LÉKAŘSKÁ FAKULTA MASARYKOVY UNIVERSITY
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Centrum molekulární biologie a genové terapie

Analýza exprese microRNA

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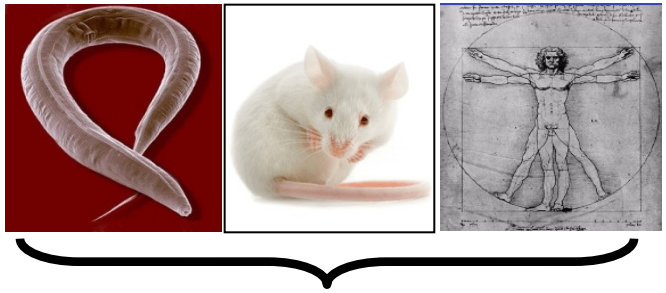
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ
Tato prezentace je spolufinancována Evropským sociálním fondem a státním rozpočtem České republiky

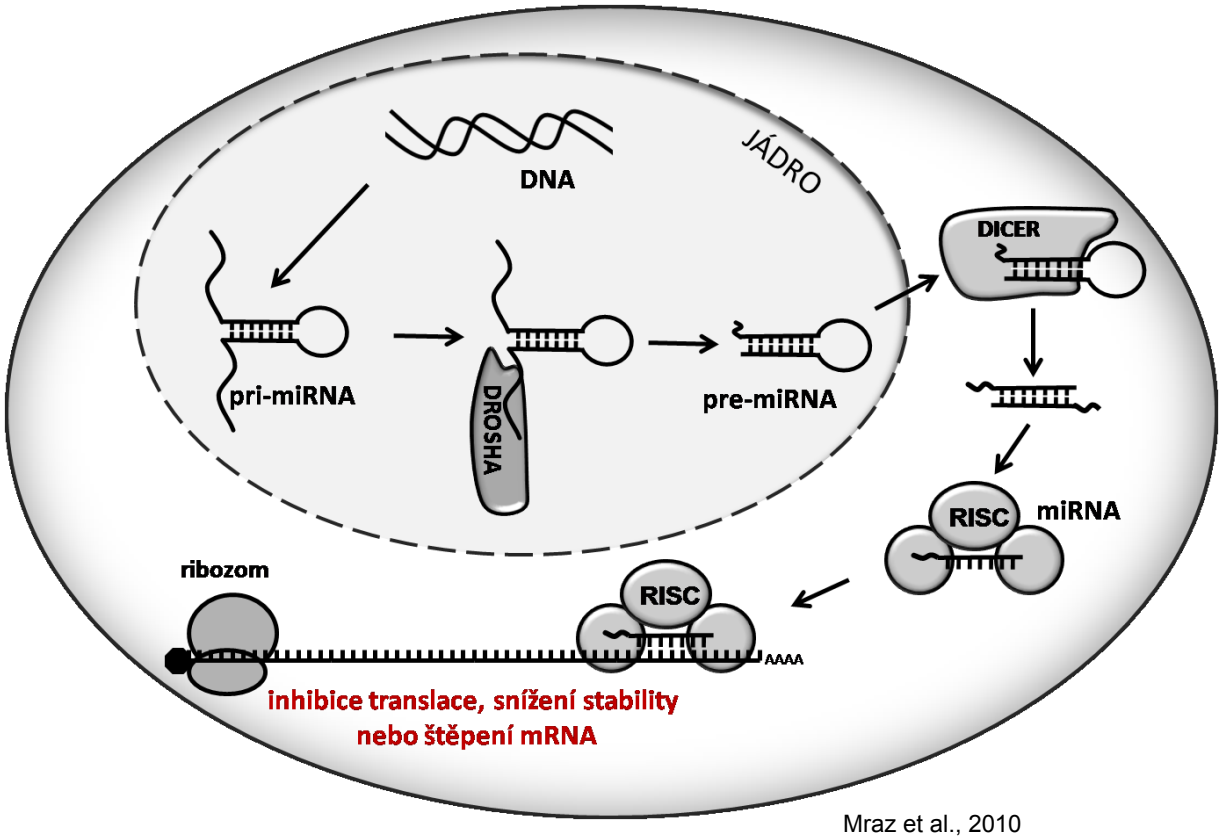
microRNA (miRNA)

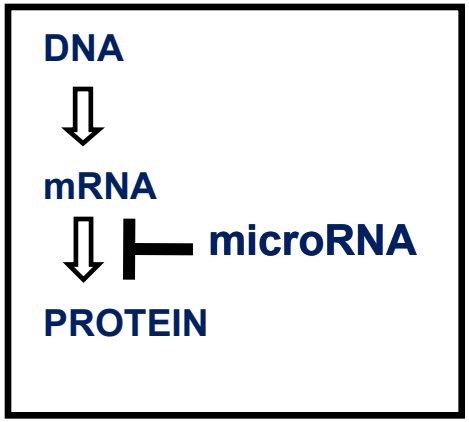
- krátké RNA molekuly ~22 nukleotidů
- komplementární vazba k cílové mRNA
- inhibují translaci a snižují stabilitu mRNA

- Lidské miRNA geny: cca 2000



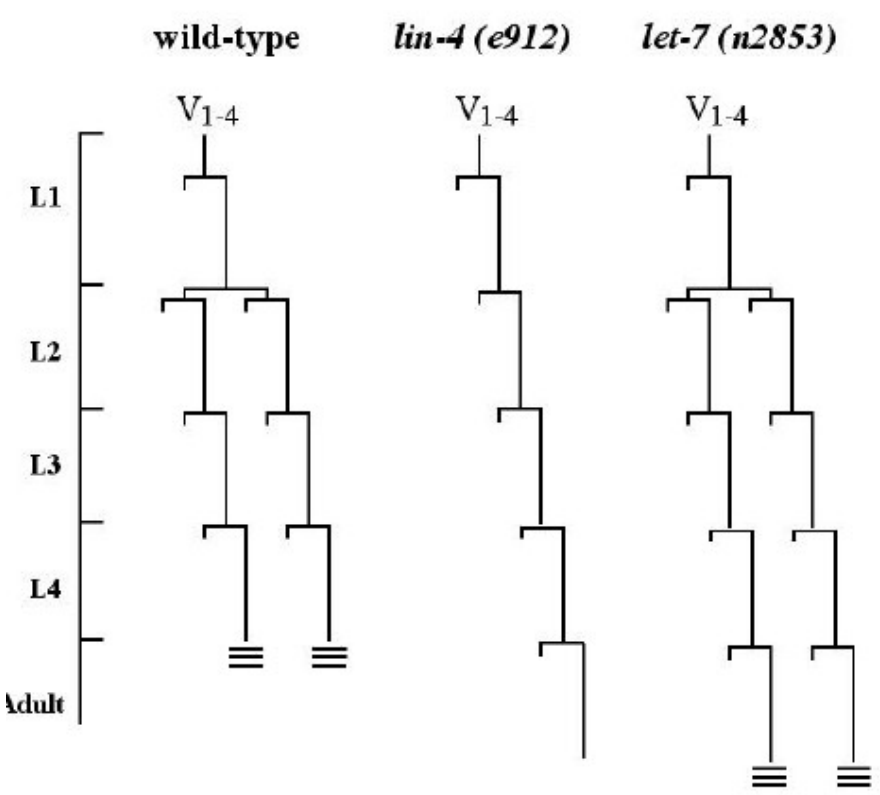
Stovky evolučně konzervovaných microRNA

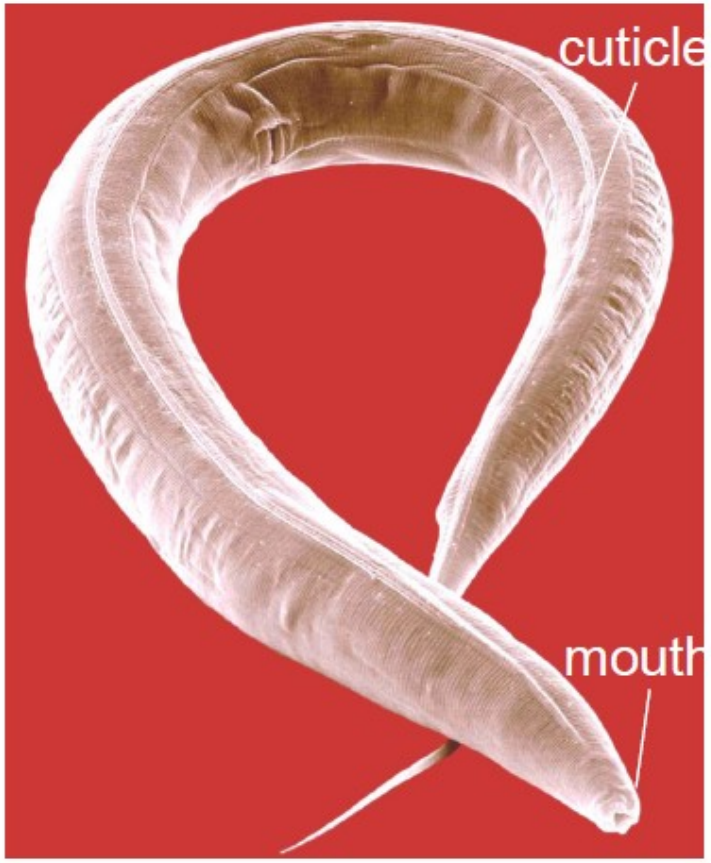




Mraz et al., 2010

MicroRNAs were discovered by V. Ambros and G. Ruvkun in *C. elegans*

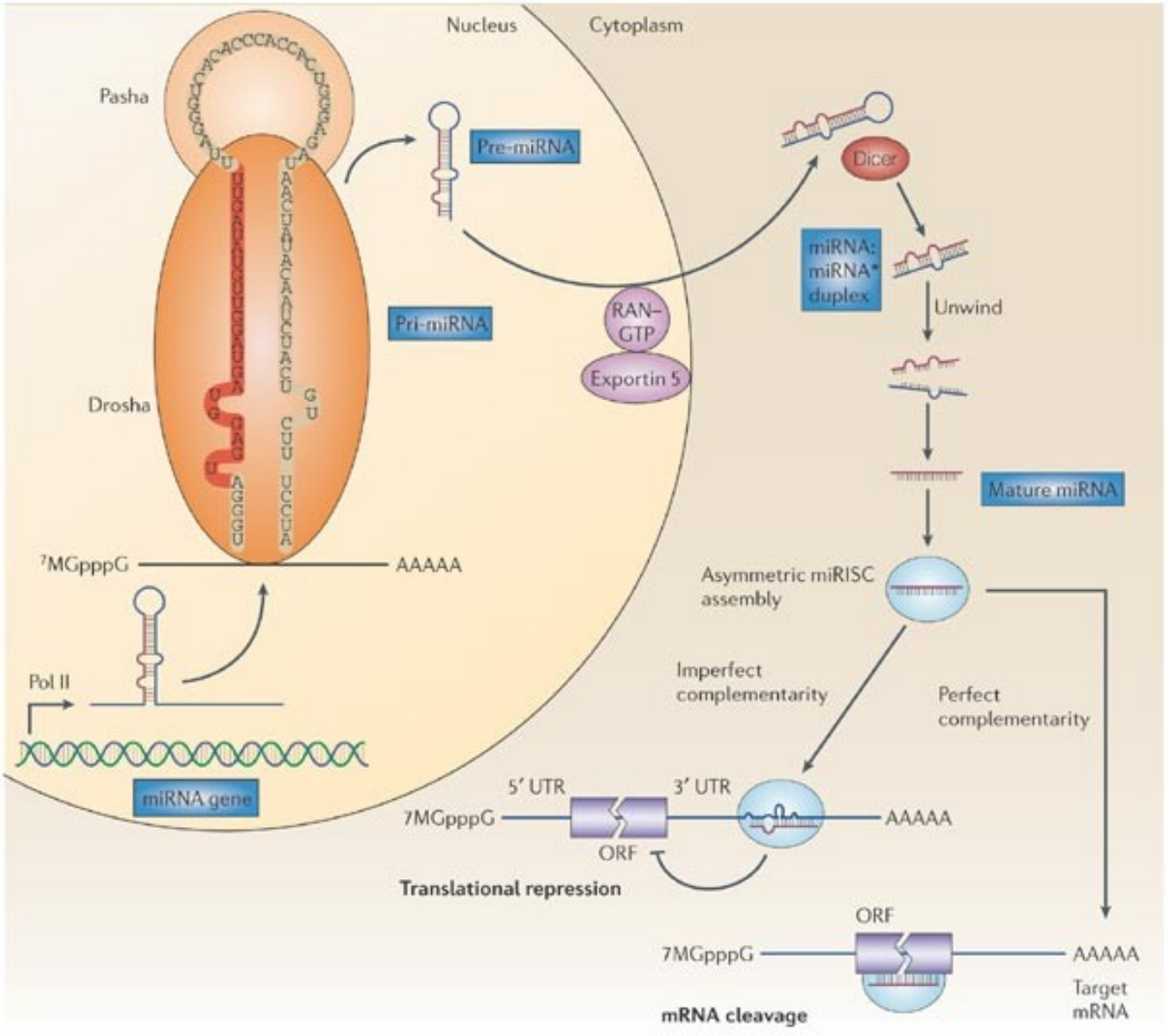


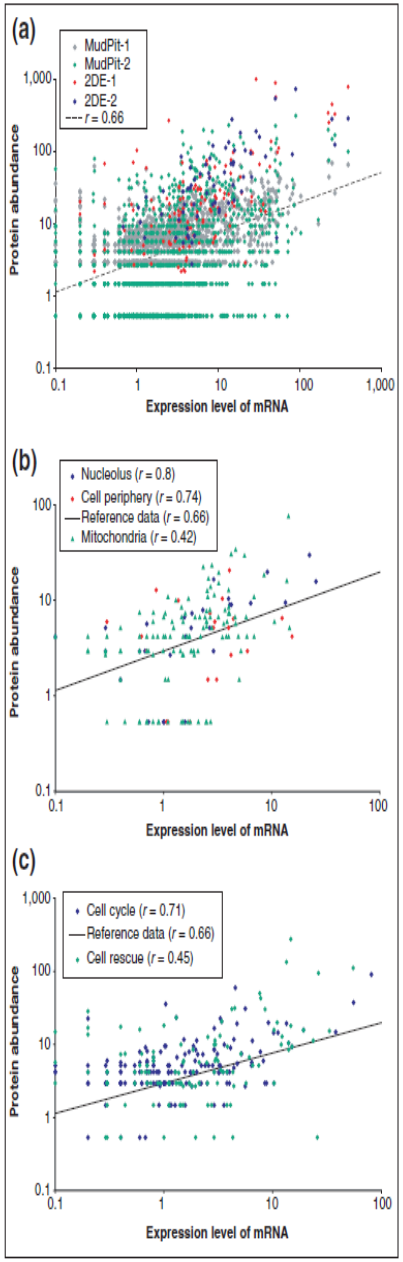


Lee et al. 1993 *Cell*

Reinhart et al. 2000 *Nature*

courtesy of F. Slack





mRNA neznamená, že v buňce bude i protein

Historicky vždy velká neshoda mezi daty z expresních čipů a expresí proteinů (Western Blot)

DNA

⇩

mRNA

⇩ ⊢ **microRNA**

PROTEIN

Specifika analýzy exprese microRNAs:

- velmi malé molekuly – 22nt – specifikum izolace, specifické značení i design sond
- malé zastoupení ve vzorku – separace microRNA
- v lidském genovu cca 2000 genů
- některé mají velmi podobnou sekvenci – rozdíl 1nt
- pre-miR, pri-miR, mature-miR
- málo se ví o jejich funkcích – obtížná interpretace výsledků
- zatím málo zkušeností a standardizace

- Isolace
- Microarrays
- Identifikace miRNA (deep sequencing, cloning a Northern blot)
- Real-Time PCR

1/ Izolace a stabilita microRNA

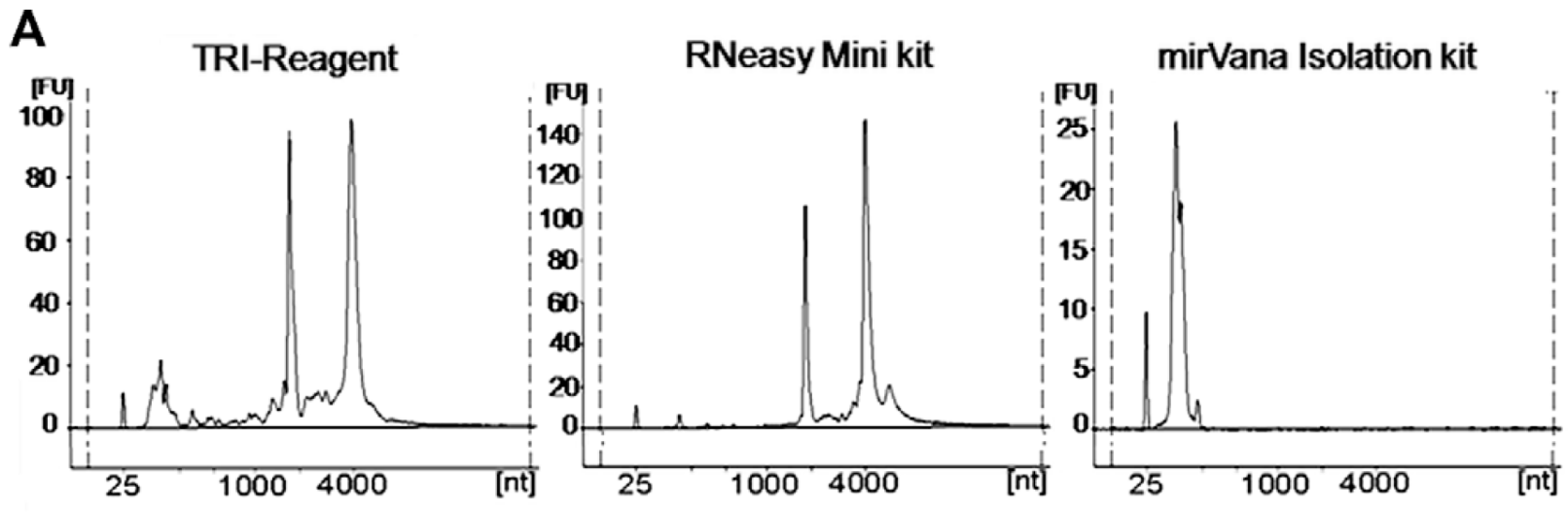
Problémy: velikost 22nt, celkově cca 0,01% z celkové RNA

Izolace:

TRIzol/TriReagent
miRvana (Ambion)
PureLink (Invitrogen)
a další

Obohacení:

PAGE
FlashPAGE Fractionator (Ambion)



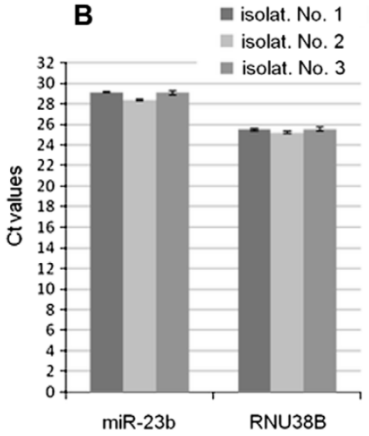
Mraz et al., 2009

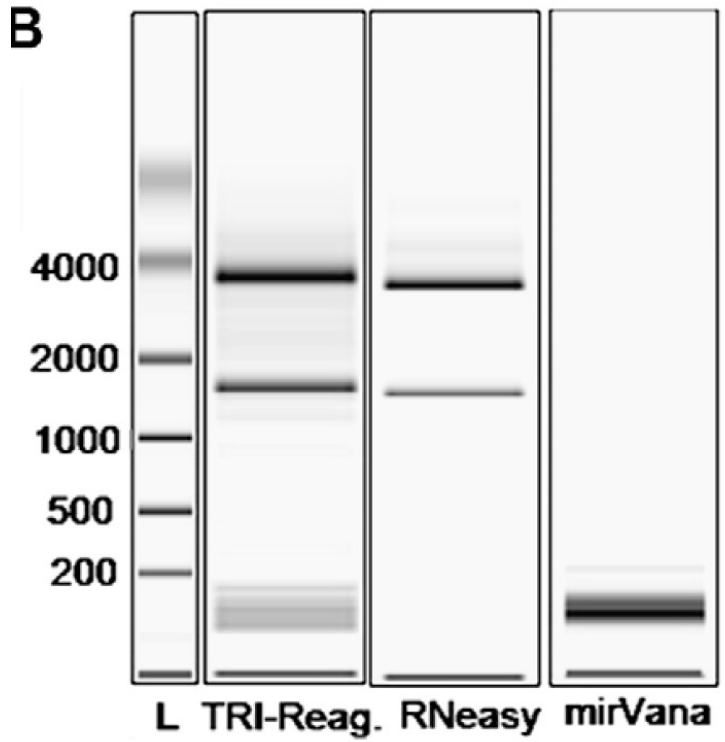
Izolace:

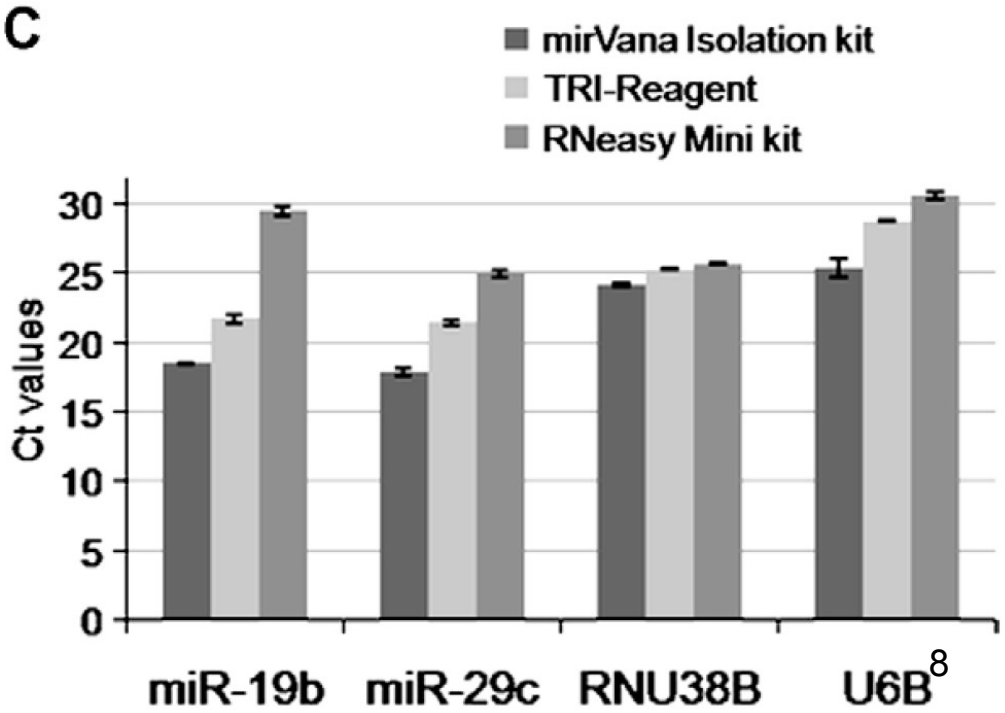
TRIzol/TriReagent
miRvana (Ambion)
PureLink (Invitrogen)
a další

Izolace:

TRIReagent/TRIzol
is the „gold standard“
(Mraz et al., 2009)







Mraz et al., 2009

Obohacení:

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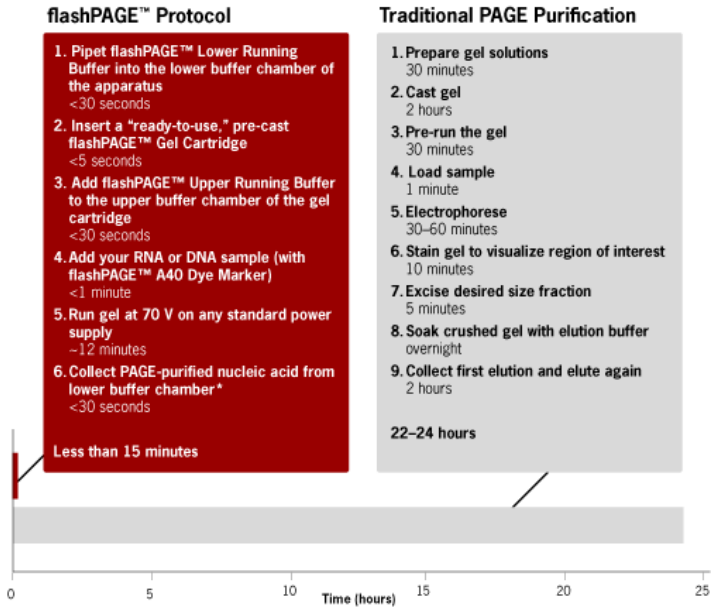
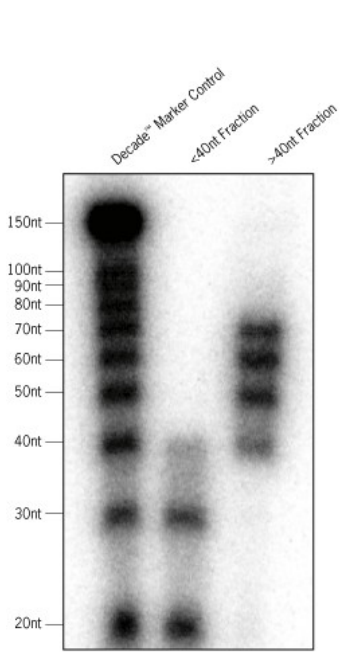
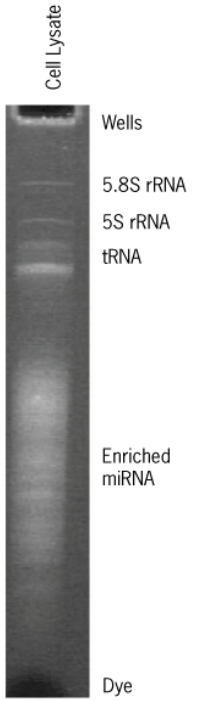
FlashPAGE Fractionator (Ambion)



15min 20hours

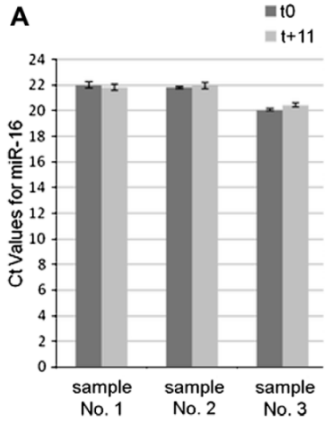




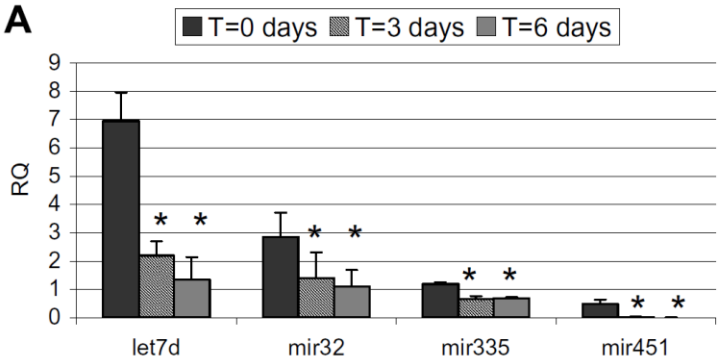
Stabilita microRNA :

Stabilita po izolaci

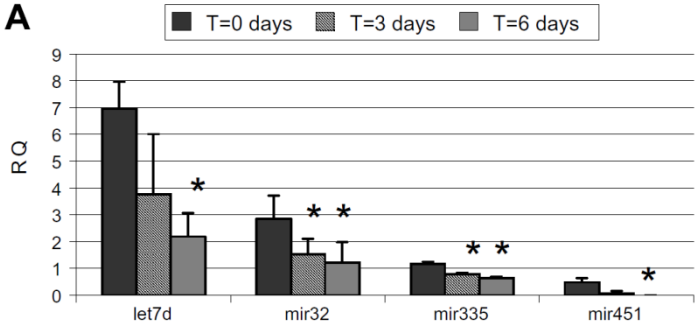
Stabilita v FFPE (formalin-fixed parafin-embedded tissue)



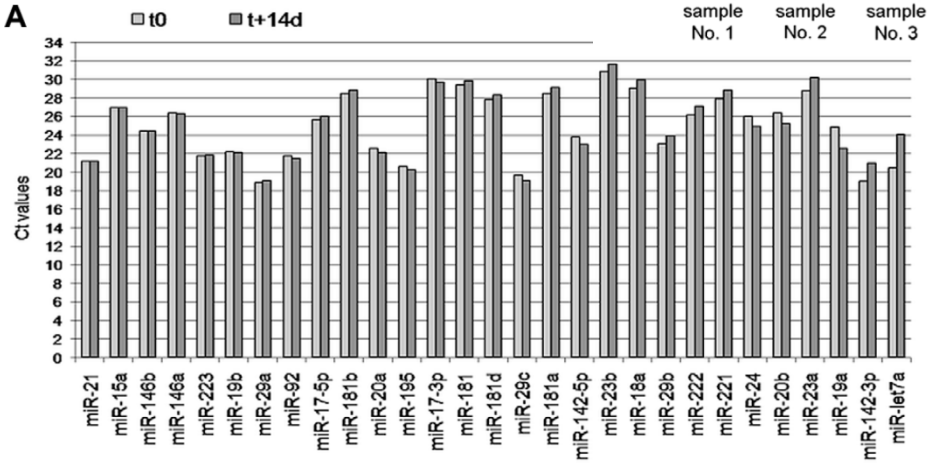
RNA

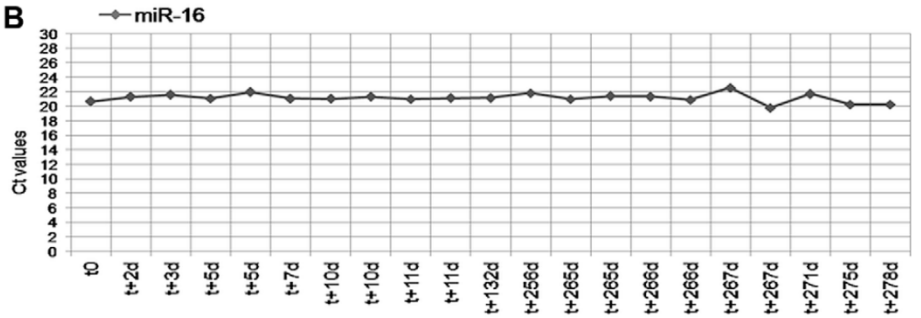


cDNA



Bravo et al., 2007

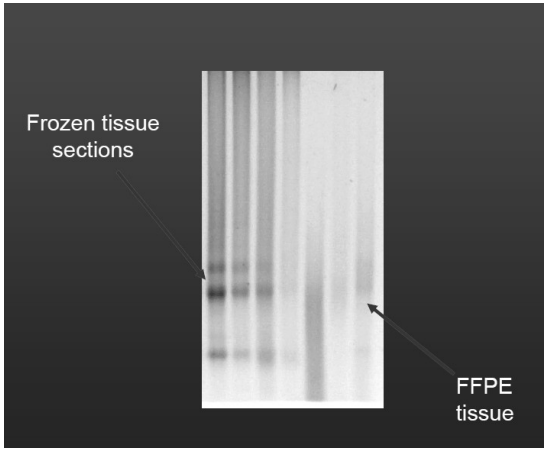


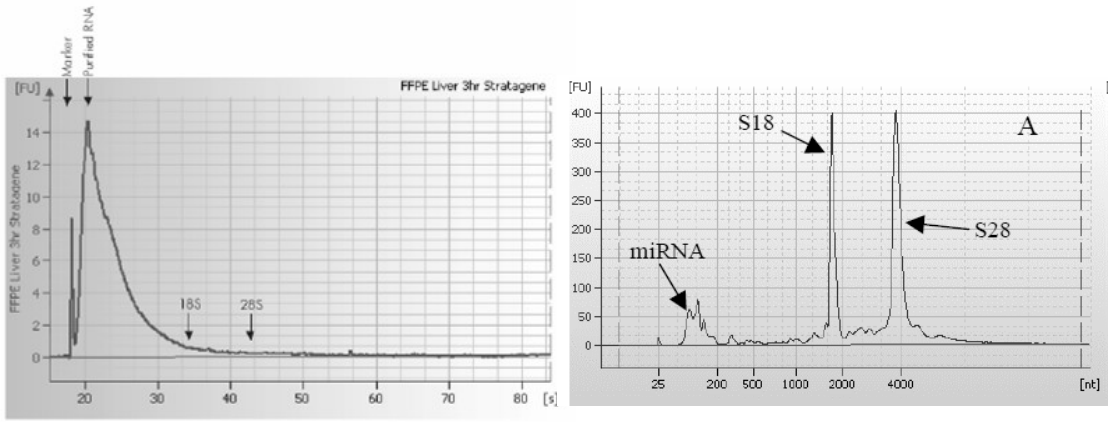


Mraz et al., 2009

Stabilita microRNA :

Stabilita v FFPE (formalin-fixed parafin-embedded tissue)





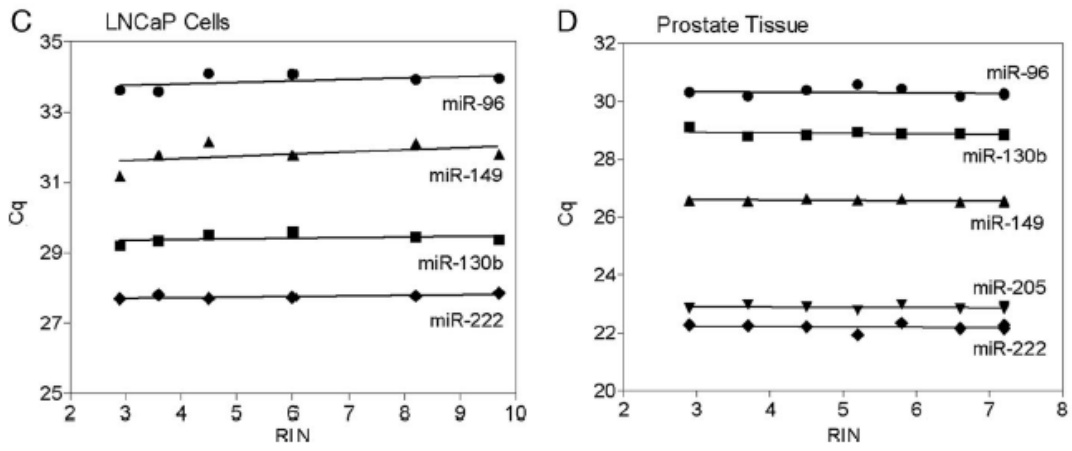


Fig. 2. Influence of RNA integrity on miRNA gene expression.
(A), miR-141, miR-155, miR-200c, and miR-210 in RNA samples from ccRCC cell line Caki-2. (B), miR-141, miR-155, miR-200c, and miR-210 in RNA samples from the renal tissue pool. (C), miR-96, miR-130b, miR-149, and miR-222 in RNA samples from PCa cell line LNCaP. (D), miR-96, miR-130b, miR-149, miR-205, and miR-222 in RNA samples from the prostate tissue pool. For further details, including regression line characteristics, 95% CIs of the slopes, and *P* values indicating significant deviations from 0, see Table 5 in the online Data Supplement.

Jung et al., 2010

Expression microarrays pro microRNAs:

- velmi malé molekuly – 22nt – specifikum izolace, specifické značení i design sond
- malé zastoupení ve vzorku – separace microRNA
- v lidském genovu cca 2000 genů – menší počet sond na čipu
- některé mají velmi podobnou sekvenci – rozdíl 1nt
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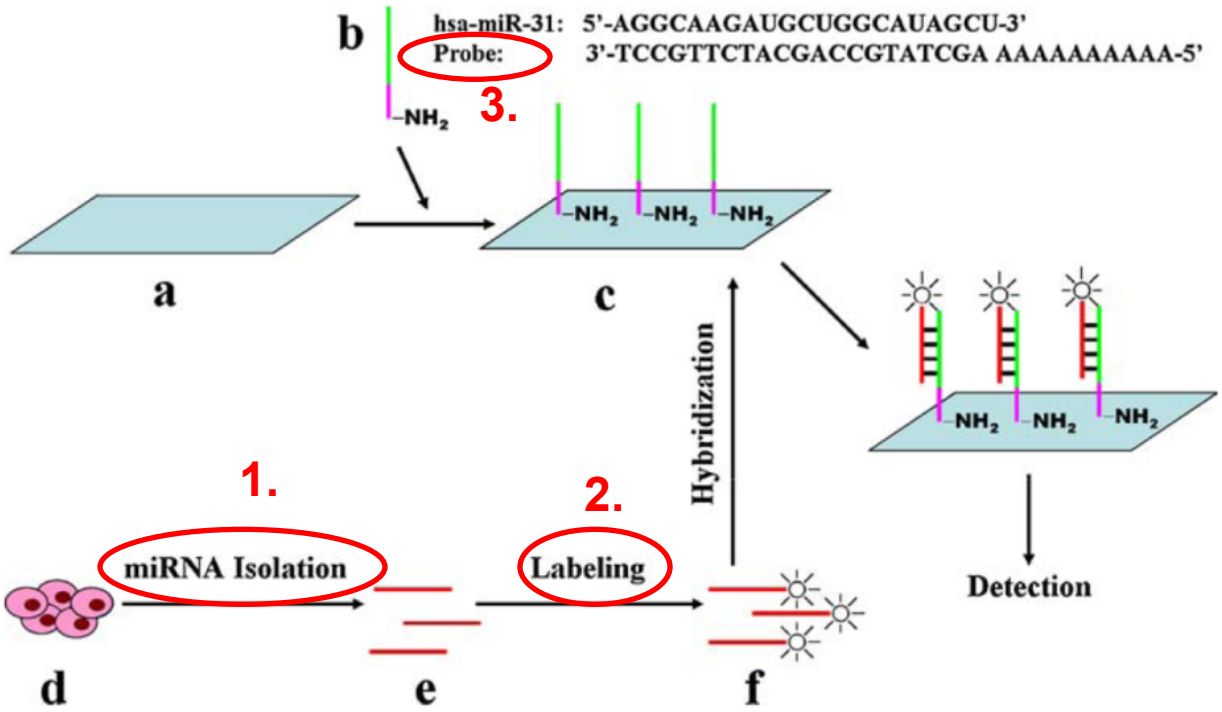


Li and Ruan, 2009

3/ Labeling – značení:

- Není možný labeling pomocí značených polyT při reverzní transkripci
- Přímé značení (direct labeling) – většinou nějaká fluorescenční barva
- Nepřímé značení (indirect labeling) – probíhá nějaká reverzní transkripce/PCR

Přímé značení:

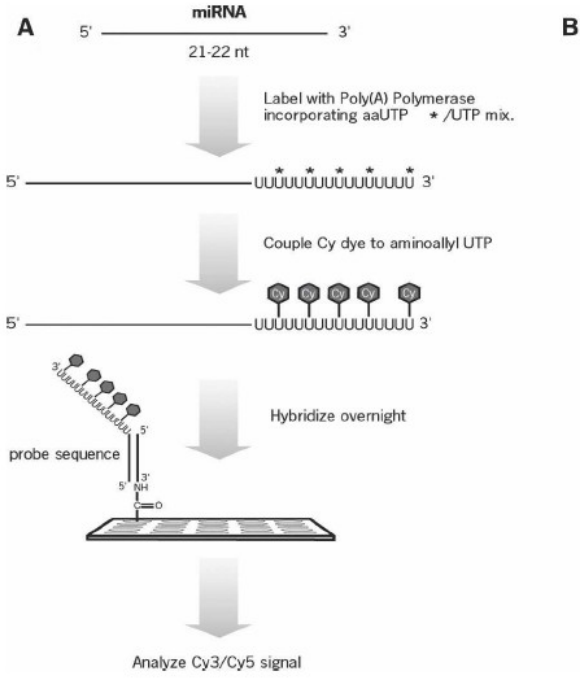
Jednoduché, rychlé a „čím méně kroků tím méně vnesených chyb a variability“

1/ Značení guaninu v microRNA

Flurochromem vážícím se na guanin jsou označeny miRNA (Ulysis Alexa Flour 546/647)
Všechny lidské miRNA obsahují guanin, ale v různém množství
Nemožnost usuzovat na vzájemnou expresi různých miRNA (různý obsah guaninu)
(Babak et al., 2004)

2/ Značení pomocí Poly (A) polymerázy

Můžu se rozhodnout jak dlouhý bude poly(A)
a tím ovlivnit sílu signálu
(Shingara et al., 2005)



3/ značení chemickou metodou

3‘OH skupina je oxidována na dialdehyd
Následuje reakce s Biotin-X-hydraxidem –> Biotinilovaná miRNA
Vazba fluorescenční molekuly-quantum dot
(Liang et al., 2005)

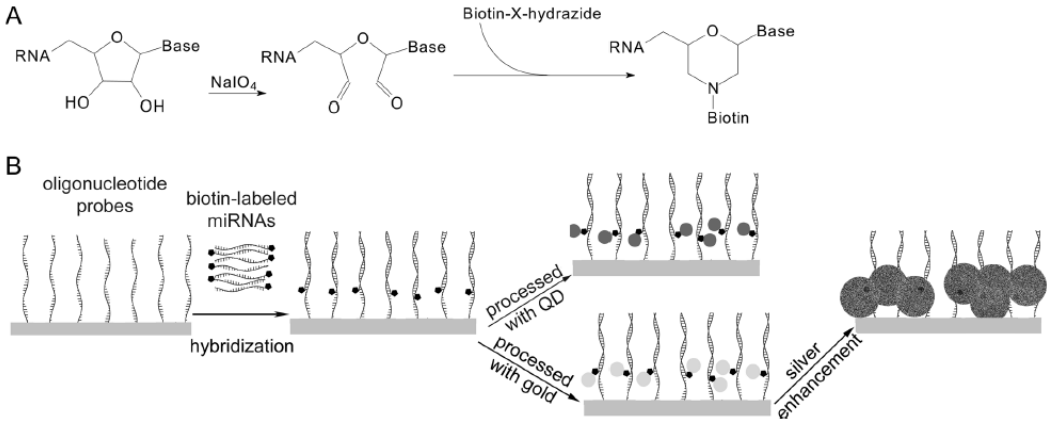


Figure 1. Schematic principles of the miRNA profiling microarray. (A) Principle of labeling miRNA at the 3′ terminus with biotin. (B) Principle of the miRNA profiling microarray detected with QD or colorimetric method.

4/ značení pomocí T4 ligasy

Krátký značený oligonukleotid
je připojen T4 ligásou k 3‘konci
Výhodou je přednostní vazba
na RNA o velikosti 18-30bp ->total RNA
(Thomson et al., 2004; Castoldi et al., 2007)

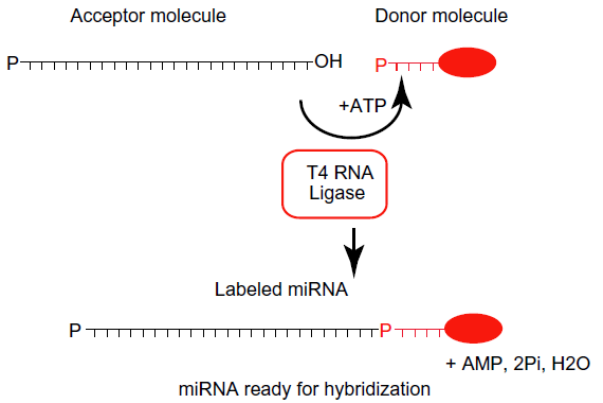


Fig. 2. Schematic representation of the miRNA labeling principle: a short Cy-dye labeled RNA-linker (donor molecule) is ligated to the single-stranded miRNA (acceptor molecule) by T4 RNA ligase in the presence of ATP.

Nepřímé značení:

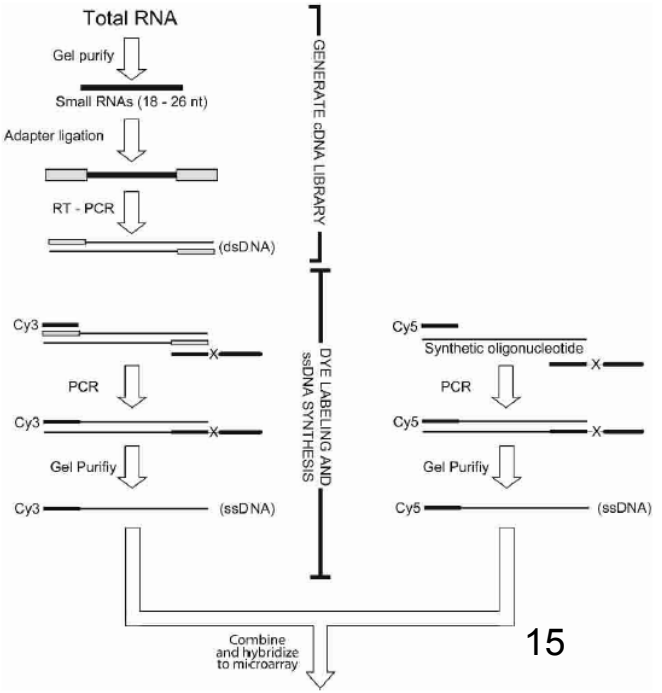
Značen je produkt reverzní transkripce či PCR
Výhody: cDNA je pak stabilní a lze uchovat, Pre-amplifikace a tím snadnější detekce méně exprimovaných miRNA

1/ značení revezního transkriptu miRNA

Reverzní transkripce pomocí náhodných 8-merů značených 2 biotiny (3‘-(N)8 – (A)12-biotin-(A)12-biotin-5‘ (Liu et al., 2004)
Reverzní transkripce pomocí náhodných neznačených 7-merů, následně označeny s pomocí terminální transferázy a biotin-dideoxy-UTP (Sun et al., 2004)
Nebezpečí chyb z nespecifické vazby primeru

