

Introducing:



Q uantiMir

Small RNA Quantitation System

What QuantiMir does:

The new QuantiMir RT Kit tags and converts small RNAs into cDNA, ready to use as template for real-time qPCR

- Measure and profile MicroRNA expression levels from any tissue source
- Detect and quantify siRNAs from knockdown experiments
- Validate the existence of newly discovered MicroRNA species

*No more messy Northern blots or RNase protection assays!
Say “good-bye” to radioactivity!*

Stop wasting your precious RNA on a single MicroRNA detection per RT reaction!

How QuantiMir Works

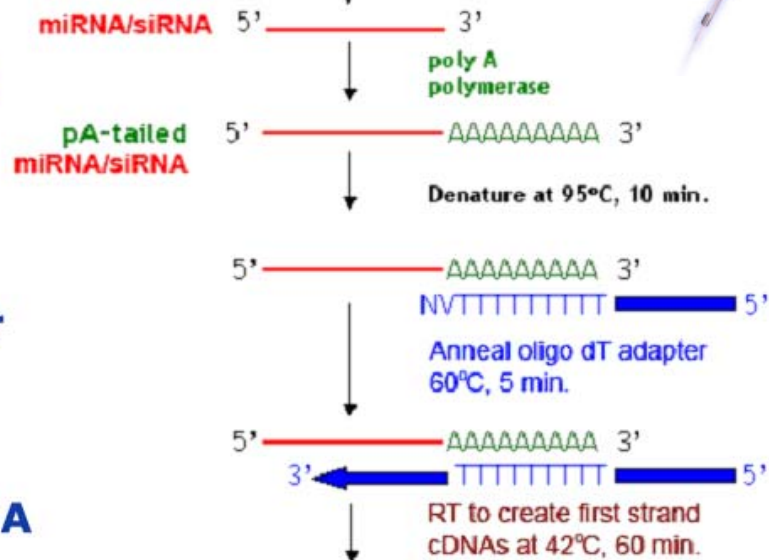
Single tube 3 Step Assay

- 1 Tag small RNA
- 2 Anneal adapter
- 3 Convert to cDNA

DONE !

