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Single ES cell profiling reveals unique microRNA expression signatures and heterogeneities

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Outline

- Introduction
- TaqMan® MicroRNA Assays
- Expression profiles of microRNAs in ES and differentiated cells
- Single ES cell miRNA and mRNA profiling
- Summary

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MicroRNAs

- Small, single-stranded, endogenous, non-coding RNAs of ~ 22nt
- Found in animals, plants, and some viruses
- Identified 470 human miRNAs, estimated 600~800 miRNA genes in human
- Highly conserved (>80% identity between human and mice)
- Wide ranges of expression: from few to ~250,000 copies per cell
- Translational repression
- Regulate crucial biological processes

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Micro RNAs, Macro significance

- Development
  - Timing: lin-4, let-7
  - Neuron: miR-7, -23a, -430
  - Flower: miR-172
- Proliferation: miR-101, -181
- Apoptosis: miR-14, -15, -16
- Diseases
  - Cancers: miR-10, -17, -18, -19a, -19b-1,-20, -92-1, -101,-372, -373
  - Diabetes: miR-375
- Epigenetic: let-7a-3, miR-127, -223

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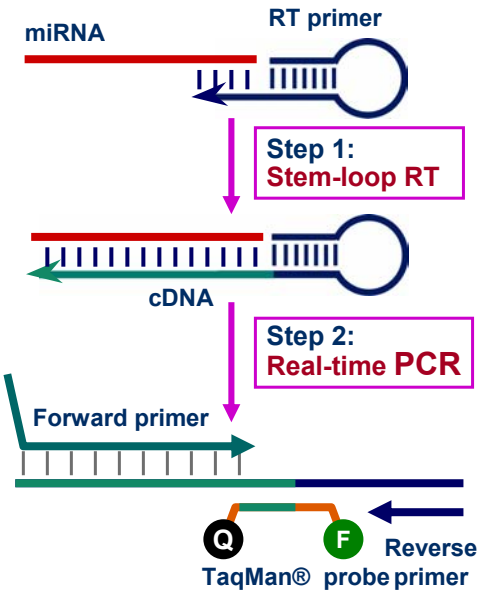

TaqMan® MicroRNA Assays

Four oligos per miRNA

1. RT primer
2. Forward primer
3. Reverse primer
4. TaqMan® probe

Two enzymes required

1. Reverse transcriptase
2. AmpliTaq Gold® DNA Polymerase

Chen *et al*. Nucleic Acid Res. v33: e179, 2005

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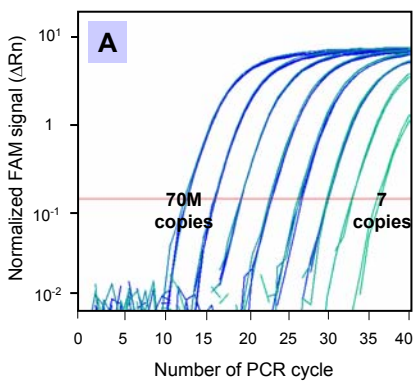
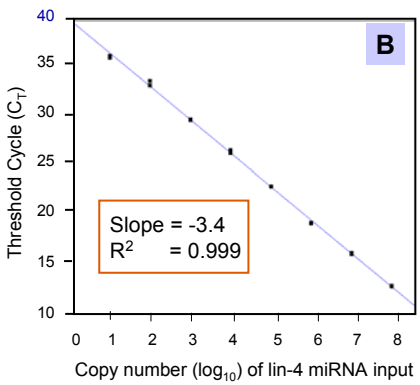
MicroRNA quantitation: efficiency and dynamic range

- Synthetic lin-4 miRNA input from 7 to 70M copies in TaqMan® MicroRNA Assay
- High efficiency, very sensitive and up to 7-log dynamic range