2/ značení produktu RT-PCR

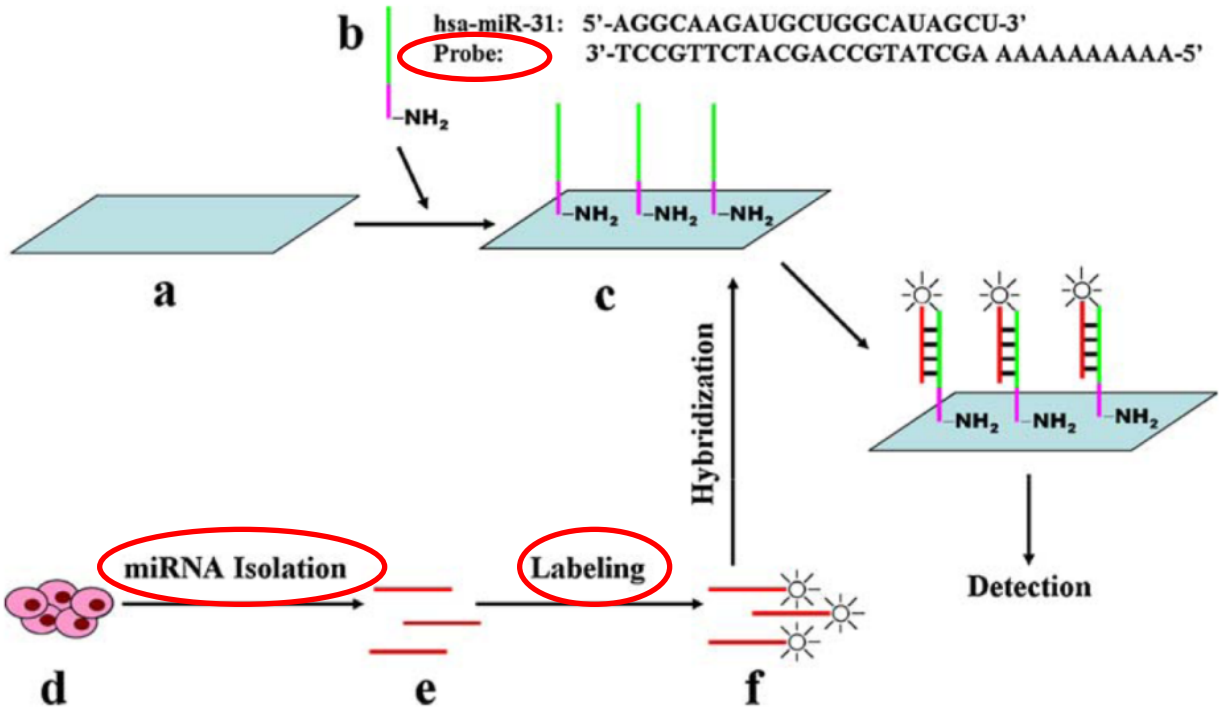
Výhoda: snadná pre-amplifikace
Dva adaptory
fluorescenčně-značený primer (k adaptoru)
(Miska et al., 2004)
Nevýhoda: antisense strand přiromen při hybridizaci
Rešením je různá délka sense a antisense ->PAGE
(Baskerville, 2005)



3/ značení in vitro transkriptu

Jeden z adaptorů je promotor T7 RNA polymerázy
(Barad et al. 2004)

3/ Microarrays/ Próby:
Problémy: krátké RNA, malé rozdíly v sekvenci, Tm



Tm – melting temperature určité próby
T – hybridizační teplota

Tm<T .....nižší efektivita vazby miRNA
Tm>T .....vyšší efektivita vazby miRNA

- Je třeba navrhnout próby tak,aby měly všechny podobnou Tm
- To se u „dlouhých“ mRNA řeší vhodným výběrem oblasti genu k němuž bude sonda komplementární nebo délkou sondy
- navíc některé miRNA jsou téměr sekvenčně totožné

ÚPRAVA DÉLKY

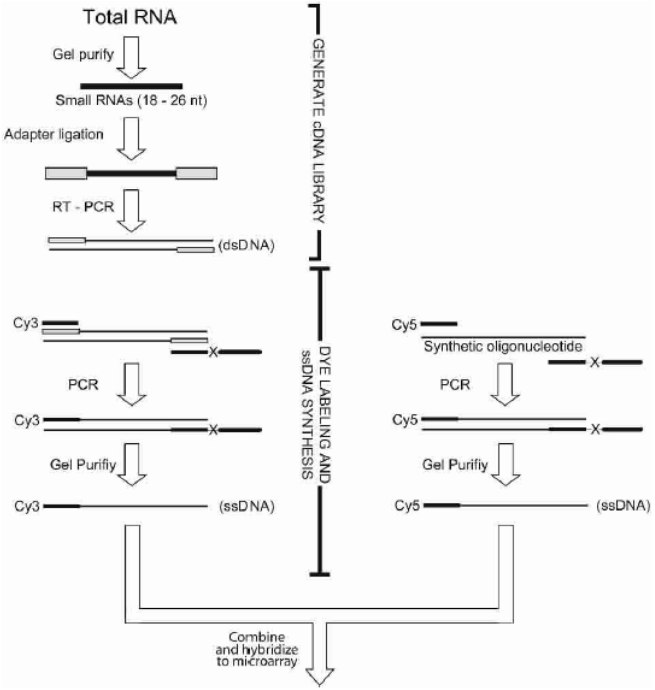
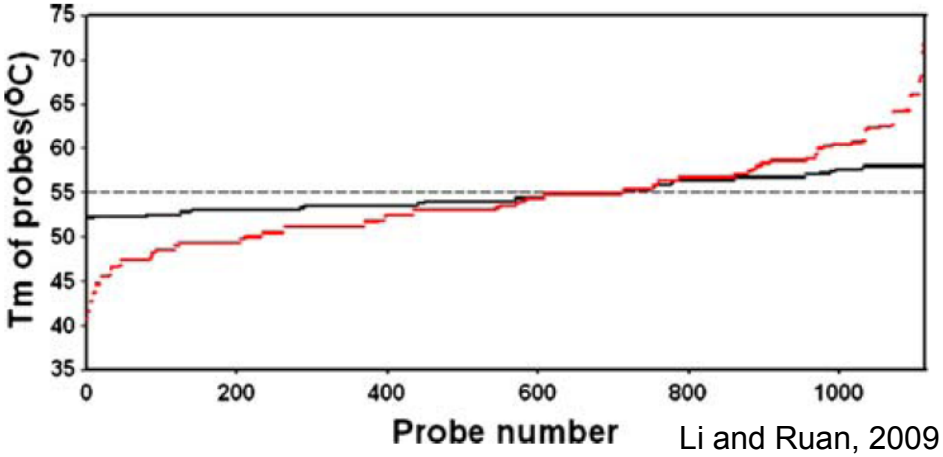


FIGURE 1. Microarray sample preparation and reference oligonucleotide synthesis. Small RNAs were fractionated on a polyacrylamide gel, and oligonucleotide primers were then ligated to the 5′ and 3′ ends of the small RNA library (Lau et al. 2001). A cDNA library was generated through reverse transcription, and the product was amplified using PCR. Using a pair of modified oligonucleotide primers in a second PCR, the sense strand of the library was fluorescently labeled and the antisense strand was selectively lengthened (Williams and Bartel 1995). The sense strand of the asymmetric duplex was purified away from the antisense strand in a denaturing gel, and this purified dye-labeled ssDNA sample was used for hybridization and detection on the array. At each feature, the signal from the miRNA sample was compared to that from a reference sample, which had been generated by amplifying and labeling synthetic oligonucleotides using the same strategy as for the miRNA sample.

Baskerville and Bartel, 2005

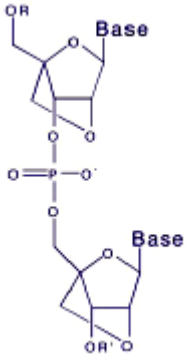


Li and Ruan, 2009

Fig. 2 Tm (melting temperature) distribution for microRNA probes for human, rat and mouse. *Red and black curves* represent the Tm distributions of the raw and normalized probes, respectively

ÚPRAVA SÍLY VAZBY NUKLEOTIDŮ

LNA próby (Locked Nucleic Acid)
ribózový kruh je „uzamčen“ methylenovým můstkem mezi atomy 2´-O a 4´-C



Použití LNA pro některé báze v próbě

SÍLA VAZBY:
LNA vs DNA próba
Tm až 72^{0}C
(Castoldi et al., 2006)

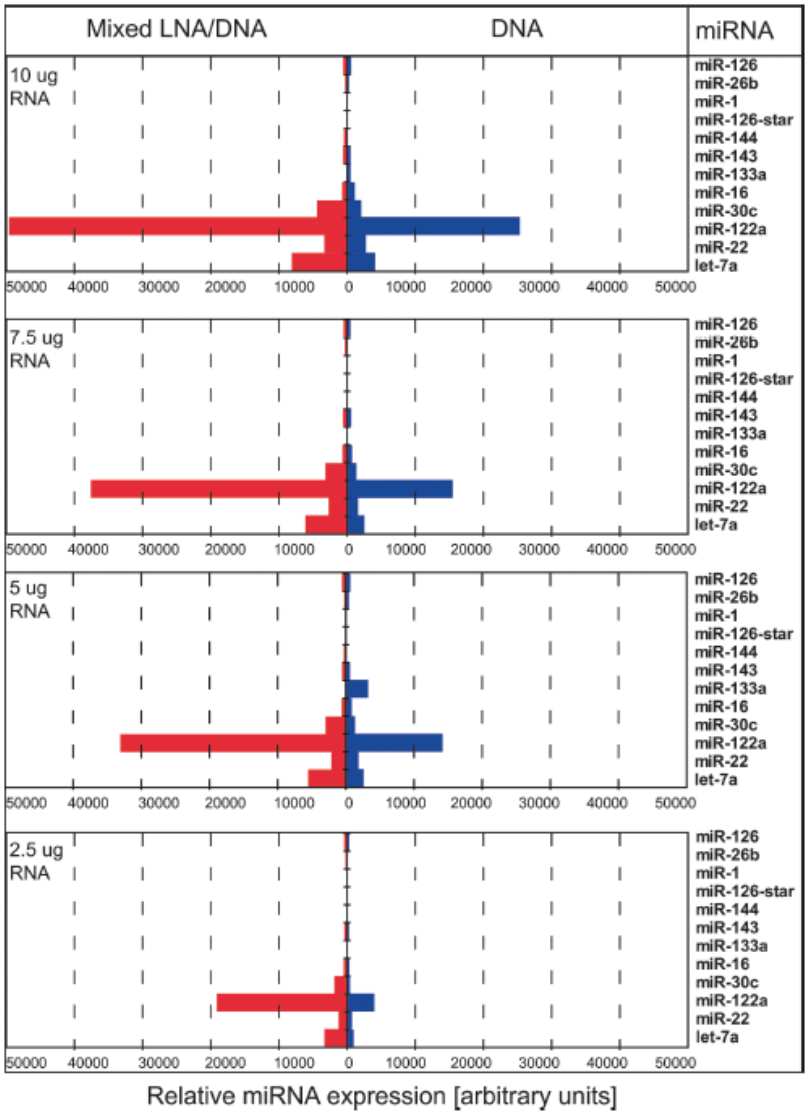
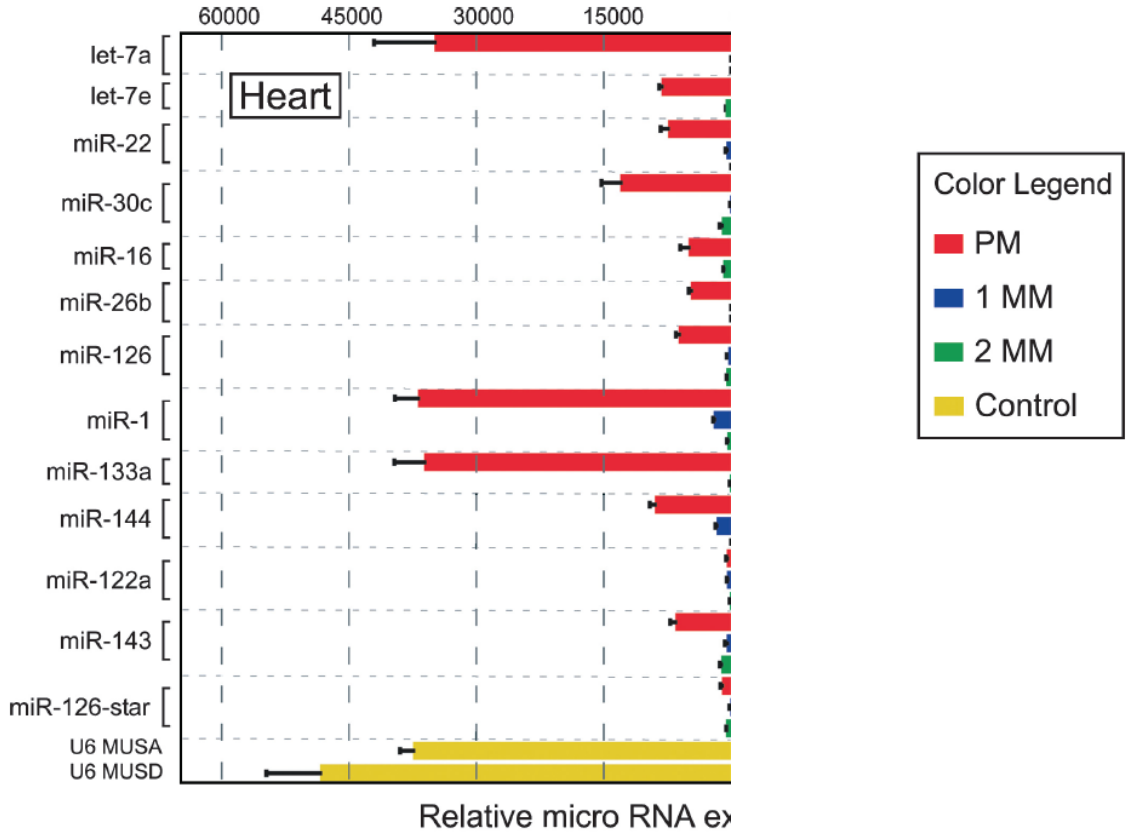


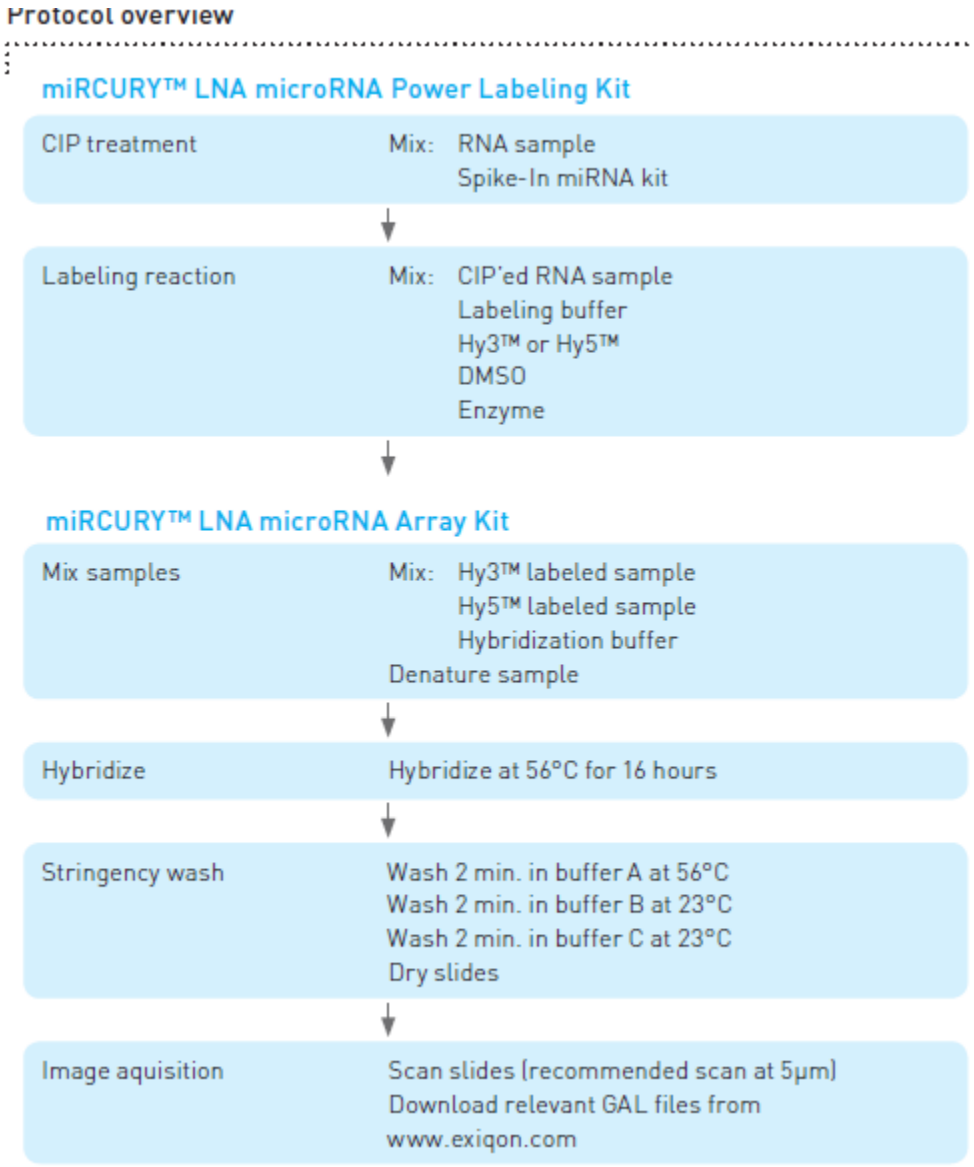
FIGURE 1. Mixed DNA/LNA capture probes display increased sensitivity for miRNA detection. miRNA expression was assessed in murine liver using a test set of LNA-modified (*left*) or unmodified DNA oligonucleotide capture probes (*right*). Decreasing amounts of total RNA were used as input material for miRNA analysis. Data are presented as median intensity (four replicas per miRNA capture probe; a representative experiment is shown).

SPECIFITA VAZBY: LNA vs DNA próba

(Castoldi et al., 2006)

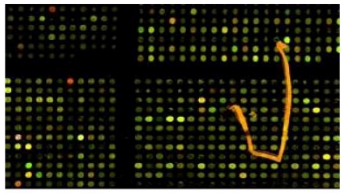


miRCURY LNA Array, Exiqon : 3 dny

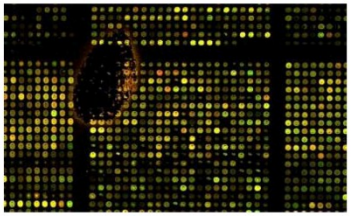


Co se nemusí podařit:

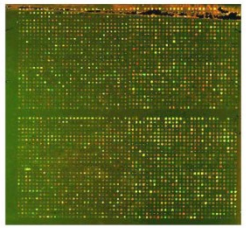
Nekvalitní RNA
Nepodaří se značení
Nepodaří se hybridizace
Nepodaří se promývání
Technická variabilita čipů je větší než ta biologická
Nepodaří se validace dat pomocí RT-PCR, atd



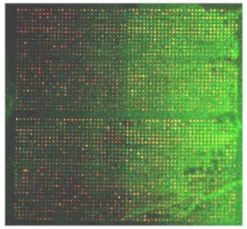
Fiber or scratch?



Bubble



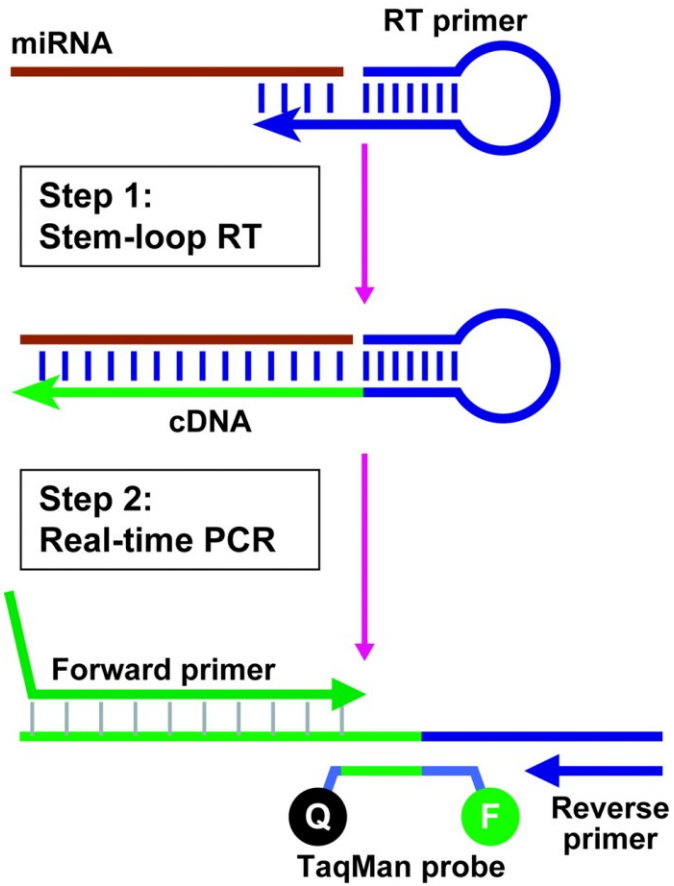
Edge effect

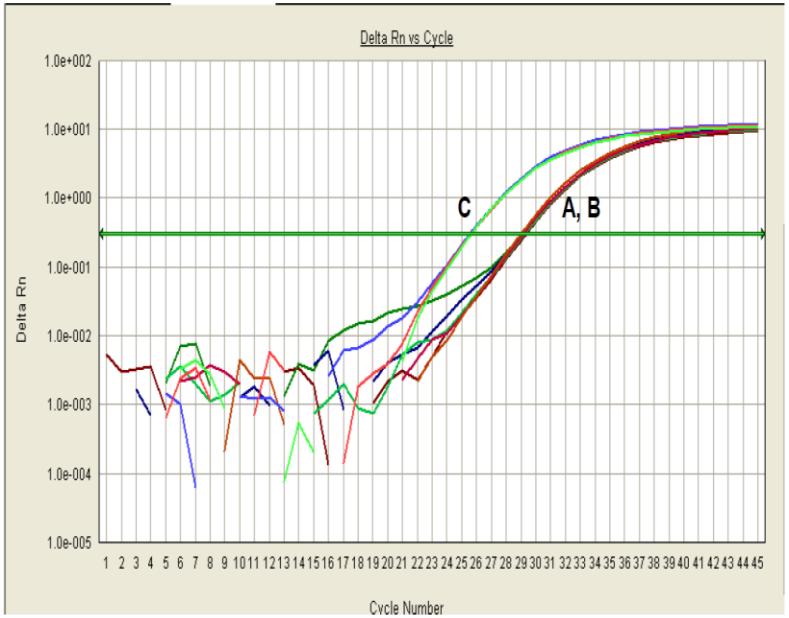


Background haze

Práce s miRNA čipy je velmi obtížná. Všeobecně nižší míra standardizace. Obtížná interpretace získaných dat z pohledu biologického smyslu např. deregulace několika miRNA (nádor vs. zdravá tkáň apod.)

