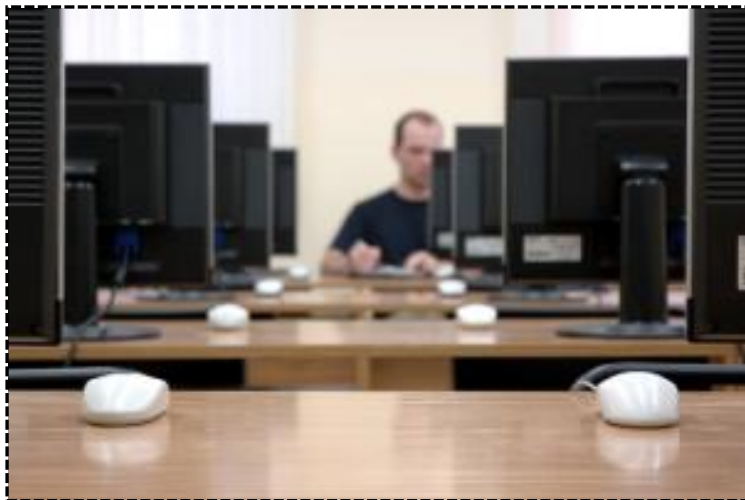


Dissecting microRNA function through integrative genomics

SysKid workshops, annual meeting and symposium
January 26, 2011

Jo Vandesompele, PhD
Biogazelle, CEO
Ghent University, professor

courses
webinars
consultancy

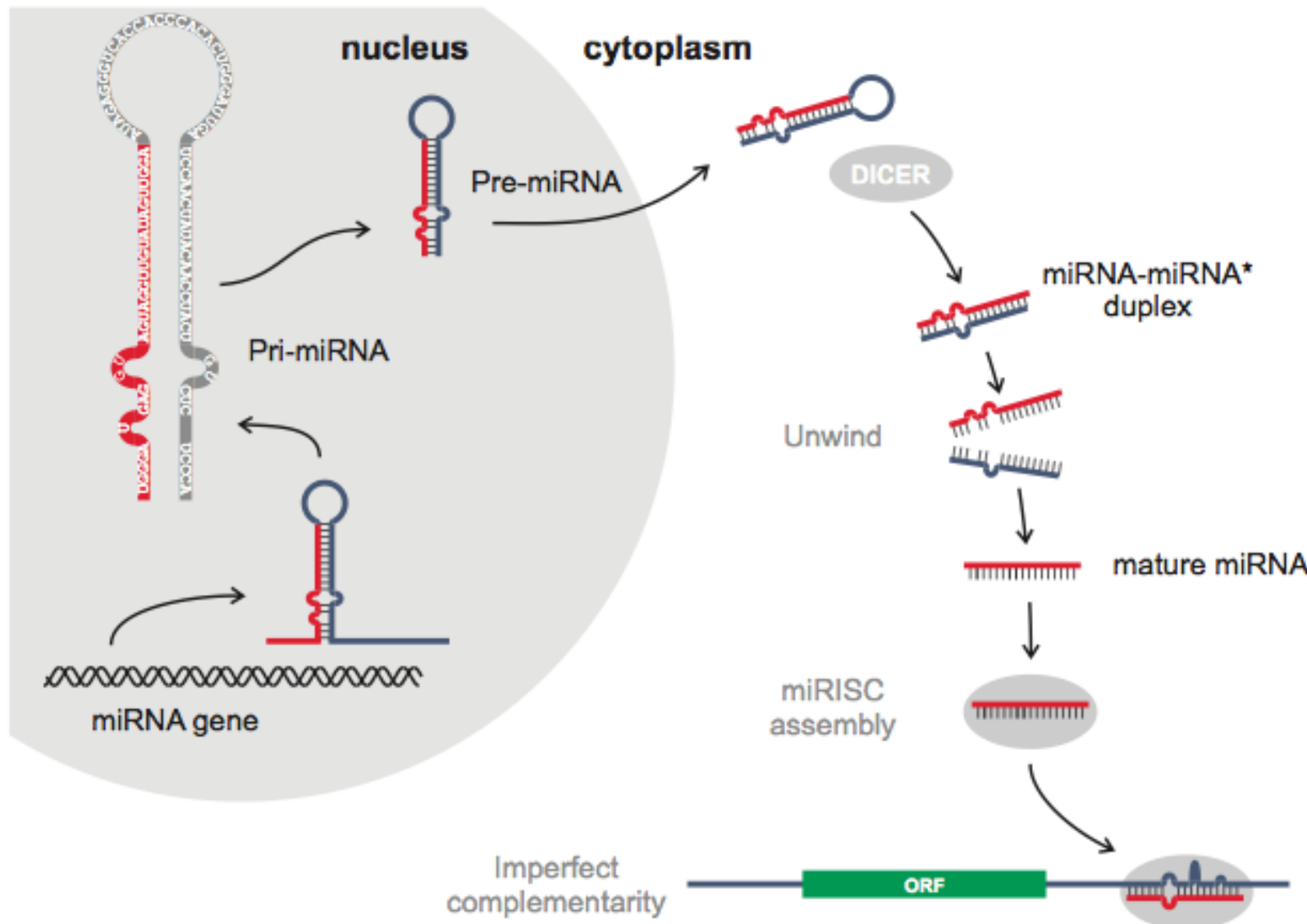


laboratory services



state-of-the-art
analysis software

qbase PLUS



- 21-23 bp negative regulators of gene expression
- predominantly bind 3'UTR
 - ▶ translational inhibition
 - ▶ mRNA degradation

hybridisation based (microarray or beads in solution)

- Exiqon probeset | miRCURY arrays | flexmiR beads
- Ambion mirVana probeset
- Invitrogen NCode probeset
- Agilent Human miRNA Microarray
- Affymetrix

PCR based

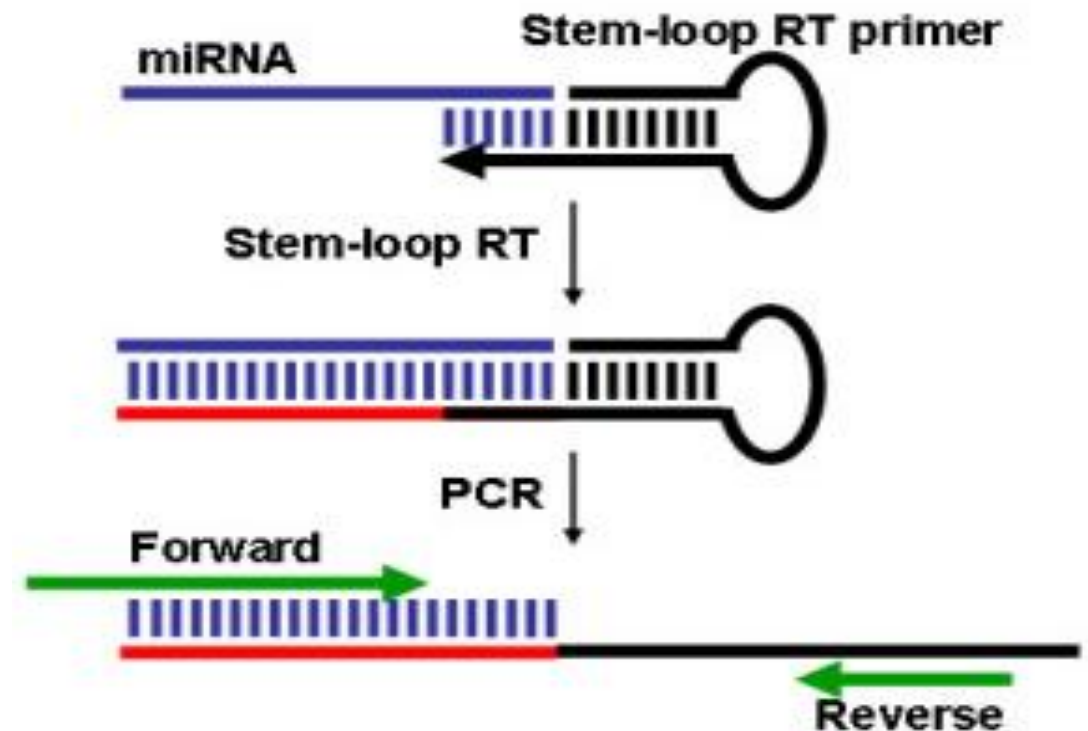
- Applied Biosystems stem-loop RT-PCR
- Exiqon miRCURY LNA microRNA PCR System
- Invitrogen NCode miRNA RT-PCR
- Qiagen miScript primer set

- sensitivity
- specificity

sequencing based (small RNA seq)

