

microRNA profiling

(points to consider)

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“... results suggested that there may be **no robust mRNA markers** that show consistent differential expression between tumors and normal tissues of different lineage.

Surprisingly, it has been recently shown that **miRNA expression signatures can be extremely informative** for cancer diagnosis. ... These experiments indicate that even within individual malignancies, miRNAs can be used to distinguish among different mechanisms of tumorigenesis.”

JM Cummins & VE Velculescu: miRNA profiling for cancer diagnosis.
Oncogene **25** (2006) 6220-6227



miRNA introduction

- miRNAs are short (~22 nt) non-coding regulatory RNAs (ncRNA)
- miRNAs represent a major regulatory gene family in plants, animals and viruses (~30 % regulated genes)
- miRNAs comprise 1 %-5 % of transcripts
- miRNAs post-transcriptionally regulate gene expression by specific base-pairing to mRNAs, in a combinatorial fashion
- miRNAs have the same activity as transcription factor, however miRNAs do not require ‘translation’

miRNAs function

- Up to date, several thousand miRNAs have been cloned/predicted
- Target/function is known only for a fraction of them

miRNA	Function	Known targets	Species
<i>lin-4</i>	Developmental timing	<i>lin-14, lin-28</i>	<i>Ce</i>
<i>let-7</i>	Developmental timing	<i>lin-41, hbl-1</i>	<i>Ce</i>
<i>lsey-6</i>	Neuronal patterning	<i>cog-1</i>	<i>Ce</i>
<i>miR-273</i>	Neuronal patterning	<i>die-1</i>	<i>Ce</i>
<i>bantam</i>	Cell death, proliferation	<i>hid</i>	<i>Dm</i>
<i>miR-14</i>	Cell death, fat storage	Caspase?	<i>Dm</i>
<i>miR-181</i>	Haematopoiesis	?	<i>Mm</i>
<i>miR-196</i>	Development?	<i>HoxB8, HoxC8, HoxD8, HoxA7</i>	<i>Mm</i>
<i>miR-143</i>	Adipocyte differentiation	?	<i>Hs</i>
<i>miR-375</i>	Insulin secretion	Myotrophin	<i>Mm</i>

Ce, Caenorhabditis elegans; Dm, Drosophila melanogaster; Hs, Homo sapiens; Mm, Mus musculus.

Kim, Nat. Rev. Mol. Cell Biol. (2005)

- miRNAs are tissue and developmental stage specific
- miRNAs control cell proliferation and apoptosis (oncogene/tumor suppressor)
- miRNAs affect neuronal patterning and modulation of hematopoietic lineage differentiation
- miRNAs dysfunction is linked to cancers and other (human) diseases

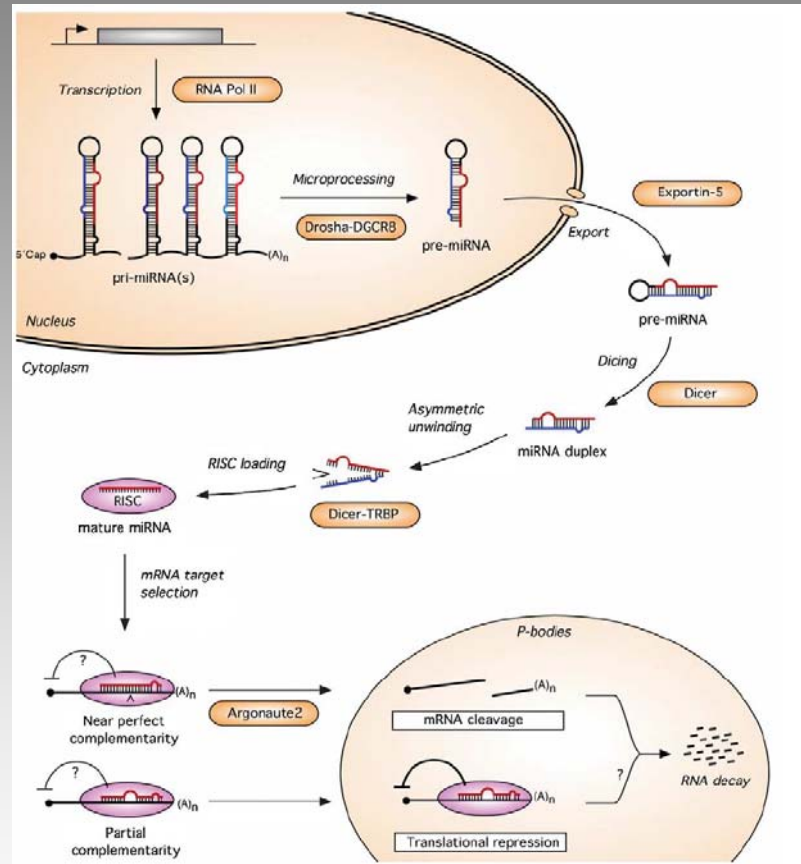
miRNAs targets and “antitargets”

Category	Description	# Genes	p(over) in Targets	p(under) in Antitargets
GO:0009887	Organogenesis	646	2.1E-34	7.3E-26
GO:0007399	Neurogenesis	364	2.2E-23	5.4E-19
GO:0007165	Signal transduction	791	2.7E-19	2.5E-14
GO:0030154	Developmental process	1155	1.1E-06	1.1E-06
GO:0009718	Cellular process	1155	1.1E-06	1.1E-06
GO:0045119	Regulation of transcription	470	1.7E-05	2.0E-05
GO:0002009	Morphogenesis of an epithelium	104	1.0E-08	3.0E-08
GO:0007422	Peripheral nervous system development	95	4.5E-08	3.9E-07
GO:0009795	Embryonic morphogenesis	101	1.1E-07	5.2E-07
GO:0007498	Mesoderm development	135	3.5E-07	2.0E-04
GO:0030529	Ribonucleoprotein complex	200	3.7E-06	1.3E-11
GO:0005840	Ribosome	128	2.4E-05	1.1E-11
GO:0006412	Cellular process	1155	1.1E-06	1.1E-06
GO:0016070	Cellular process	1155	1.1E-06	1.1E-06
GO:0016591	Cellular process	1155	1.1E-06	1.1E-06
GO:0006119	Oxidative phosphorylation	61	1.8E-02	2.3E-04
GO:0006281	DNA repair	70	2.2E-02	4.7E-04
GO:0000502	Proteasome complex (sensu Eukarya)	37	2.6E-02	4.1E-04
GO:0006259	DNA metabolism	203	2.8E-02	3.9E-03
GO:0008380	RNA splicing	78	3.9E-02	1.4E-02