TaqMan-based real-time PCR quantification of mature miRNAs





Chen, C. et al. Nucl. Acids Res. 2005 33:e179; doi:10.1093/nar/gni178

Nucleic Acids Research

Konstrukce cDNA knihovny malých RNA

Cloning nebo deep sequencing
Validace pomocí Northern blot

3’ RNA adapter ligation

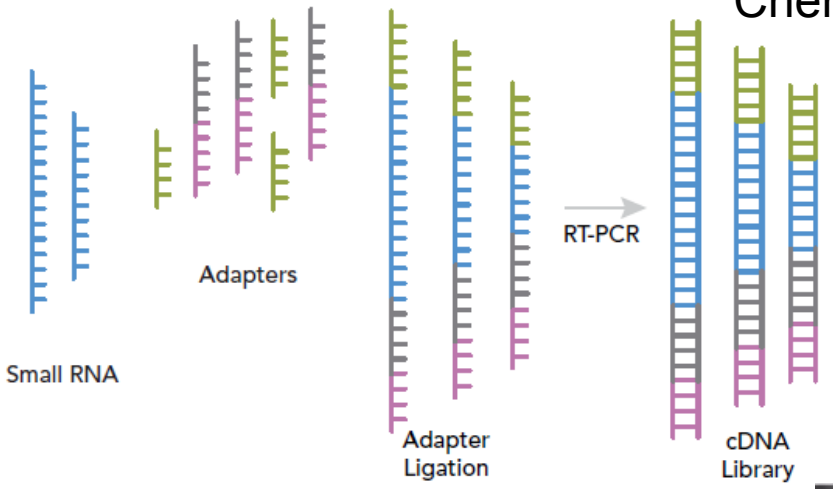
5’ RNA adapter ligation

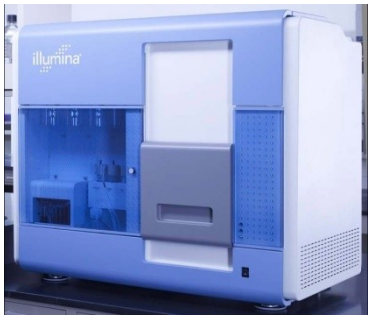
RT-PCR amplification

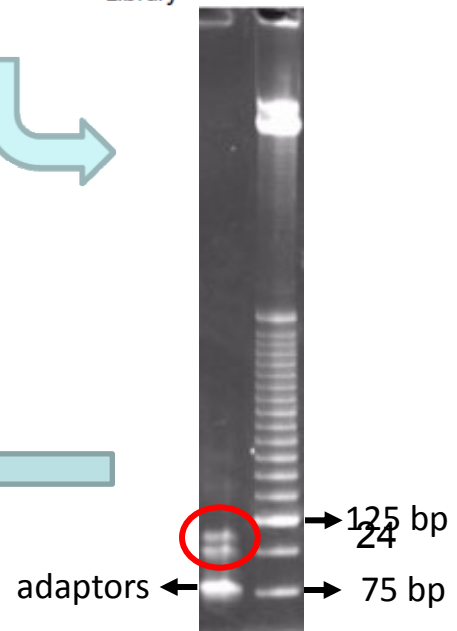
Purify small RNA library

Cluster generation and sequencing (Cluster Station and Genome Analyzer)

Cheng et al., 2007



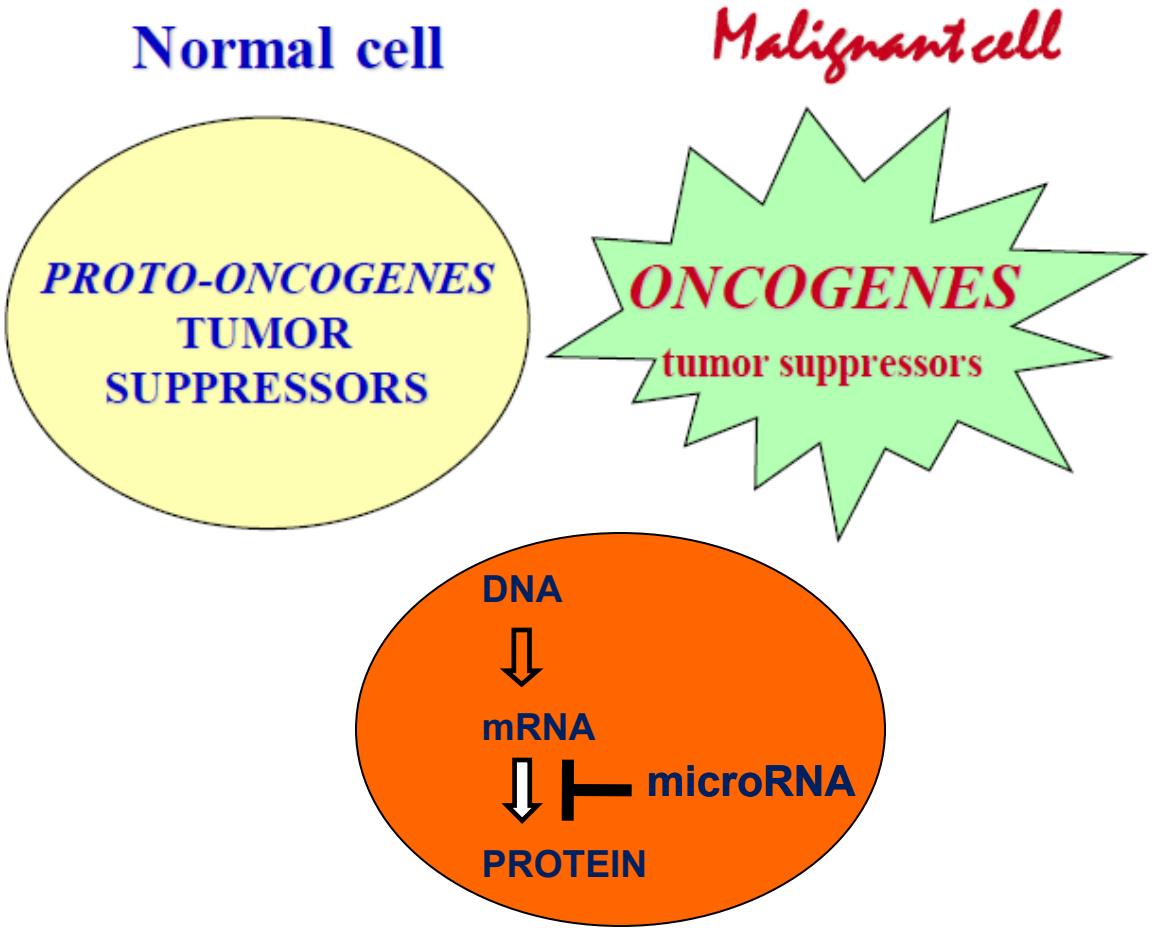




Year 2K "Central dogma" of molecular oncology

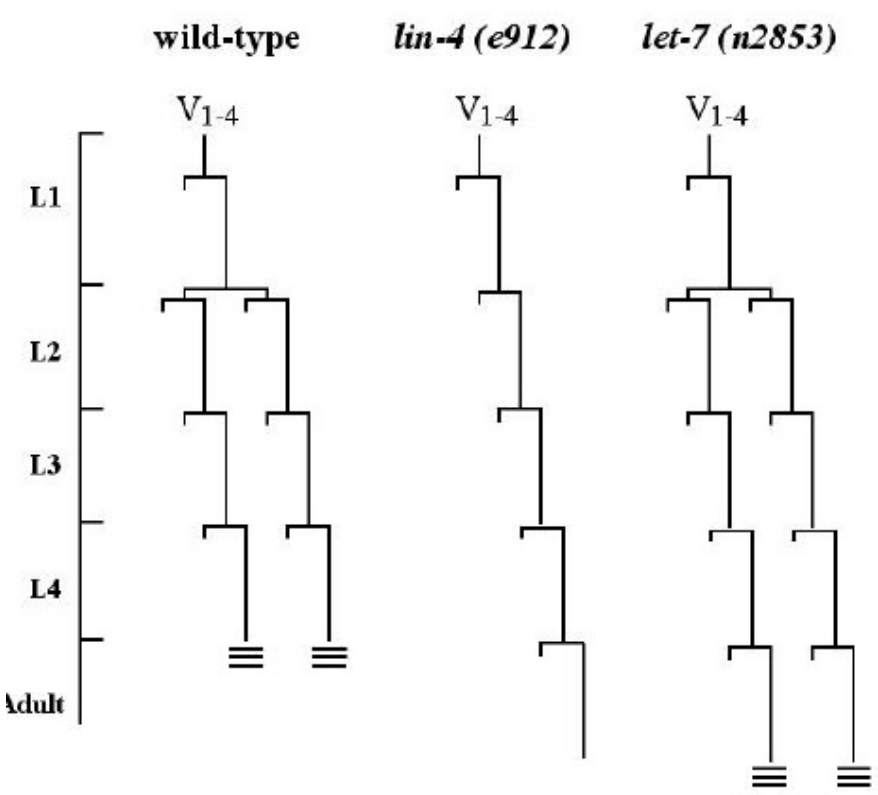
Cancer is the GENETIC DISEASE with the most complex mechanism.

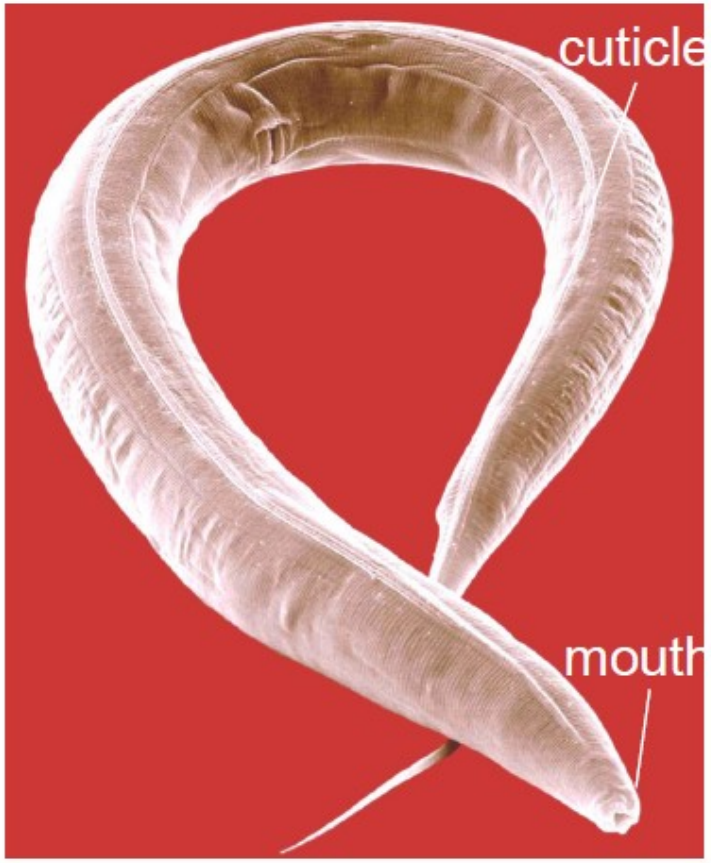
Oncogenes and Tumor-suppressors are the two types of PROTEINS deregulated in cancer cells.



courtesy of G. Calin

MicroRNAs were discovered by V. Ambros and G. Ruvkun in *C. elegans*





Lee et al. 1993 *Cell*

Reinhart et al. 2000 *Nature*

courtesy of F. Slack

microRNA genes map to cancer loci

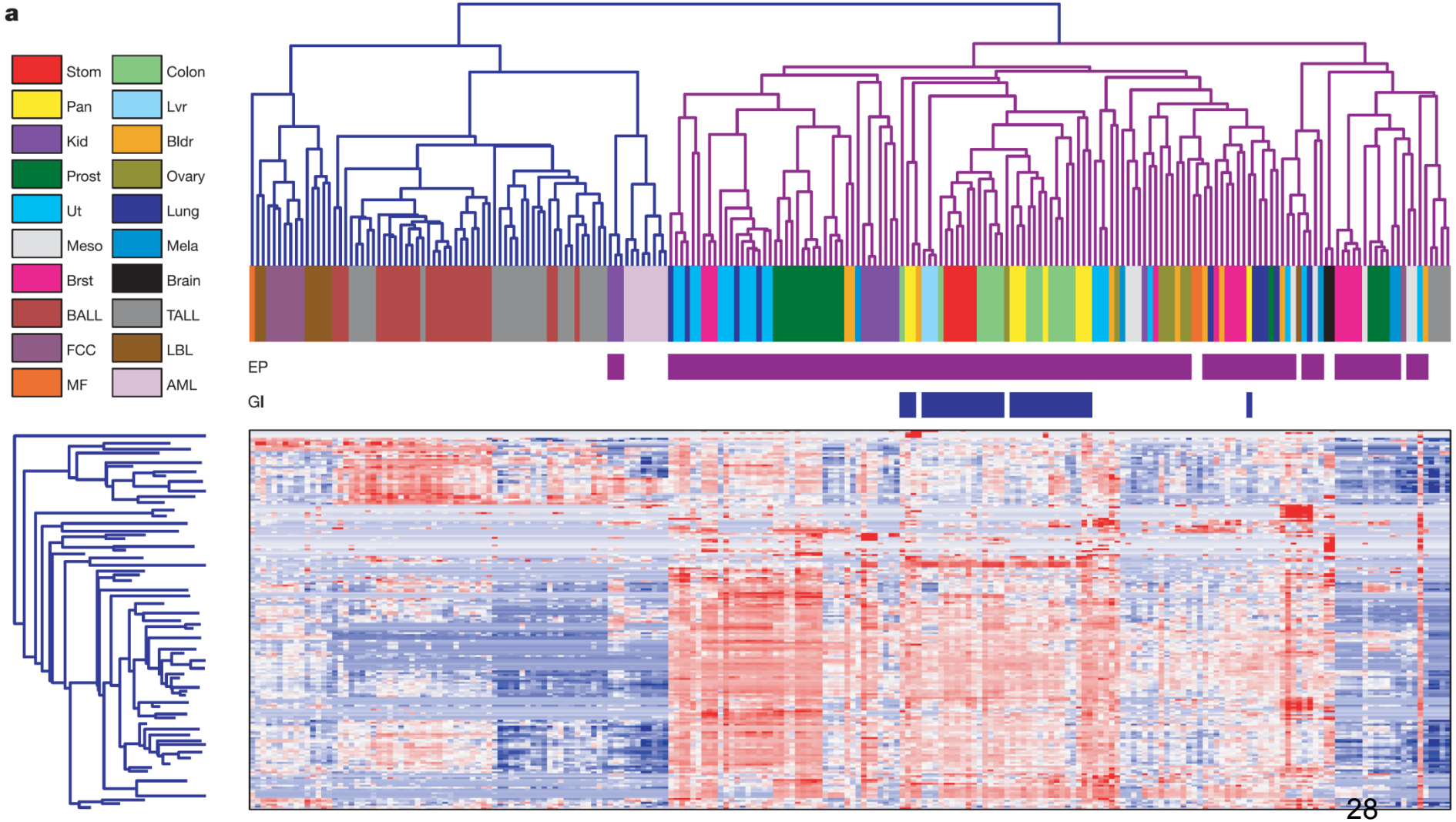
Table 2. Examples of miRs located in minimal deleted regions, minimal amplified regions, and breakpoint regions involved in human cancers

Chromosome	Location (defining markers)	Size, Mb	miR	Hystotype	Known OG/TS
3p21.1–21.2-D	ARP-DRR1	7	*let-7g/miR-135–1*	Lung, breast cancer	—
3p21.3(AP20)-D	GOLGA4-VILL	0.75	*miR-26a*	Epithelial cancer	—
3p23–21.31(MDR2)-D	D3S1768-D3S1767	12.32	*miR-26a; miR-138–1*	Nasopharyngeal cancer	—
5q32-D	ADRB2-ATX1	2.92	*miR-145/miR-143*	Myelodysplastic syndrome	—
9q22.3-D	D9S280-D9S1809	1.46	*miR-24–1/mir-27b/miR-23b; let-7a-1/let-7f-1/let-7d*	Urothelial cancer	PTC, FANCC
9q33-D	D9S1826-D9S158	0.4	*miR-123*	NSCLC	—
11q23-q24-D	D11S927-D11S1347	1.994	*miR-34a-1/miR-34a-2*	Breast, lung cancer	PPP2R1B
11q23-q24-D	D11S1345-D11S1328	1.725	*miR-125b-1/let-7a-2/miR-100*	Breast, lung, ovary, cervix cancer	—
13q14.3-D	D13S272-D13S25	0.54	*miR-15a/miR-16a*	B-CLL	—
13q32–33-A	stSG15303-stSG31624	7.15	*miR-17/miR-18/miR-19a/miR-20/ miR-19b-1/miR-92–1*	Follicular lymphoma	—
17p13.3-D	D17S1866-D17S1574	1.899	*miR-22; miR-132; miR-212*	HCC	—
17p13.3-D	ENO3-TP53	2.275	*miR-195*	Lung cancer	TP53
17q22-t(8;17)	miR-142s/c-MYC		*miR-142s; miR-142as*	Prolymphocytic leukemia	c-MYC
17q23-A	CLTC-PPM1D	0.97	*miR-21*	Neuroblastoma	—
20q13-A	FLJ33887-ZNF217	0.55	*miR-297–3*	Colon cancer	—
21q11.1-D	D21S1911-ANA	2.84	*miR-99a/let-7c/miR-125b*	Lung cancer	—

D, deleted region; A, amplified region; NSCLC, non-small-cell lung cancer; HCC, hepatocellular carcinoma; PTC, patched homolog (*Drosophila*); FANCC, Fanconi anemia, complementation group C; PPP2R1B, protein phosphatase 2, regulatory subunit A (PR 65), β isoform. miRs in a cluster are separated by a slash. For references, see Table 6.

Calin, G.A., *et al.*, 2004. *PNAS* 101:2999-3004

microRNA exprese je schopná rozlišit původ nádoru



Lu et al. Nature 435: 834, 2005

A microRNA expression signature of human solid tumors defines cancer gene targets

Stefano Volinia*†‡, George A. Calin*‡, Chang-Gong Liu*, Stefan Ambs§, Amelia Cimmino*, Fabio Petrocca*, Rosa Visone*, Marilena Iorio*, Claudia Roldo*, Manuela Ferracin¶, Robyn L. Prueitt§, Nozumu Yanaihara§, Giovanni Lanza¶, Aldo Scarpa‖, Andrea Vecchione**, Massimo Negrini¶, Curtis C. Harris§, and Carlo M. Croce*††

*Department of Molecular Virology, Immunology, and Medical Genetics and Cancer Comprehensive Center, Ohio State University, Columbus, OH 43210; §Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; †Telethon Facility–Data Mining for Analysis of DNA Microarrays, Department of Morphology and Embryology, and ¶Department of Experimental and Diagnostic Medicine and Interdepartmental Center for Cancer Research, University of Ferrara, 44100 Ferrara, Italy; ‖Department of Pathology, University of Verona, 37100 Verona, Italy; and **Department of Histopathology, Sant'Andrea Hospital, and University of Rome ''La Sapienza,'' 00185 Rome, Italy

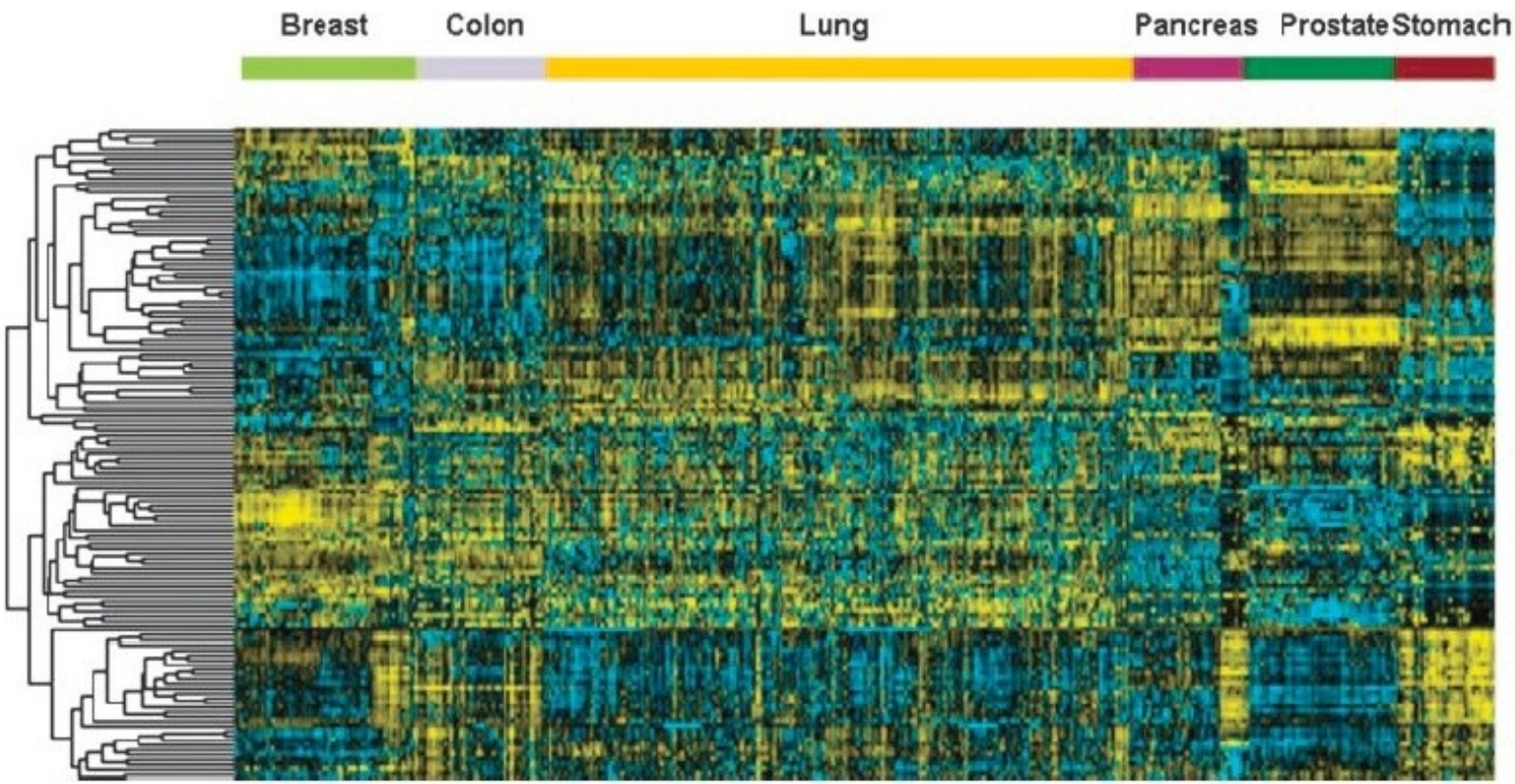
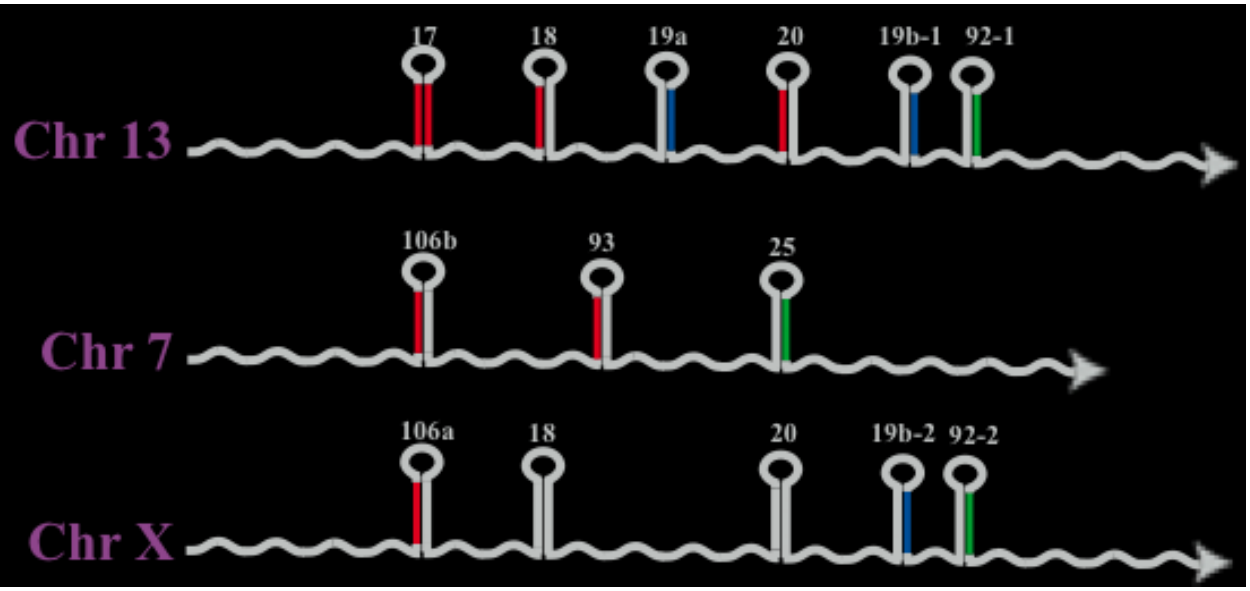
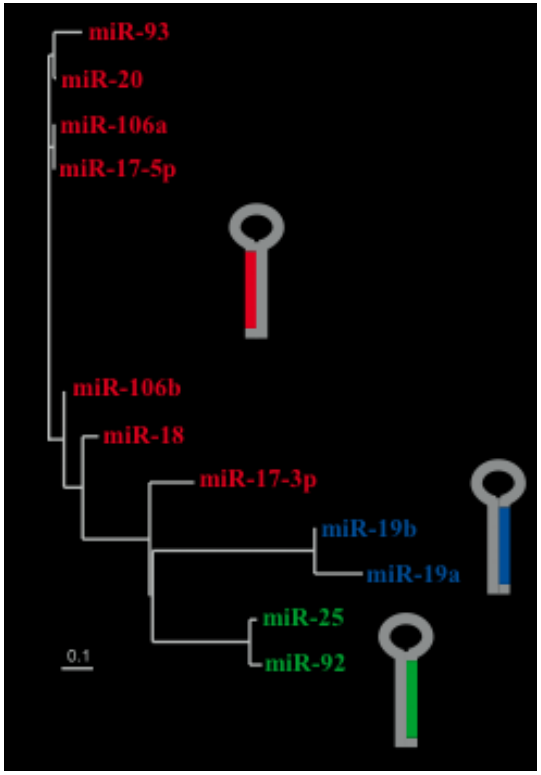


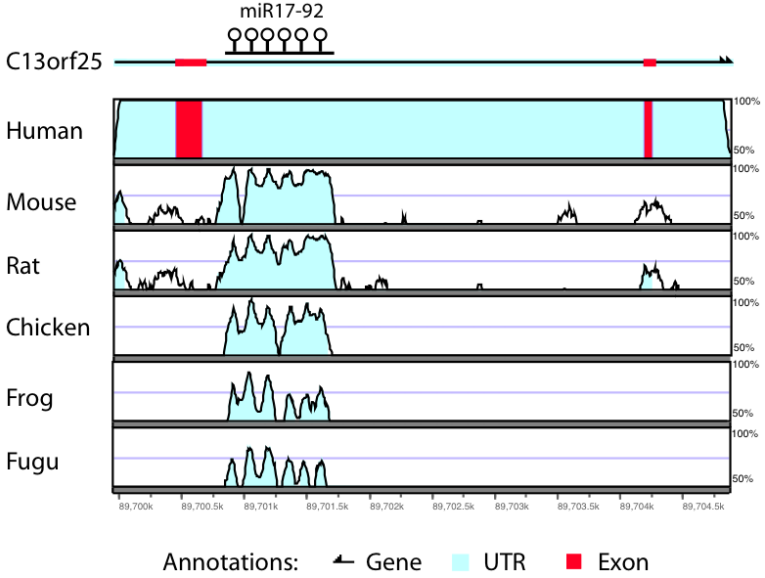
Table 2. The miRNAs shared by the signatures of the six solid cancers

miR	*N*	Tumor type
miR-21	6	Breast, colon, lung, pancreas, prostate, stomach
miR-17-5p	5	Breast, colon, lung, pancreas, prostate
miR-191	5	Colon, lung, pancreas, prostate, stomach
miR-29b-2	4	Breast, colon, pancreas, prostate
miR-223	4	Colon, pancreas, prostate, stomach
miR-128b	3	Colon, lung, pancreas
miR-199a-1	3	Lung, pancreas, prostate
miR-24-1	3	Colon, pancreas, stomach
miR-24-2	3	Colon, pancreas, stomach
miR-146	3	Breast, pancreas, prostate
miR-155	3	Breast, colon, lung
miR-181b-1	3	Breast, pancreas, prostate
miR-20a	3	Colon, pancreas, prostate
miR-107	3	Colon, pancreas, stomach
miR-32	3	Colon, pancreas, prostate
miR-92-2	3	Pancreas, prostate, stomach
miR-214	3	Pancreas, prostate, stomach
miR-30c	3	Colon, pancreas, prostate
miR-25	3	Pancreas, prostate, stomach
miR-221	3	Colon, pancreas, stomach
miR-106a	3	Colon, pancreas, prostate

The list includes 21 commonly up-regulated microRNAs in 3 or more (*N*) types of solid cancers (P value $= 2.5 \times 10^{-3}$).