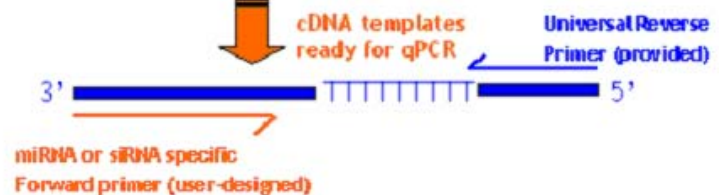
**Less than
2 hr process!**

1 μ g - 200 pg
Total RNA



cDNA pool of anchor-tailed RNA

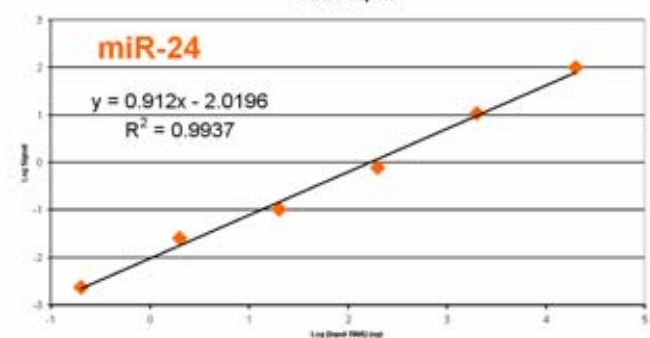
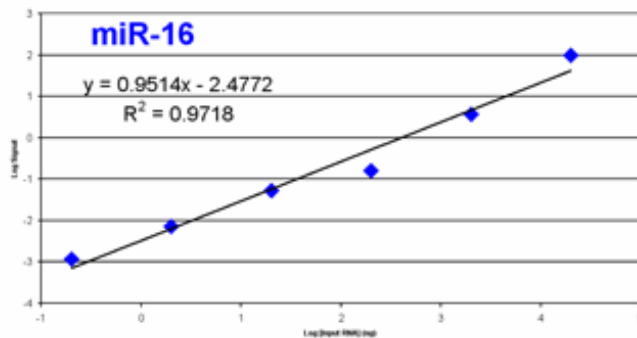
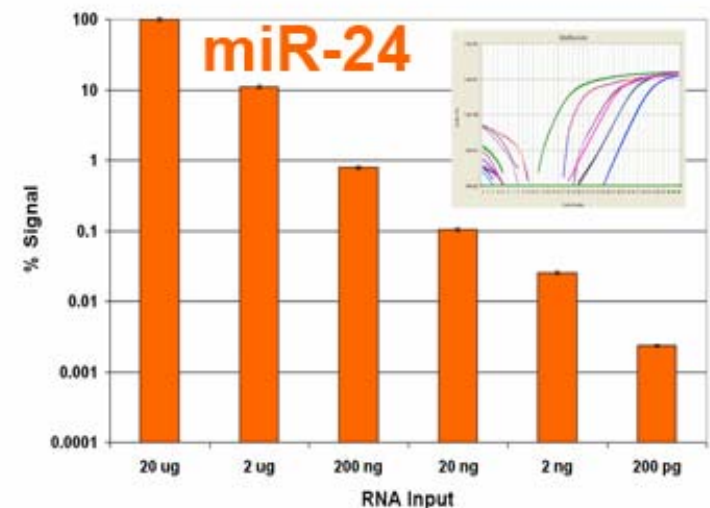
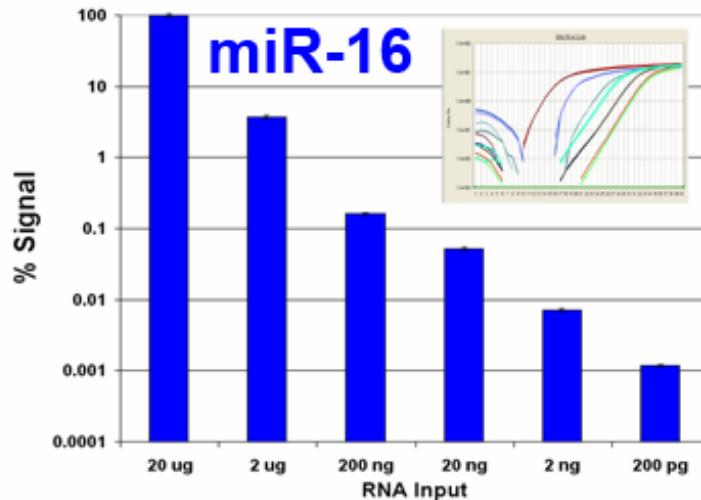
3' ————— TTTTTTTTTT ————— 5'



Extreme Sensitivity

Start with as little as **200 pg** total RNA:
No purification or fractionation required

- Biopsies
- LCM
- Rare samples



High Specificity

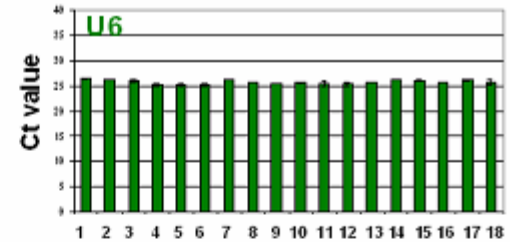
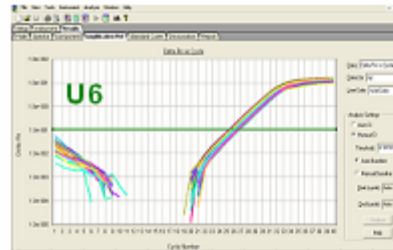
Determine Tissue Expression Profiles