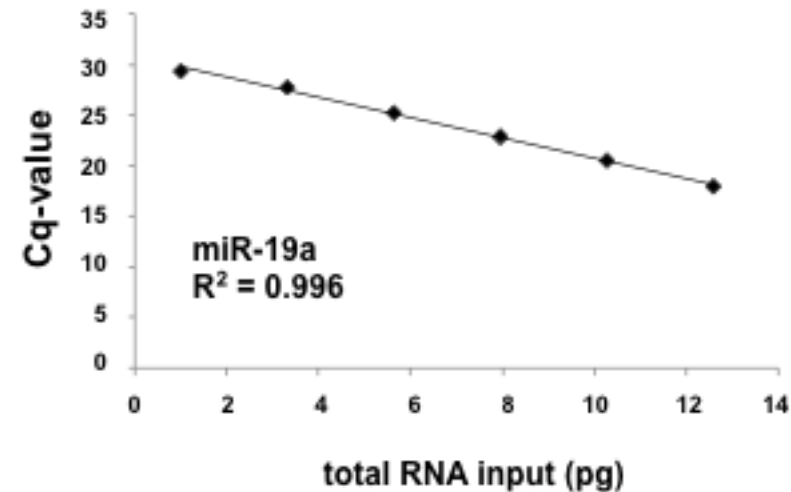
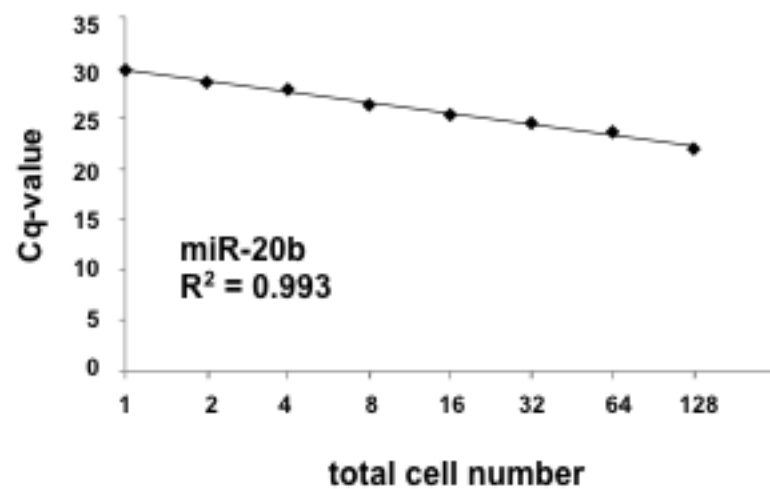
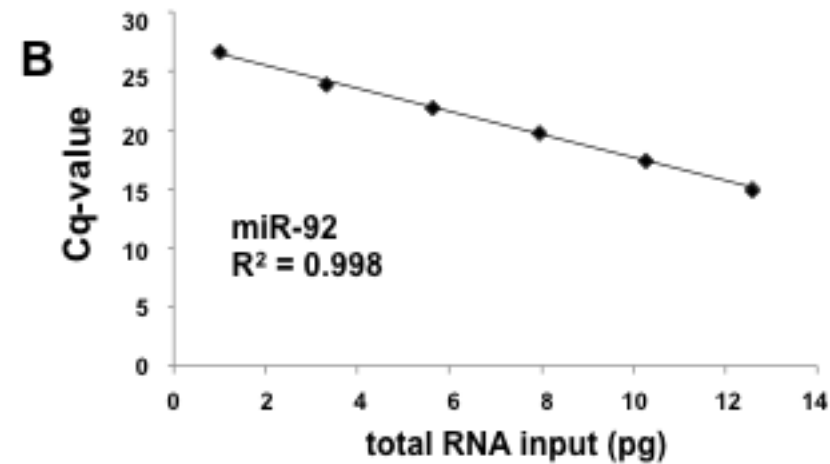
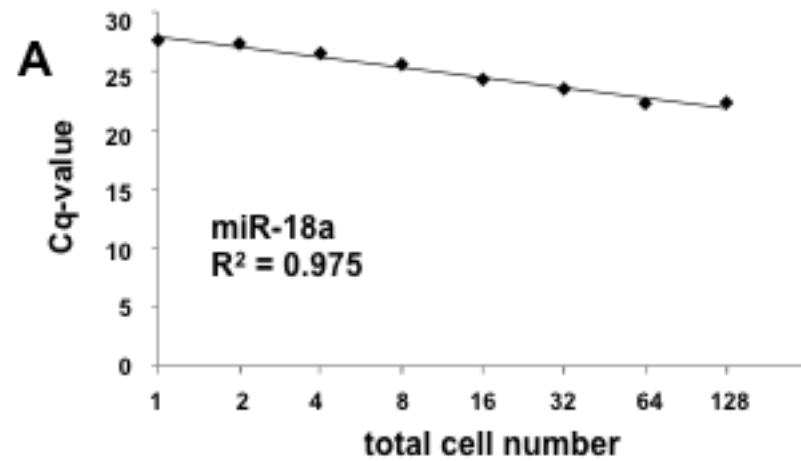
- Megaplex stem-loop reverse transcription using 60 ng total RNA
- limited-cycle pre-amplification (PreAmp)
- hydrolysis probe based qPCR quantification of 755 miRNAs

- higher sensitivity
- minimal amplification bias (Mestdagh et al., Nucleic Acids Research, 2008)
- profiled +2500 samples



- qPCR plate setup: gene maximization (Hellemans et al., Genome Biology, 2007)
 - ▶ 755 human miRNAs
 - ▶ a different miRNA in each well of a 384 well plate (no replicates)
 - ▶ 1 sample analyzed in two 384 well plates
- sample input
 - ▶ 60 ng total RNA (PreAmp)
 - ▶ 3 µg total RNA
- liquid handling Tecan Evo and Innovadyne Nanodrop Express
- qPCR reactions on Applied Biosystems 7900HT
- quality control
 - ▶ average Cq for each 384 well plate
 - ▶ number of expressed miRNAs
- data-analysis using qbase^{PLUS}

fresh frozen tissue, cultured cells, FFPE, serum/plasma, sputum



Published online 21 October 2008

*Nucleic Acids Research, 2008, Vol. 36, No. 21 e143
doi:10.1093/nar/gkn725*

High-throughput stem-loop RT-qPCR miRNA expression profiling using minute amounts of input RNA

Pieter Mestdagh¹, Tom Feys¹, Nathalie Bernard², Simone Guenther², Caifu Chen², Frank Speleman¹ and Jo Vandesompele^{1,*}

¹Center for Medical Genetics, Ghent University Hospital, 9000 Ghent, Belgium and ²Applied Biosystems, Foster City, 94404 CA, USA

Received July 22, 2008; Revised September 3, 2008; Accepted October 1, 2008

removal of experimentally induced noise

- input quantity: RNA quantity, cDNA synthesis efficiency, ...
- input quality: RNA integrity, RNA purity, ...

gold standard is the use of multiple stably expressed reference genes

- which genes?
- how many?
- how to do the calculations?

framework for qPCR gene expression normalisation using the reference gene concept

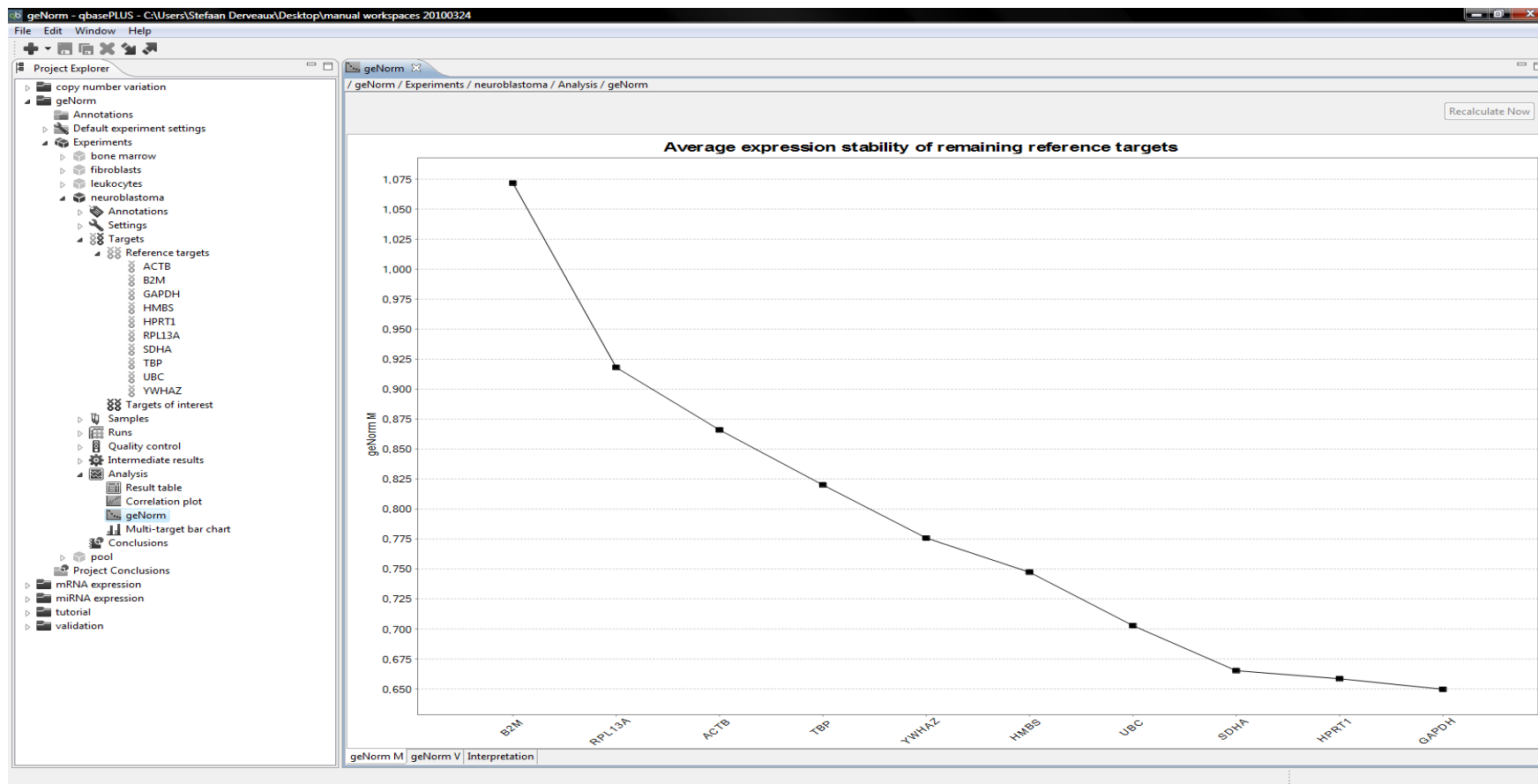
- quantified errors related to the use of a single reference gene (> 3 fold in 25% of the cases; > 6 fold in 10% of the cases)
- developed a robust algorithm for assessment of expression stability of candidate reference genes
- proposed the geometric mean of at least 3 reference genes for accurate and reliable normalisation
- Vandesompele et al., Genome Biology, 2002

