

# Characterization of miRNA expression in hESC lines using NCode™ SYBR GreenER qRTPCR

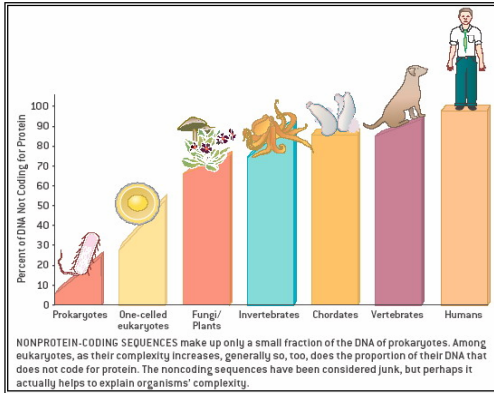


**Mark Landers**  
**Epigenetics**  
**Invitrogen**

## Presentation Overview

- **Brief overview of the biology**
- **NCode™ SYBR GreenER miRNA qRTPCR**
  - Platform
  - qRTPCR kit and performance
- **miRNA profiling of hESC cell lines**
  - miRNA expression profiles in hESC
  - Relative Quantitation of miRNA levels in hESC lines

# NC-RNA: The Key to Eukaryotic Complexity?



Organism	Percent of Transcriptional Output	
	Protein Coding RNA	Non Coding RNA
<i>E. coli</i>	84	16
<i>S. cerevisiae</i>	71	29
<i>C. elegans</i>	27	73
<i>D. melanogaster</i>	13	87
<i>H. sapiens</i>	2	98

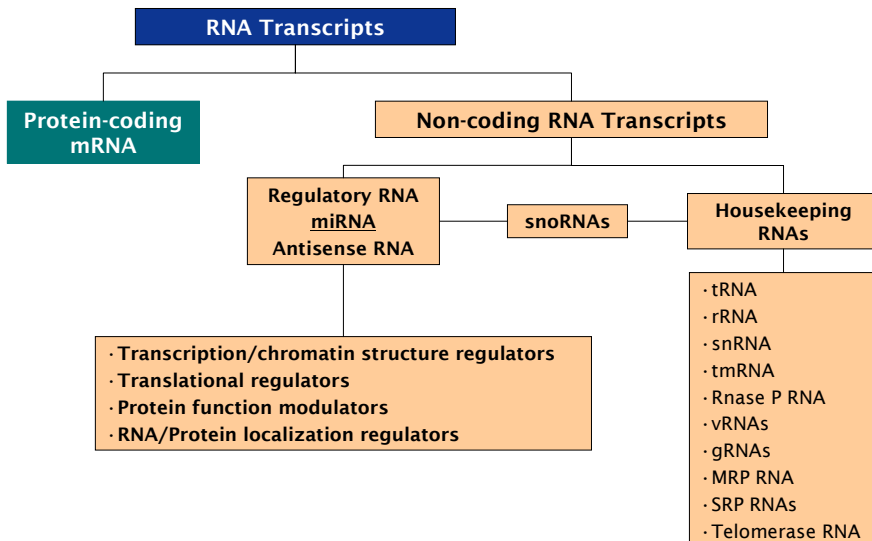
## Possible roles in:

- Cancer
- Neurological disorders
- Host-pathogen interactions

## Data suggesting role in diverse mechanisms:

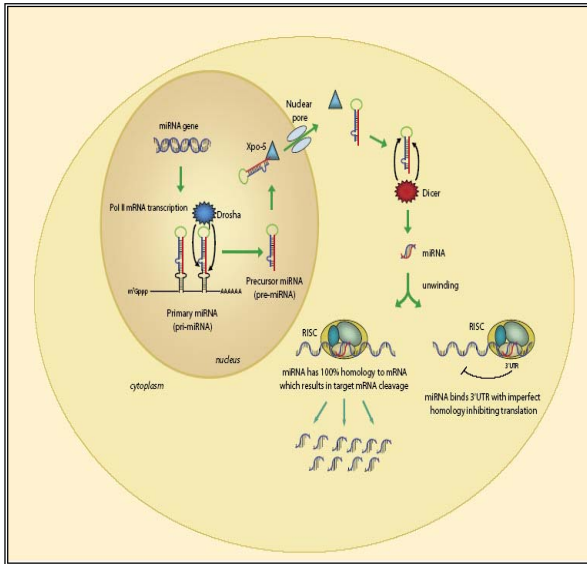
- RNAi
- DNA methylation
- Chromatin structure/remodeling

# Coding vs. Non-coding RNA



NC-RNAs compose majority of transcription in complex genomes

# miRNA Biogenesis and Action



## I. Evolutionarily conserved

- > ~500-1000+ in humans
- > Recent evidence suggests far more could exist...

