LÉKAŘSKÁ FAKULTA MASARYKOVY UNIVERSITY
Interní hematoonkologická klinika LF MU a FN Brno
Centrum molekulární biologie a genové terapie

Analýza exprese microRNA

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Principal Investigator CEITEC MU

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

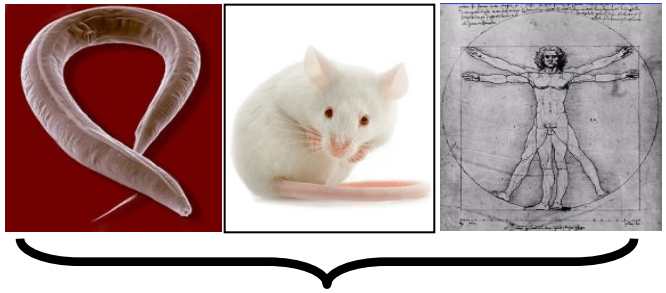
- Náhradni termín přednášky Dr. Borise Tichého 13/11 nebo 27/11?

Nic si nepište…vše bude online

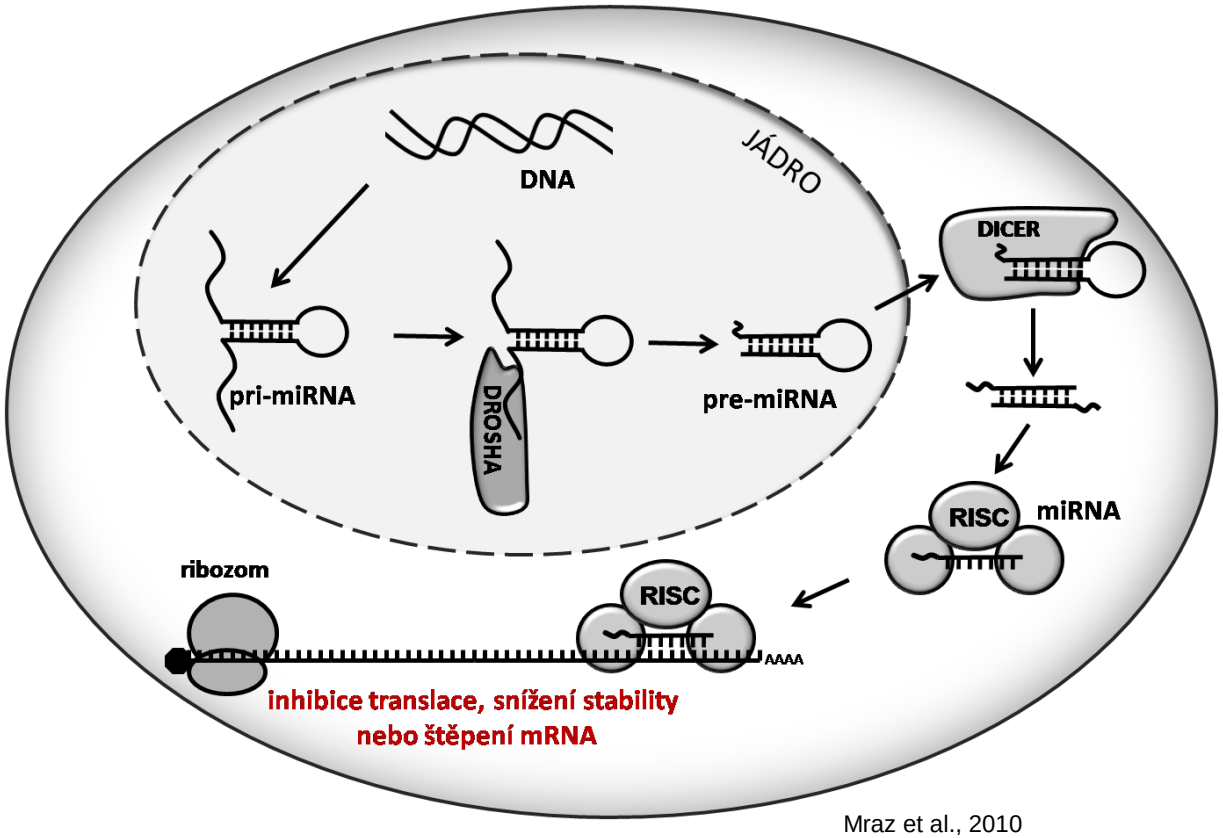
microRNA (miRNA)

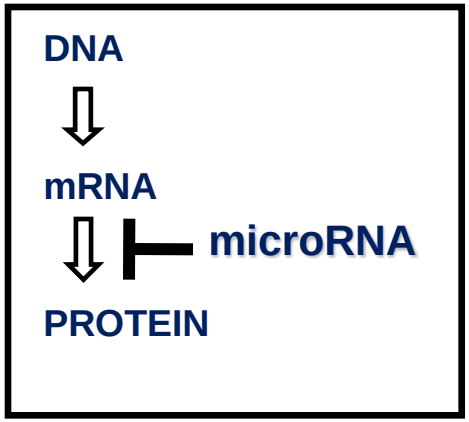
- krátké RNA molekuly
  ~22 nukleotidů
- komplementární vazba k cílové mRNA
- inhibují translaci a snižují stabilitu mRNA