Research

Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes

Jo Vandesompele, Katleen De Preter, Filip Pattyn, Bruce Poppe, Nadine Van Roy, Anne De Paepe and Frank Speleman

- ranking of candidate reference genes according to their stability
- determination of how many genes are required for reliable normalization
- <http://www.genorm.info>



- robust – insensitive to outliers
- maximal reduction of experimental variation
- accurate assessment of small expression differences
- statistically more significant results

geNorm is the *de facto* standard for reference gene validation and normalization

- > 2,500 citations of our geNorm technology
- > 13,000 geNorm software downloads in 100 countries





	classic geNorm	improved geNorm
platform	Excel Windows	qbase ^{PLUS} Win, Mac, Linux
speed	1x	20x
interpretation	-	+
ranking best 2 genes	-	+
handling missing data	-	+
raw data (Cq) as input	-	+

small-RNA controls

- classic normalization strategy
- small nuclear RNAs, small nucleolar RNAs
- 18 available from Applied Biosystems
 - not proven to be universally stably expressed
 - have only chemical structure in common with miRNA

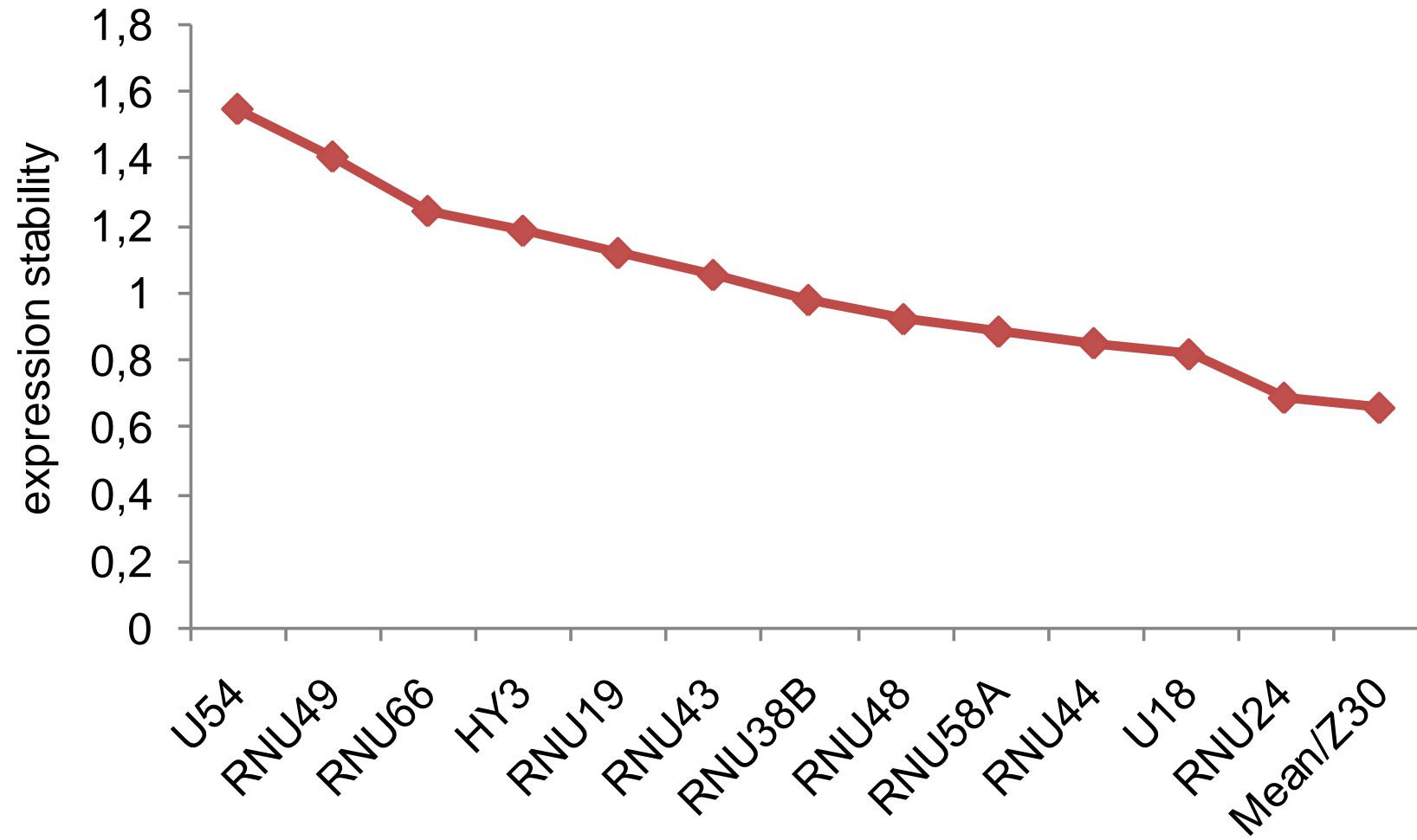
global mean normalization

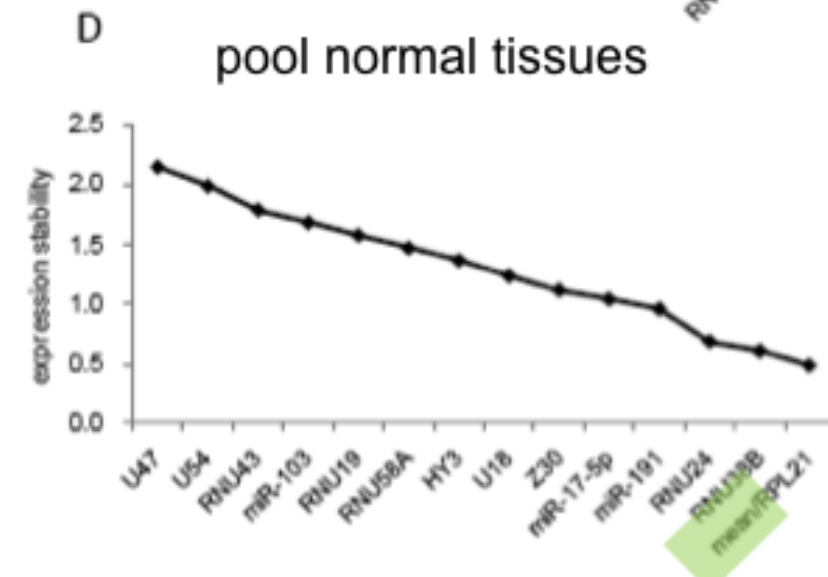
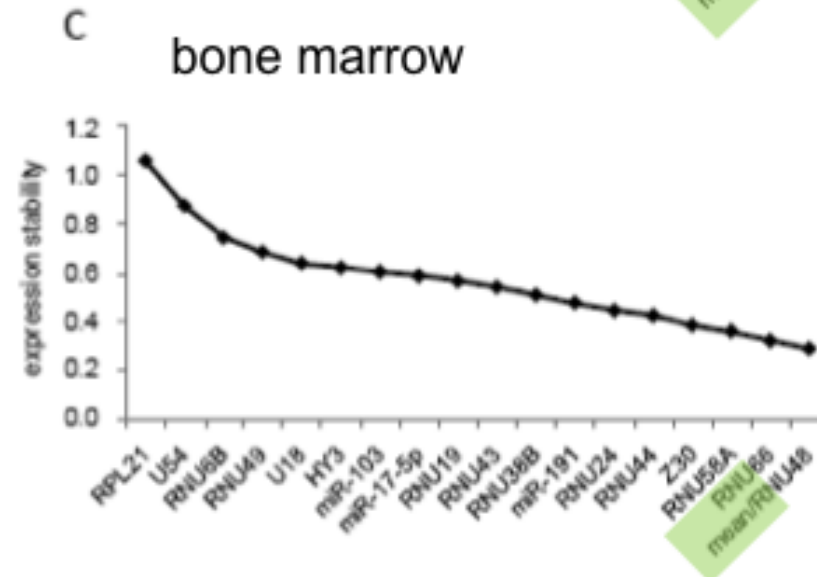
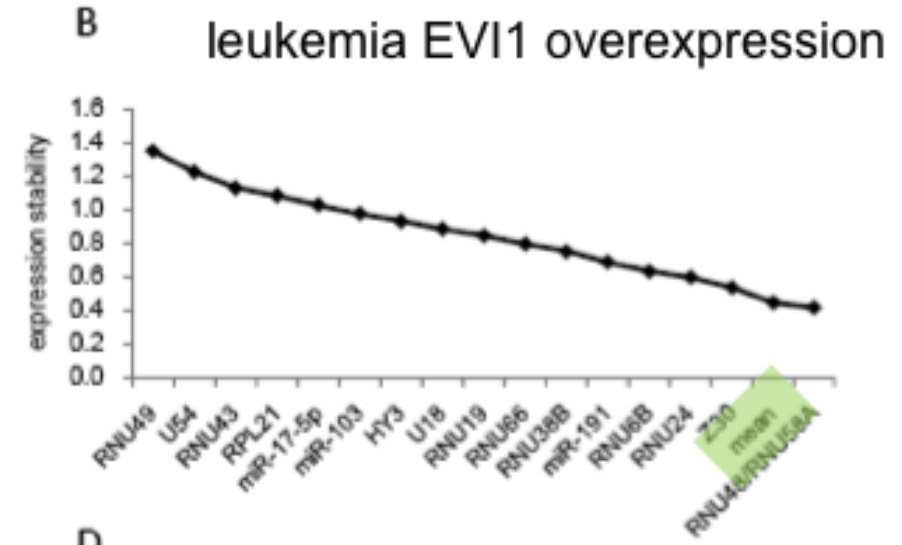
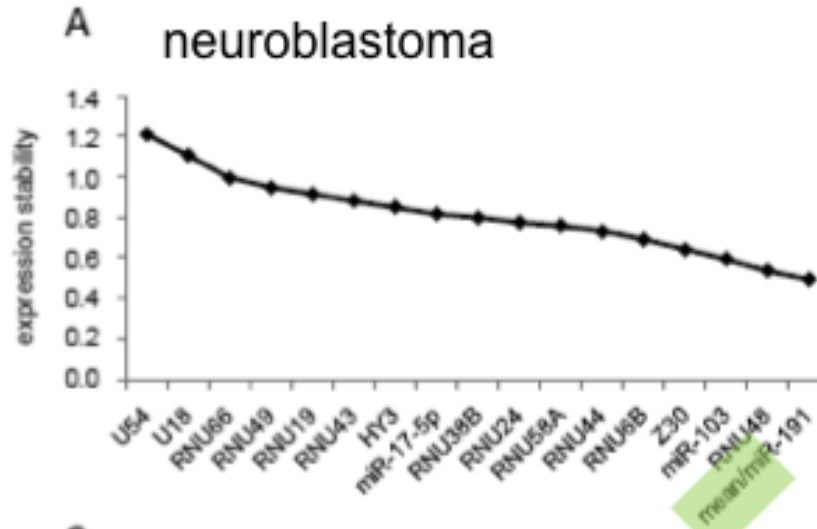
- calculate the average expression level across all miRNAs
- works if large number of miRNAs are measured
- novel, robust, better

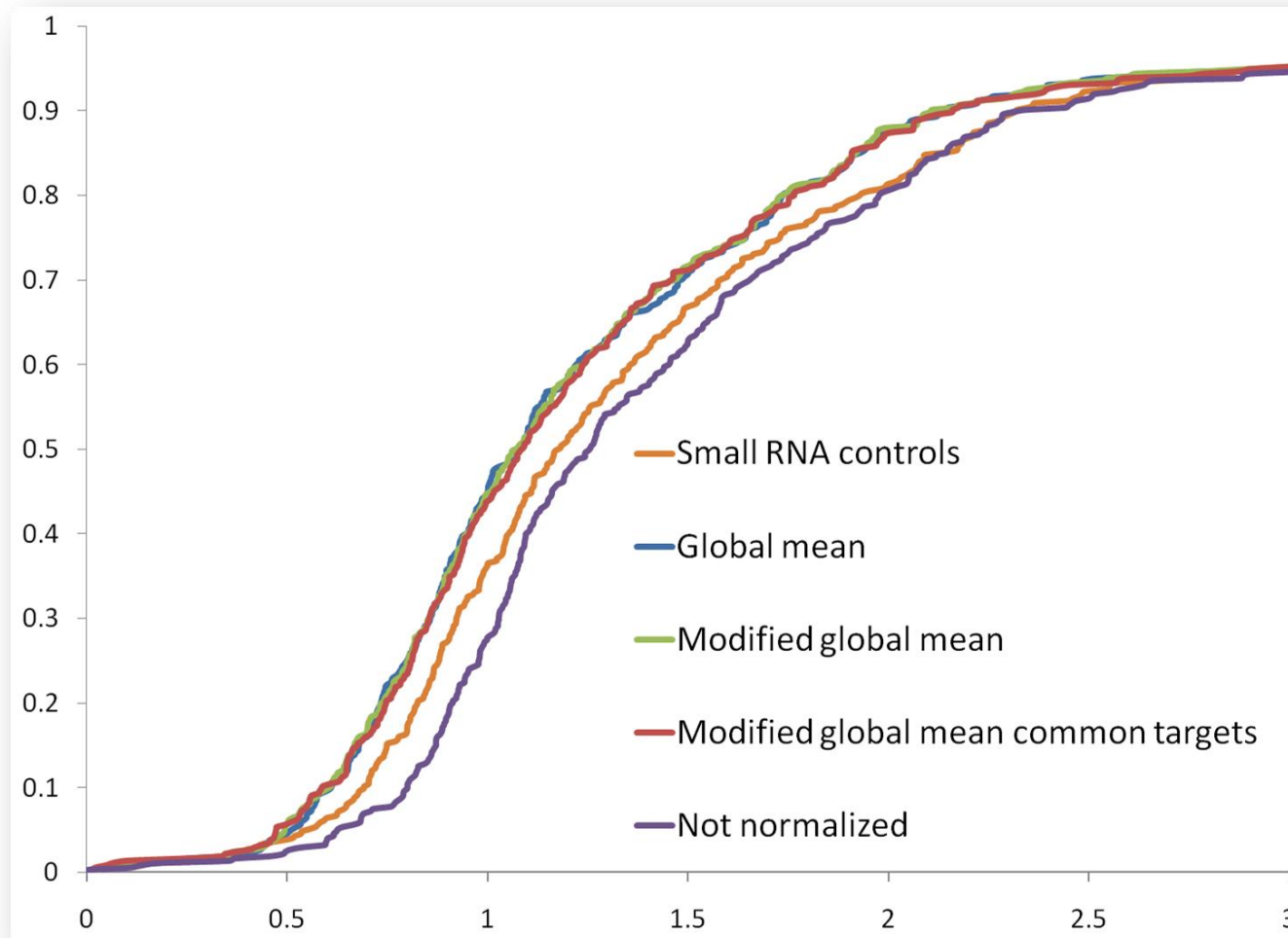
How 'stable' is the mean compared to controls?

- geNorm analysis using controls and mean as input variables
- exclusion of potentially co-regulated controls