## II. 20-24 nt in length

## III. Interaction with mRNA target suppressing protein expression

- > Perfect-mRNA degradation
- > Imperfect-translational suppression

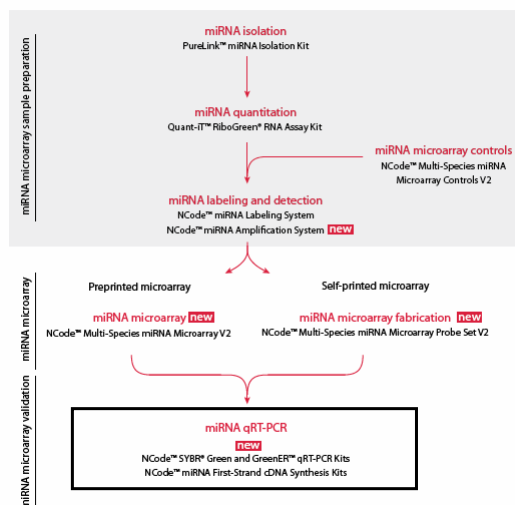
## IV. Influence most human protein-coding genes

- > 1/3 mRNAs targeted by miRNAs
- > Remainder of mRNAs "on the run"

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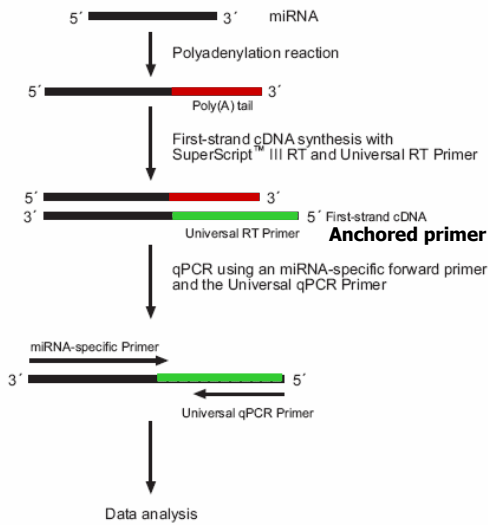
# miRNA Profiling Workflow

## miRNA profiling workflow overview



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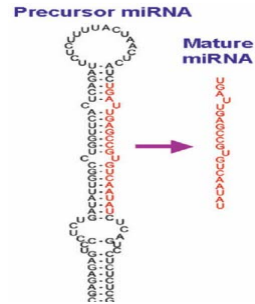
## NCode™ SYBR® Green miRNA qRT-PCR Kits



### 1. Flexible system

- Only 1 primer needed
- No special probes
- Quick assay design
- Permits analysis of other small RNAs

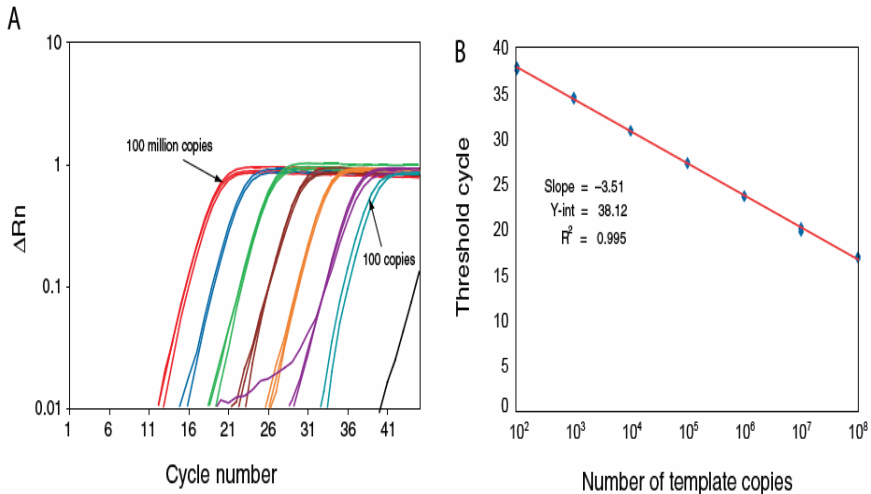
### 2. Inexpensive



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## NCode™ SYBR® Green miRNA qRT-PCR Kit