- Lidské miRNA geny: cca 2000



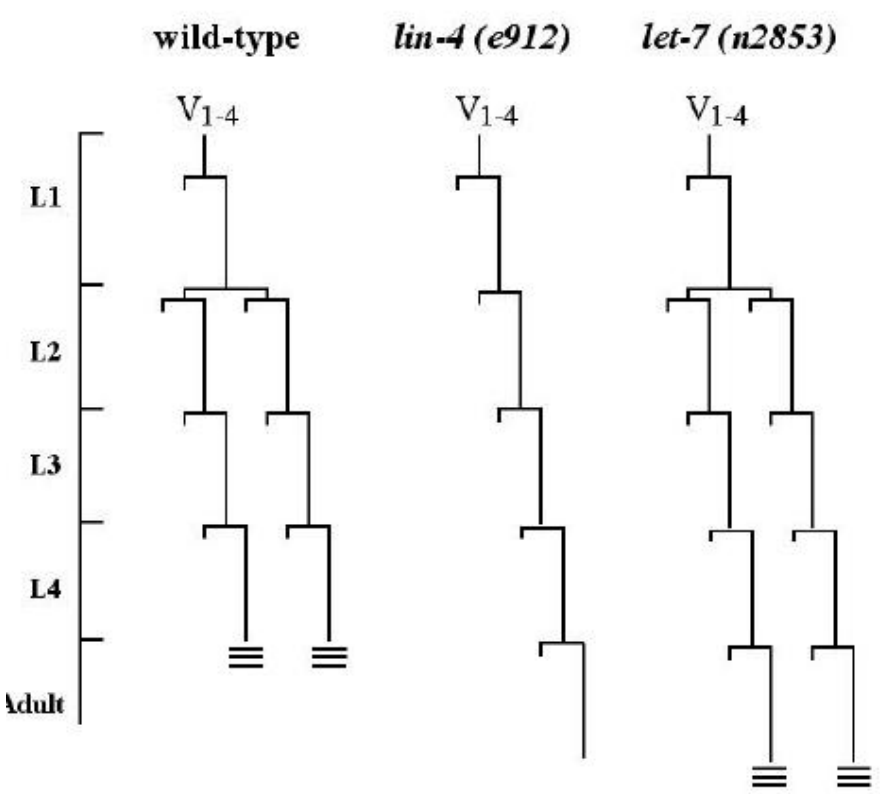
Stovky evolučně konzervovaných microRNA

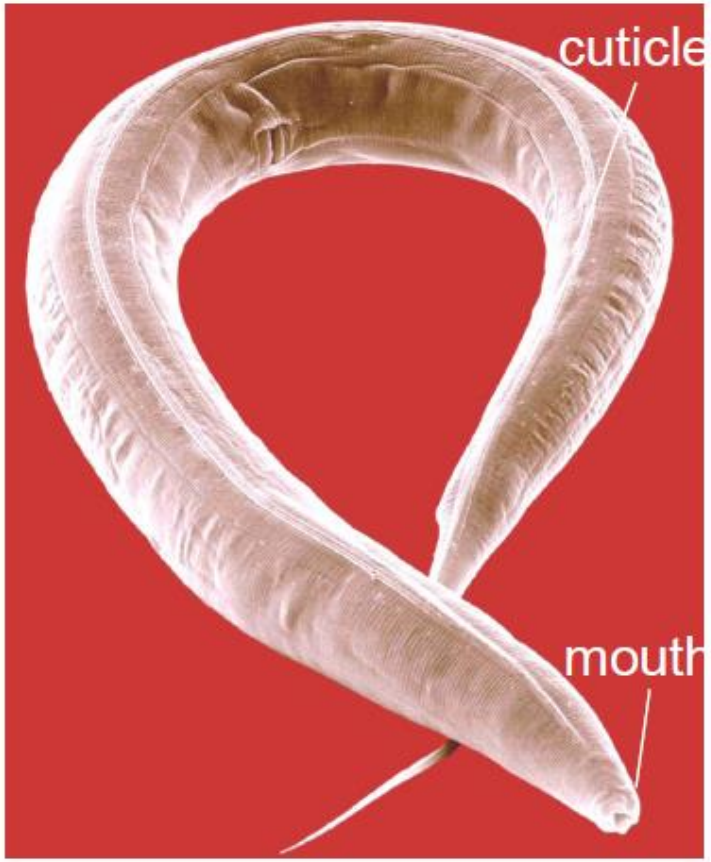




Mraz et al., 2010

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

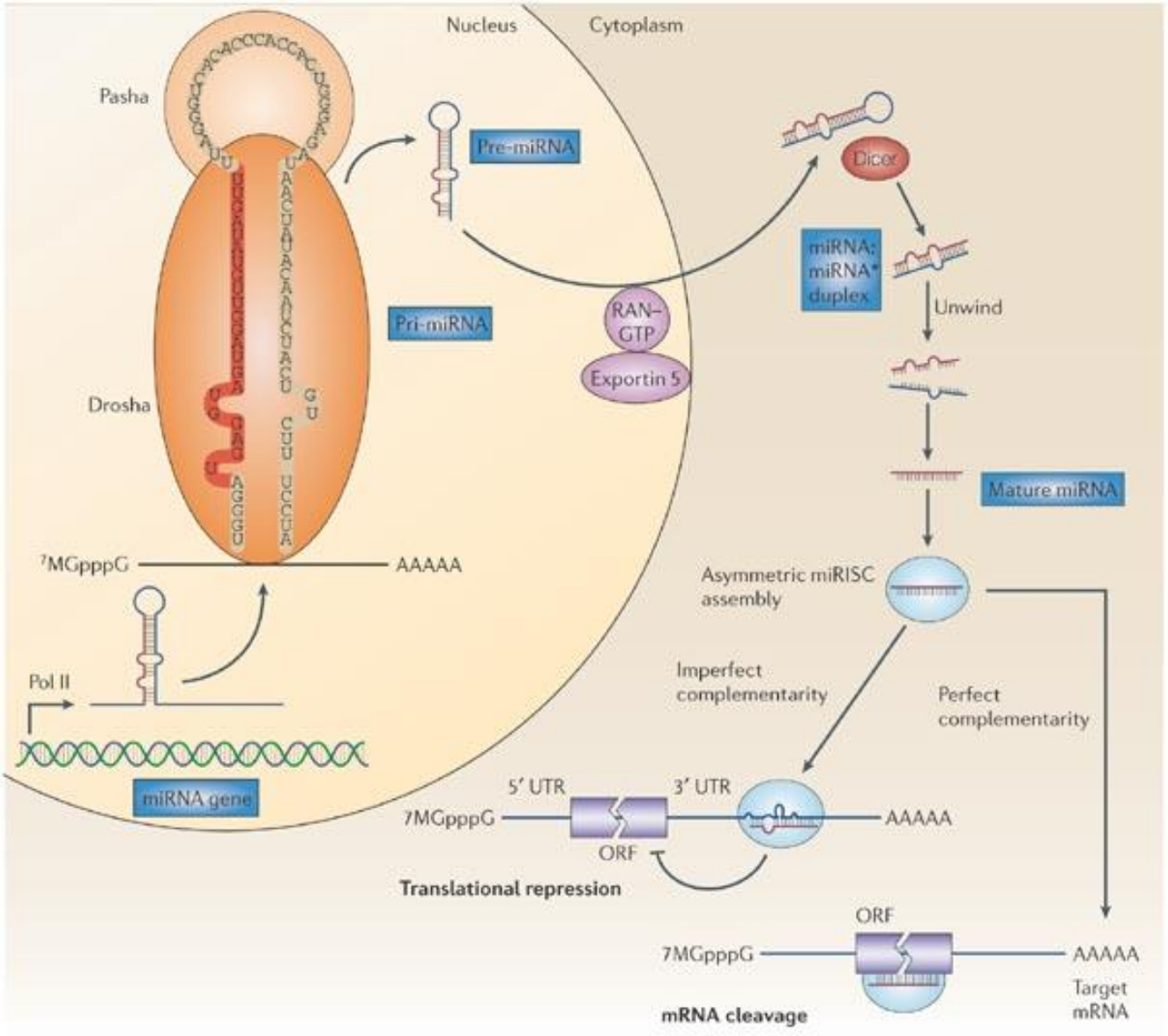


