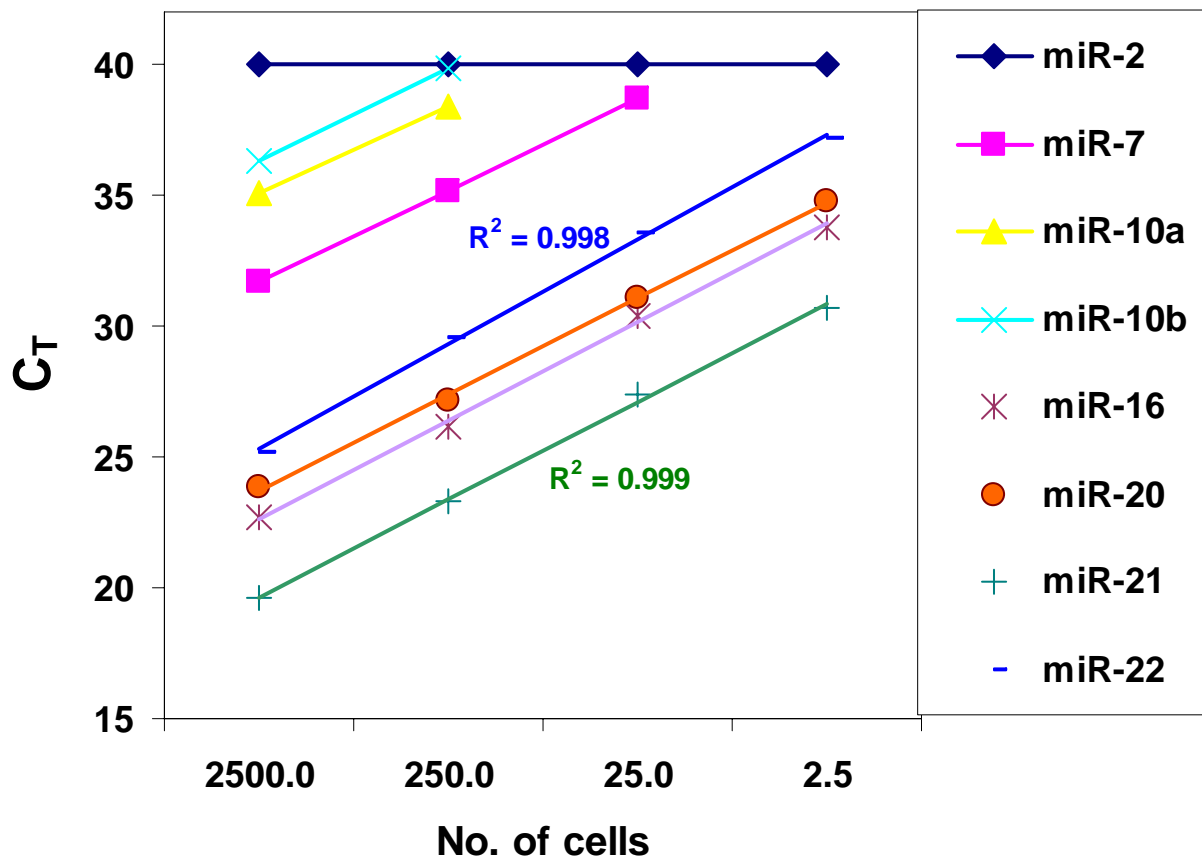
Dynamic range:  $\geq 10^4$  using mouse lung total RNA