Developmental processes

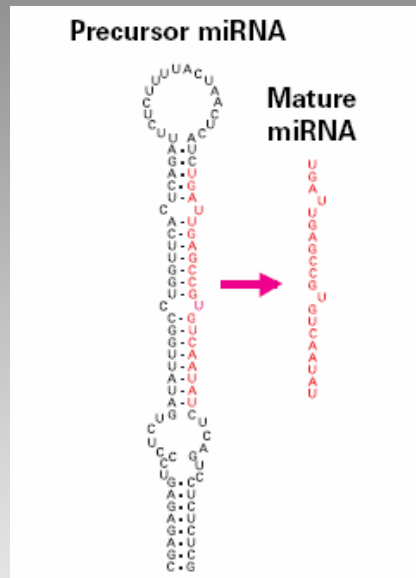
Basic cellular processes

Complex biogenesis of miRNAs



Wienholds & Plasterk, *FEBS Letters* (2005)

miRNA profiling



T_m between 45°C and 74°C

miRNAs may differ just by one base

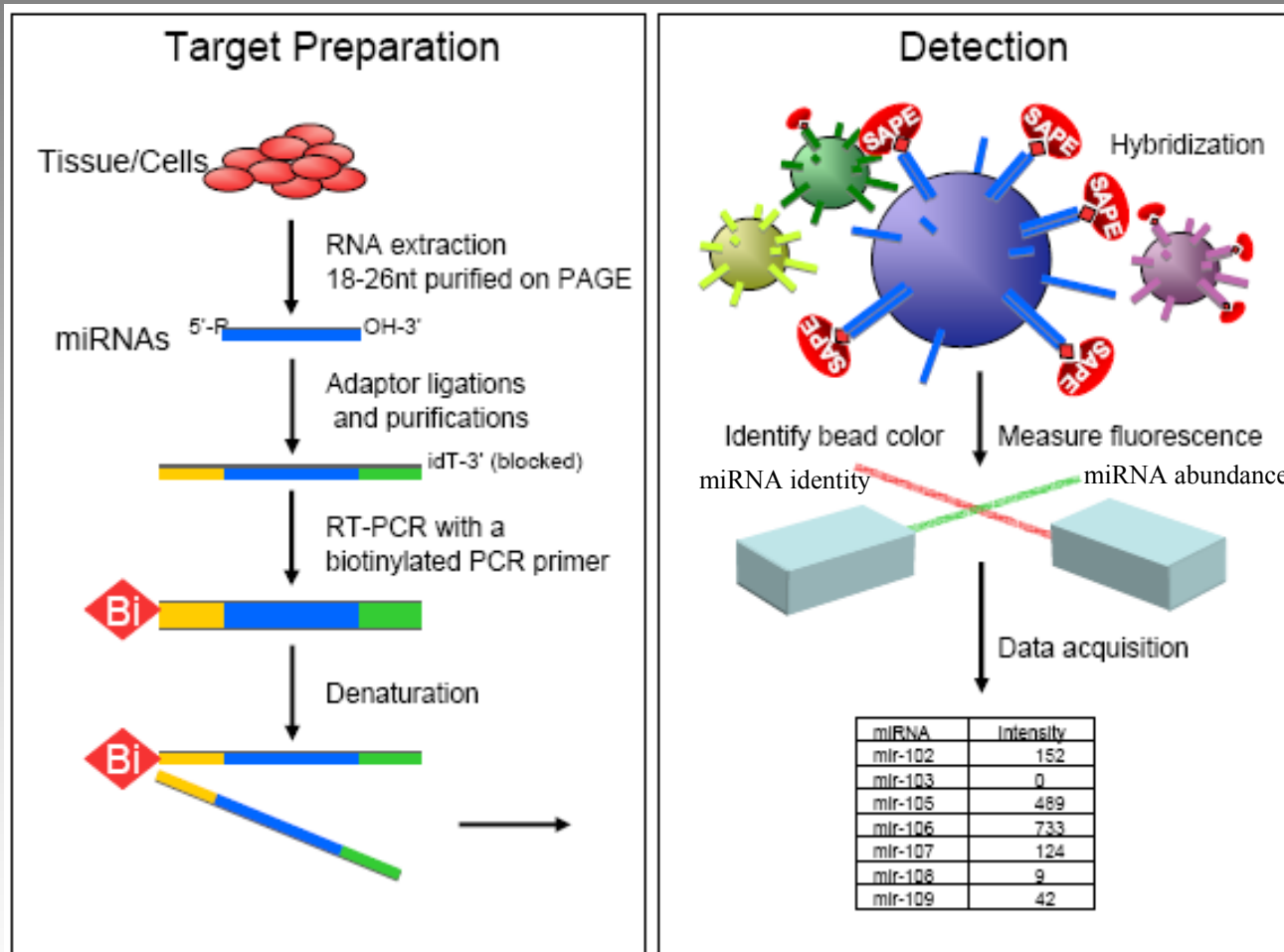
miRNA profiling toolbox

<i>Method</i>	<i>Advantages</i>	<i>Disadvantages</i>	<i>References</i>
Cloning (miRAGE)	Open ended; possibility for discovery of new miRNAs	Cost of sequencing	Cummins <i>et al.</i> (2006), Lagos-Quintana <i>et al.</i> (2001), Lagos-Quintana <i>et al.</i> (2003), Lau <i>et al.</i> (2001), Lee and Ambros (2001)
Northern blot	Gold standard; ability to assay miRNA precursor	Low throughput; limited sensitivity for low-abundance species	Lau <i>et al.</i> (2001)
Microarrays	Low cost; high throughput	Closed-ended	Babak <i>et al.</i> (2004), Barad <i>et al.</i> (2004), Bentwich <i>et al.</i> (2005), Calin <i>et al.</i> (2004a), Liang <i>et al.</i> (2005), Liu <i>et al.</i> (2004), Nelson <i>et al.</i> (2004), Shingara <i>et al.</i> (2005), Thomson <i>et al.</i> (2004)
Real-time PCR	Low cost; high throughput; superior detection of low-abundance species	Closed-ended	Chen <i>et al.</i> (2005), Raymond <i>et al.</i> (2005), Schmittgen <i>et al.</i> (2004), Shi and Chiang (2005)
Bead-based hybridization <i>In situ</i> detection	Cost; superior hybridization Ability to visualize miRNA levels in tissue context	Closed-ended Low throughput, closed-ended	Lu <i>et al.</i> (2005) Kloosterman <i>et al.</i> (2006)
Single molecule detection	Speed	Closed-ended; high cost	Neely <i>et al.</i> (2006)