Normalize with
U6 snRNA
present in every tissue

miR-1
Heart + Sk. Muscle only

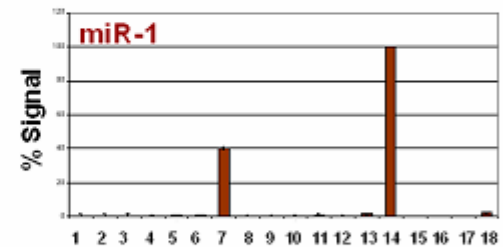
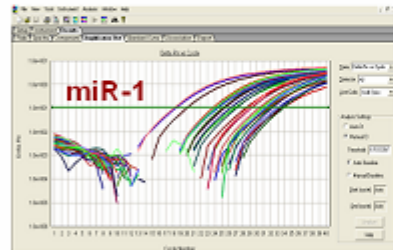
miR-122a
Liver only

A.

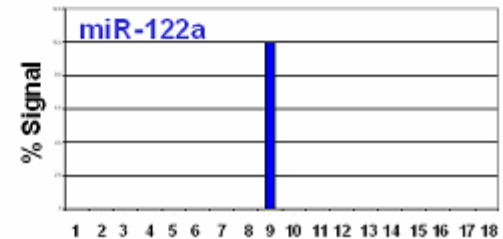
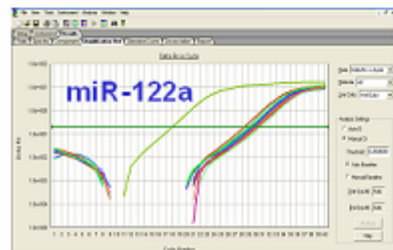


1 adipose	7 heart	13 prostate
2 bladder	8 kidney	14 skeletal muscle
3 brain	9 liver	15 small intestine
4 cervix	10 lung	16 spleen
5 colon	11 ovary	17 testes
6 esophagus	12 placenta	18 thymus

B.

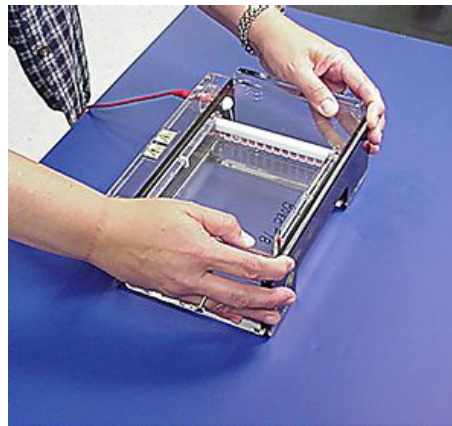
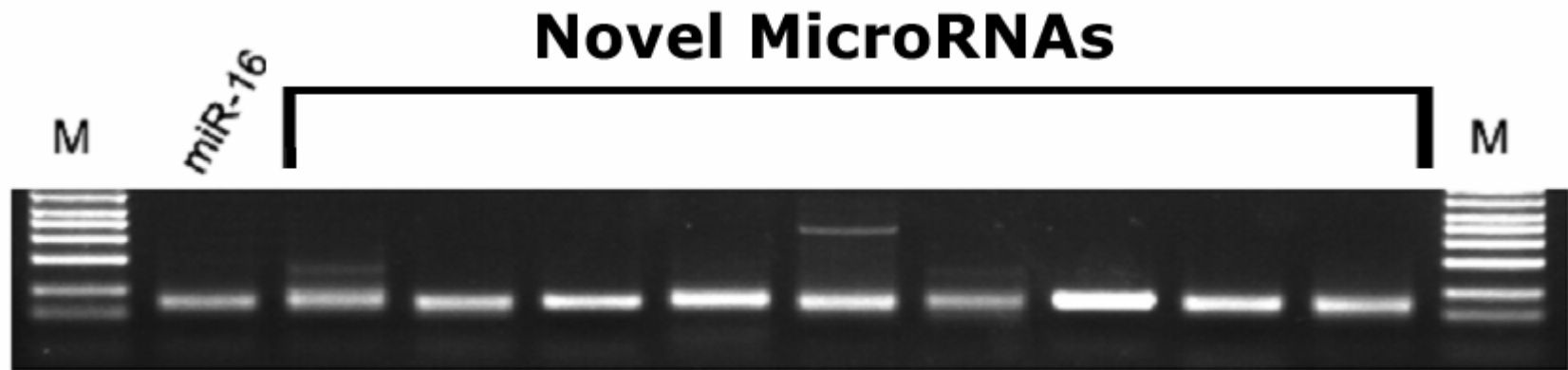


C.