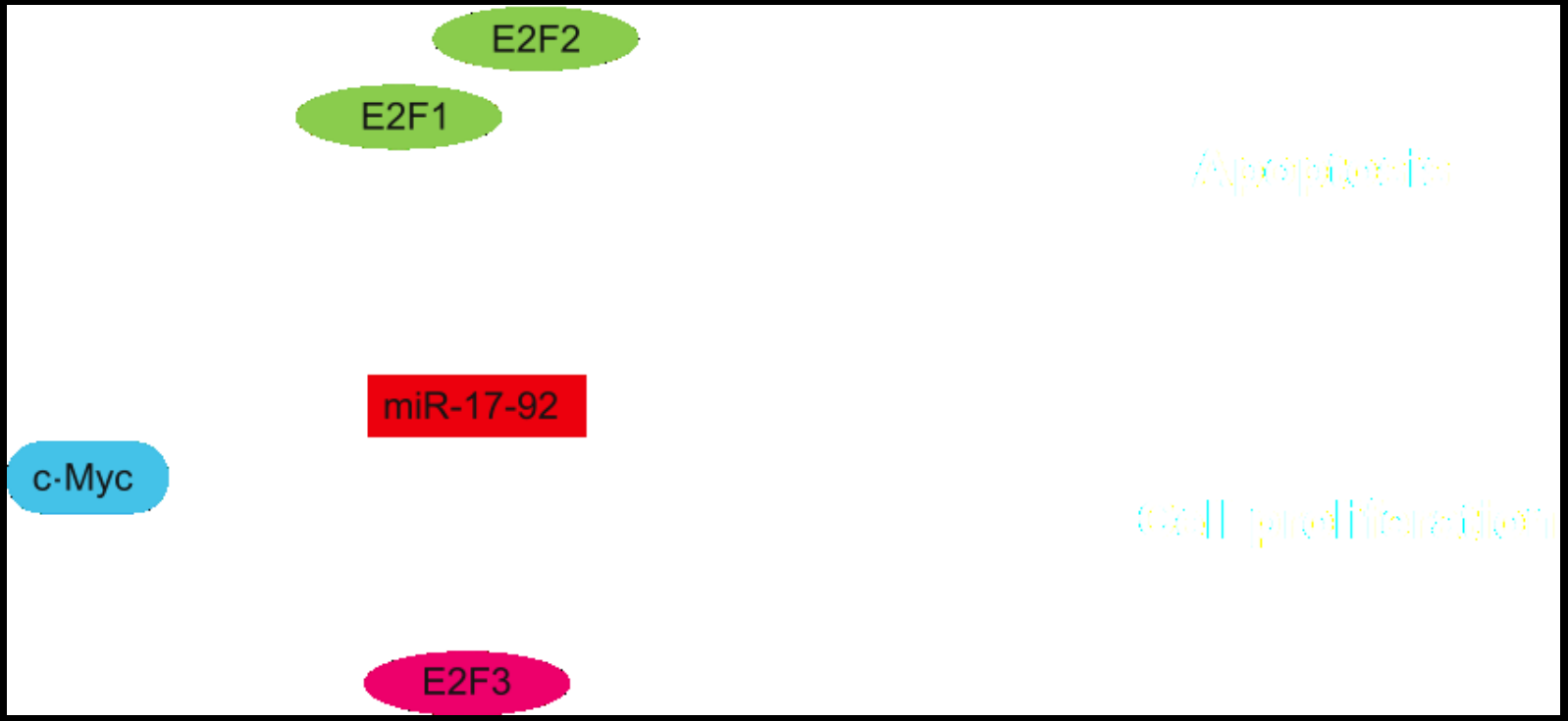




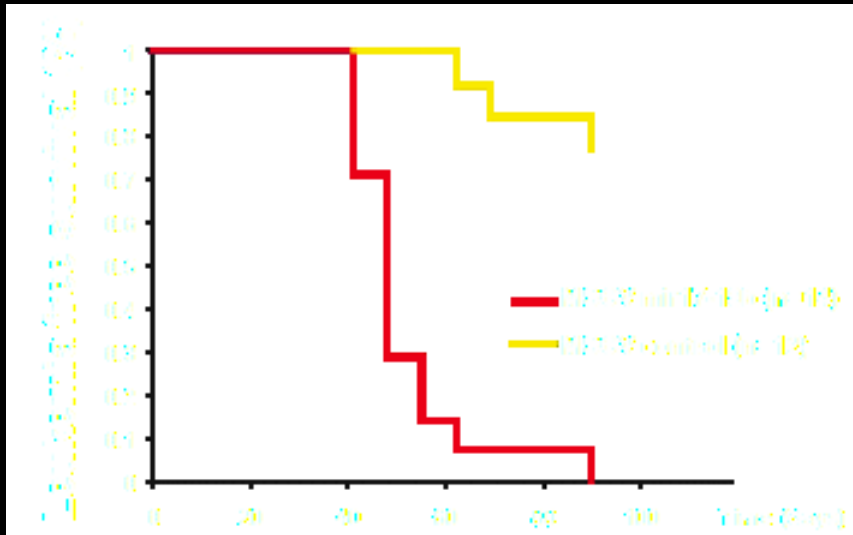


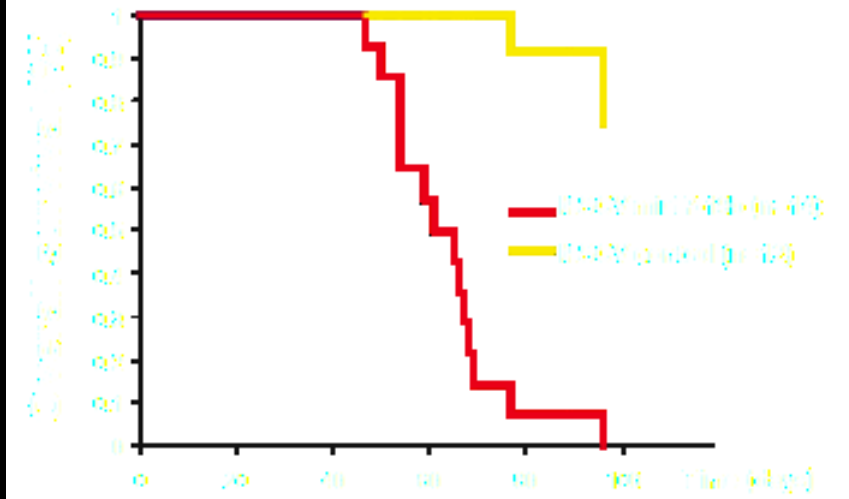
courtesy of S. Hammond

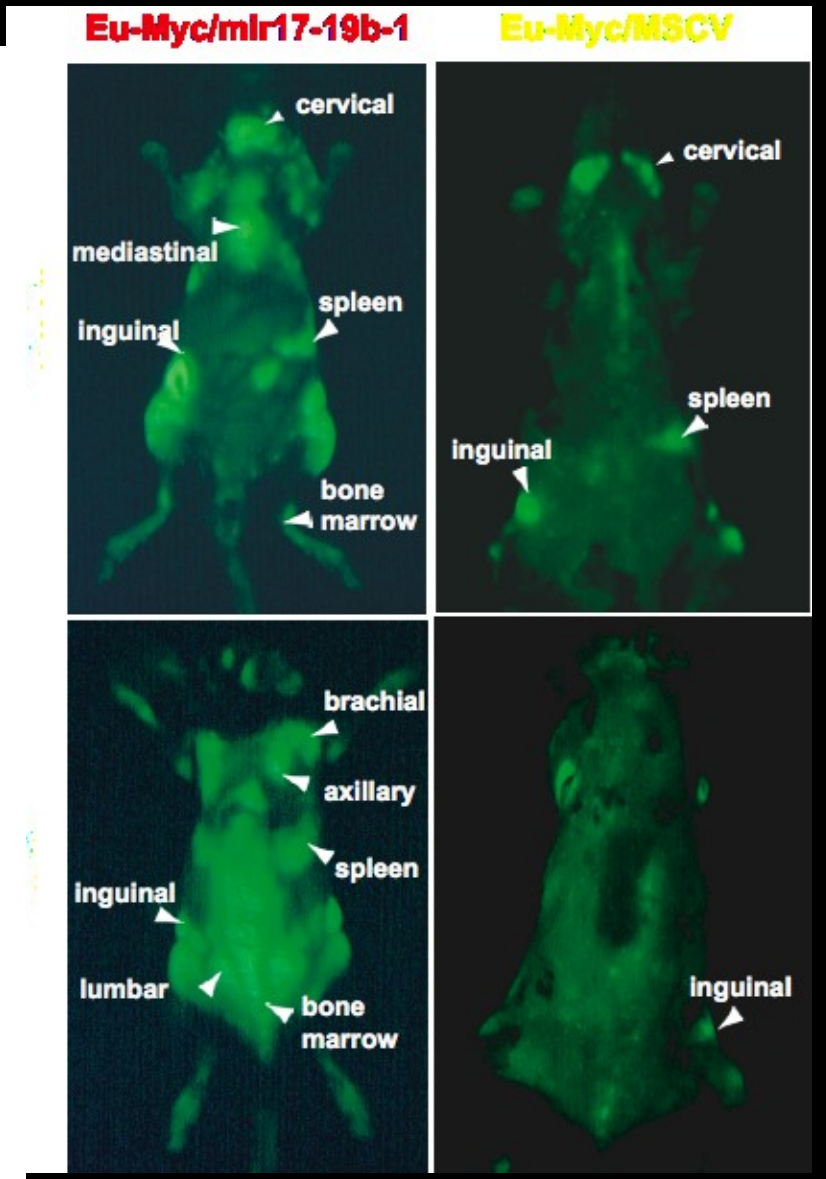
miR-17-92 is regulated by E2F transcription factors



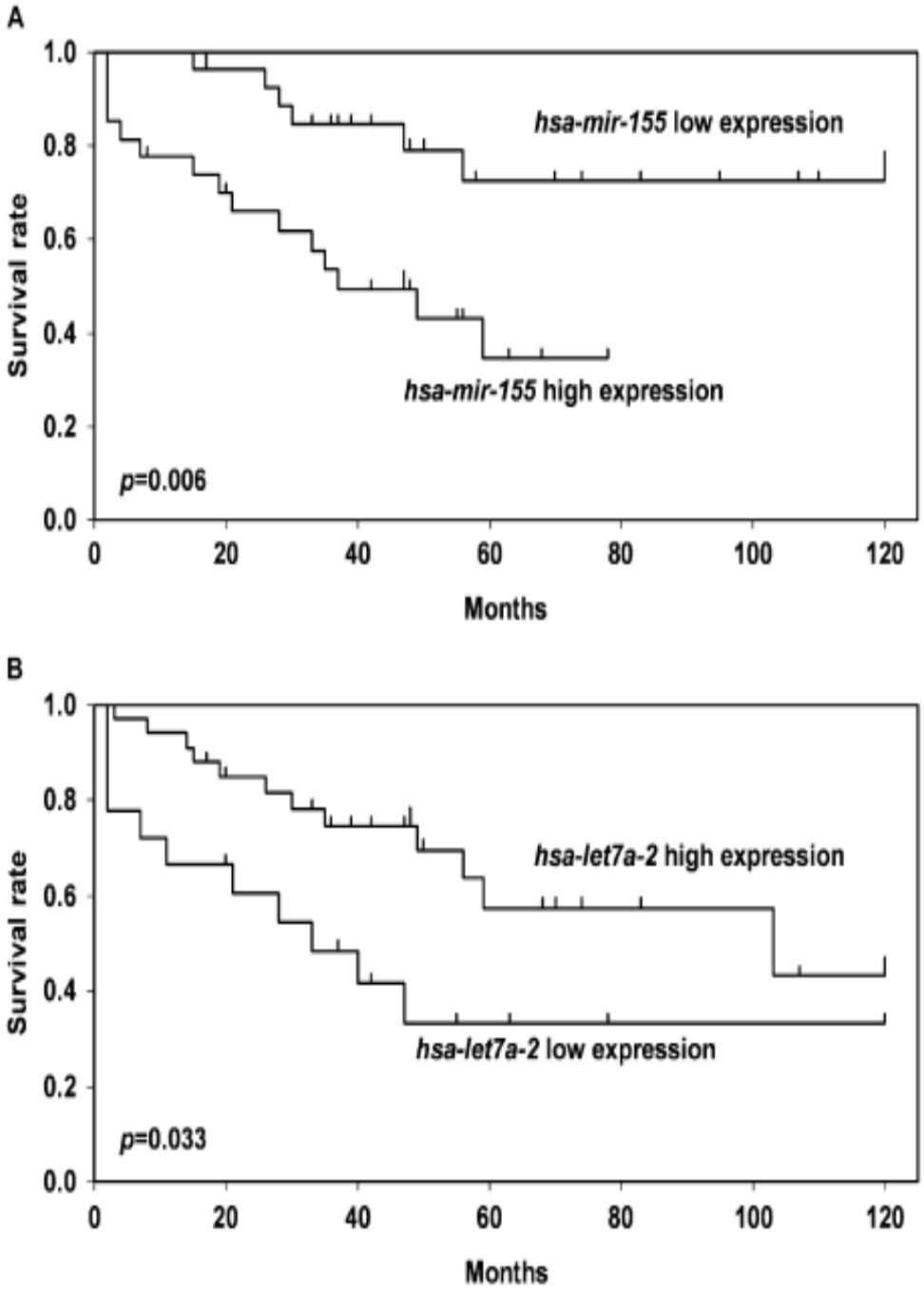
Expression of miR17-19b Results in B-cell Lymphoma







He, et al Nature 435(7043):828-33 2005


A
1.0
0.8
0.6
0.4
0.2
0.0
Survival rate
hsa-mir-155 low expression
hsa-mir-155 high expression
p=0.006
0
20
40
60
80
100
120
Months
B
1.0
0.8
0.6
0.4
0.2
0.0
Survival rate
hsa-let7a-2 high expression
hsa-let7a-2 low expression
p=0.033
0
20
40
60
80
100
120
Months

A unique miRNA signature is associated with lung cancer prognosis

Table 5. Postoperative survival of patients with lung adenocarcinoma in relation to clinicopathological characteristics and miRNA expression analyzed by microarray analysis

Variable	Subset	Hazard ratio (95% confidence interval)	p
Univariate analysis (n = 65)			
Age	age ≥ 67/age < 67	1.41 (0.67–3.06)	0.348
Sex	male/female	1.36 (0.64–2.93)	0.413
Stage	II–IV/I	2.51 (1.29–6.82)	0.010
Smoking history	current/former	1.32 (0.63–2.79)	0.456
hsa-mir-155 (n = 55)	high/low	3.42 (1.42–8.19)	0.006
hsa-let-7a-2 (n = 52)	low/high	2.35 (1.08–6.86)	0.033
Multivariate analysis (n = 55)[a,b]			
Age	age ≥ 67/age < 67	1.92 (0.71–5.17)	0.195
Sex	male/female	1.23 (0.47–3.22)	0.669
Stage	II–IV/I	3.27 (1.31–8.37)	0.013
Smoking history	current/former	1.49 (0.51–4.34)	0.457
hsa-mir-155	high/low	3.03 (1.13–8.14)	0.027

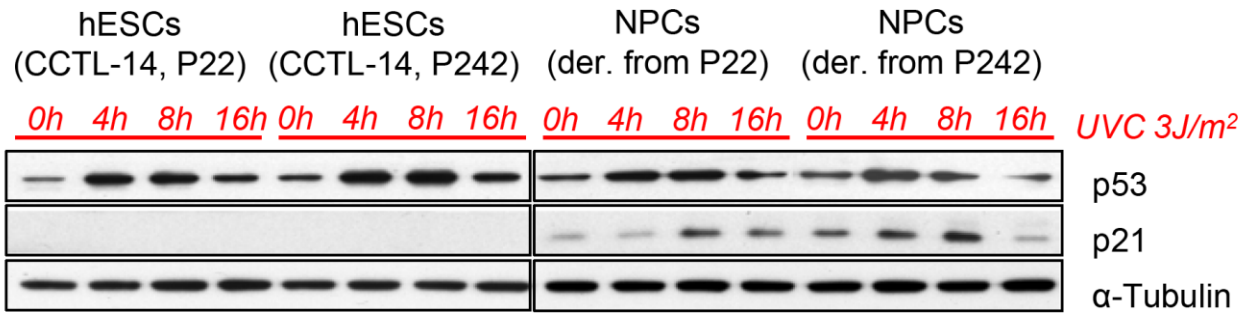
[a]Multivariate analysis, Cox proportional hazard regression model.
[b]*hsa-let-7a-2* low/high was not statistically significant (p = 0.129).

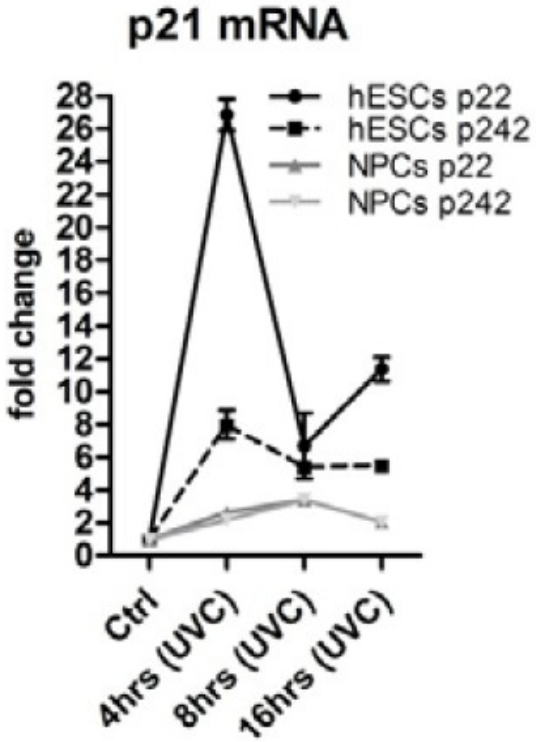
(Yanaihara et al, Cancer Cell, 2006)

MICRORNAS EXPRESSION IN THE REGULATION OF P21 IN HUMAN EMBRYONIC STEM CELLS

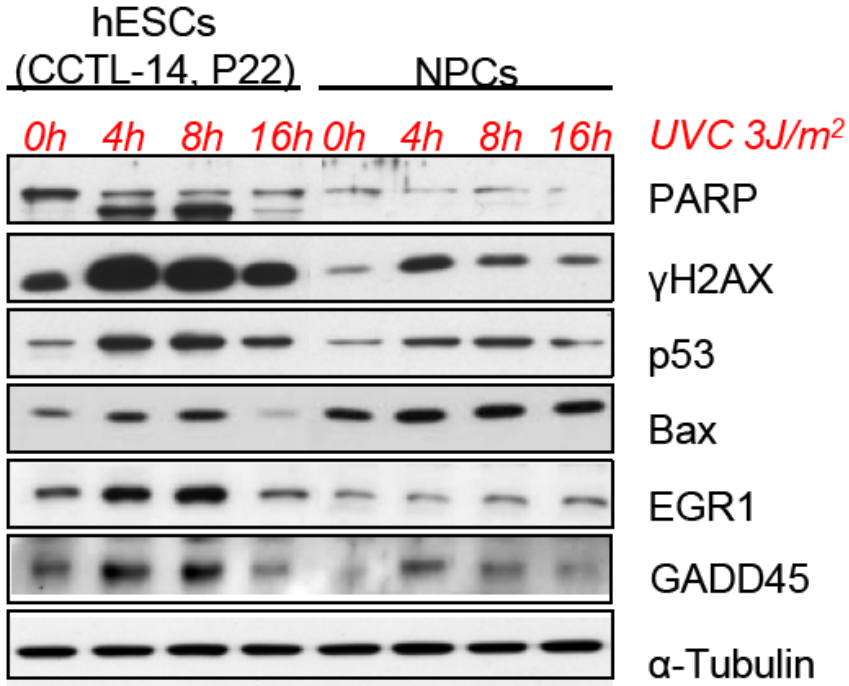
Dasa Dolezalova
Marek Mraz

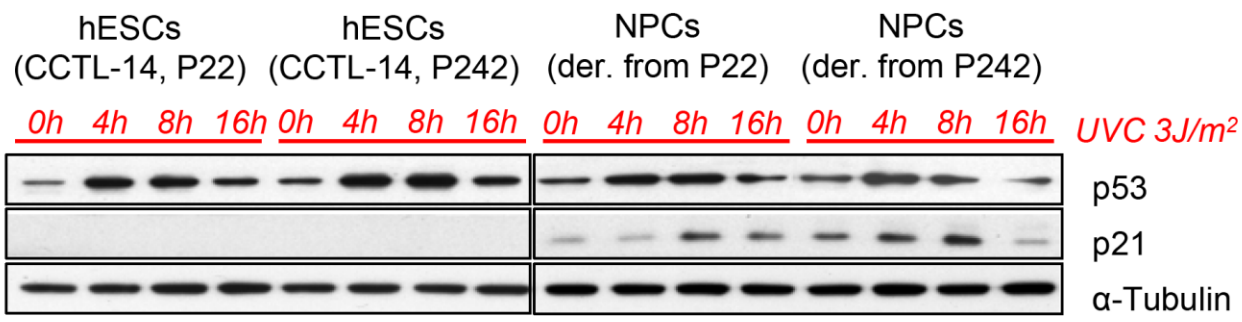
MicroRNAs expression in the regulation of p21 in human embryonic stem cells