Abbreviations: miRAGE, miRNA serial analysis of gene expression; miRNA, micro-RNAs. Open-ended methods enable the detection of new miRNA genes, whereas closed-ended methods assay a predetermined set of miRNAs.

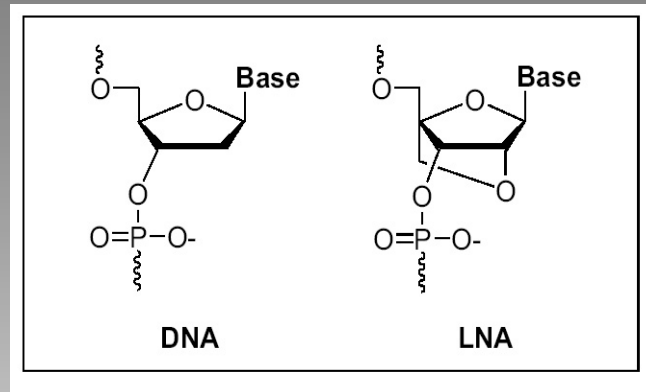
Cummins & Velculescu, Oncogene (2006)

Bead-based miRNA detection



Lu et al., Nature (2005)

DNA vs LNA (Locked Nucleic Acid)



- LNA spiking increases base-pairing T_m

LNA-DNA: $\Delta T_m = +1 - +8^\circ\text{C}/\text{insertion}$

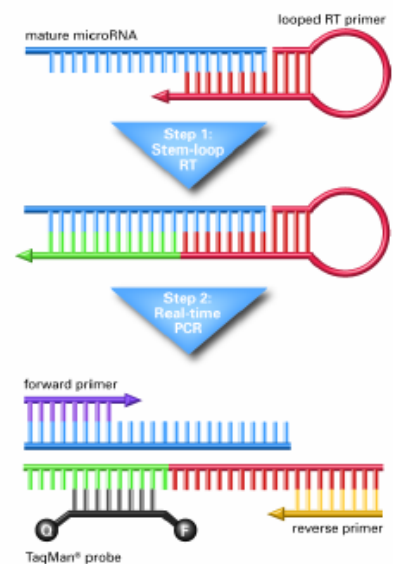
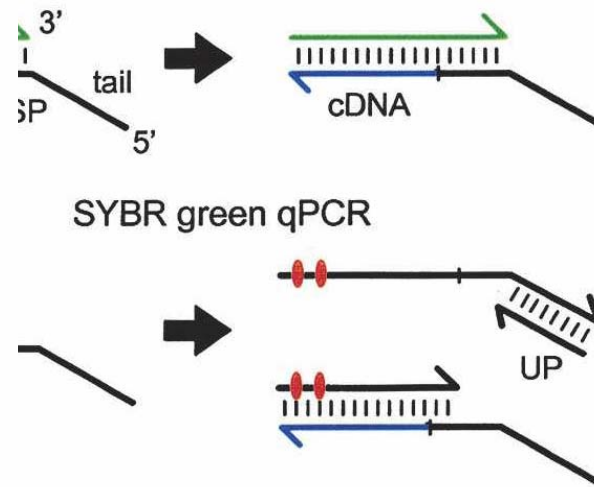
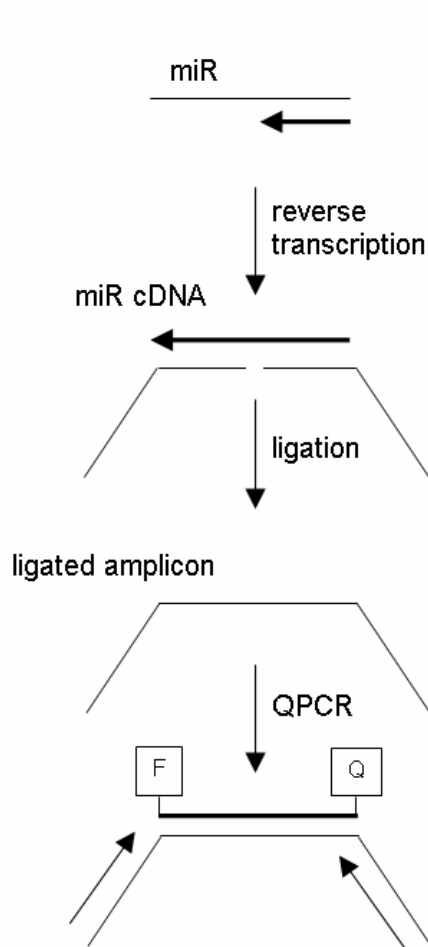
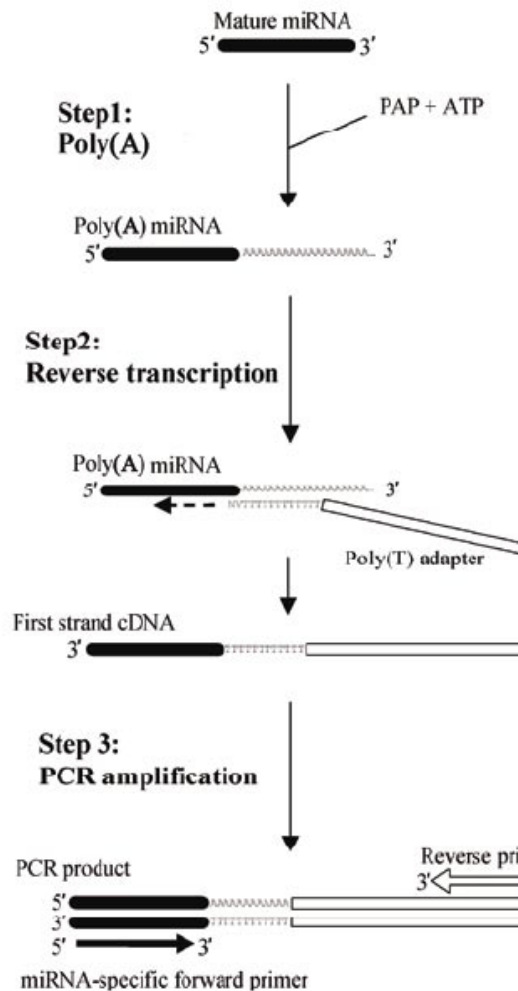
LNA-RNA: $\Delta T_m = +2 - +10^\circ\text{C}/\text{insertion}$