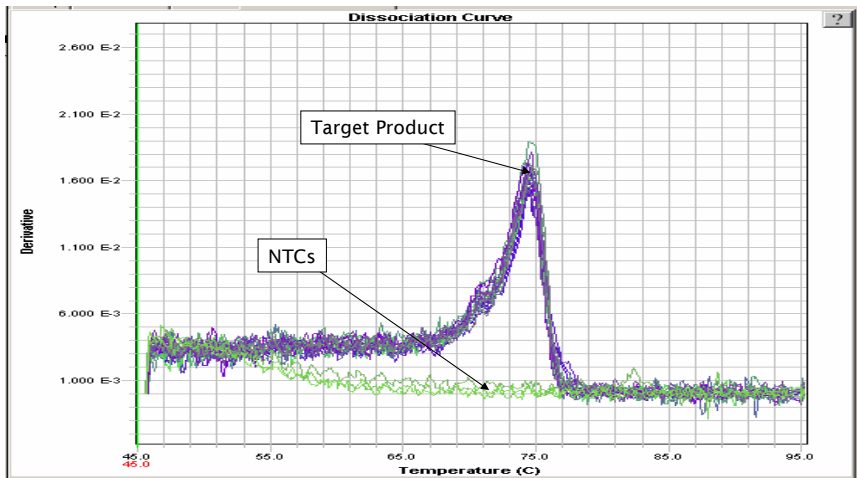


Accurate quantitation over a broad dynamic range of input sample

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## NCode™ SYBR® Green miRNA qRT-PCR Kit

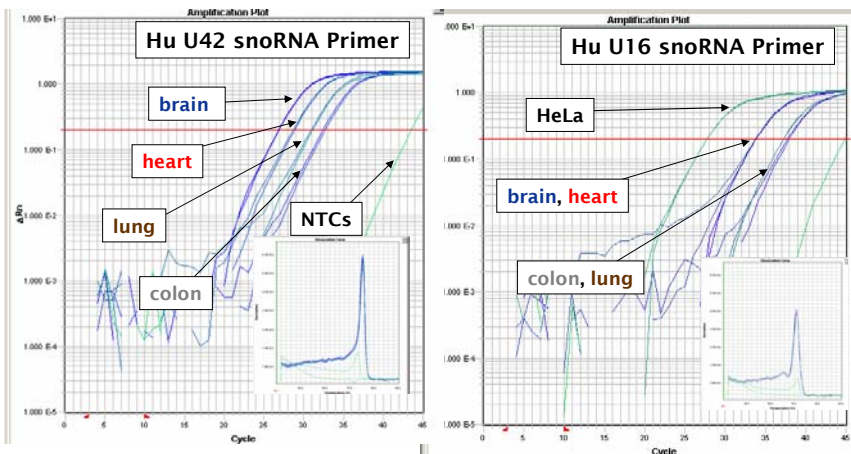


Specific amplification of miRNA targets

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## Quantification of snoRNAs with NCode™ qRT-PCR

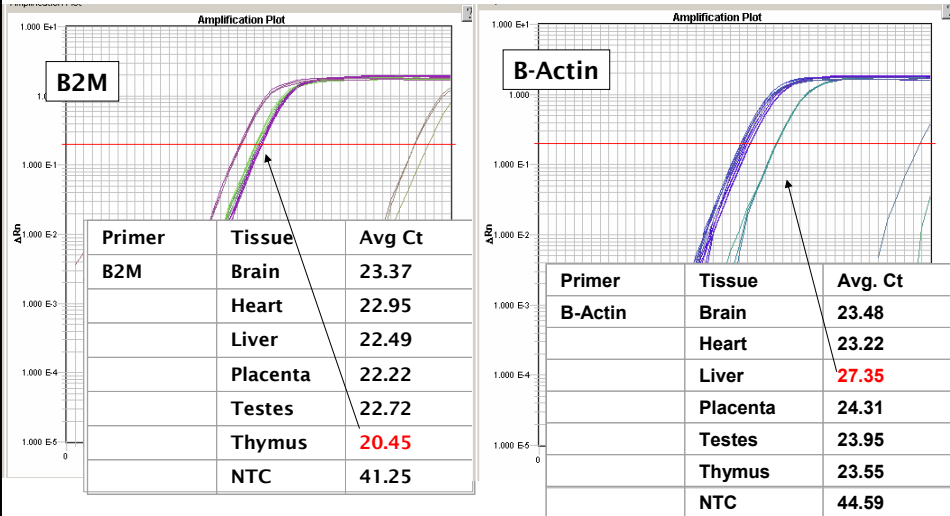


Quantify a range of small RNAs using the NCode™ qPCR system

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## Housekeeping Genes as Reference Controls