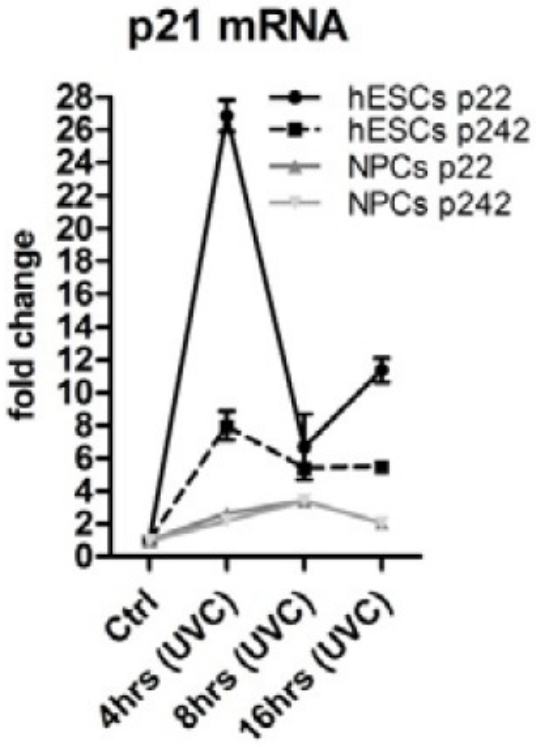




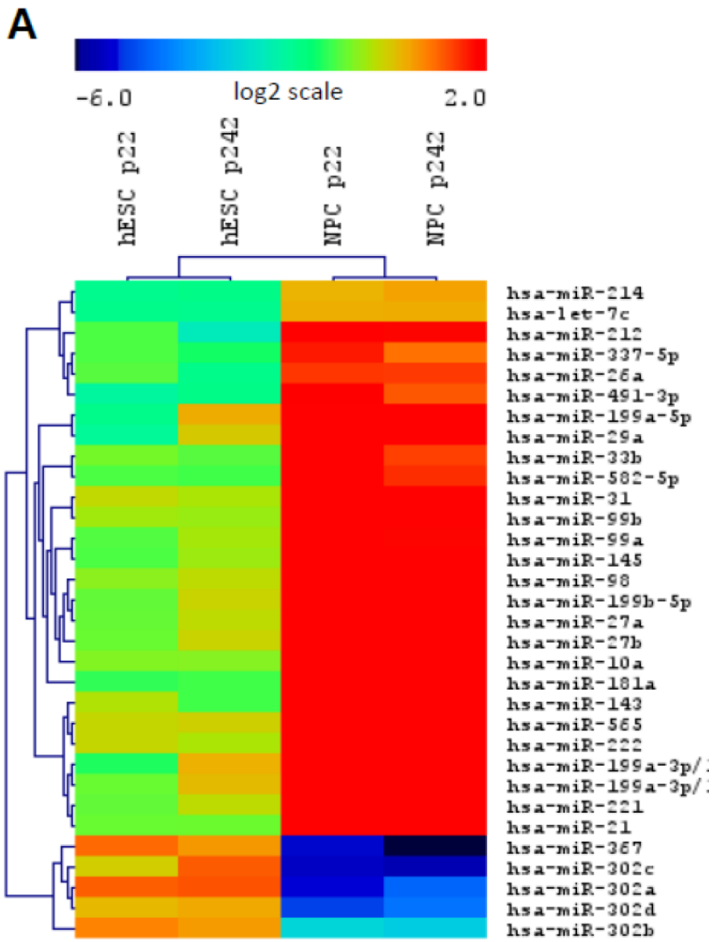
MicroRNAs expression in the regulation of p21 in human embryonic stem cells

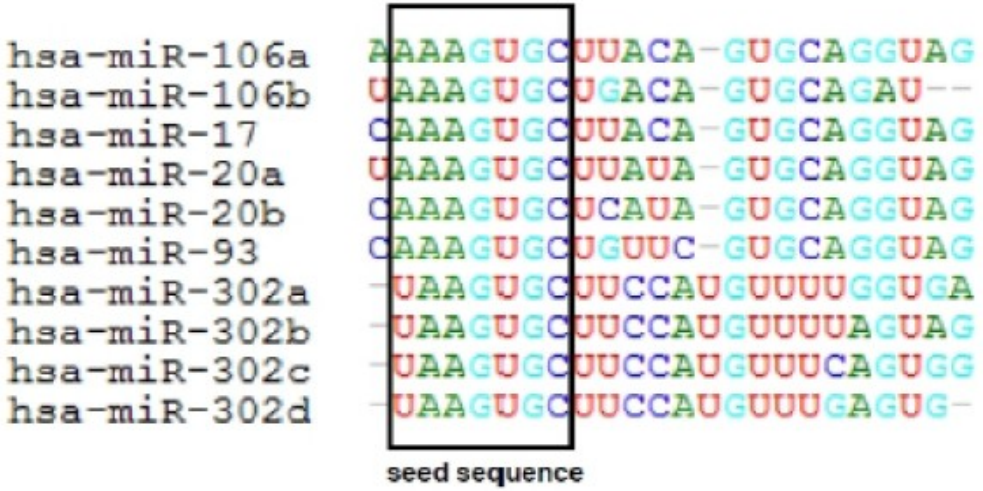




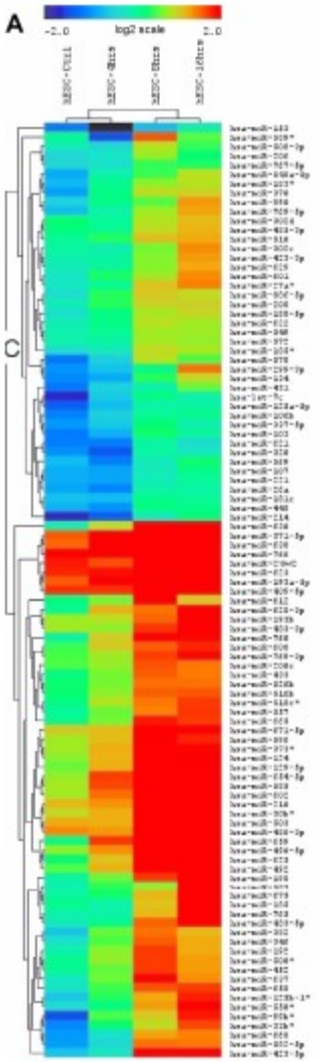


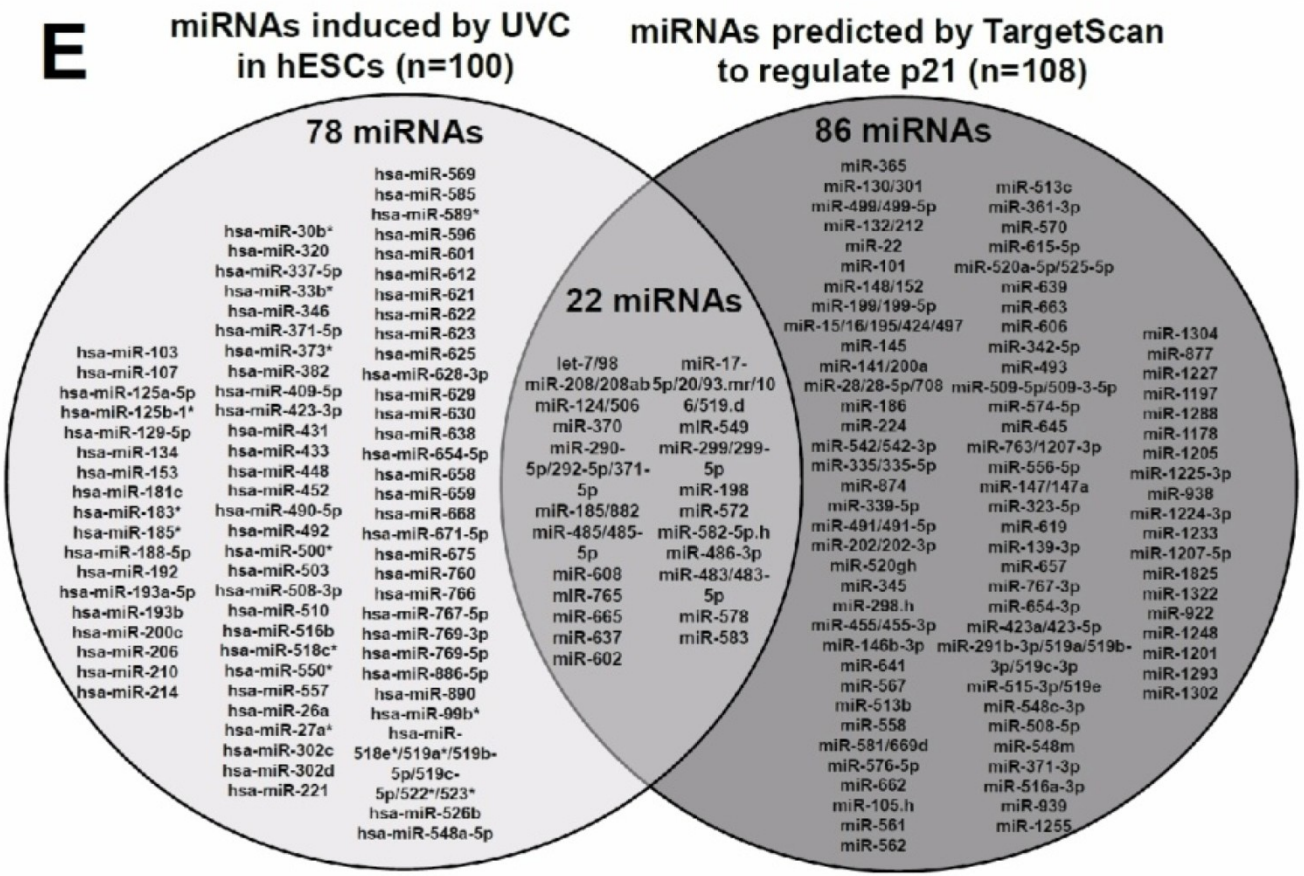
Differences in microRNA expression in hESC vs NPCs



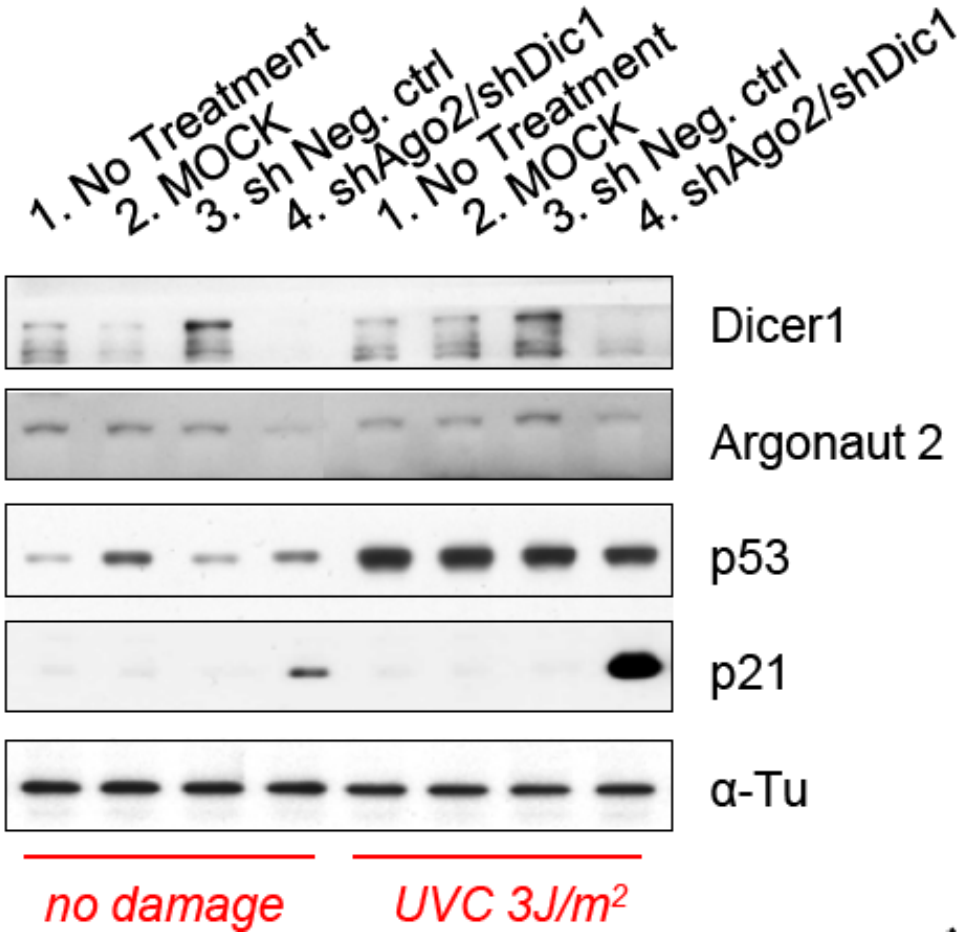


Induction of microRNAs after DNA damage in hESCs

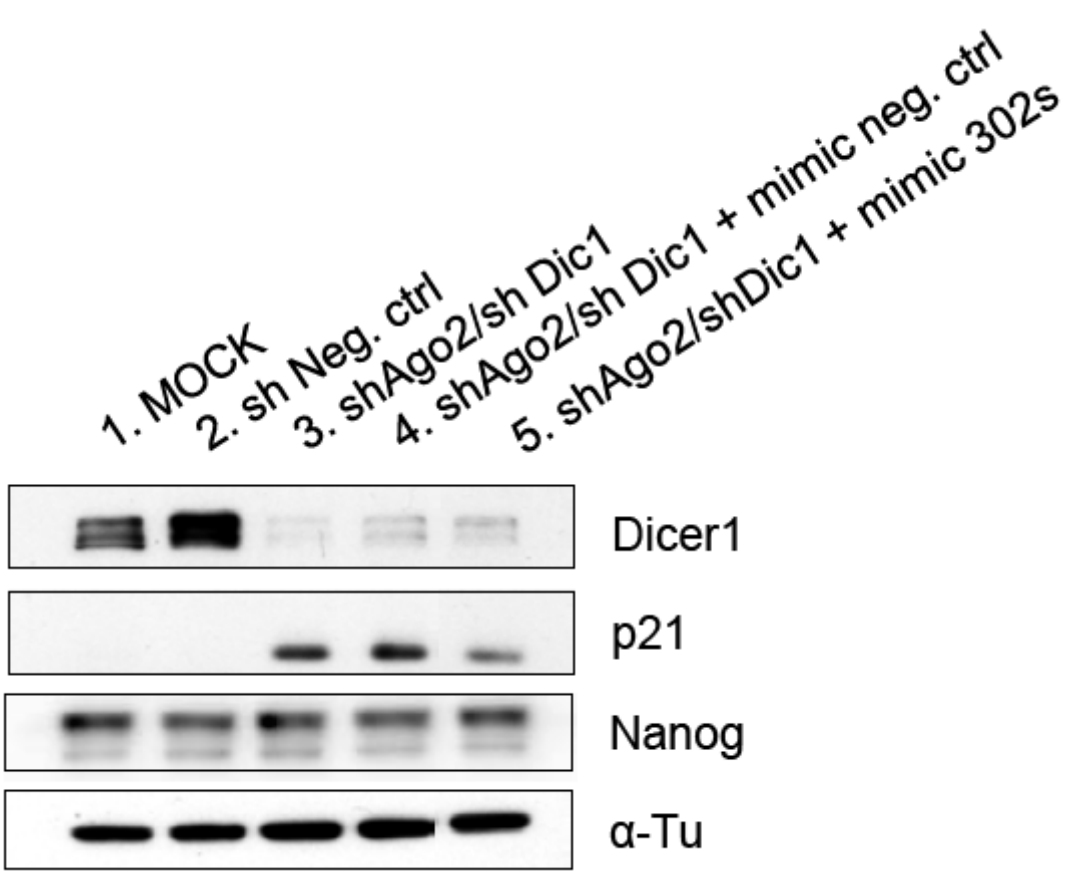


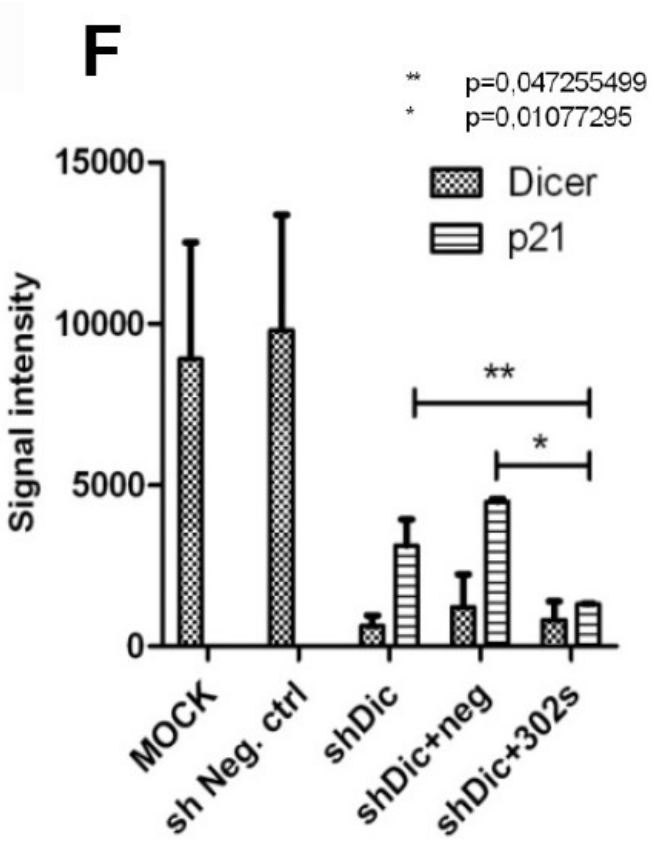


MicroRNA pathway regulates p21

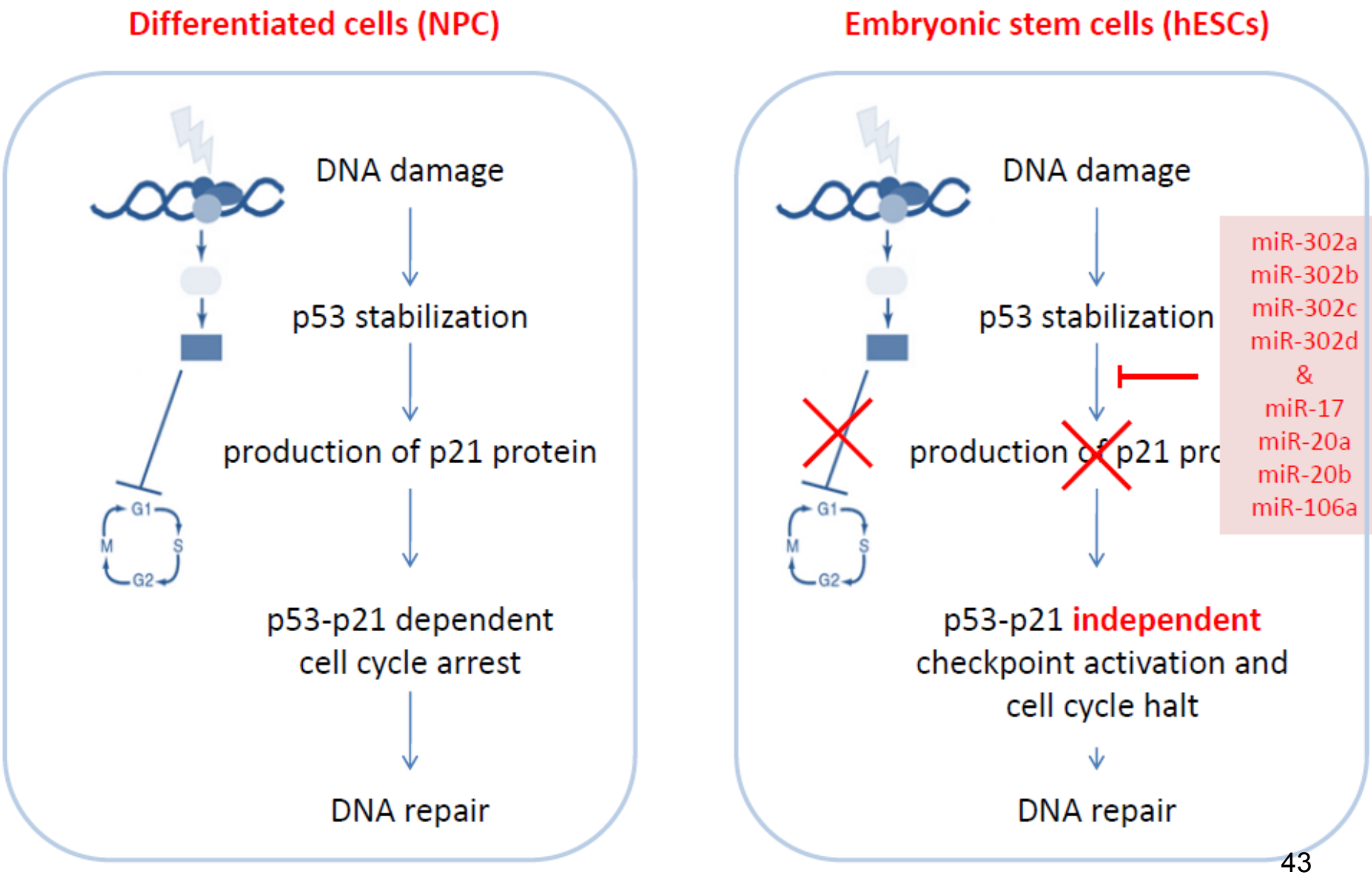


MicroRNA pathway regulates p21: miR-302s contribute





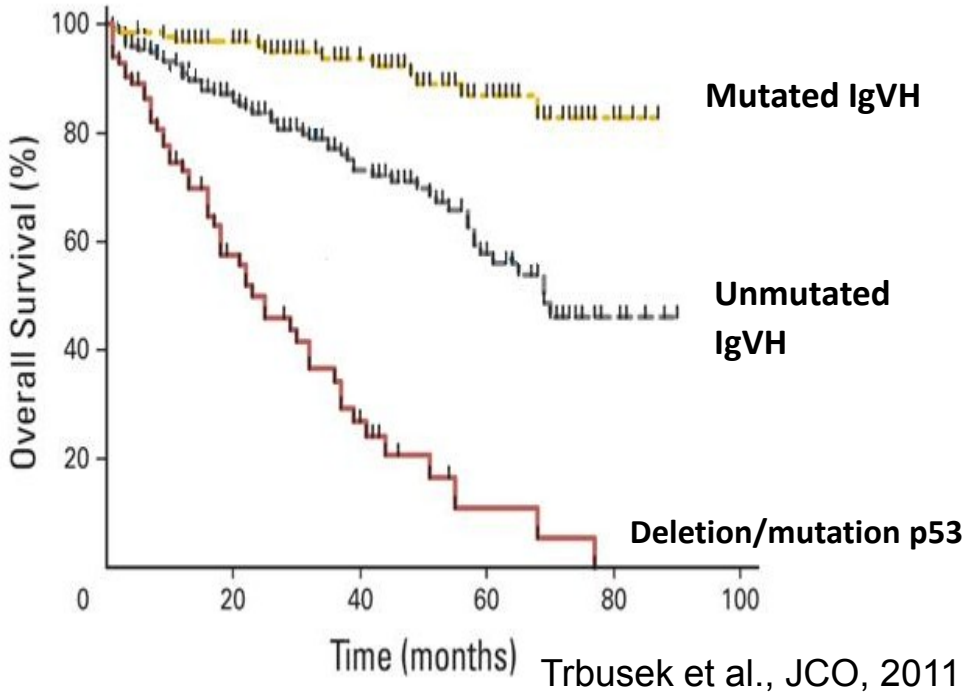
Conclusion


Differentiated cells (NPC)
DNA damage
p53 stabilization
production of p21 protein
G1
M
S
G2
p53-p21 dependent
cell cycle arrest
DNA repair
Embryonic stem cells (hESCs)
DNA damage
p53 stabilization
production of p21 pro
miR-302a
miR-302b
miR-302c
miR-302d
&
miR-17
miR-20a
miR-20b
miR-106a
G1
M
S
G2
p53-p21 independent
checkpoint activation and
cell cycle halt
DNA repair