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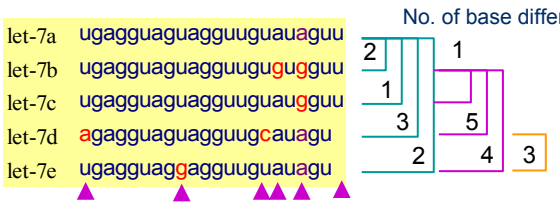
Assay specificity: Cross-reactivity

Discriminate miRNAs only different in a few nucleotides

miRNA assay	Synthetic miRNA template					Relative detection (%)
	let-7a	let-7b	let-7c	let-7d	let-7e	
let-7a	100.0	0.3	3.7	0.0	0.0	
let-7b	0.0	100.0	0.3	0.0	0.0	
let-7c	0.0	2.5	100.0	0.1	0.0	
let-7d	0.1	0.0	0.0	100.0	0.0	
let-7e	0.0	0.0	0.0	0.0	100.0	



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

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MicroRNAs and stem cells

Stem cells are cells with unique qualities:

- Self-renewal
- Multipotency

Biomarkers of stem cells:

- mRNAs (Oct4, Nanog, Sox2, AFP et al.)
- Cell surface markers (Cd34, c-Kit, Sca-1 et al.)

Embryonic stem (ES) cells

Loss of Dicer1 causes embryonic lethality: miRNA importance in stem cells.

Can miRNAs be used as biomarkers to distinguish stem cells from differentiated cells?

To profile miRNAs in ES cells and in differentiated cells with the TaqMan® MicroRNA Assay

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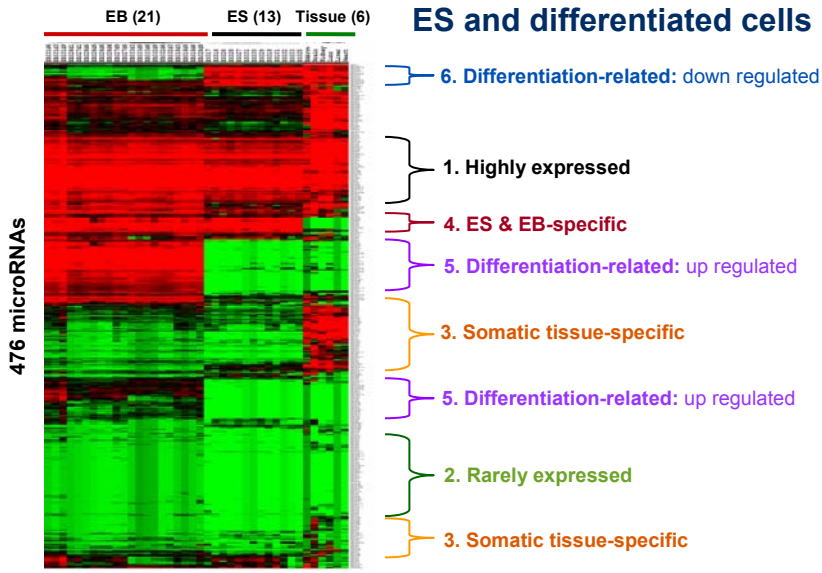
MicroRNA profiles classify mouse ES and differentiated cells



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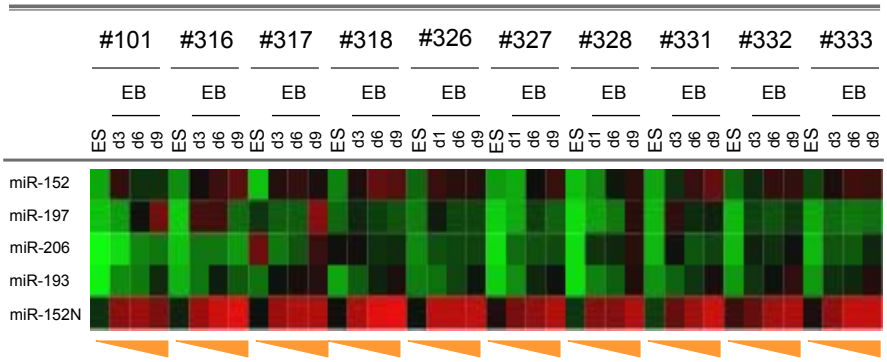
Increased expression of miRNAs that respond to differentiation time of ES cells into EB cells



These miRNAs could be used as biomarkers to determine the degree of ES differentiation.