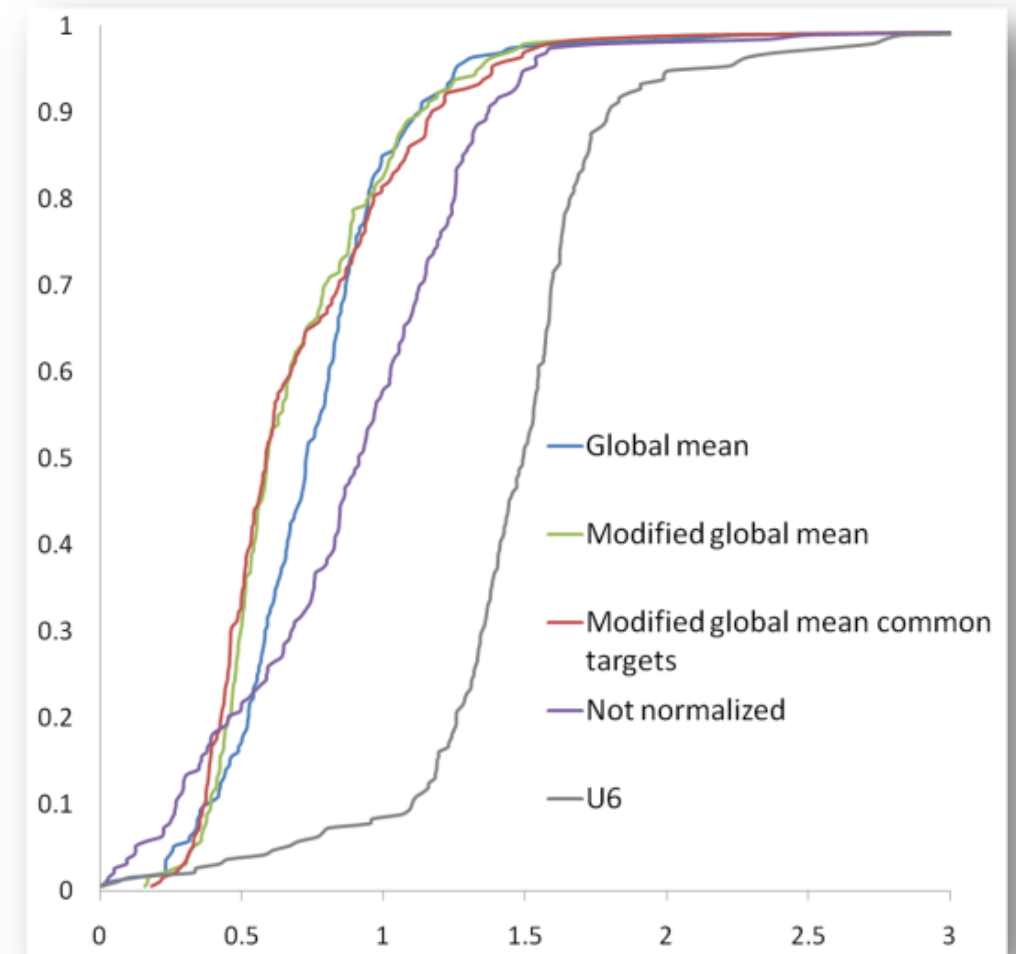
HY3	7q36
RNU19	5q31.2
RNU24	9q34
RNU38B	1p34.1-p32
RNU43	22q13
RNU44	1q25.1
RNU48	6p21.32
RNU49	17p11.2
RNU58A	18q21
RNU58B	18q21
RNU66	1p22.1
RNU6B	10p13
U18	15q22
U47	1q25.1
U54	8q12
U75	1q25.1
Z30	17q12
RPL21	13q12.2



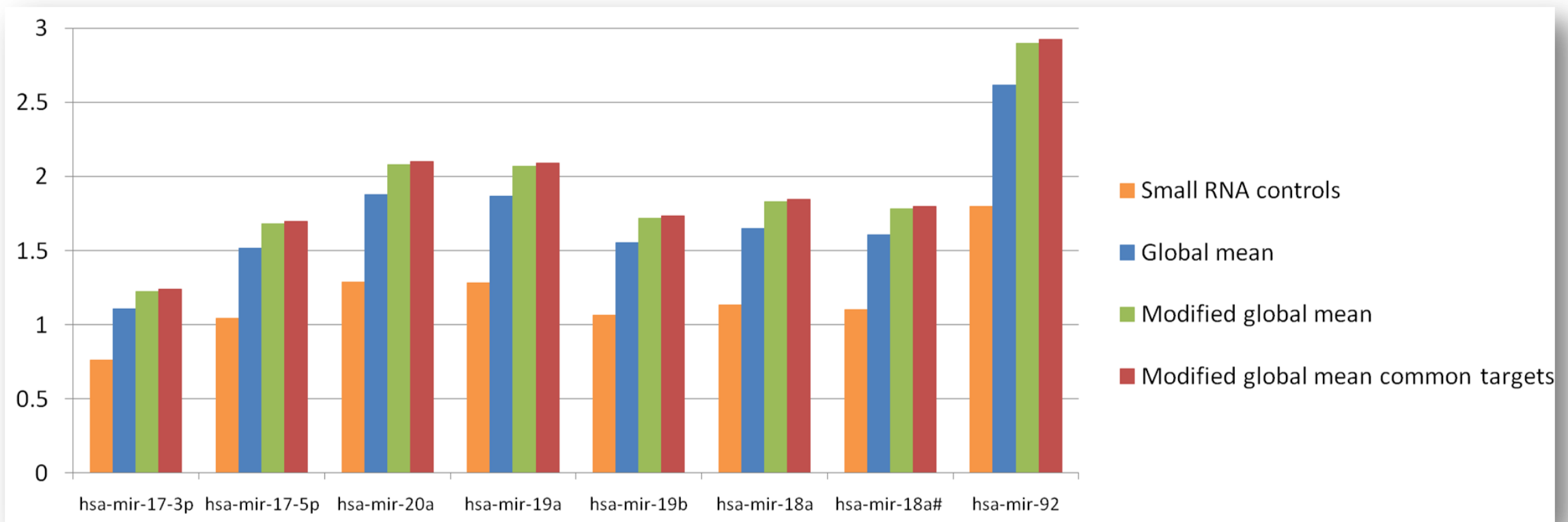




neuroblastoma tumors

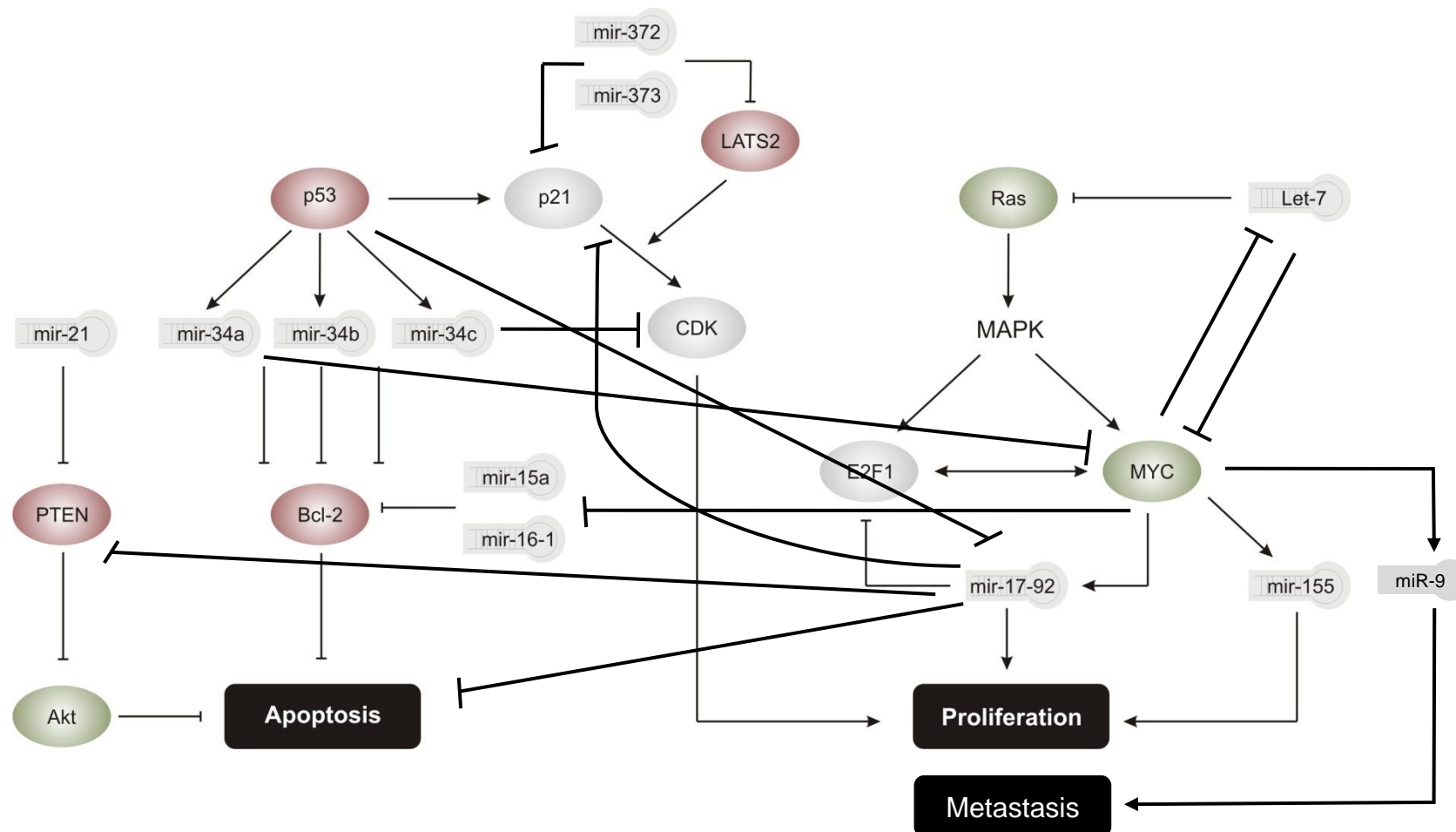


sputum (non-)smokers



- improved global mean normalization is implemented in qbase^{PLUS} (SysKid work package 2)