miR-7 CAACAAAATCACTAGTCTTCCA $T_m = 50^\circ\text{C}$

miR-7 **CAACAAAATCACTAGTCTTCCA** $T_m = 70^\circ\text{C}$

(Sigma-Proligo)

miRNA profiling by qPCR



miRNA profiling by qPCR

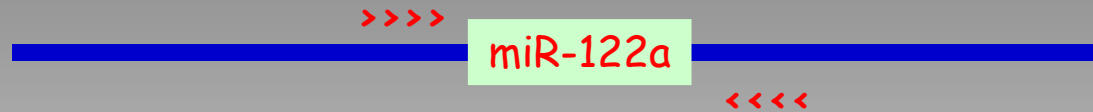
Input: 100 ng of total RNA

NCode, LNA, SYBR	let7a	mir122a	mir215
Cre-	$C_t=23.5$	$C_t=20.7$	$C_t=30.3$
Cre-	23.2	20.5	30.1
Cre+	22.6	28.4	34.4
Cre+	22.6	28.1	33.7
NCode, DNA, SYBR	let7a	mir122a	mir215
Cre-	$C_t=27.4$	$C_t=22.4$	$C_t=34.2$
Cre-	26.9	22.4	34.0
Cre+	26.4	30.3	36.7
Cre+	26.3	30.1	36.7

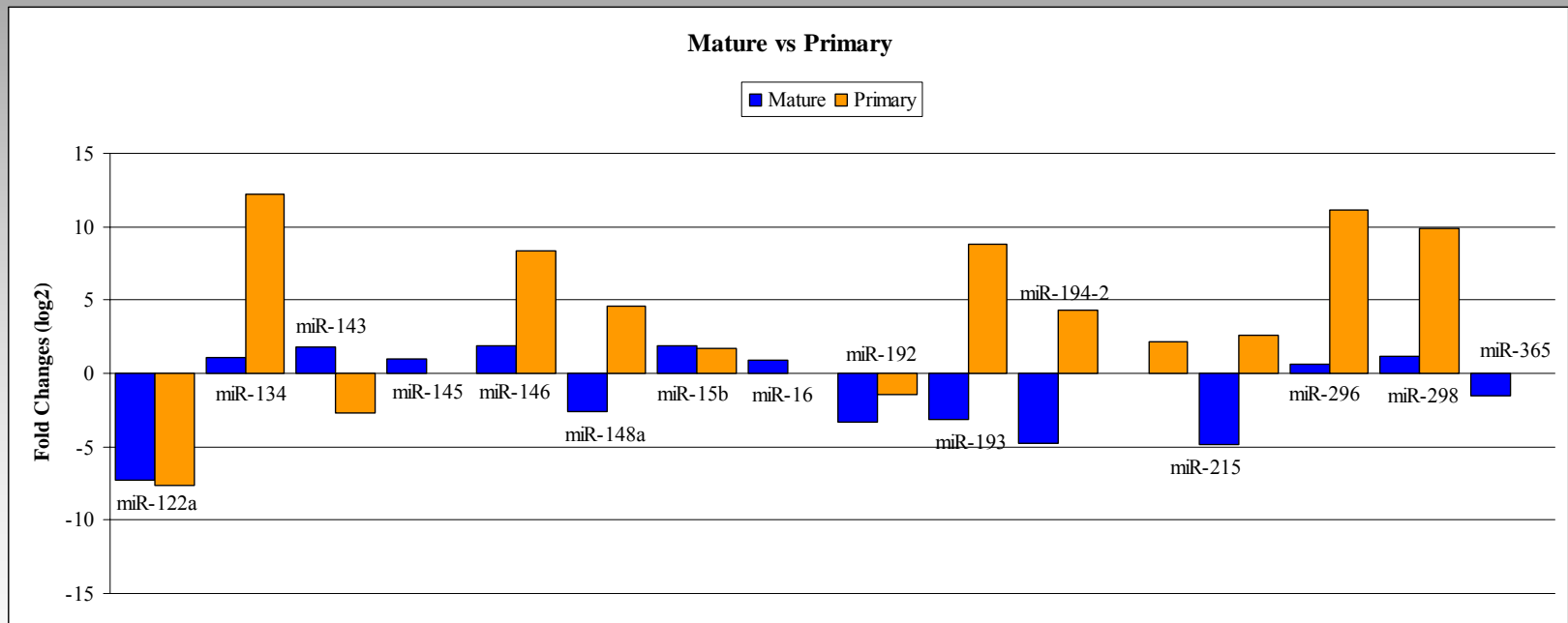
Input: 10 ng of total RNA

ABI Probes	let7a	mir122a	mir215
Cre-	$C_t=25.4$	$C_t=26.2$	$C_t=31.1$
Cre+	24.6	31.1	34.1

Primary miRNAs expression profile (principle)