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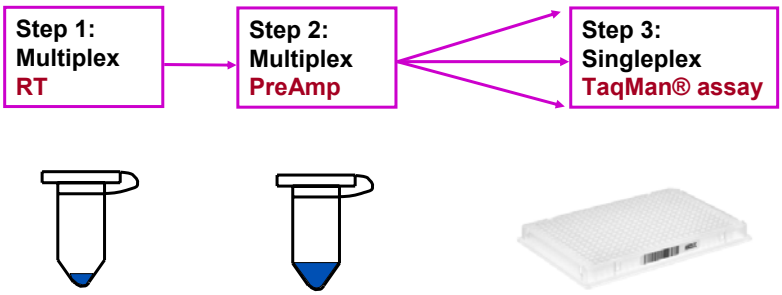
Single cell miRNA & mRNA profiling scheme



Advantages of Multiplex RT Preamp TaqMan® MicroRNA Assay:

1. Minimize RNA sample input in miRNA profiling
2. Improve through put and simplicity
3. Reduce cost
4. Better normalization
5. Profile miRNAs and mRNAs simultaneously

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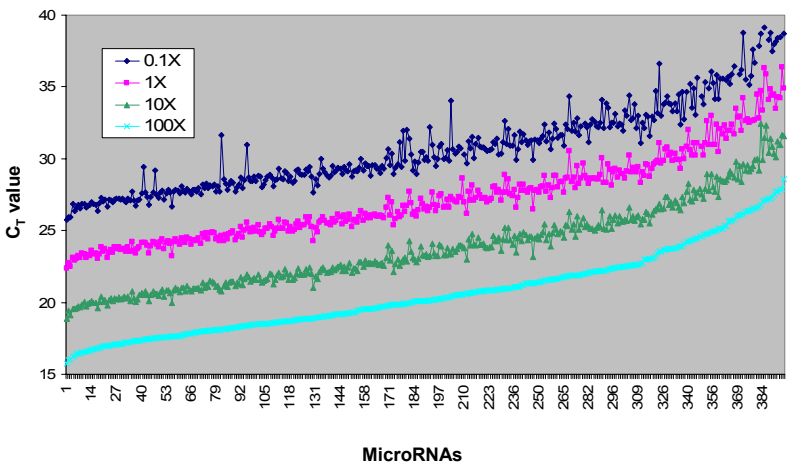
Linear response to RNA input



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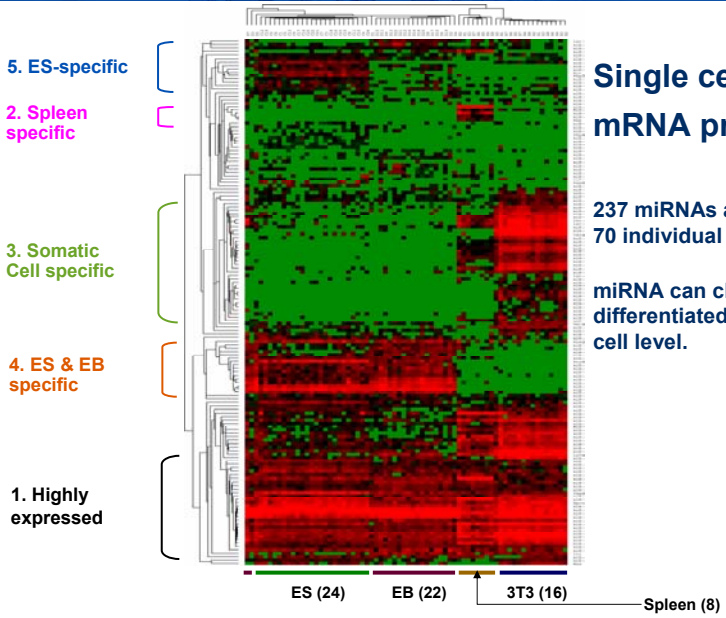

Single cell miRNA & mRNA profiling

237 miRNAs and 21 mRNAs in 70 individual single cells

miRNA can classify ES and differentiated cells at single cell level.