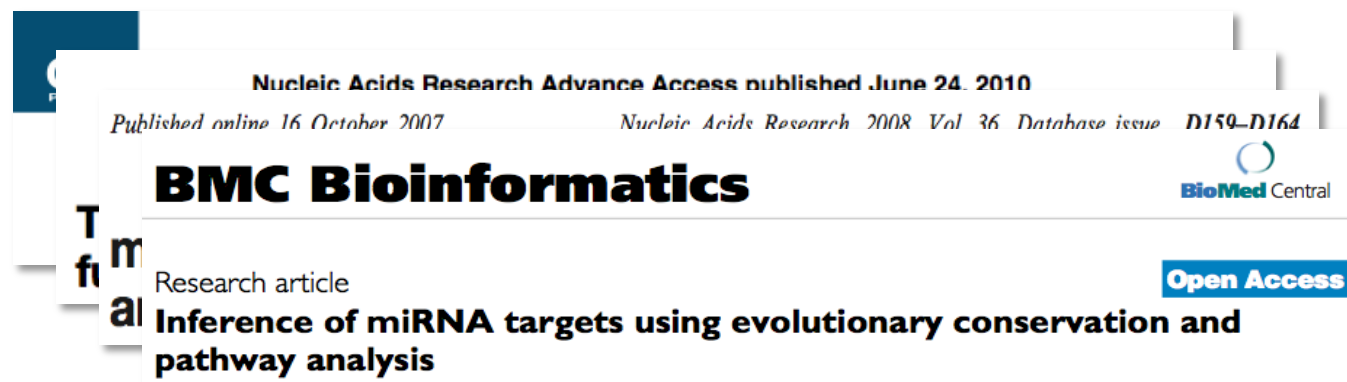
■ Mestdagh et al., BJMO, 2008



experimental tools for high-throughput miRNA target identification

- miRNA perturbation followed by microarray or mass spectrometry
- RIP-ChIP, HITS-CLIP, ...
 - for one or few miRNAs
 - need to prioritize candidate miRNAs up front

in silico tools for functional miRNA annotation

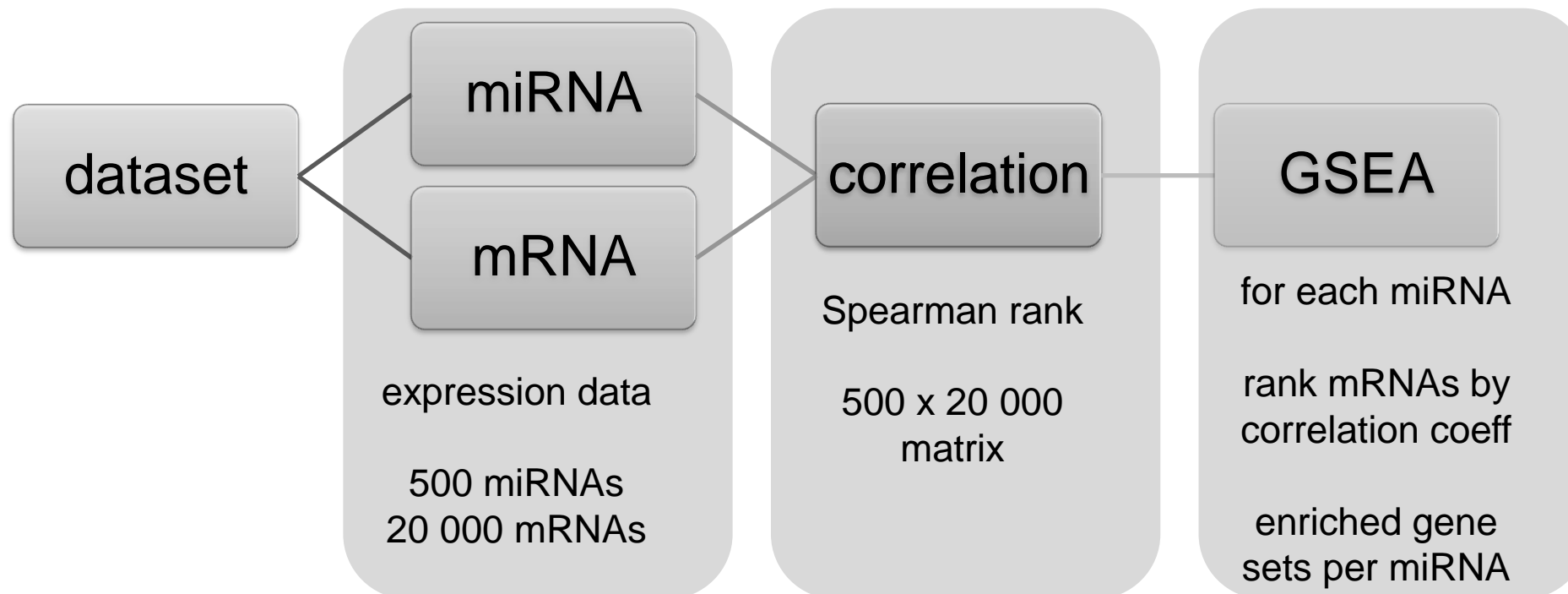


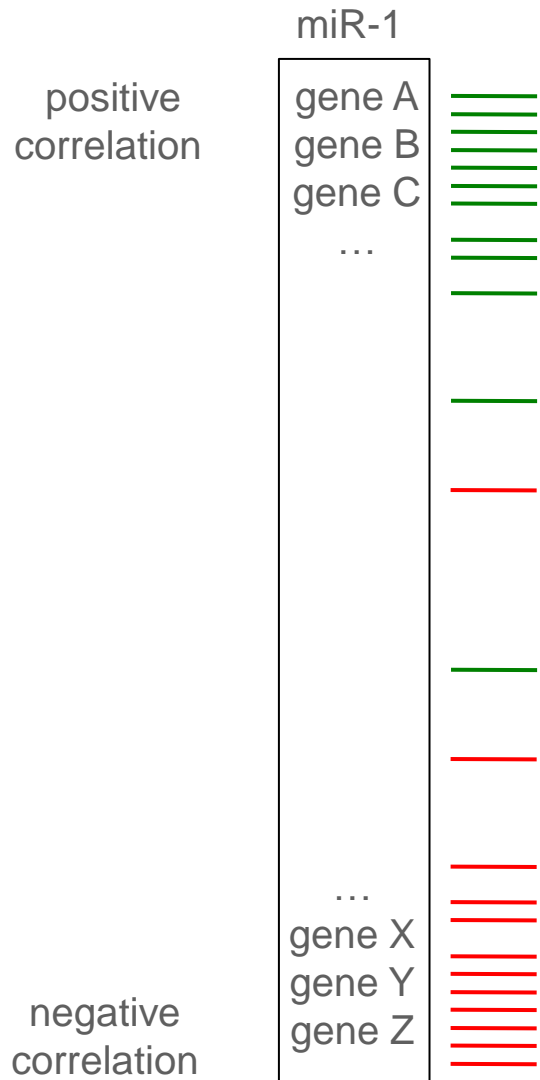
- based on target prediction (high number of false positives, not tissue-specific)

integrated approach for functional miRNA annotation

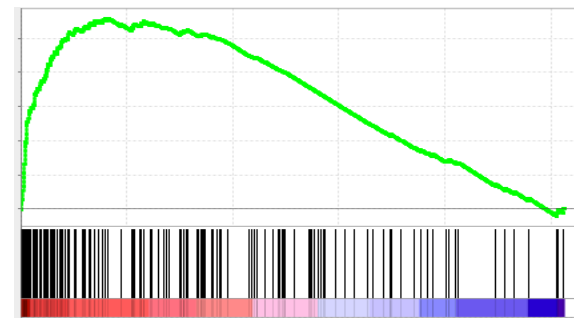
- mRNA expression – miRNA expression – target prediction – miRNA network models
- tissue specific
- higher confidence (integration of multiple levels of information)