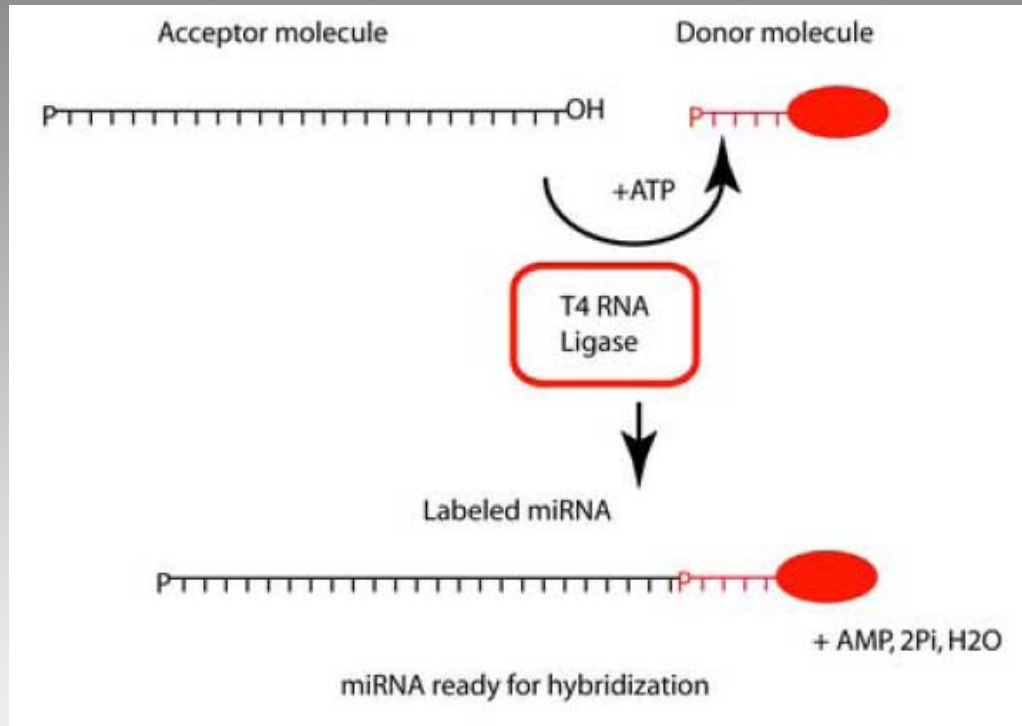
Primary miRNAs expression profile



miChip

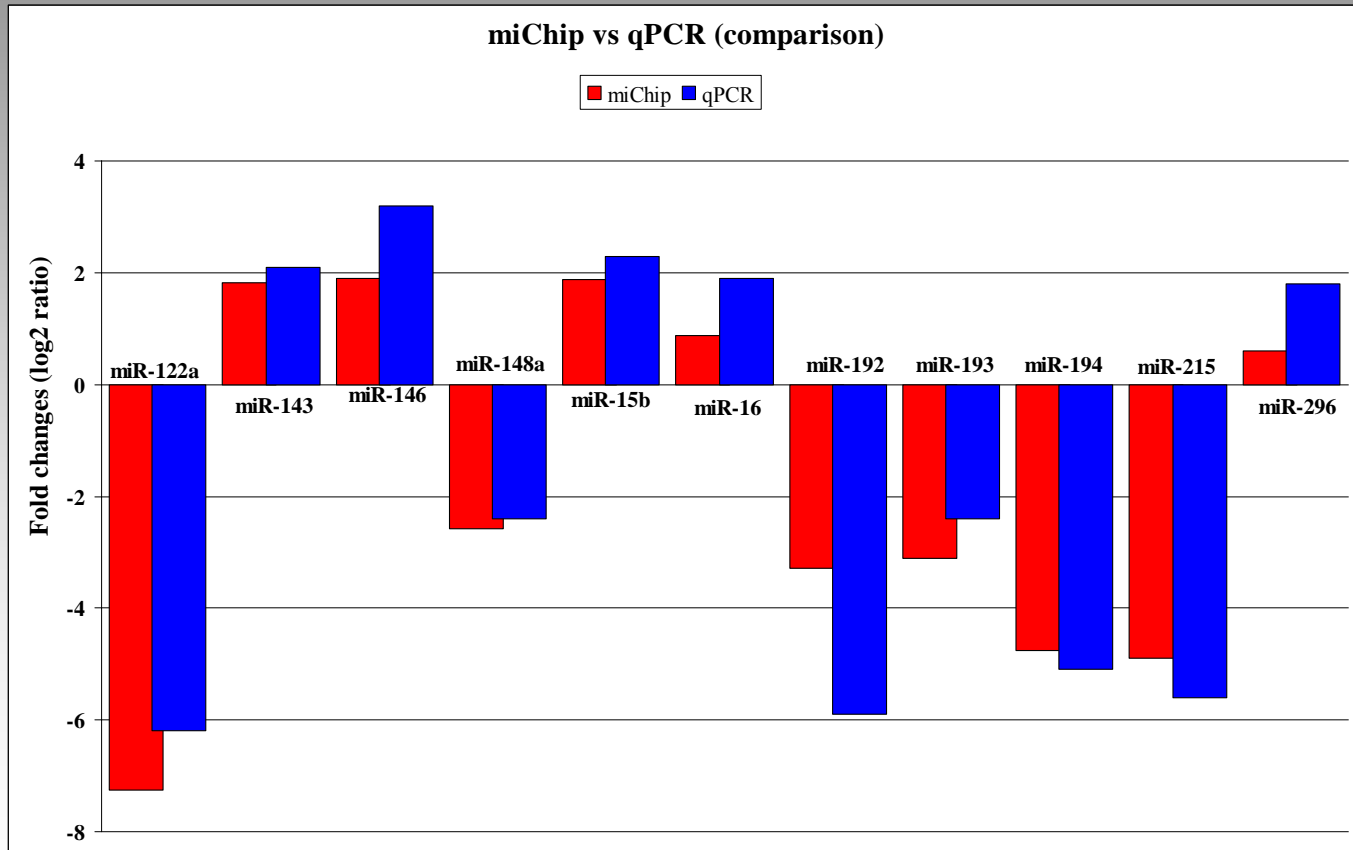
- Genome-wide profiling of mature miRNAs
- LNA modified capture probes
 - Uniform melting temperature (LNA content, length) => possibility to establish normalized hybridization conditions suitable for all miRNAs
 - Accurate and sensitive miRNA detection
 - High detection specificity