Chronic lymphocytic leukemia (CLL)

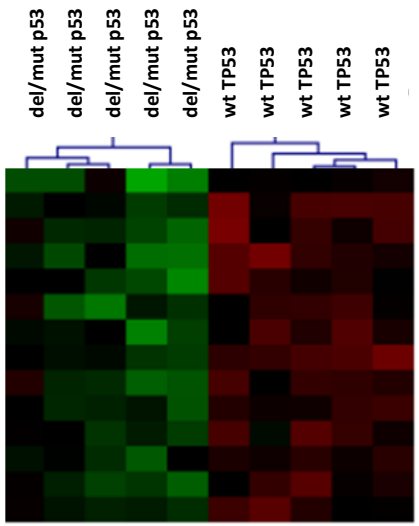
- ❑ Prototype disease
- ❑ Most frequent leukemia in adults
- ❑ Extremely variable survival (months vs. dozens of years)
- ❑ No unifying genetic abberations (most frequently del 13q14)

miR-15-16 are deleted in 60% of CLL cases
(Calin et al., 2002)

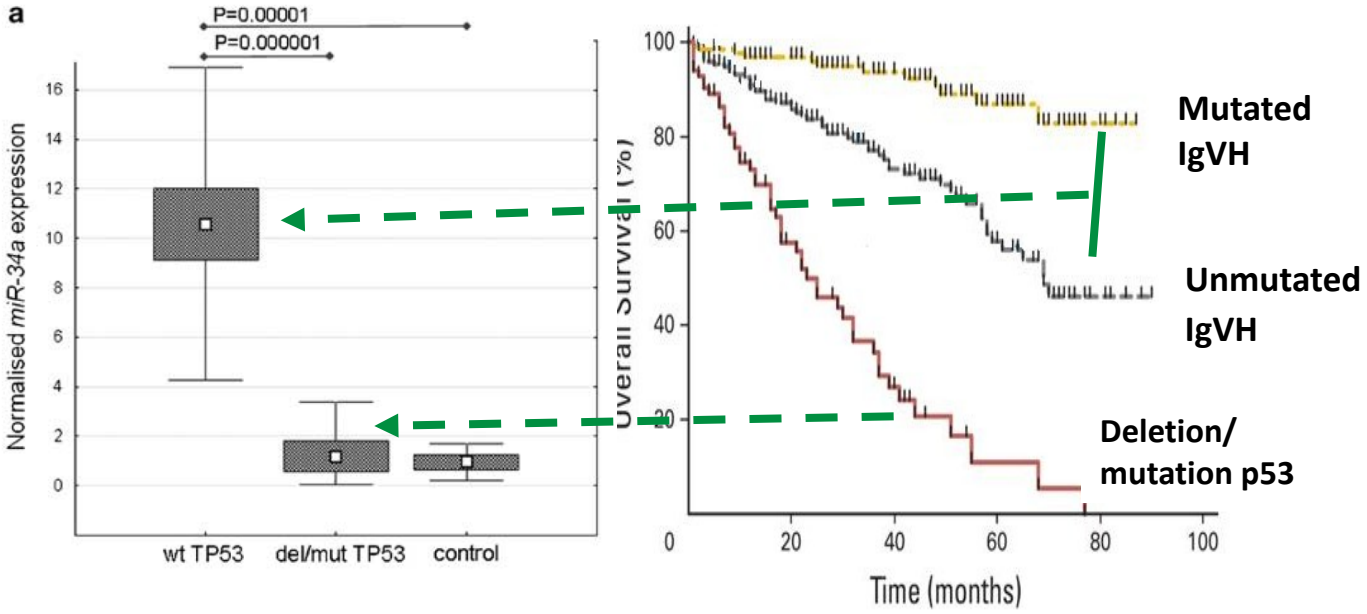


Trbusek et al., JCO, 2011

(i) microRNAs and DNA-damage response in CLL

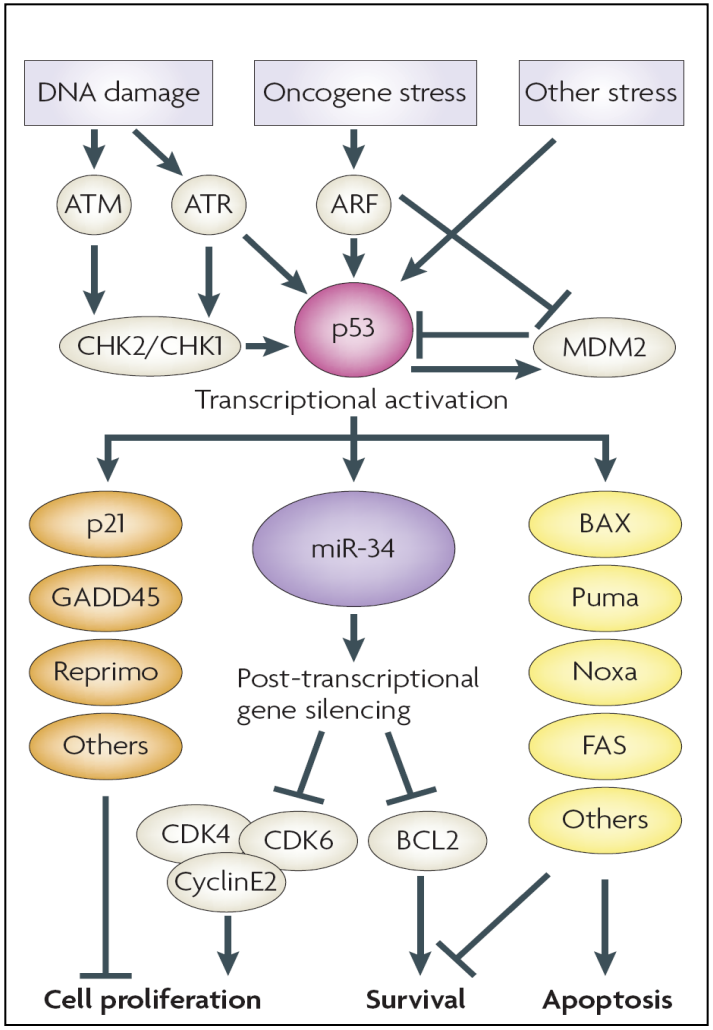


miR-34a



Mraz et al., Leukemia 2009
Mraz et al., Leukemia and Lymphoma, 2009

(i) microRNAs and DNA-damage response in CLL



He et al., 2007

- ***miR-34a* is transcriptionally activated by p53 protein** (He et al., 2007)

- ***miR-34a* is abnormally expressed in leukemia patients**

Mraz et al., Leukemia 2009
Mraz et al., Leuk Lymphoma, 2009
Asslaber et al., Blood, 2010

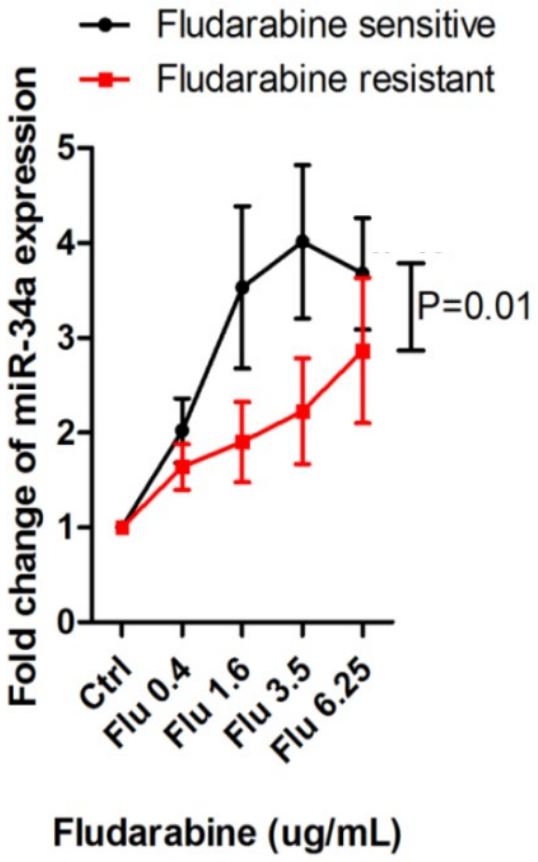
miR-34a clinical assay

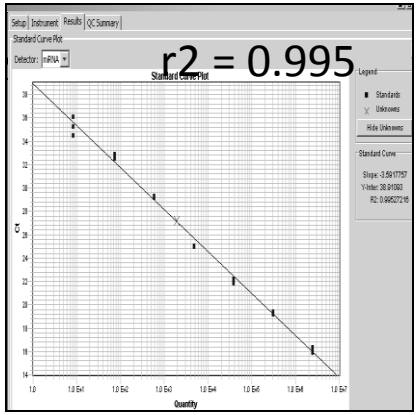
University of California, Mayo Clinic, University of Salzburg, European Research Initiative on CLL

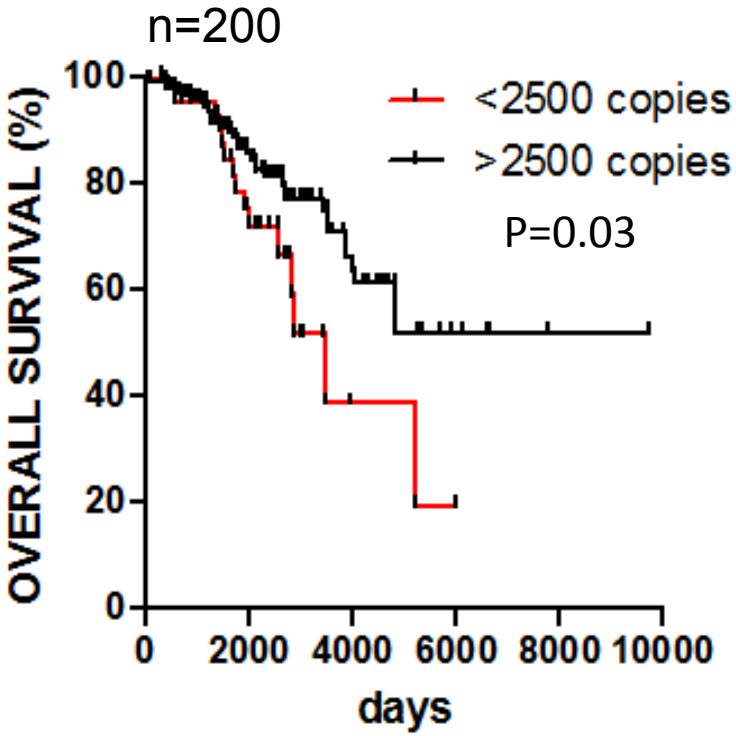
LAB RESEARCH

DIAGNOSTIC TEST

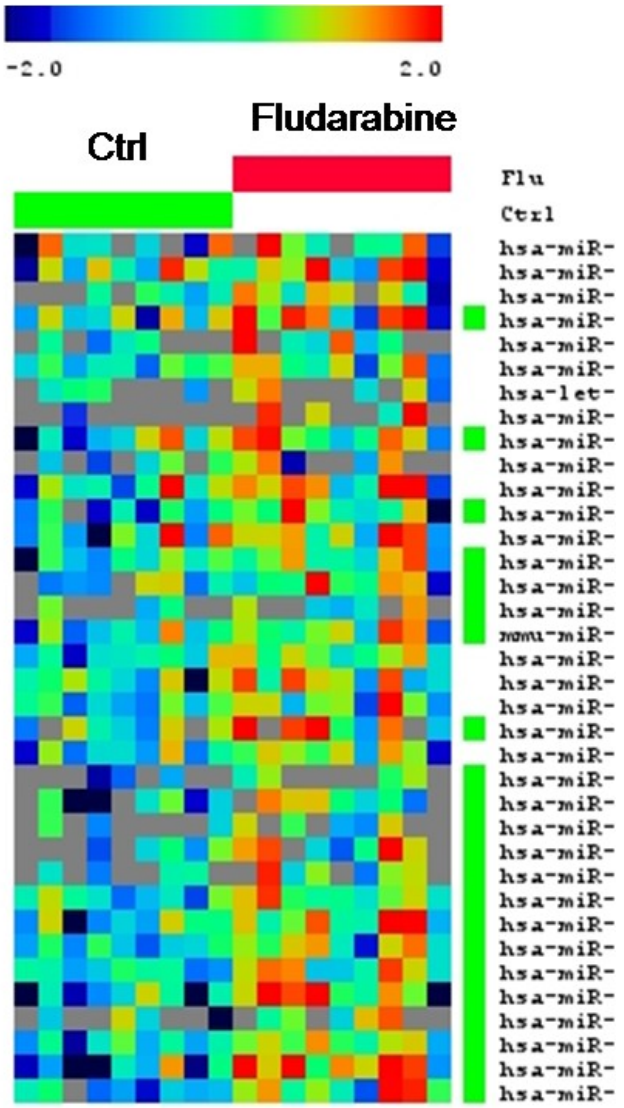
CLINICAL TRIALS





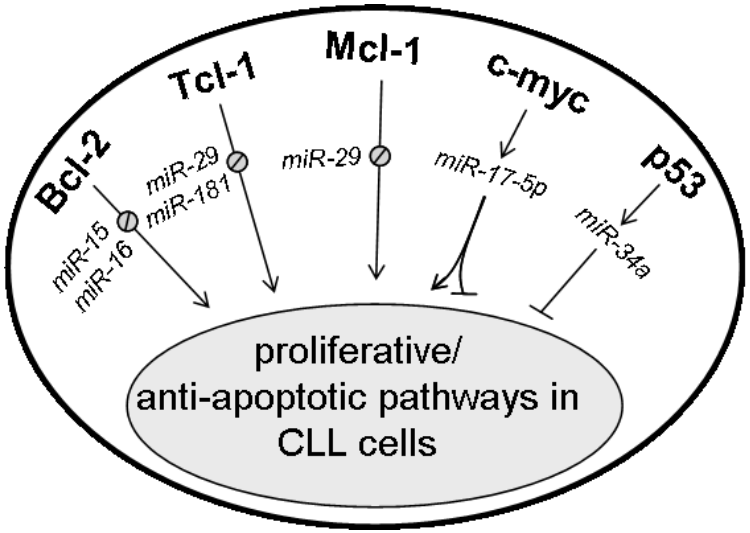


DNA damage response



- *In vivo* samples from ~100 leukemia treated patients
- large scale search for miRNAs that are activated after chemo-immuno therapy *in vivo*
- Application of miRNAs as predictive and prognostic markers

- Definování expresního profilu miRNA u pacientů s CLL a delecí/mutací *p53* genu.



Mraz et al., Leukemia 2009
Mraz et al., Leukemia&Lymphoma, 2009
Mraz et al., BBRC, 2009
Mraz et al., 2012
Mraz et al., 2013

Je to k něčemu dobré i v medicíně?

Tab. 1
Exprese microRNA u pacientů s nepříznivýni prognostikými markery (nemutované IgVH a delece/mutace p53)
(dle [1] Calin, 2005; [2] Mraz, 2009b)

MicroRNA	Chromozomální oblast	Exprese miRNA u CLL vzorků s:	
		nemutovaným IgVH (ZAP70+) vs. **mutovaným IgVH** (ZAP70-)	**delecí/mutací p53** (bez ohledu na IgVH) vs. **wild-type p53** (bez ohledu na IgVH)
miR-15a	13q14	VYSOKÁ[1]	NEZMĚNĚNA[2]
miR-16-1	13q14	VYSOKÁ[1]	NEZMĚNĚNA[2]
miR-16-2	3q26	VYSOKÁ[1]	NEZMĚNĚNA[2]
miR-23b	9q22	VYSOKÁ[1]	NEZMĚNĚNA[2]
miR-24-1	9q22	VYSOKÁ[1]	NEZMĚNĚNA[2]
miR-29a-2	7q32	NÍZKÁ[1]	NEZMĚNĚNA[2]
miR-29b-2	1q32	NÍZKÁ[1]	NEZMĚNĚNA[2]
miR-29c	1q32	NÍZKÁ[1]	**NÍZKÁ[2]**
miR-146	5q34	VYSOKÁ[1]	NEZMĚNĚNA[2]
miR-155	21q21	VYSOKÁ[1]	NEZMĚNĚNA[2]
miR-195	17p13	VYSOKÁ[1]	NEZMĚNĚNA[2]
miR-221	Xp11.3	VYSOKÁ[1]	NEZMĚNĚNA[2]
miR-223	Xq12	NÍZKÁ[1]	NEZMĚNĚNA[2]
miR-34a	1p36	NEZMĚNĚNA[2]	**NÍZKÁ[2]**
miR-17-5p	13q31	NEZMĚNĚNA[2]	**NÍZKÁ[2]**
miR-142		**NÍZKÁ[2]**	NEZMĚNĚNA[2]

- potentially usefull as **therapeutic targets**

nature

Vol 452|17 April 2008|doi:10.1038/nature06783

LETTERS

LNA-mediated microRNA silencing in non-human primates

Joacim Elmén[1]*, Morten Lindow[1]*, Sylvia Schütz[2], Matthew Lawrence[3], Andreas Petri[1], Susanna Obad[1], Marie Lindholm[1], Maj Hedtjärn[1], Henrik Frydenlund Hansen[1], Urs Berger[4], Steven Gullans[3], Phil Kearney[1], Peter Sarnow[2], Ellen Marie Straarup[1] & Sakari Kauppinen[1,5]

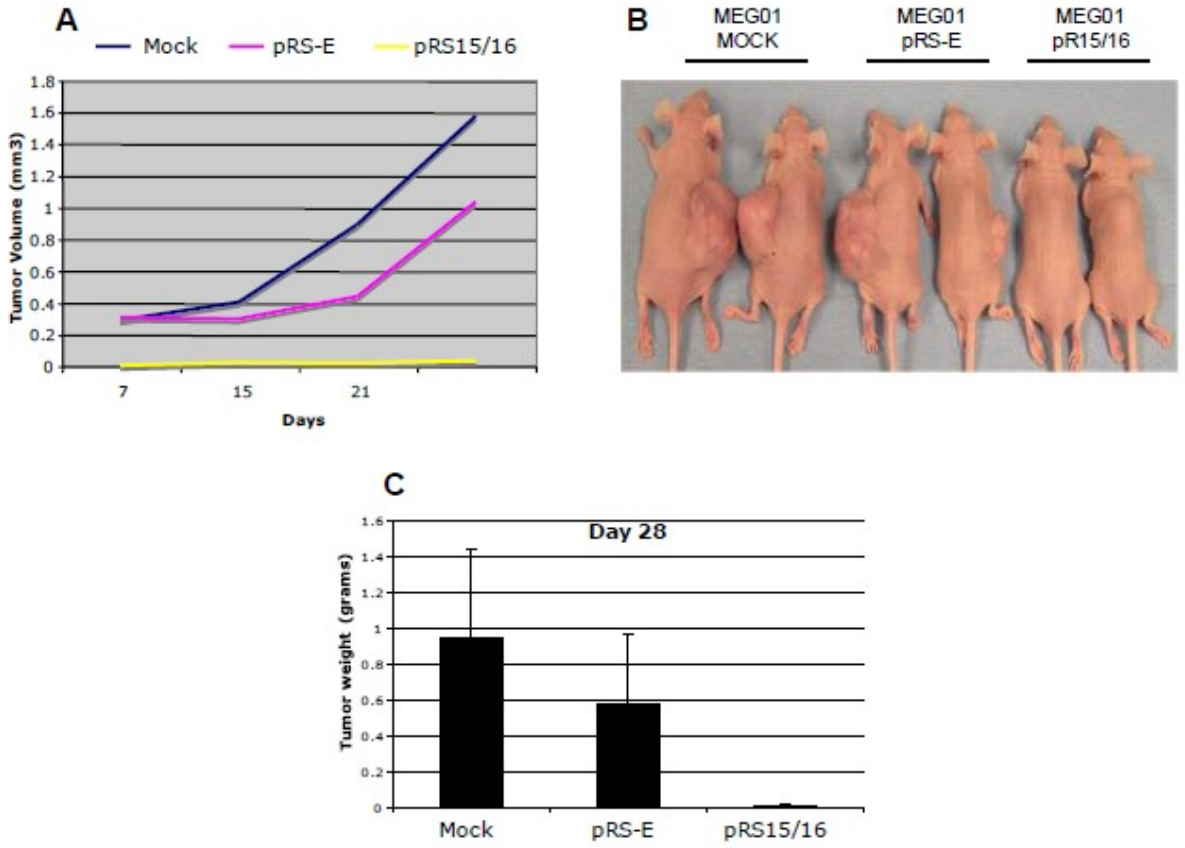
PCR based therapeutics ?!

blood

2012 120: 1678-1686
Prepublished online July 13, 2012;
doi:10.1182/blood-2012-02-410647

LNA-mediated anti–miR-155 silencing in low-grade B-cell lymphomas

Yong Zhang, Aldo M. Roccaro, Christopher Rombaoa, Ludmilla Flores, Susanna Obad, Stacey M. Fernandes, Antonio Sacco, Yang Liu, Hai Ngo, Phong Quang, Abdel Kareem Azab, Feda Azab, Patricia Maiso, Michaela Reagan, Jennifer R. Brown, To-Ha Thai, Sakari Kauppinen and Irene M. Ghobrial



Calin et al., 2008

Děkuji za pozornost

Marek Mráz
marek.mraz@email.cz

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ
Tato prezentace je spolufinancována Evropským sociálním fondem a státním rozpočtem České republiky

MicroRNA-650 Expression Is Influenced by Immunoglobulin Gene Rearrangement and Affects the Biology of Chronic Lymphocytic Leukemia

Marek Mraz[1,2], Dasa Dolezalova[3], Karla Plevova[1,2], Katerina Stano Kozubik[1,2], Veronika Mayerova[1,2], Katerina Cerna[1,2], Katerina Musilova[1,2], Boris Tichy[1,2], Sarka Pavlova[1,2], Marek Borsky[2], Jan Verner[1,2], Michael Doubek[1,2], Yvona Brychtova[2], Martin Trbusek[1,2], Ales Hampl[3], Jiri Mayer[1,2], Sarka Pospisilova[1,2]