functional annotation using GSEA

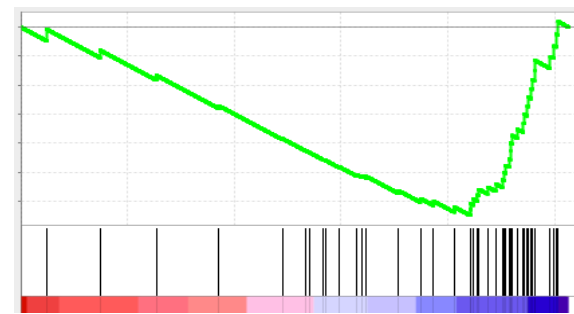


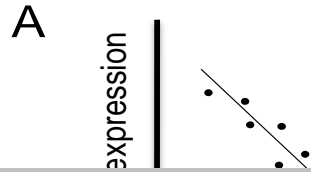


gene sets enriched among positively correlated mRNAs

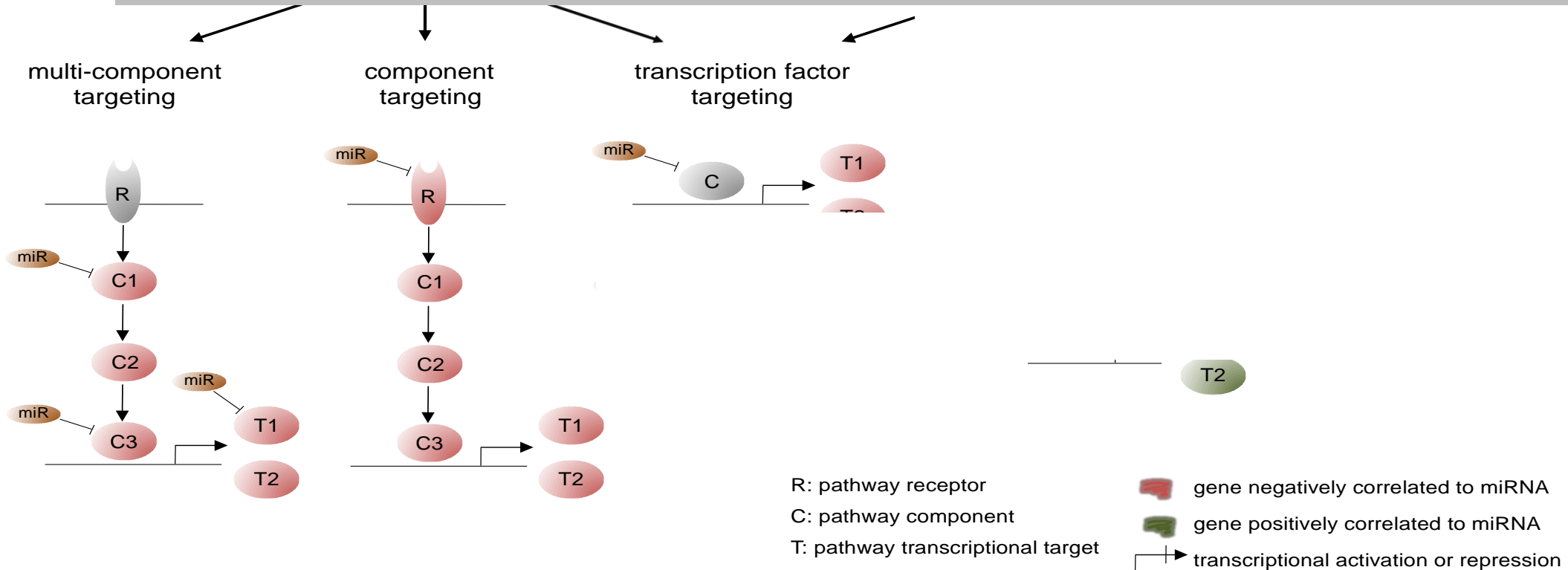


gene sets enriched among negatively correlated mRNAs



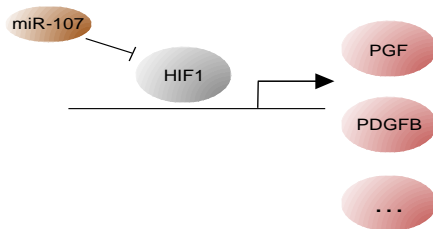
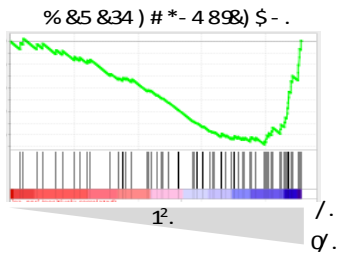


a significant miRNA – gene set association is more likely to be functional if there is evidence that links the association to one of these models



transcription factor targeting

miR-107 ~ HIF1A



P53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis

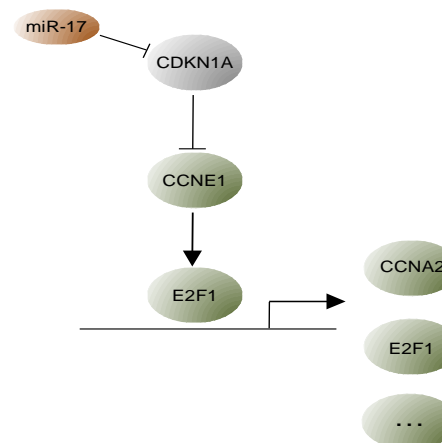
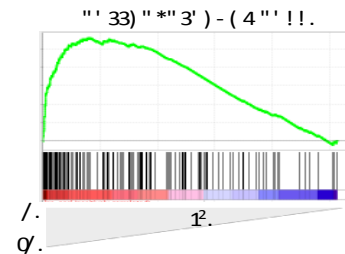
Munekazu Yamakuchi^{1,2}, Craig D. Lotterman^{1,2,3,4}, Clare Bao⁵, Ralph H. Hruban⁶, Baktiar Karim⁶, Joshua T. Mendell^{1,2}, David Huso⁷, and Charles J. Lowenstein^{1,2}

¹Department of Medicine, Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642; and ²Department of Medicine, ³The Mokusick-Nathans Institute of Genetic Medicine, ⁴Department of Pediatrics, ⁵Department of Pathology, and ⁶Department of Comparative Molecular Medicine and Pathobiology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

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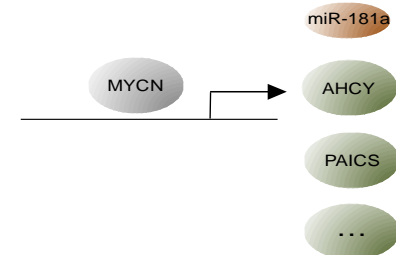
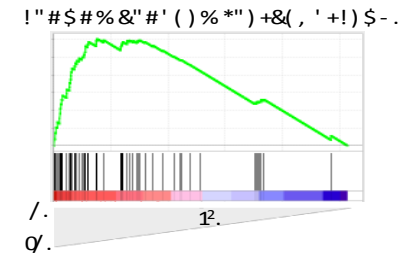
targeting negative regulator

miR-17 ~ CDKN1A



common transcriptional activator/repressor

MYCN ~ miR-181a



Oncogene (2009), 1-11
© 2009 Macmillan Publishers Limited. All rights reserved 0950-9232/09 \$32.00
www.nature.com/onc

ONCOGENOMICS

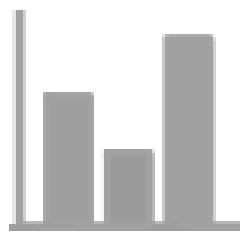
MYCN/c-MYC-induced microRNAs repress coding gene networks associated with poor outcome in MYCN/c-MYC-activated tumors

P Mestdagh¹, E Fredlund¹, F Pattyn¹, JH Schulte², D Muth³, J Vermeulen¹, C Kumps¹, S Schlierf⁴, K De Preter¹, N Van Roy¹, R Noguera⁴, G Laureys², A Schramm², A Eggert², F Westermann³, F Speleman¹ and J Vandesompele¹

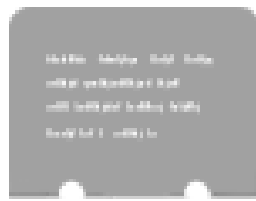
GSEA data

experimental data

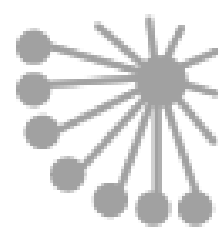
- repository of miRNA expression data and functional miRNA annotation in normal and disease tissues
- currently: genome wide miRNA expression data for 750 samples