miChip RNA labeling



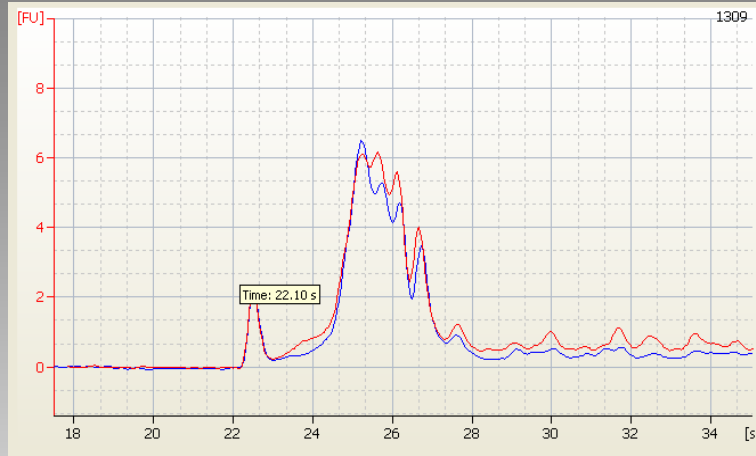
No need for miRNA enrichment or amplification,
specific for mature miRNAs (single-stranded RNA)

Validation of mature miRNAs expression



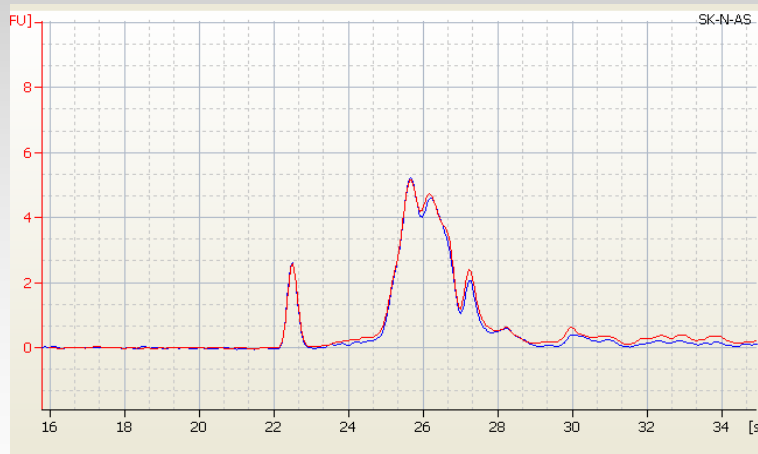
Comparing small RNA profiles of Trizol and miRNeasy