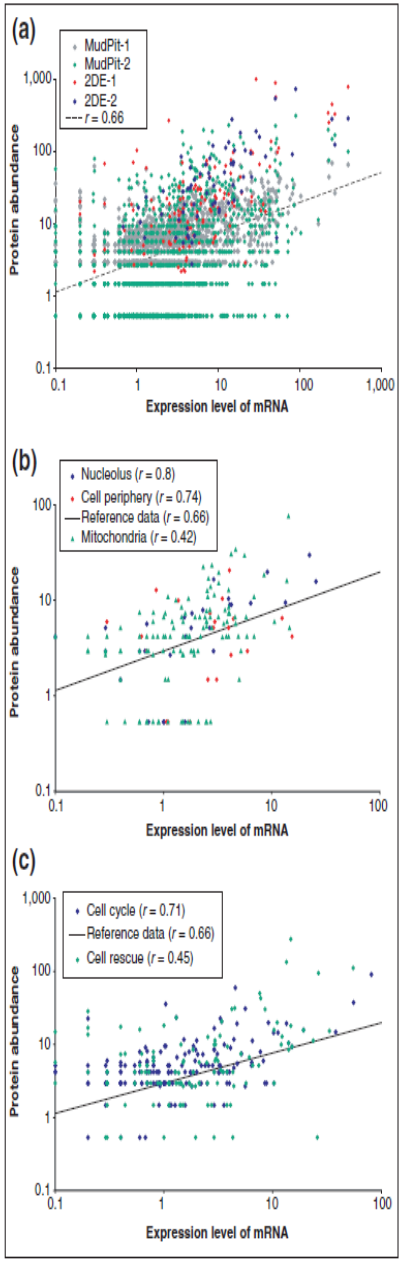


Lee et al. 1993 *Cell*

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

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

1/ Izolace a stabilita microRNA

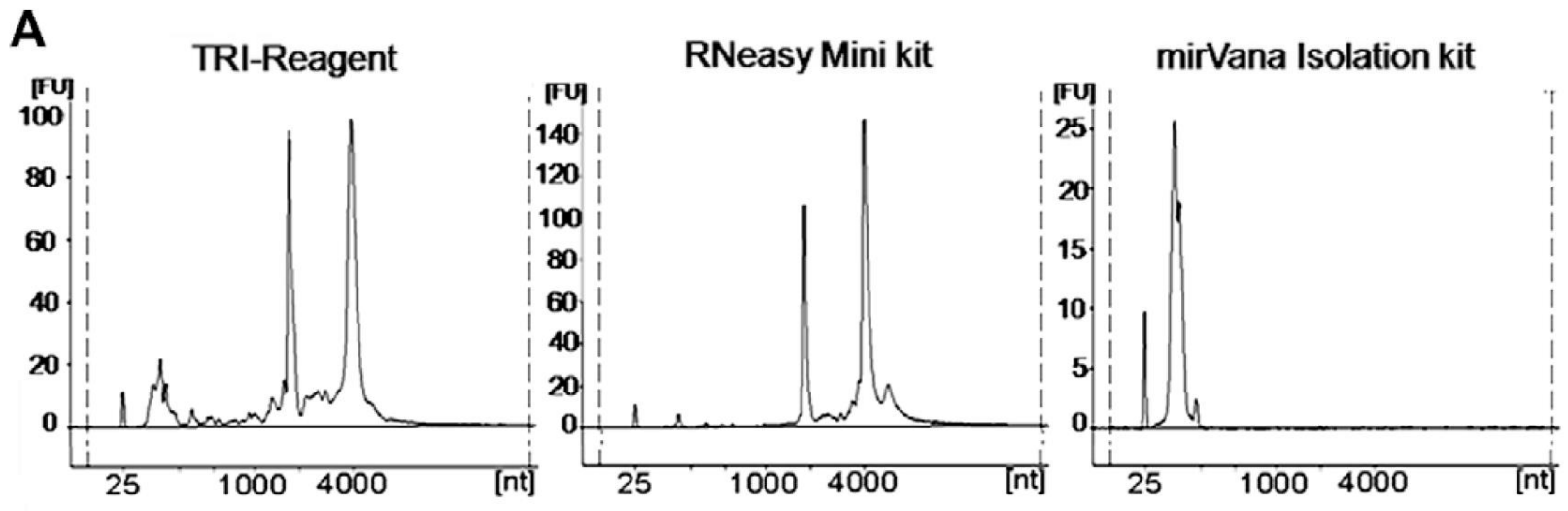