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The copy number of expressed miRNA increases with differentiation time

	ES	EB	3T3	Spleen
No. of cells analyzed	24	22	16	8
Ave. copy No./cell	210	300	1260	960
Inter-cell CV (%)	115	139	80	99

High inter-cell miRNA variation indicates the heterogeneity of single cells

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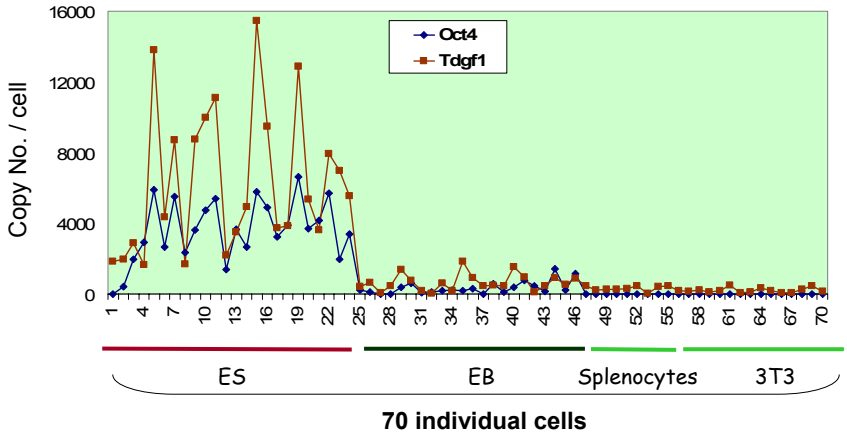
Expression pattern of ES marker genes Oct4 and Tdgf1 in single ES, EB, and somatic cells



- Oct4 and Tdgf1 are co-regulated in ES differentiation
- Variation of Oct4 and Tdgf1 among ES cell indicate the heterogeneities of ES cells

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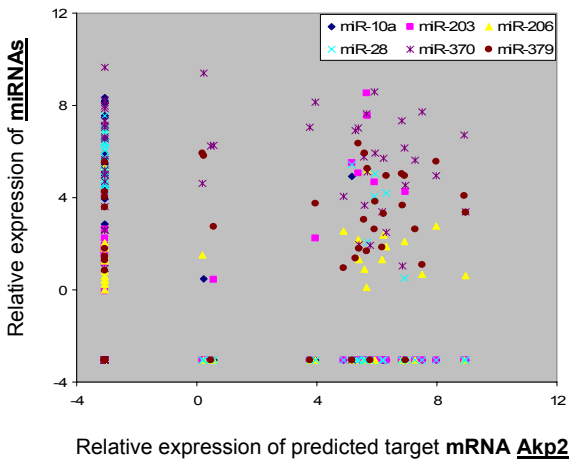
Gene expression between miRNAs and their predicted target mRNAs is NOT correlated at single cell level



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Summary

I. TaqMan® MicroRNA Assays

- miRNAs can classify ES and differentiated cells
- miRNAs could be used as biomarkers to determine the degree of ES differentiation

II. Multiplex RT PreAmp TaqMan® MicroRNA Assay

- The copy number of expressed miRNA increases with differentiation time
- ES cells are highly heterogeneous. The highest variability was observed among EB cells
- Lack of correlation between miRNAs and their predicted target mRNA expression, supporting translational repression model of miRNA mechanism

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