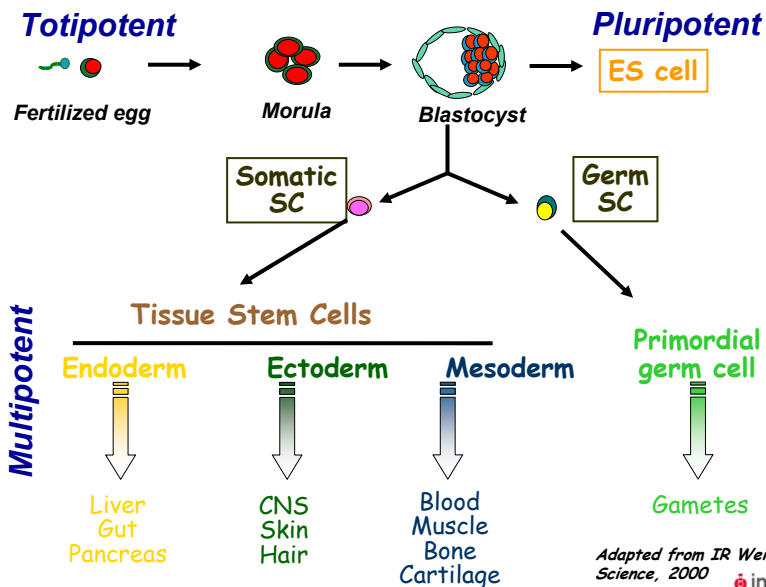


Examine housekeeping gene variation across all potential sample types

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## Stem Cells Differentiation



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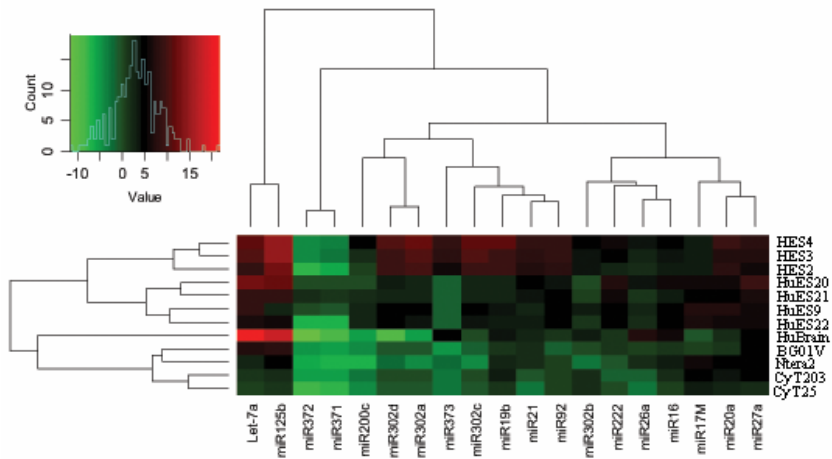
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## Top 10 Expressed miRs in hESC and hEC

BG01V	HUES9	HUES10	HUES20	HUES21	HUES22	Ntera2	2102Ep
miR_21	miR_302b	miR_302a	miR_21	miR_21	miR_302b	miR_21	miR_302b
miR_302b	miR_302d	miR_423	miR_302b	miR_302b	miR_302a	miR_20a	miR_372
miR_302a	miR_302a	miR_320	miR_302d	miR_302d	miR_302d	miR_17_5p	miR_302a
miR_302d	miR_302c	miR_494	miR_302a	miR_302a	miR_21	miR_302d	miR_373
miR_22	miR_302a*	miR_106a	miR_302c	miR_373	miR_302c	miR_106a	miR_302d
miR_125b	miR_20a	miR_302d	miR_199a*	miR_372	miR_106a	miR_302b	miR_371
miR_24	miR_17_5p	miR_296	let_7a	miR_302c	miR_20a	miR_20b	miR_21
miR_31	miR_21	miR_17_5p	miR_221	miR_302a*	miR_302a*	miR_302a	miR_302c
miR_296	miR_106a	miR_302a*	miR_125b	miR_106a	miR_20b	miR_24	miR_302a*
miR_29a	miR_20b	miR_122a	let_7f	miR_20a	miR_20a	miR_17_5p	miR_106b
hESC/EC Specific				Rare in hESC			