Problémy: velikost 22nt, celkově cca 0,01% z celkové RNA

Izolace:

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

Obohacení:

PAGE
FlashPAGE Fractionator (Ambion)



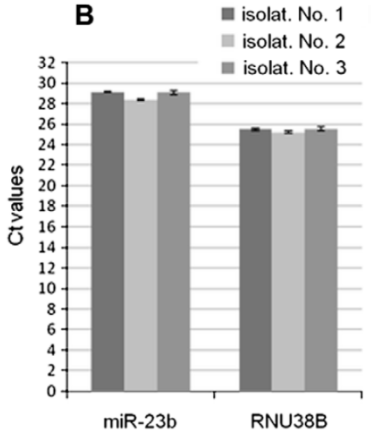
Mraz et al., 2009

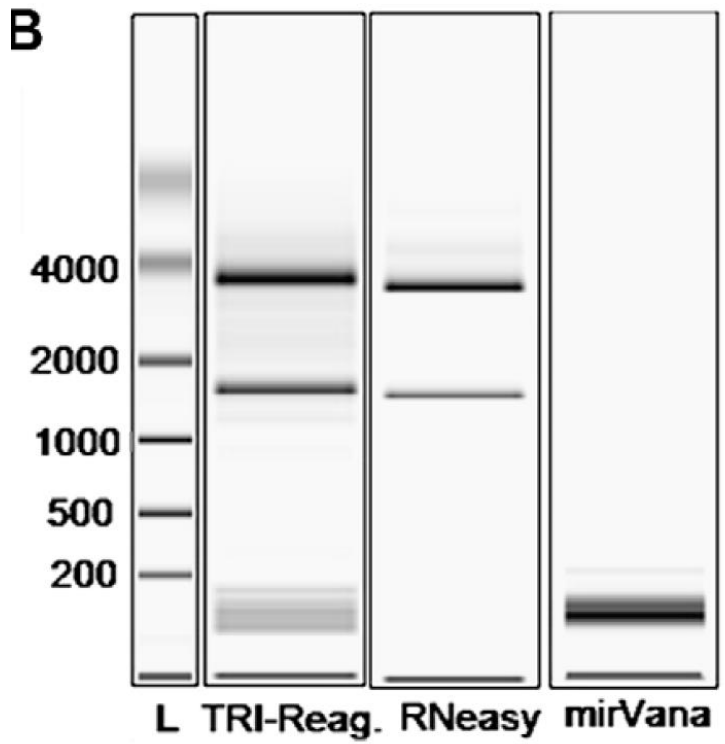
Izolace:

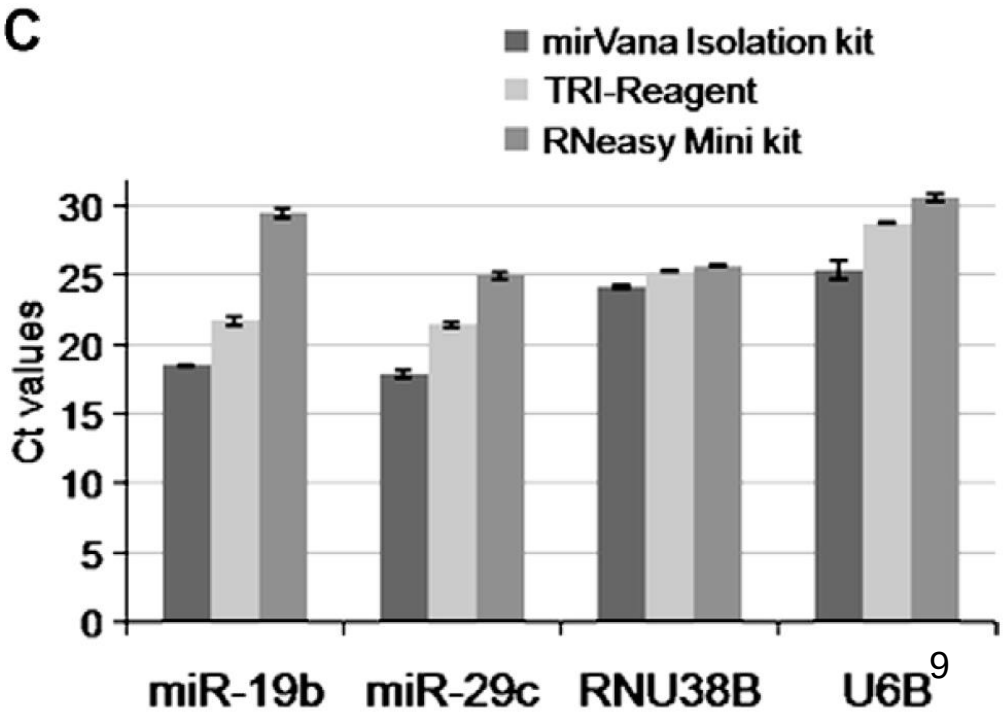
TRIzol/TriReagent
miRvana (Ambion)
PureLink (Invitrogen)
a další

Izolace:

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





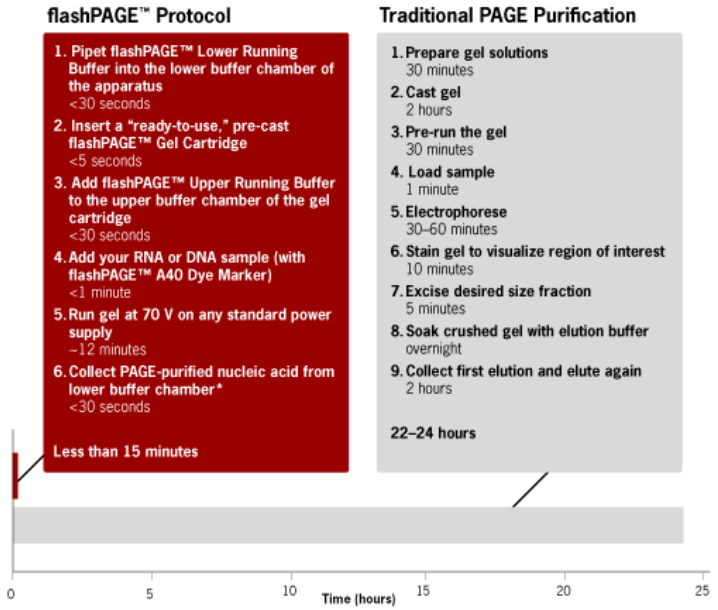


Mraz et al., 2009

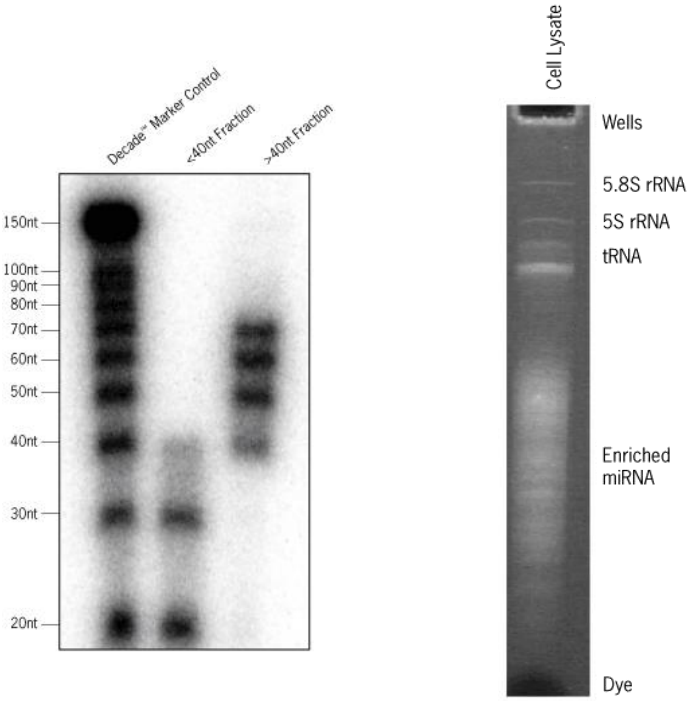
Obohacení:

PAGE

FlashPAGE Fractionator (Ambion)



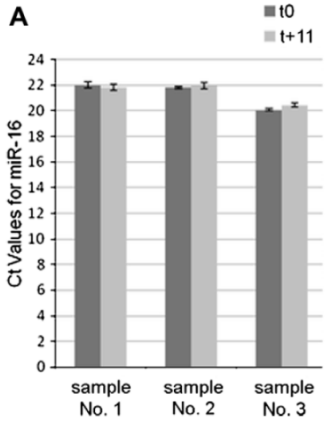
15min 20hours



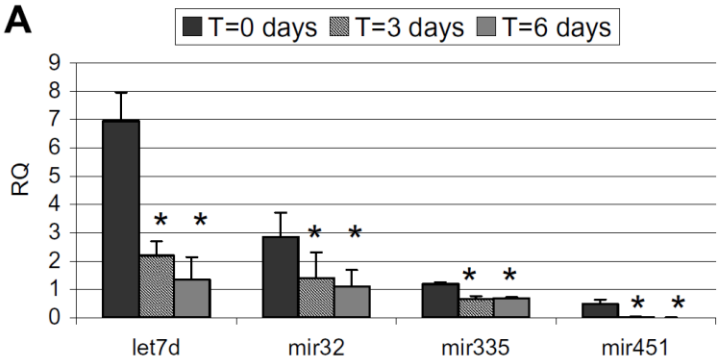
Stabilita microRNA :

Stabilita po izolaci

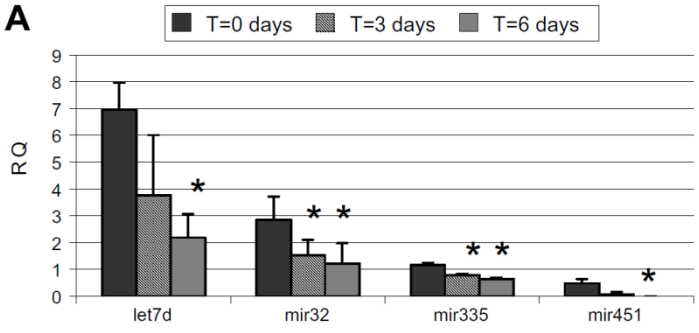
Stabilita v FFPE (formalin-fixed parafin-embedded tissue)