■ Trizol only



● SK-N-AS

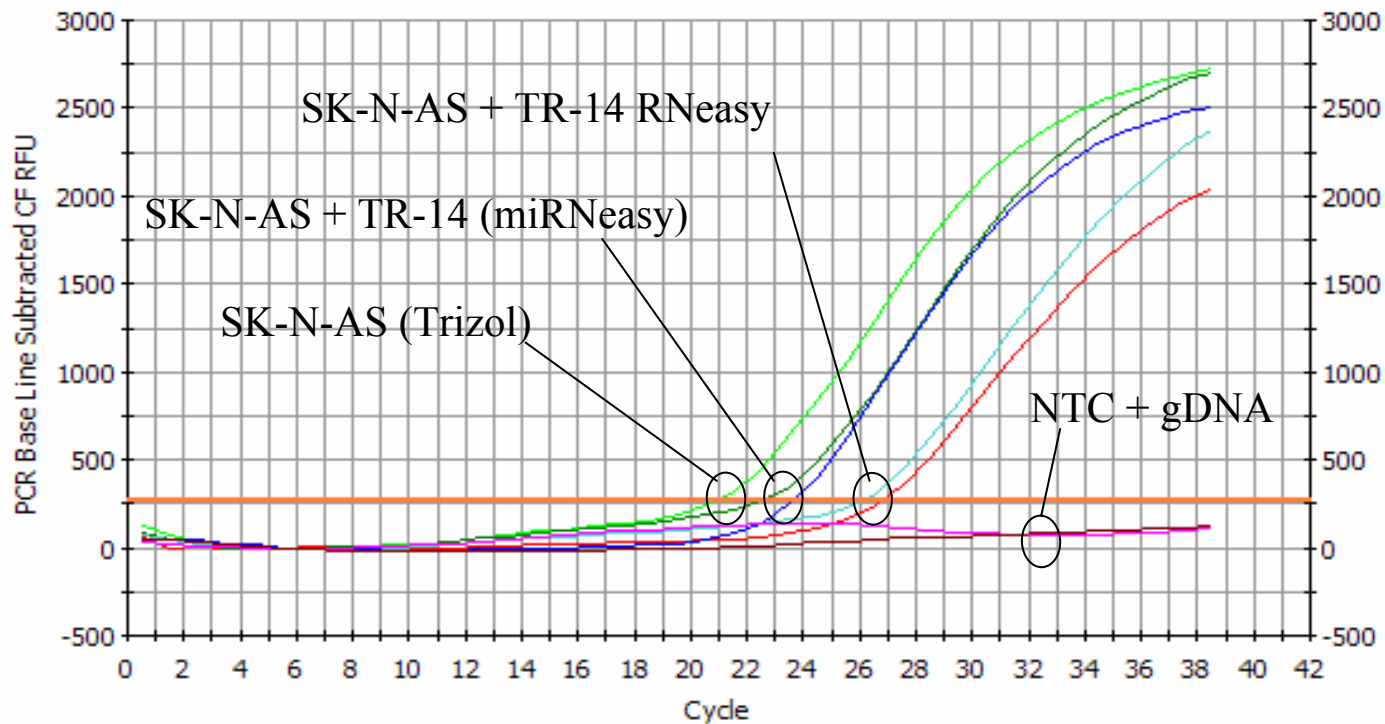
■ miRNeasy



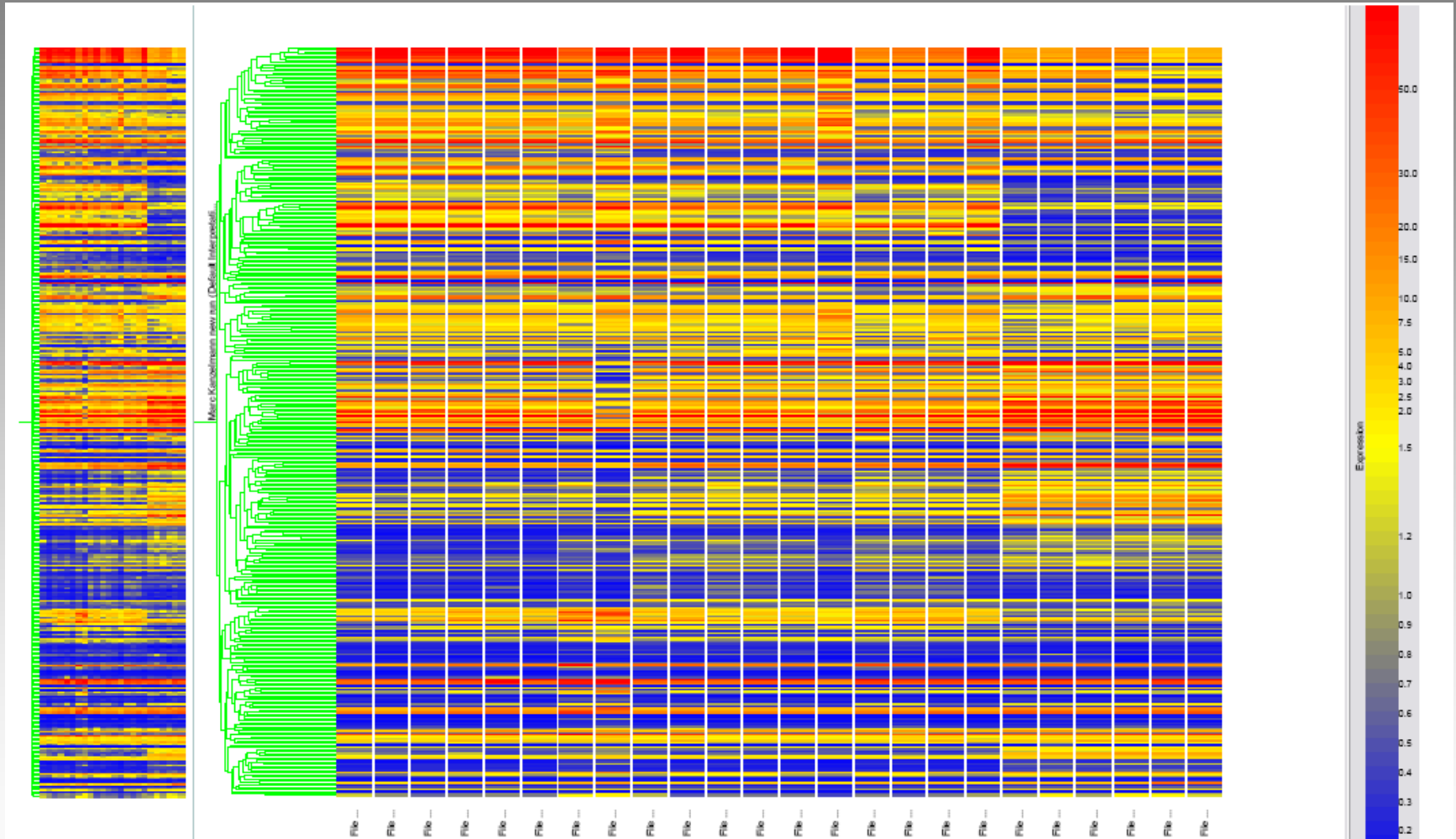
● SK-N-AS

miRNA expression (stem loop qPCR for mir-16, 10ng RNA)

- Trizol only: SK-N-AS \rightarrow Ct = 20.9
- RNeasy: SK-N-AS \rightarrow Ct = 26.8 TR-14 \rightarrow Ct = 26
- miRNeasy: SK-N-AS \rightarrow Ct = 22.5 TR-14 \rightarrow Ct = 23.6