expression analysis



miRNA2function



function2miRNA

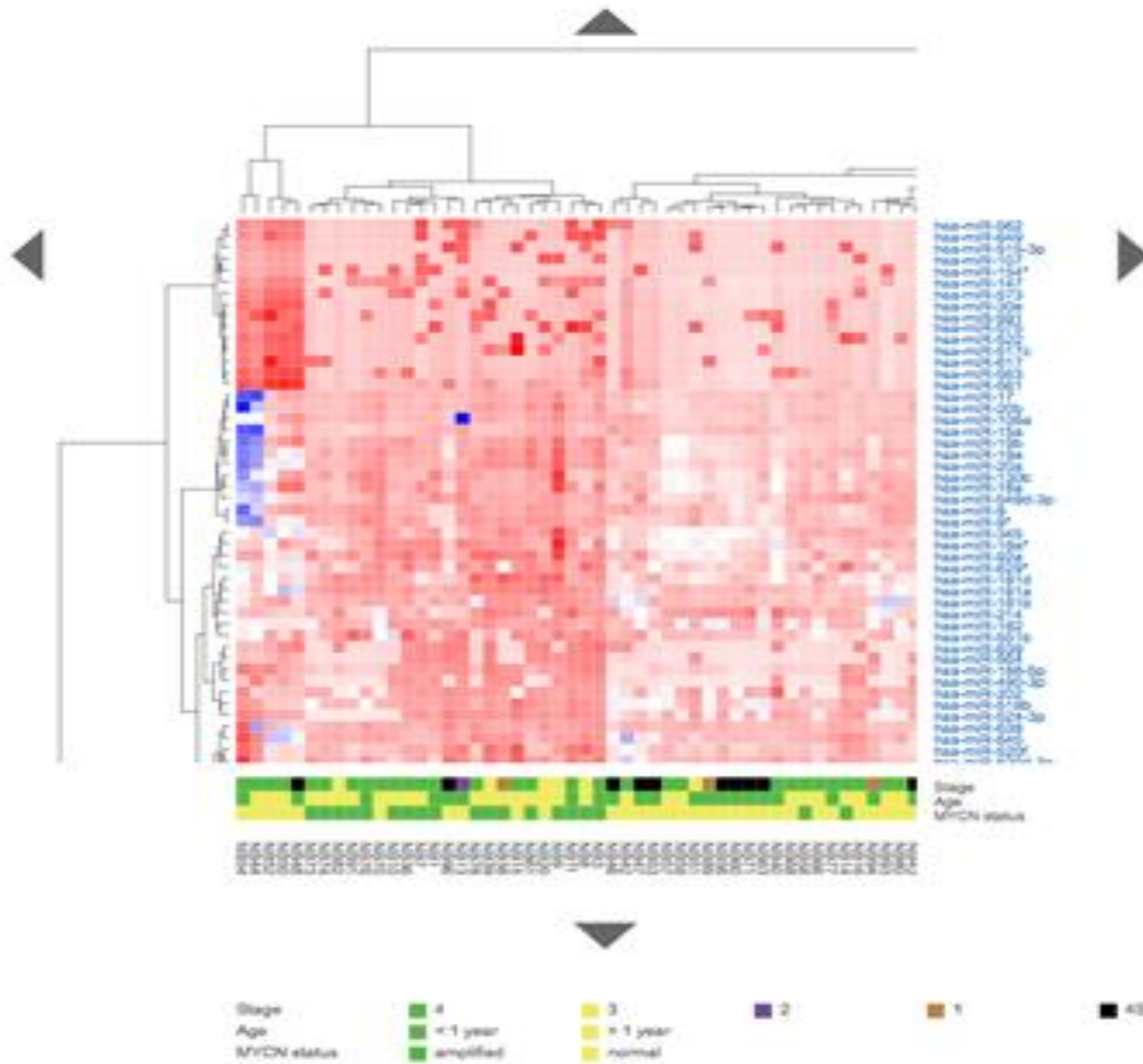


pubmed search



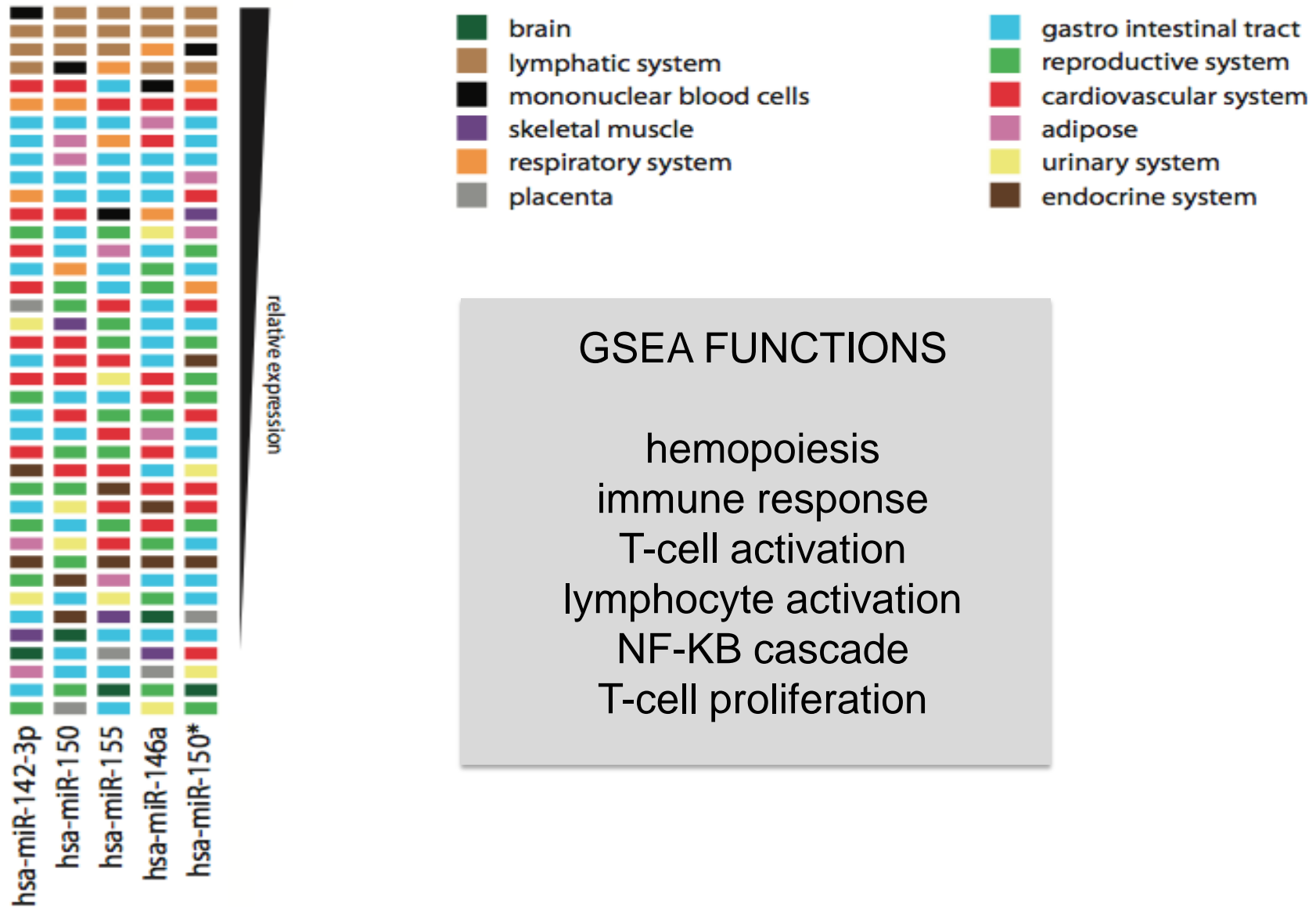
- **expression analysis**: identify differentially expressed miRNAs, visualize miRNA expression patterns
- **miRNA2function**: predict miRNA function in a specific dataset
- **function2miRNA**: retrieve miRNAs annotated to a given function
- **pubmed search**: literature knowledge mining tool with result context highlighting to retrieve experimentally validated miRNA functions

heatmap



ranked expression map





Functional annotation

Dataset : normal tissues – Mestdagh et al. (submitted) – qPCR
neuroblastoma – Mestdagh et al. (2010) – qPCR
myeloma – Gutierrez et al. (2010) – qPCR
NCI60 – Gaur et al. (2007) – qPCR

Type of correlation : Negative correlation Positive correlation

Gene set : Chemical and genetic perturbations Molecular function Biological process

Level of evidence : in at least 1 or in all the selected datasets

Gene sets enriched for targets of transcription factor targeted by selected miRNA

Gene sets enriched for targets of selected miRNA

search

Level of evidence : 1 dataset at least 2 datasets all selected datasets

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	WINTER_HYPOXIA_DN
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	PICCALUGA_ANGIOIMMUNOBLASTIC_LYMPHOMA_UP
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	LIU_PROSTATE_CANCER_DN
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	SCHUETZ_BREAST_CANCER_DUCTAL_INVASIVE_UP
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	DAVICIONI_TARGETS_OF_PAX_FOXO1_FUSIONS_UP
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	TURASHVILI_BREAST_DUCTAL_CARCINOMA_VS_LOBULAR_NORMAL_DN
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	FULCHER_INFLAMMATORY_RESPONSE_LECTIN_VS_LPS_DN
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	THUM_SYSTOLIC_HEART_FAILURE_UP
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	DIAZ_CHRONIC_MEYLOGENOUS_LEUKEMIA_DN
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	CASORELLI_APL_SECONDARY_VS_DE_NOVO_UP
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	DEURIG_T_CELL_PROLYMPHOCYTIC_LEUKEMIA_DN
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	CHARAFE_BREAST_CANCER_LUMINAL_VS_BASAL_DN
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	CHARAFE_BREAST_CANCER_LUMINAL_VS_MESENCHYMAL_DN
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	LAIHO_COLORECTAL_CANCER_SERRATED_UP

analysing overlap between miRNA target prediction algorithms

Target prediction

- miRDB
- PITA
- MicroCosm
- TargetScan
- TargetScan_cons
- DIANA
- miRecords

☐ Predicted by 7 databases

GAB1

☐ Predicted by 6 databases

ARID4B	ATAD2	C1orf63	CRIP1	DIP2A	DOCK4	E2F5	ERBB3	FOXQ1
KIAA1191	LAPTM4A	MAP3K8	MAP3K9	MAPK9	METAP1	MINK1	MYCN	NUP35
POLQ	POU6F1	PTPN4	SACS	SC4MOL	SPTY2D1	STK17B	TLE4	TMUB2
TRIM8	TRIP11	TRPV6	TSHZ3	VSX1	WEE1			

- ☐ Predicted by 5 databases
- ☐ Predicted by 4 databases
- ☐ Predicted by 3 databases
- ☐ Predicted by 2 databases
- ☐ Predicted by 1 databases

Biogazelle

- Barbara D'haene
- Jan Hellemans
- Gaëlle Van Severen

Life Technologies

- Caifu Chen
- Dana Ridzon
- Linda Wong

SysKid

Ghent University

- Pieter Mestdagh
- Steve Lefever
- Annelies Fiew
- Filip Pattyn
- Maté Ongenaert
- Joëlle Vermeulen
- Anne De Paepe
- Frank Speleman