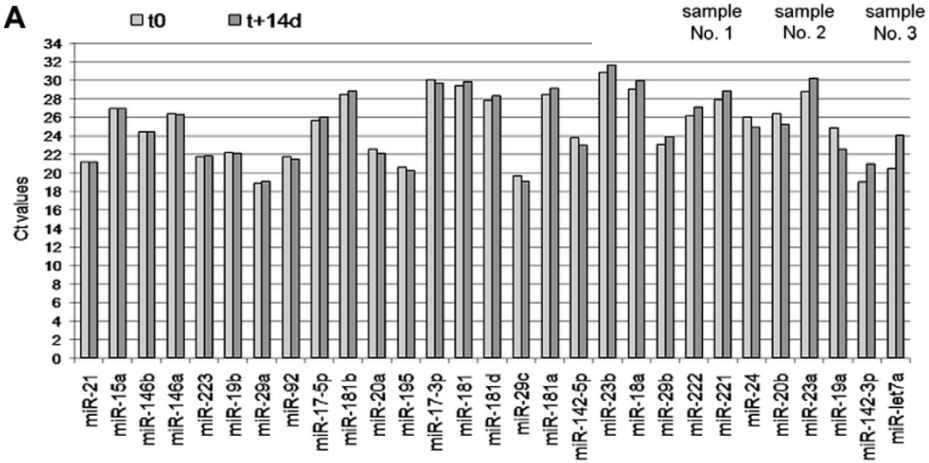
RNA

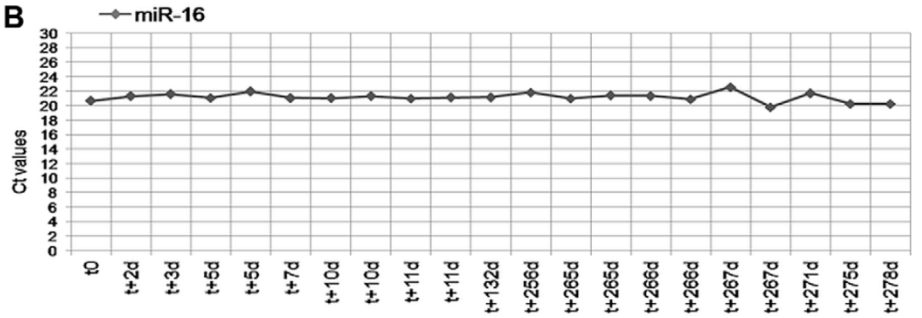


cDNA



Bravo et al., 2007

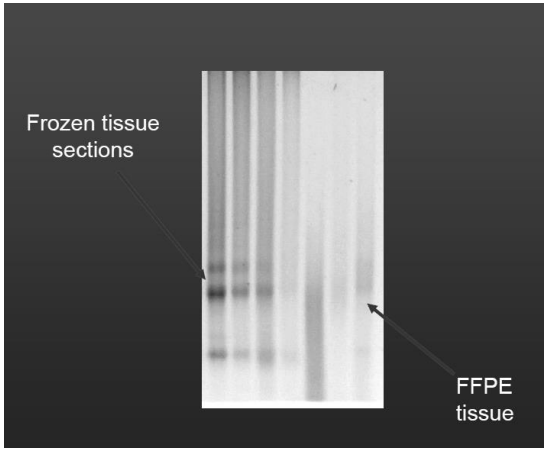


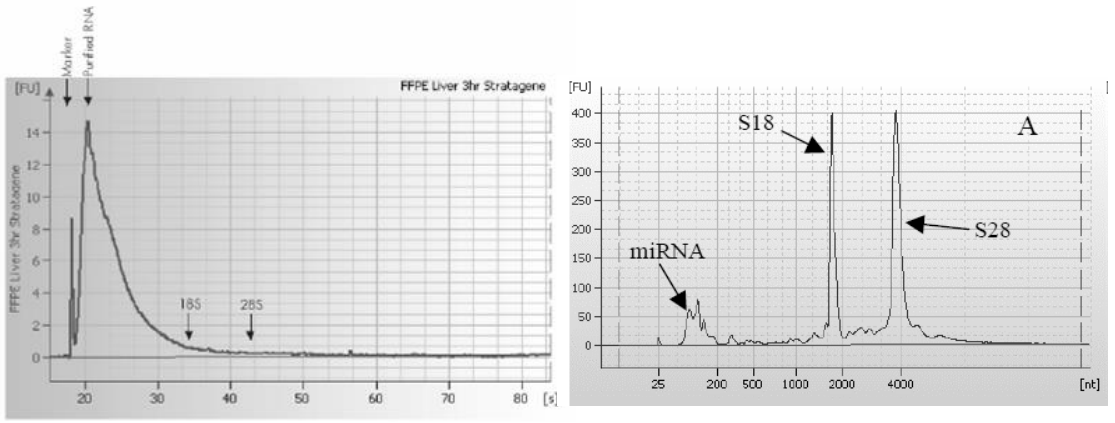


Mraz et al., 2009

Stabilita microRNA :

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





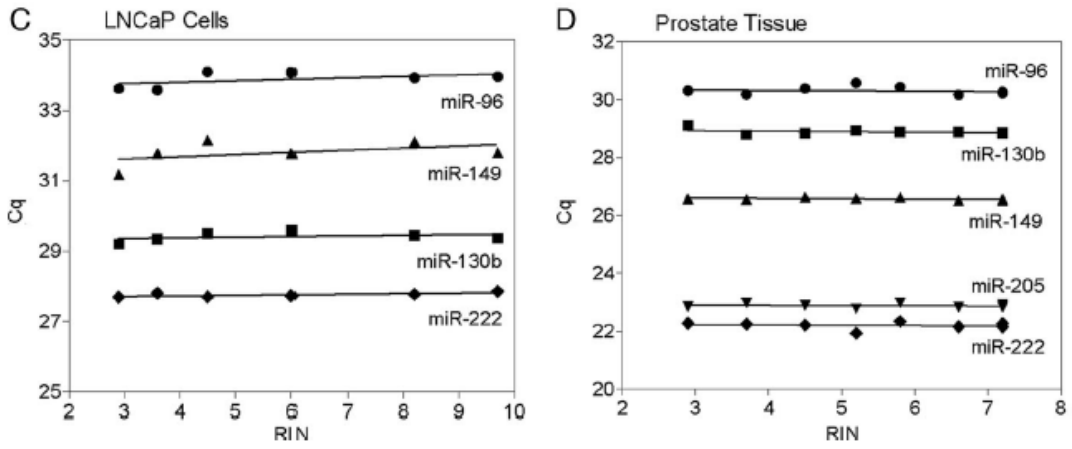


Fig. 2. Influence of RNA integrity on miRNA gene expression.