# miRNA Quantitation-Cell lysate

Detection sensitivity: up to **single cells**

Dynamic range:  $\geq 10^3$  using OP9 cell lysate



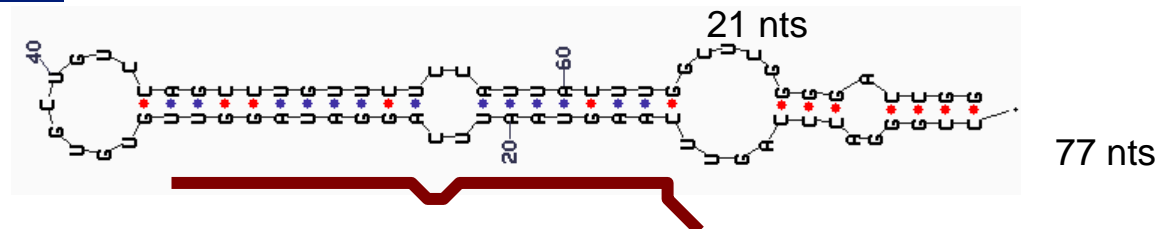


# Assays Are Specific for Mature miRNAs

ID	Synthetic target input (No. copies)		TaqMan miRNA Assay (C <sub>T</sub> )
	Mature miRNA	Precursor	
miR-26b	1.5×10 <sup>8</sup>	0	<b>16.5</b>
	0	1.5×10 <sup>8</sup>	<b>27.4</b>
	0	0	ND
let-7a	1.5×10 <sup>8</sup>	0	<b>16.5</b>
	0	1.5×10 <sup>8</sup>	<b>29.5</b>
	0	0	ND

Mature miRNA miR-26b: UUCAAGUAAUUCAGGAUAGGU

miR-26b Precursor:

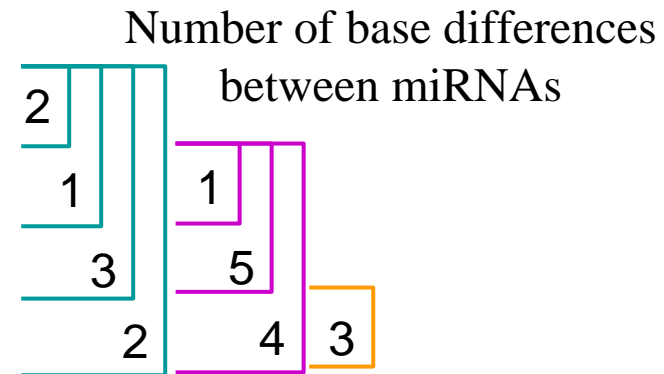


# Single-base Discrimination

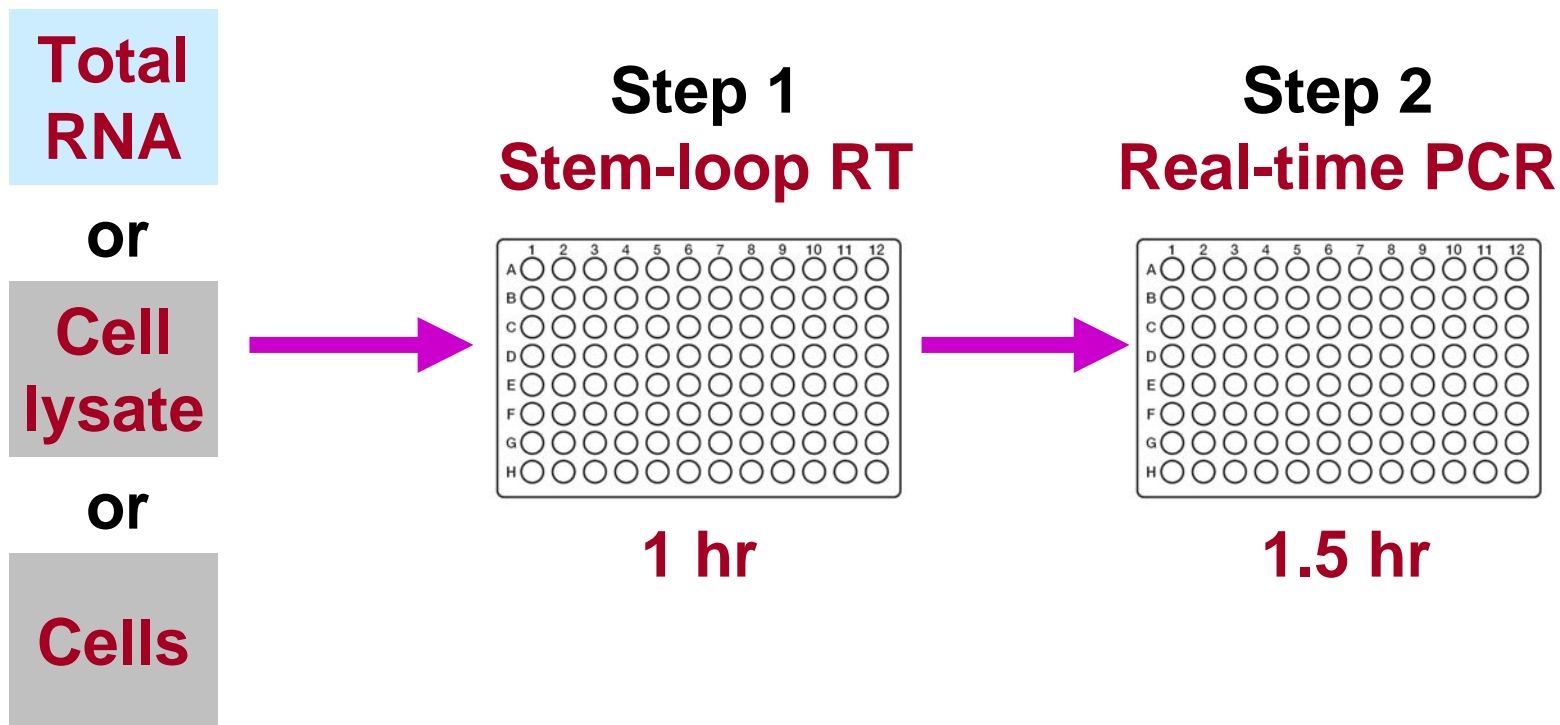
		Synthetic miRNA target					Relative detection (%)
		let-7a	let-7b	let-7c	let-7d	let-7e	
miRNA assay	let-7a	100	0.3	3.7	0.0	0.0	Relative detection (%)
	let-7b	0.0	100	0.3	0.0	0.0	
	let-7c	0.0	2.5	100	0.1	0.0	
	let-7d	0.1	0.0	0.0	100	0.0	
	let-7e	0.0	0.0	0.0	0.0	100	

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

let-7a ugagguaguagguuguauaguu  
 let-7b ugagguaguagguuguguguu  
 let-7c ugagguaguagguuguauaguu  
 let-7d agagguaguagguugcauagu  
 let-7e ugagguaggagguuguauagu



# TaqMan<sup>®</sup> miRNA Assay Flowchart



# Mouse miRNA Body Map

miRNA ID	Copy number per cell							Average	Fold-change
	Brain	Heart	Liver	Lung	Thymus	Ovary	Embryo		
let-7a	2010	1420	700	2390	1420	3120	1050	1730	5
miR-16	10240	13520	3890	22080	32090	11100	5210	14020	6
miR-20	70	300	130	580	1990	420	620	590	28
miR-21	670	2540	4450	7970	3550	5310	390	3550	20
miR-22	290	1020	310	590	130	560	40	420	26
miR-26a	7470	4360	3240	10680	2160	6880	1390	5170	8
miR-29a	4410	1040	730	7740	790	2950	20	2530	387
miR-30a	120	160	70	370	20	130	40	130	19
miR-34a	1240	140	90	430	200	490	60	380	21
miR-200b	20	1	10	210	40	130	30	60	233
miR-323	80	0	0	0	0	0	30	20	2377
miR-324-5p	270	30	10	100	50	80	150	100	27
Average	2240	2040	1140	4430	3540	2600	750	<b>2400</b>	263

- **Copy number per cell** is estimated based on standard curve of lin-4 synthetic miRNA assuming 15 pg/cell.
- A total of 10 ng RNA (or equivalent ~667 cells) used in PCR.

# Potential Applications

- Expression profiling
- Biomarker discovery
- MicroRNA profiles for compound screening
- miRNA target discovery

A pancreatic islet-specific miRNA regulates insulin secretion

Poy et al., 2004. *Nature*

miR-375 was shown to regulate the secretion of insulin and could constitute a novel pharmacological target for diabetes treatment.