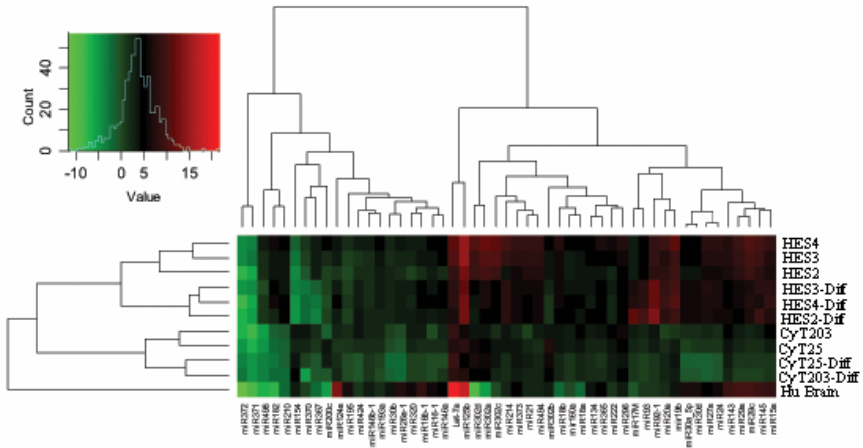
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## qRT-PCR: miRNA expression in hESC



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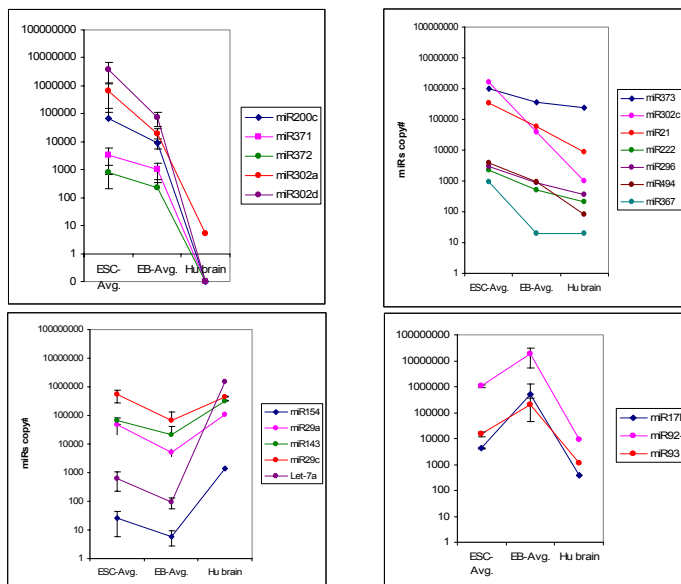
## qRTPCR: miRNA expression in ESC vs Differentiated cells



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## Relative miR copy number: hESC vs. EB & Adult brain



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## miRNAs specific to hESC or EB based on Ncode qRT-PCR profiles of five independent hESC cell lines

miRNA	ESC	EB	Adult Tissue
<i>miR200c, 371, 372, 302a, 320d</i>	<b>High</b>	Low	<i>Absent</i>
<i>miR373, 302c, 21, 222, 296, 494, 367</i>	<b>High</b>	Low	<i>Lower</i>
<i>miR154, 29a, 143, 29c, Let7a</i>	<b>High</b>	Low	<b>High</b>
<i>miR17M, 92, 93</i>	Low	<b>Higher</b>	Low
<i>miR16, 134, 145, 30a_5p</i>	Same	Same	Same

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Chris Adams  
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### Collaborators

#### *Rutgers University, NJ*

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## NCode™ qPCR Kits

- NCode™ SYBR® GreenER™ miRNA qRT-PCR Kit
  - Advanced SYBR® GreenER™ technology
  - 10 poly(A) rxns, 20 cDNA rxns, 100 qPCR rxns
- NCode™ SYBR® Green miRNA qRT-PCR Kit
  - 10 poly(A) rxns, 20 cDNA rxns, 100 qPCR rxns
- NCode™ miRNA First-Strand cDNA Synthesis Kit
  - 10 reaction kit
  - 50 reaction kit