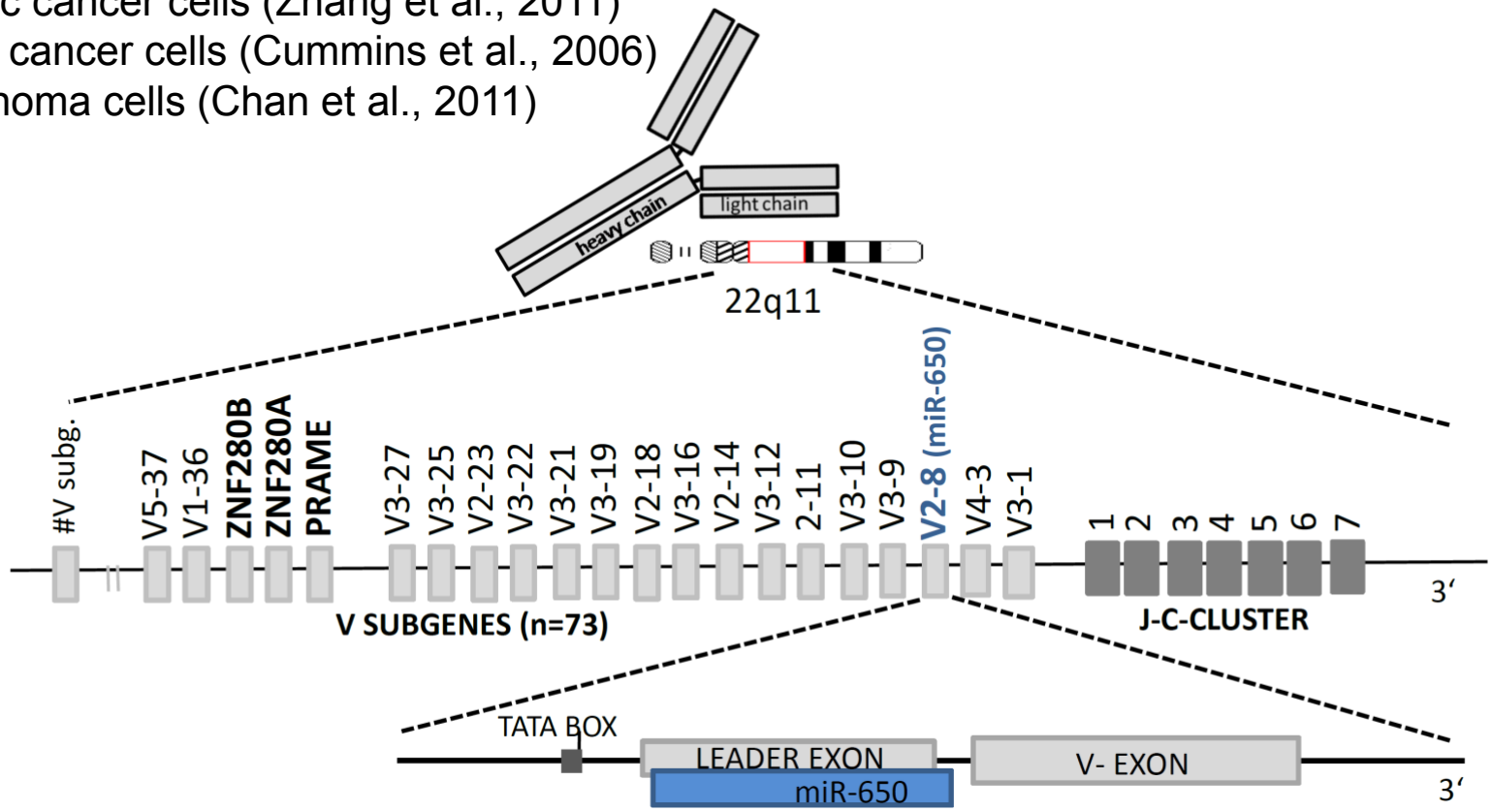
***Blood**, epub ahead of print*

IgL locus structure

miR-650 identified by miRAGE clonig approche in colorectal cells (Cummins et al., 2006)

miR-650 is expressed in:

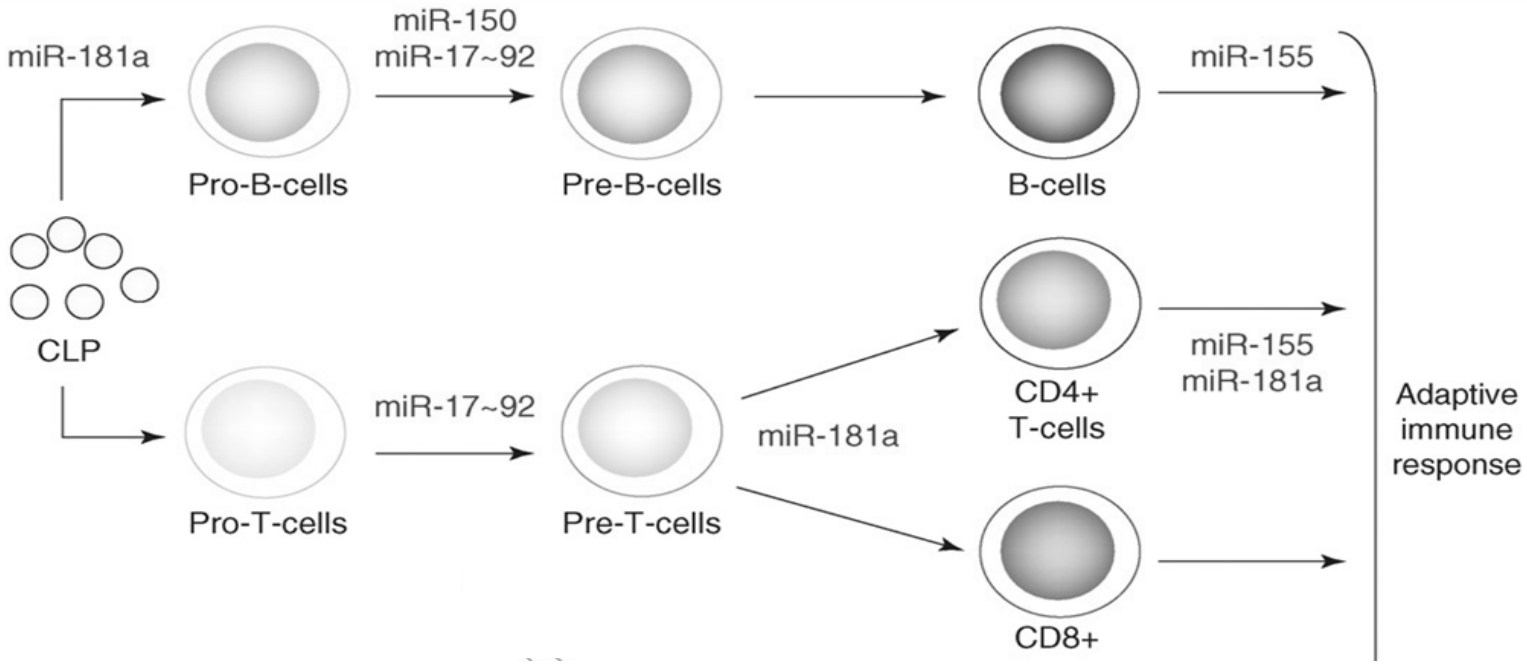
- ❑ breast cancer cells (Chien et al., 2011)
- ❑ gastric cancer cells (Zhang et al., 2011)
- ❑ colon cancer cells (Cummins et al., 2006)
- ❑ melanoma cells (Chan et al., 2011)



Das et al., 2009

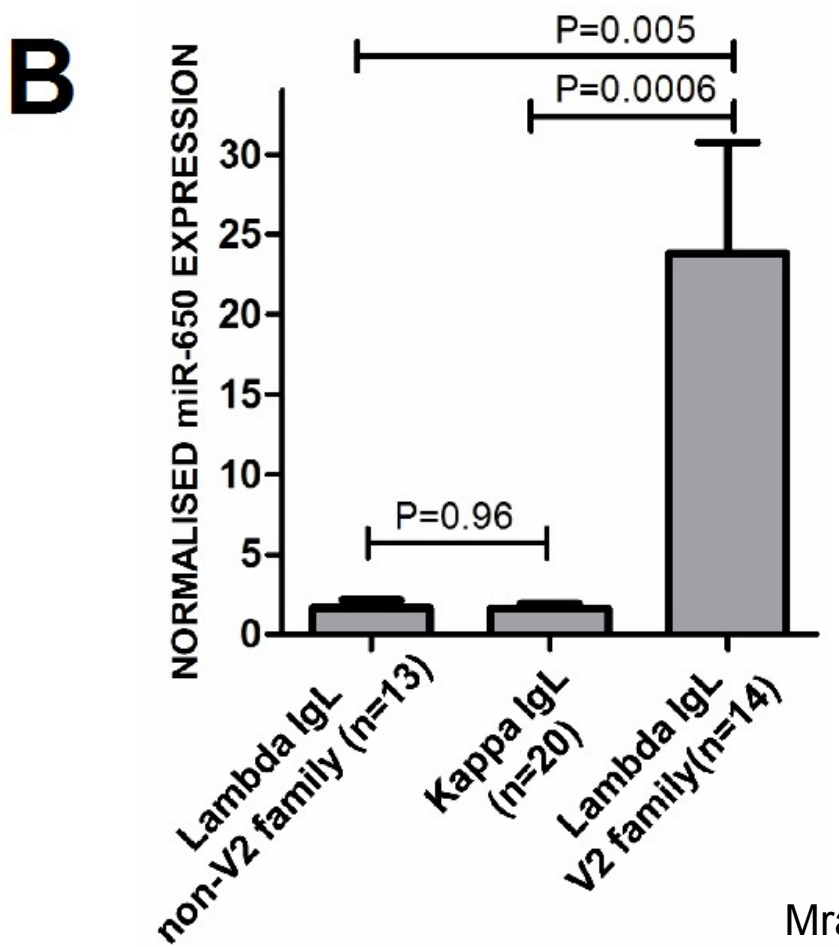
MicroRNAs regulate production of imunoglobulins

- Associated with IgG production and V(D)J recombination *(Koralov, 2008;Vigorito, 2007)*



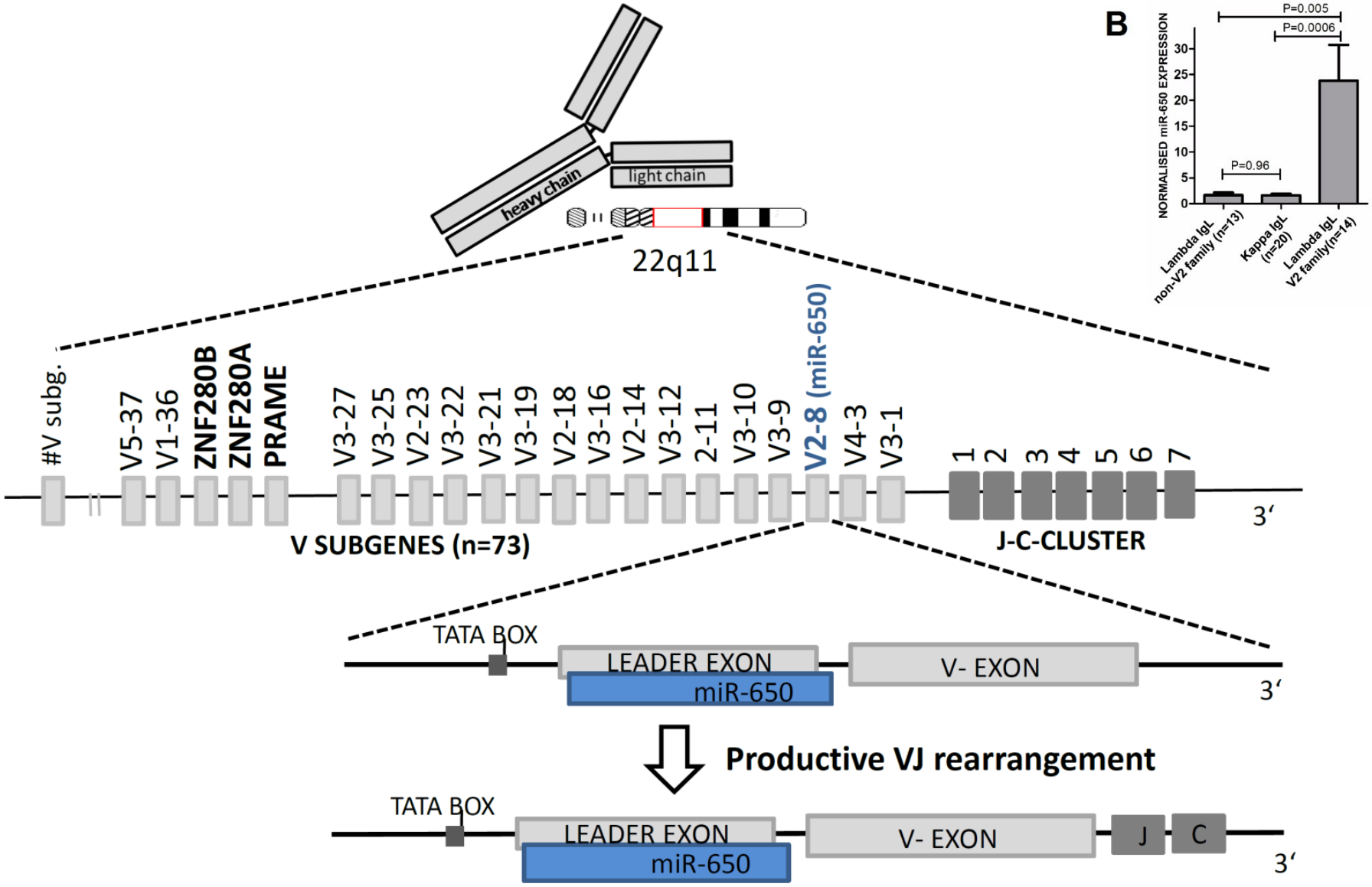
Lindsay, 2008

High expression of miR-650 in cells utilizing: V2-8, V2-14, V2-5, V2-18, V2-23 subgenes for IgL



Mraz et al., Blood, 2012

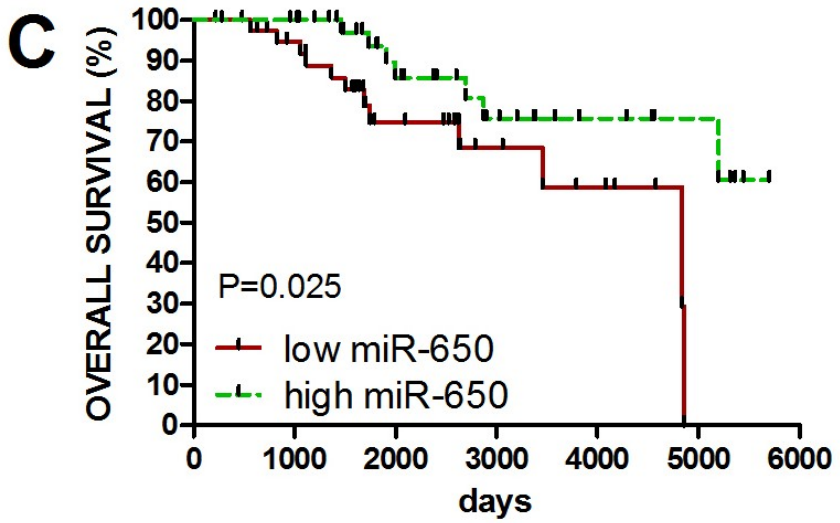
Lambda locus rearrangement utilizing V2 family is coupled with activation of miR-650 expression

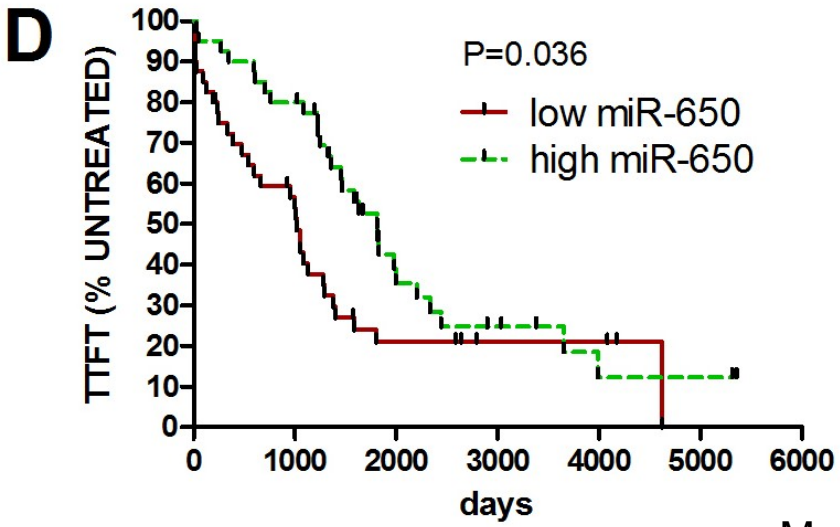


Mraz et al., Blood, 2012

Expression miR-650 is associated with CLL prognosis

higher expression is favorable

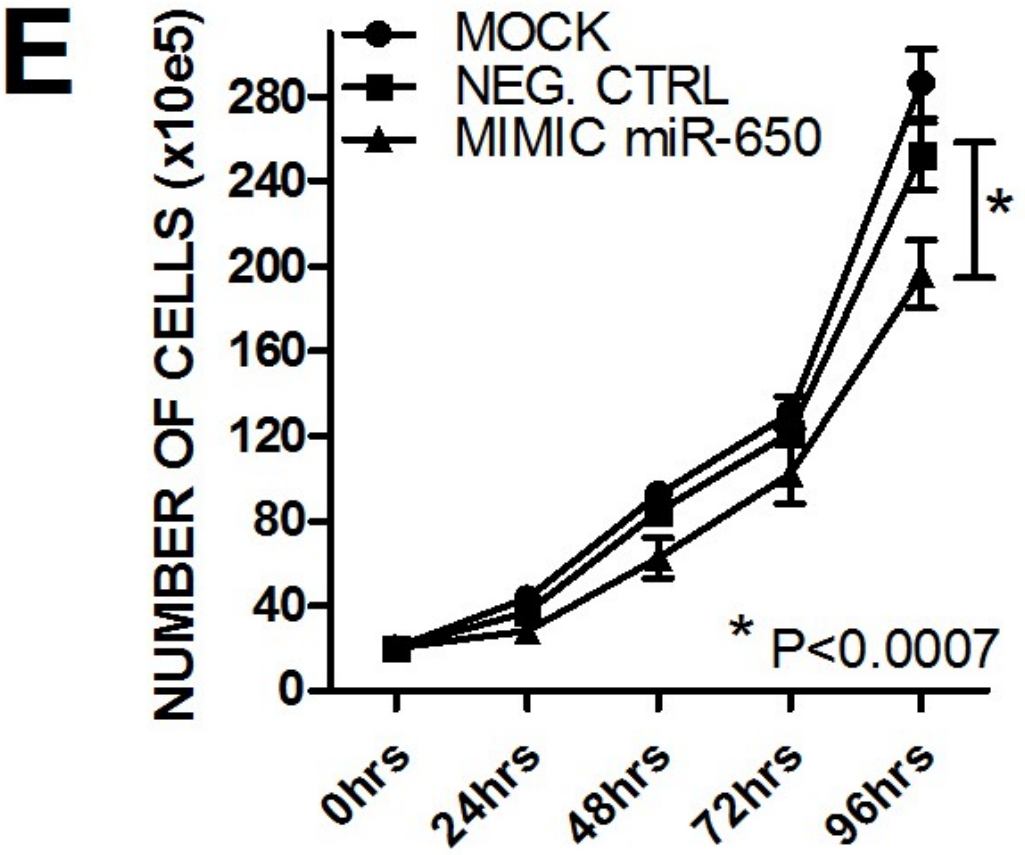




Mraz et al., Blood, 2012

miR-650 reduces the proliferative capacity of B-cell proliferation

miR-650 inhibits cell cycle progression by regulating p16INK4-mediated pathway (Chien et al., 2011)

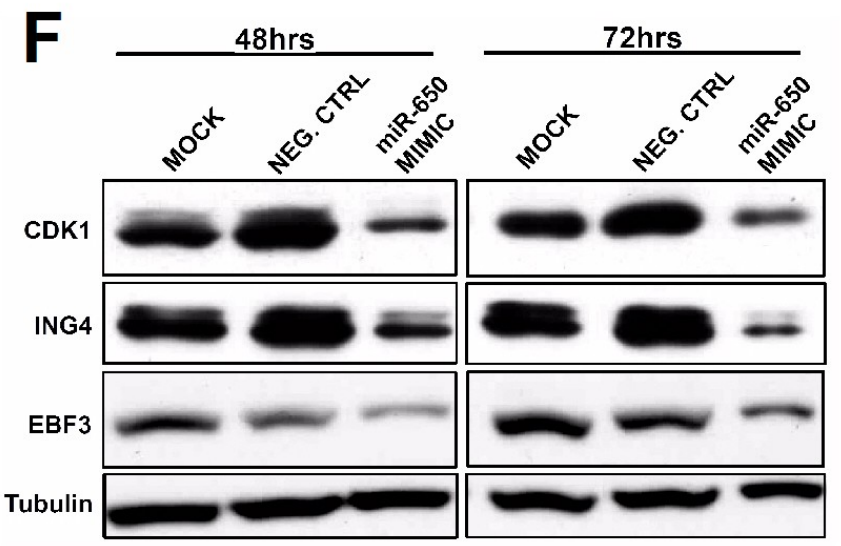


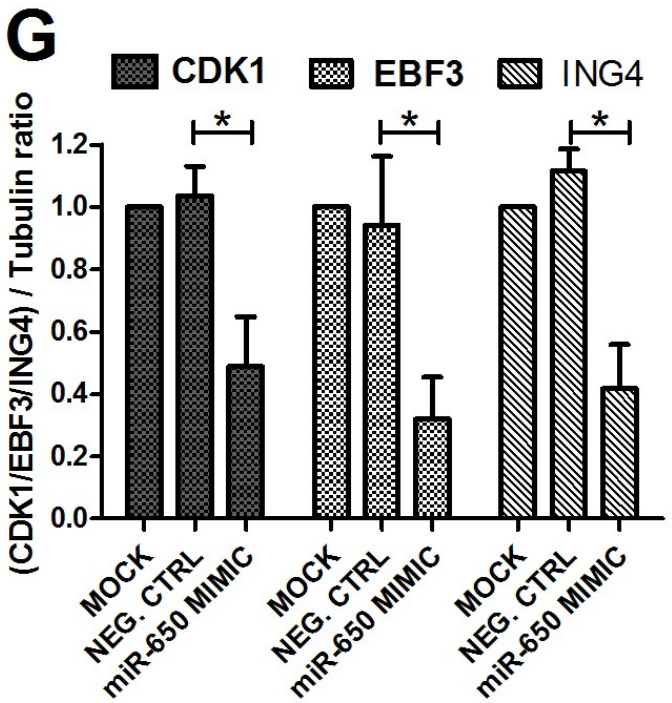
Mraz et al., Blood, 2012

miR-650 regulates CDK1, ING4 and EBF3 in B-cells

- ~50% of predicted targets of miR-650 are expressed in B-cells with numerous being important in B-cells physiology (Gene Ontology and Functional analysis by DAVID tool).

- miR-650 regulates the Cyclin Dependent Kinase 1 (CDK1) in breast cancer cells (Chien etal., 2011)
- Inhibitor of Growth 4 (ING4) in gastric cancer cells (Zhang et al., 2011)
- The EBF3 protein is a putative target with the highest score (TargetScan)





Mraz et al., Blood, 2012

Conclusion (I)

miR-650

- **V2 family of subgenes for lambda lgL includes homologues of miR-650**
- **coupled activation of miR-650 expression with lgL expression**
- **miR-650 is associated with CLL prognosis**
- **miR-650 regulates proliferation of B-cells**
- **targets for miR-650:**

CDK1 (Cyclin Dependent Kinase 1)

ING4 (Inhibitor of Growth 4)

EBF3 (early B cell factor)

DISCRIPTION OF A UNIQUE MECHANISM OF MICRORNA EXPRESSION COUPLED WITH IMMUNOGLUBULIN EXPRESSION