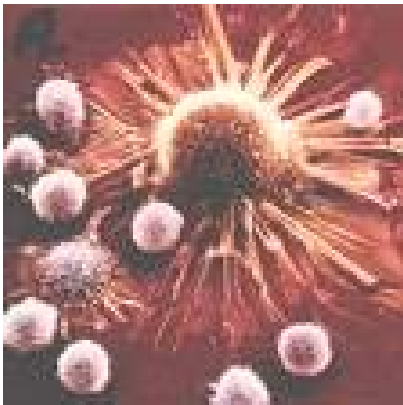
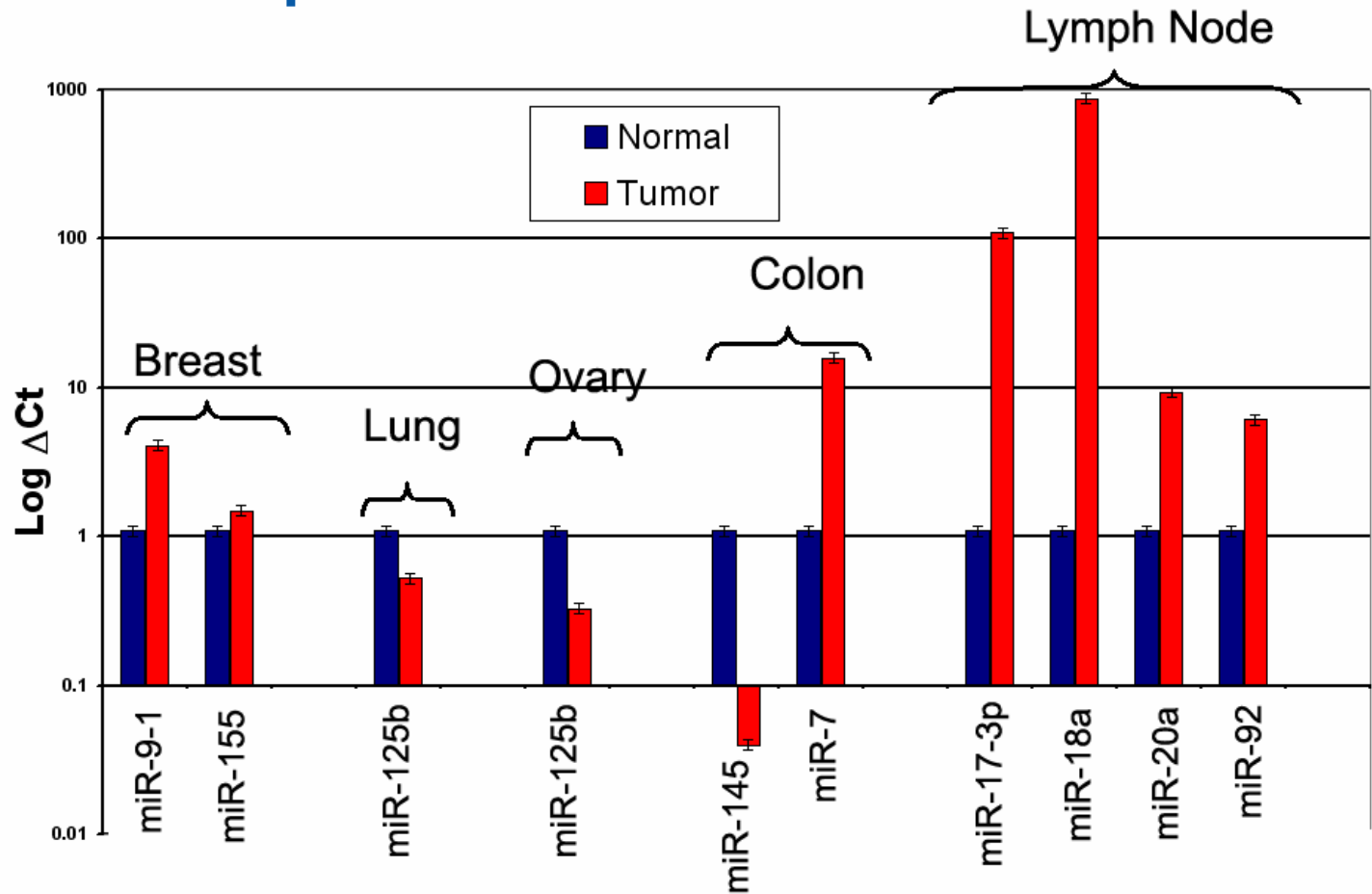
Application: Validate New MicroRNAs