# MicroRNAs and ES cells

- **Can miRNA profiles distinguish ES cells from others?**
  - Does miRNA expression change upon differentiation? How?
  - Are there miRNAs specific to the cell type or differentiation stage?
  - Can they be used to monitor spontaneous differentiation of ES cells and evaluate the cell identity?
  - Do alterations in miRNA expression result in alterations in stem cell potential/cell fate?

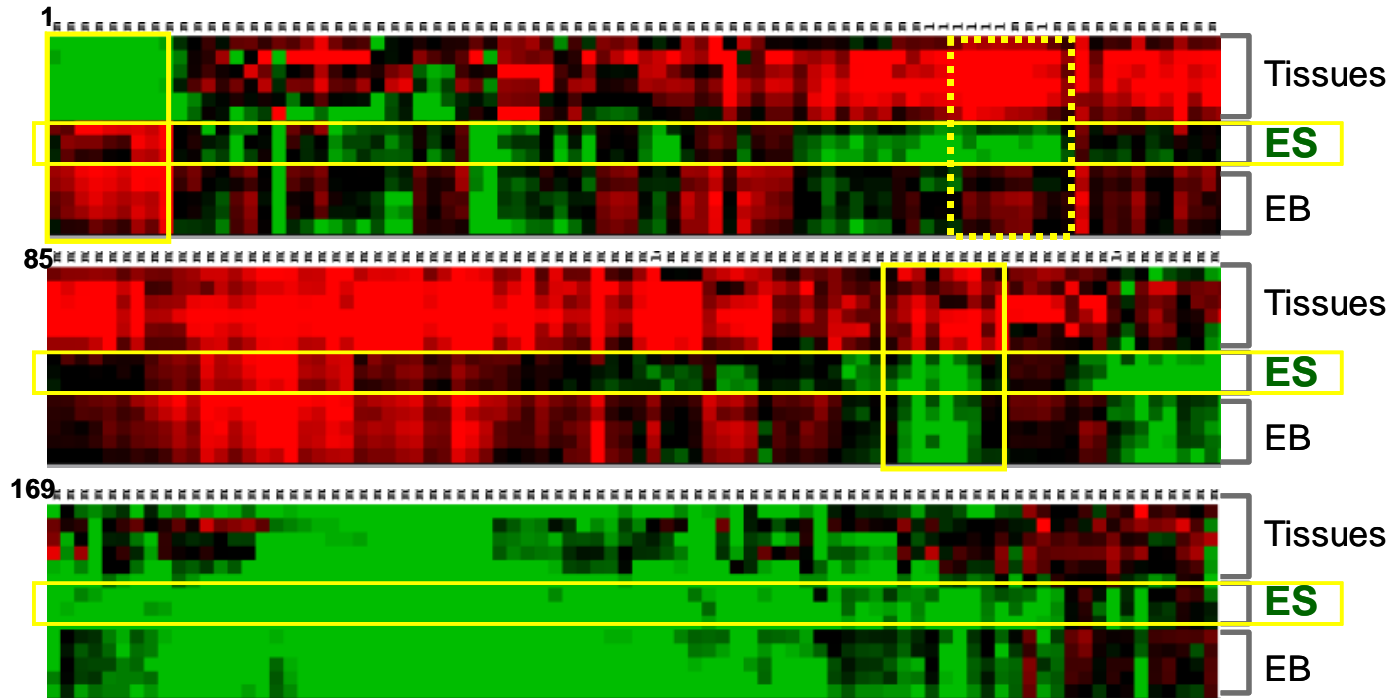
# Expression of 252 miRNAs in mouse ES and differentiated cells and tissues

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

# TaqMan<sup>®</sup>-based miRNA profiles classify ES cells, differentiated embryoid bodies (EBs) and tissues

- Increased expression ( $\geq 2$ -fold) observed in 138 miRNAs & decreased expression ( $\geq 2$ -fold) only in 3 miRNAs after ES cell differentiation into EBs.