Effect of purification method on miRNA profile



Acknowledgement

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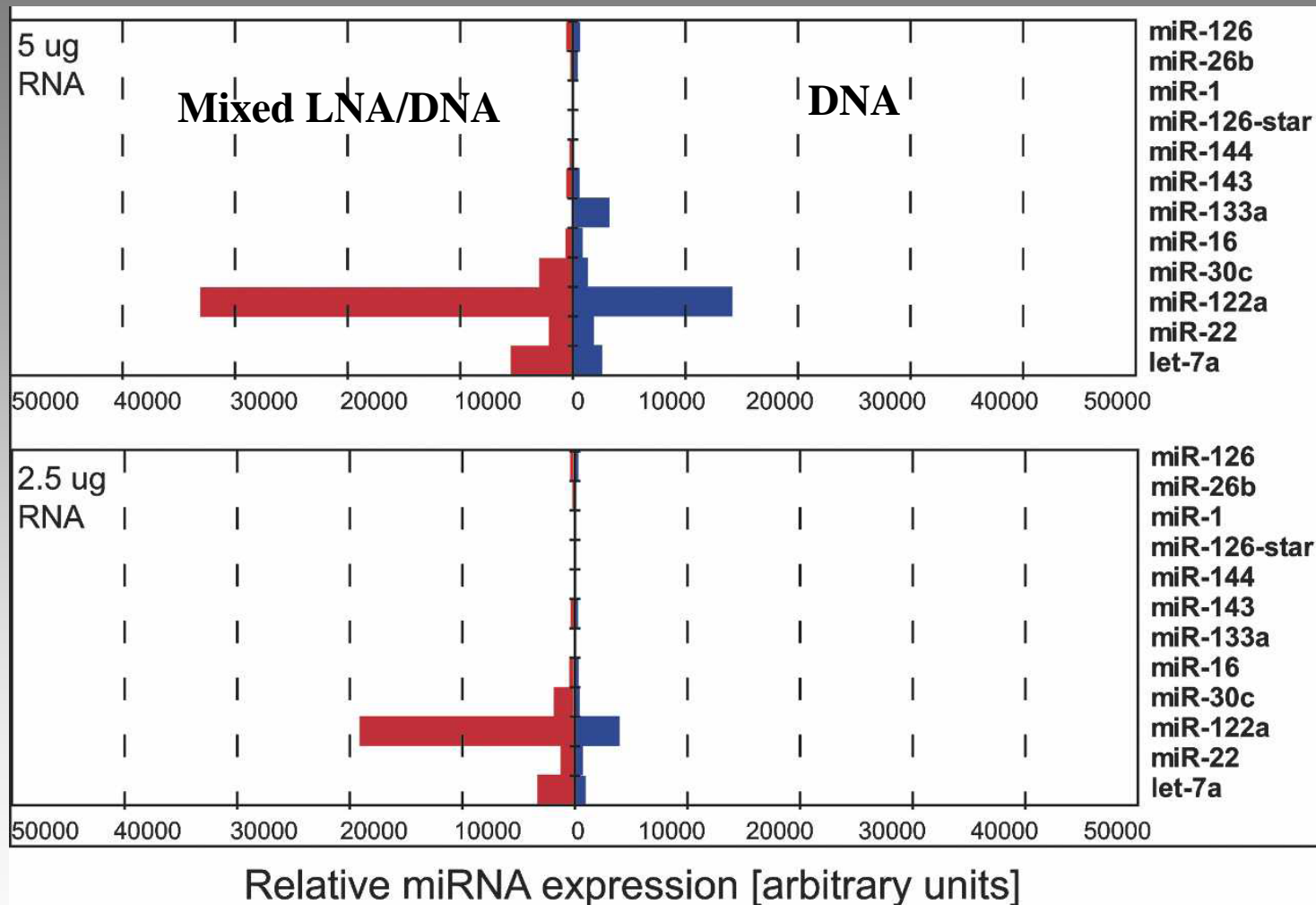
Pieter Mestdagh (University of Ghent)

Exiqon, ABI, Invitrogen, Sigma



Thank you!

miRNAs profiling, sensitivity

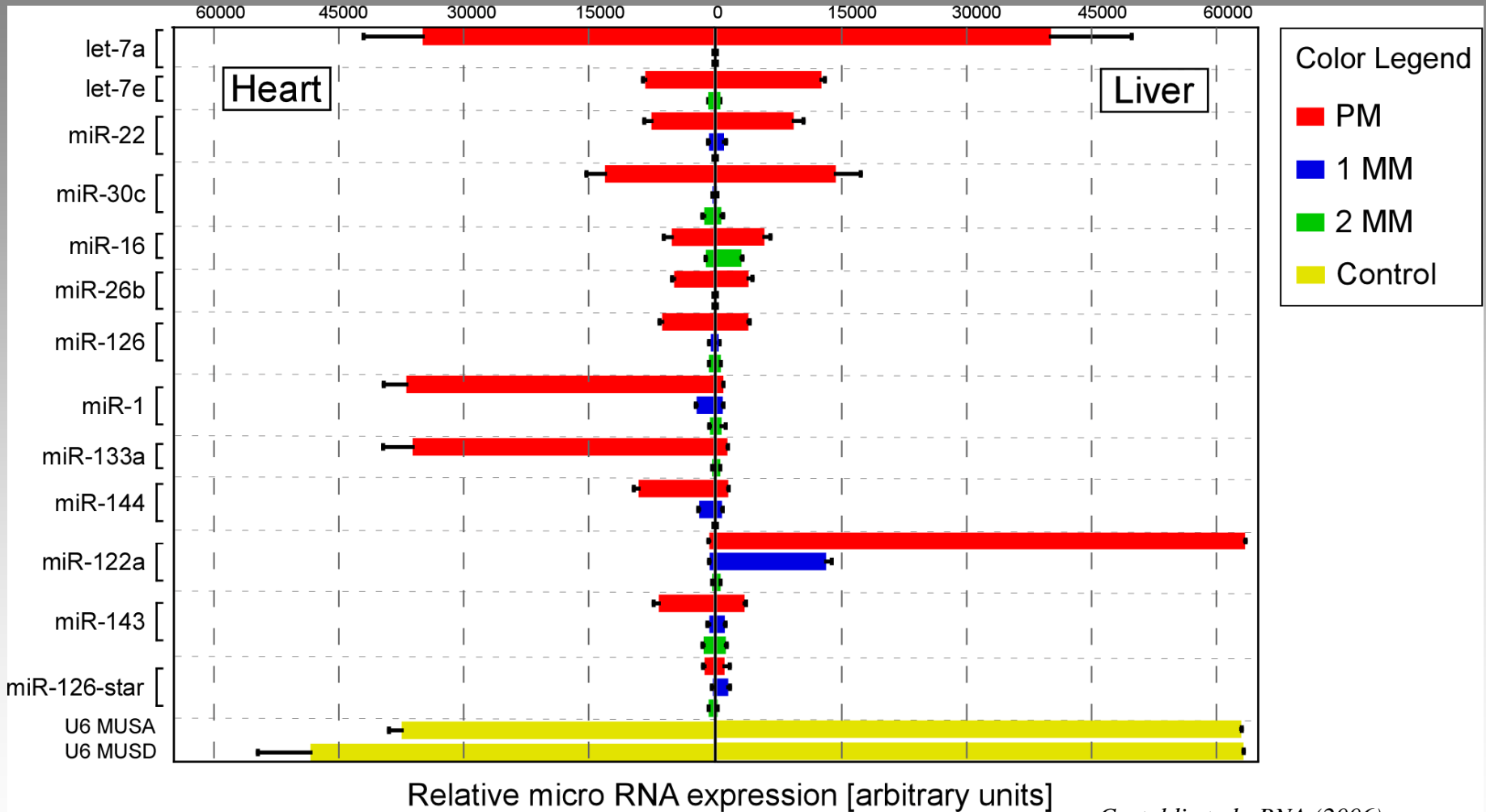


Increased sensitivity for miRNA detection

Castoldi et al., RNA (2006)



miRNAs profiling, specificity



Castoldi et al., RNA (2006)