End-point RT-PCR using QuantiMir cDNA



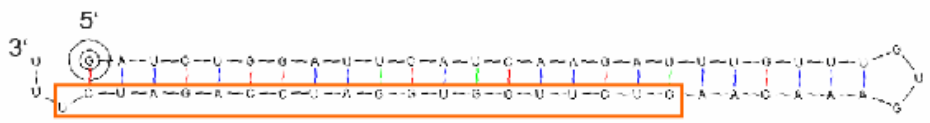
Application: MicroRNA Quantitation

Profile MicroRNA Expression in Cancer



Application: Quantify siRNA expression from Knockdown experiments

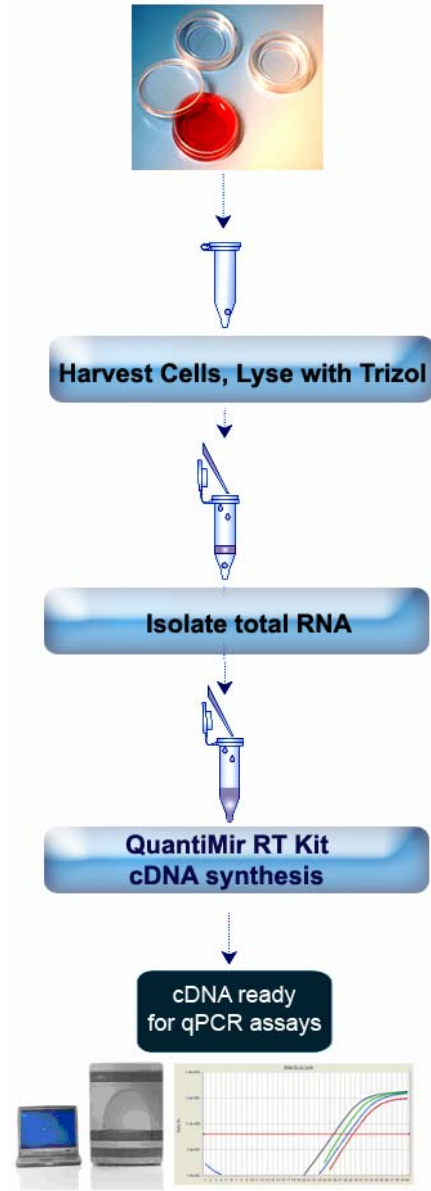
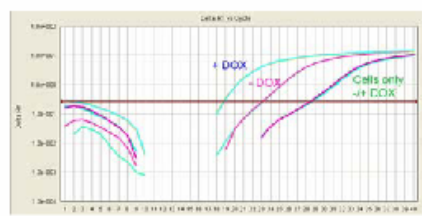
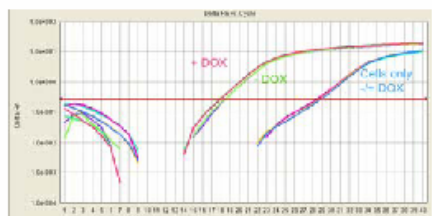
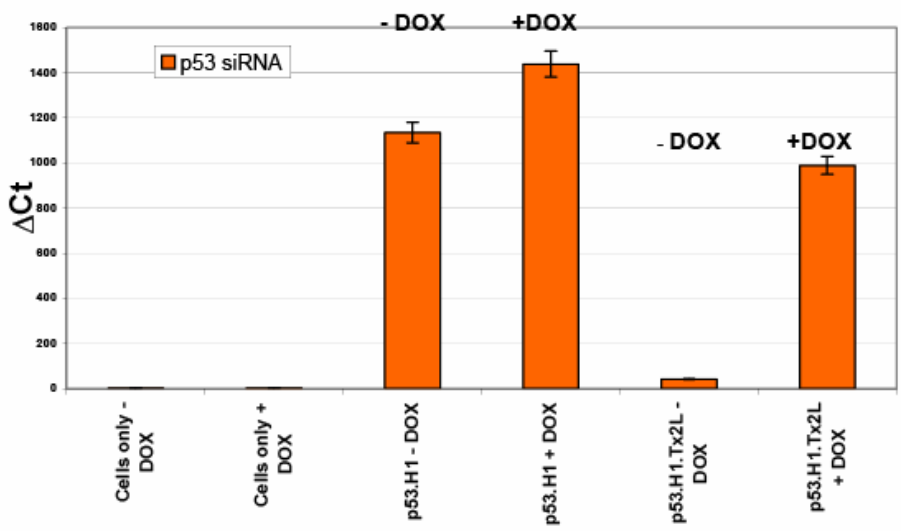
shRNA Expression Molecule:
p53.siRNA