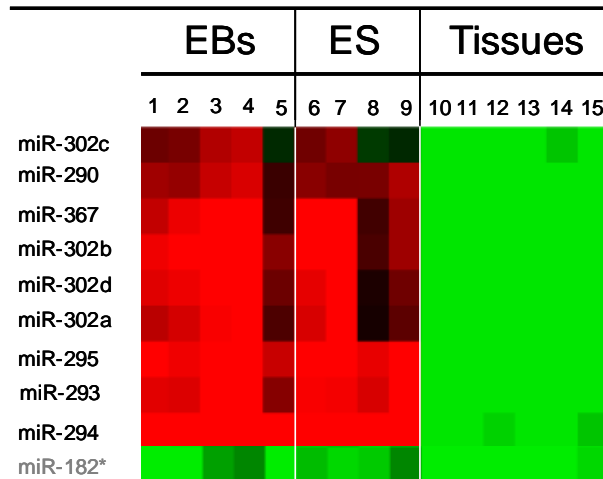
■ Highly expressed    
 ■ ■ ■ Intermediately expressed    
 ■ Nearly unexpressed

Total RNA input was normalized based on the  $C_T$  values of 18S rRNA. The fold-change was calculated based on  $C_T$  changes of mean medium  $C_T$  minus individual  $C_T$ .