5' – GATCTGGATTCATCAAGATTTGTTTGTGAAACAA**GTCTTGGTGGATCCAGATC**TT – 3'

shRNA Expression Constructs

1. p53.H1
2. p53.H1.T2xL (contains 2 copies of Tet repressor in Promoter)



Easy Forward Primer Design

Only need to design 1 Primer

Hsa-miR-16

Mature sequence MIMAT0000069	
Accession	MIMAT0000069
ID	hsa-miR-16
Sequence	14 - uagcagcacg uaaa <u>uuuggcg</u> - 35
	Get sequence
Evidence	experimental; cloned [1,5,7], Northern [1,6]

Simple: Directly use sequence of mature miRNA as forward primer in oligo design.

The mature miRNA sequence 5' – **uagcagcacg**uaaa**uuuggcg** – 3' can be simply converted to a DNA sequence and used directly as the forward primer for end-point and qPCR analysis.

Forward primer for hsa-miR-16:

5' – **TAGCAGCACGTAAATATTGGCG** – 3'

T_m = 58.9 °C, 45% GC and length = 22 bases.

QuantiMir Kit Contents

Kit contains everything you need,
including miR-16 and U6 snRNA + Controls

40 μ l	5X PolyA polymerase Buffer	10 μ l Poly A Reaction (enough for 20 reactions)
10 μ l	PolyA polymerase	
20 μ l	25 mM MnCl ₂	
30 μ l	5 mM ATP	
10 μ l	3' Oligo dT Adaptor	20 μ l RT Reaction (enough for 20 reactions)
80 μ l	5X Reverse Transcriptase Buffer	
20 μ l	Reverse Transcriptase	
30 μ l	0.1 M Dithiothreitol (DTT)	
40 μ l	dNTP Mix	End-point or qPCR Assay (enough for 500 reactions)
25 μ l	3' Universal Reverse PCR Primer	
50 μ l	5' Human U6 Control Forward Primer (10 μ M)	
50 μ l	5' Human miR-16 Control Forward Primer (10 μ M)	
1.2 ml	RNase-free Water	

End up with 20 μ l cDNA, enough to perform 40
Real-time qPCR reactions per RT reaction.

System Biosciences (SBI)
1616 North Shoreline Blvd.
Mountain View, CA 94043

ORDERING INFORMATION:

Phone: 650-968-2200
Toll-Free: 888-266-5066
Fax: 650-968-2277

info@systembio.com
www.systembio.com