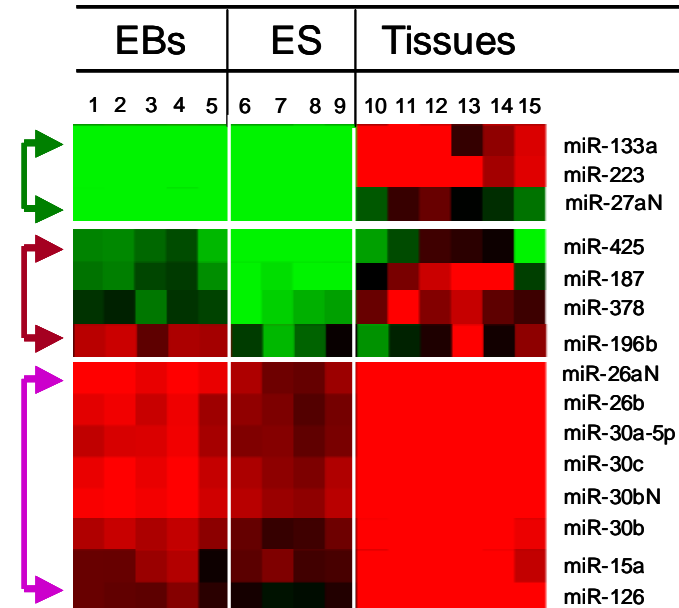


# Mouse MicroRNA Markers

## ES cell-specific



## Differentiation-specific



### Embryoid bodies (EBs)

1. EB35d9\_Ad
2. EB60d9\_NAd
3. EB60d6\_Ad
4. EB60d6\_Nad
5. EB35d6\_Ad

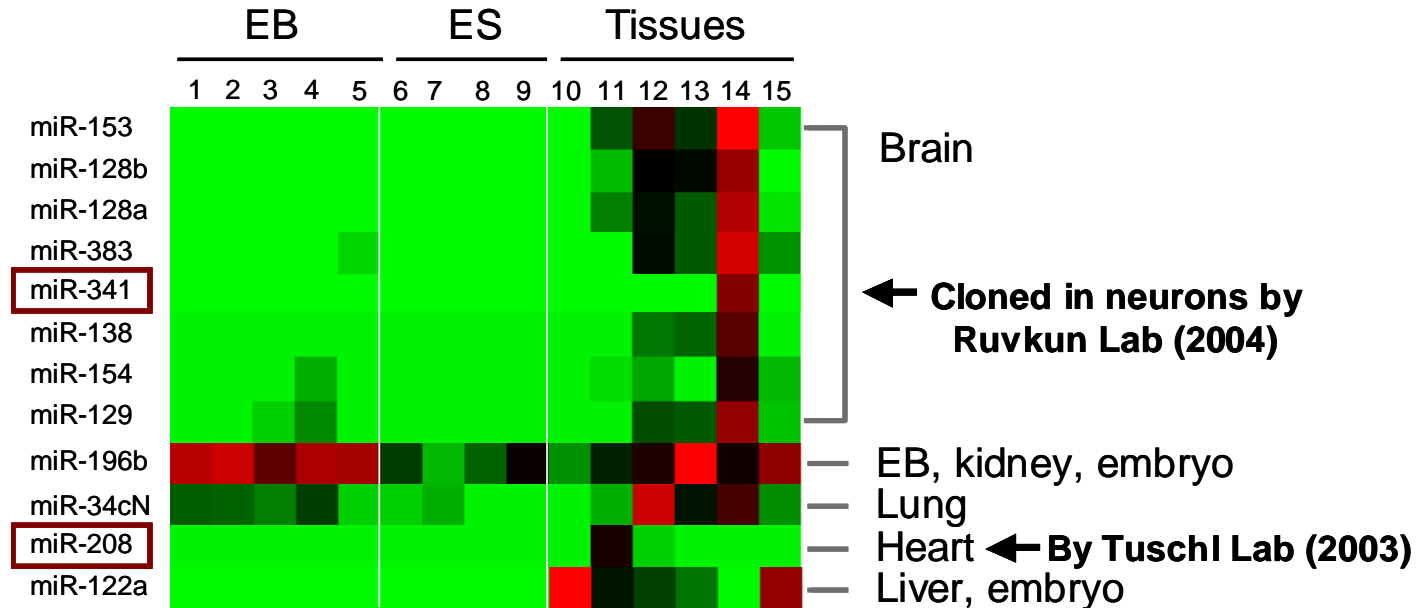
### ES lines:

6. ES60b
7. ES60a
8. ES35
9. ES60c

### Mouse tissues:

- |           |            |
|-----------|------------|
| 10. Liver | 13. Kidney |
| 11. Heart | 14. Brain  |
| 12. Lung  | 15. Embryo |

# Mouse tissue-specific miRNAs



### EBs & ES lines:

1. EB35d9\_Ad
2. EB60d9\_NAd
3. EB60d6\_Ad
4. EB60d6\_NAd
5. EB35d6\_Ad
6. ES60b
7. ES60a
8. ES35
9. ES60c

### Tissues:

10. Liver
11. Heart
12. Lung
13. Kidney
14. Brain
15. Embryo

## Other Potential Applications

- **Quantitation of siRNAs**
  - **Stability in vivo**
  - **Efficacy of siRNAs**
  - **Sense vs antisense**
- **Quantitation of shRNAs**
  - **Monitor expression levels of shRNAs/siRNAs**
  - **Optimize the shRNA vectors**
- **MicroRNA profiling in a single cell**

# Pre-amplification based TaqMan<sup>®</sup> miRNA assays detect 192 miRNAs in a *single* ES cell

No. input cells	Mouse ES			NIH/3T3		
	1	2	DMEM	1	2	DMEM
miR-16 C <sub>T</sub>	30.3	28.9	ND	28.1	26.9	ND
delta C <sub>T</sub> (1 vs 2 cells)	1.4			1.2		

\* This is a part of collaboration with **Dr. Azim Surani** and Fuchou Tang at the University of Cambridge. ND represents non-detectable amplification.

# Summary

- A total of 252 mouse and human miRNAs were examined in 4 mouse ES lines, 5 differentiated EBs, and 6 tissues. Of 238 human miRNAs, 215 (90%) were detected in mouse tissues.
- TaqMan® miRNA assay performance
  - Quantitative and specific
  - Sensitive and reproducible
  - Fast and easy to use.
- MicroRNA expression profiles can classify the ES cells, differentiated EBs and adult tissues. The ES expression levels globally increase upon differentiation.
- We have identified a set of ES- and differentiation-specific miRNAs that could be used as molecular markers to classify ES lines and to monitor their spontaneous differentiation.
- Pre-amplification based TaqMan® miRNA assays are being developed to quantify miRNAs from single cells. miRNA expression signatures in single cells will be useful in determining tissue/cell identity and monitoring the ES cell differentiation.

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- **Other AB colleagues:**

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## NOTES

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