(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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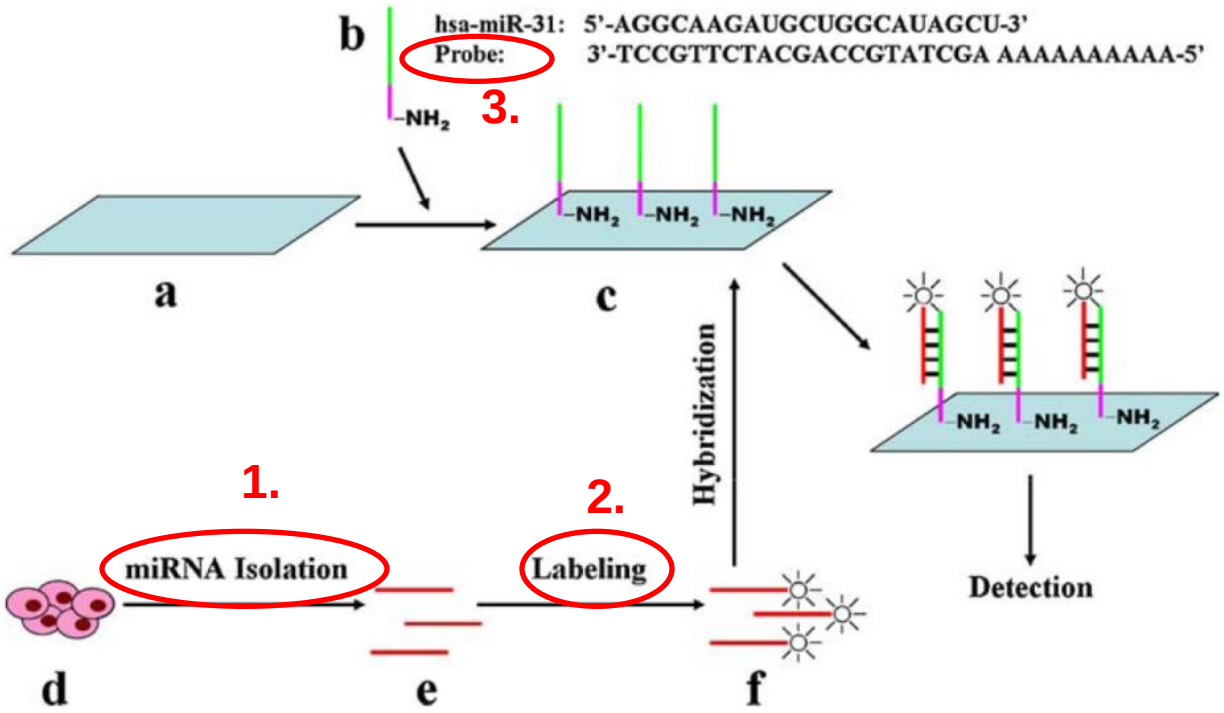
- zatím málo zkušeností a standardizace



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:

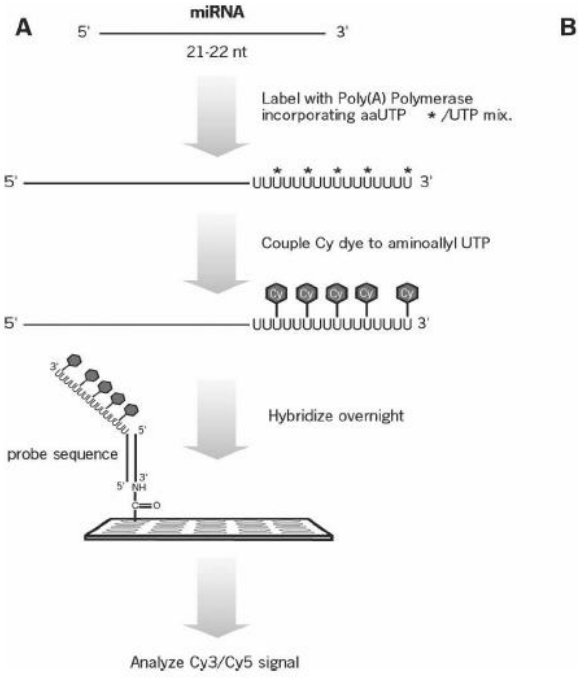
Jednoduché, rychlé a „čím méně kroků tím méně vnesených chyb a variability“

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)



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)

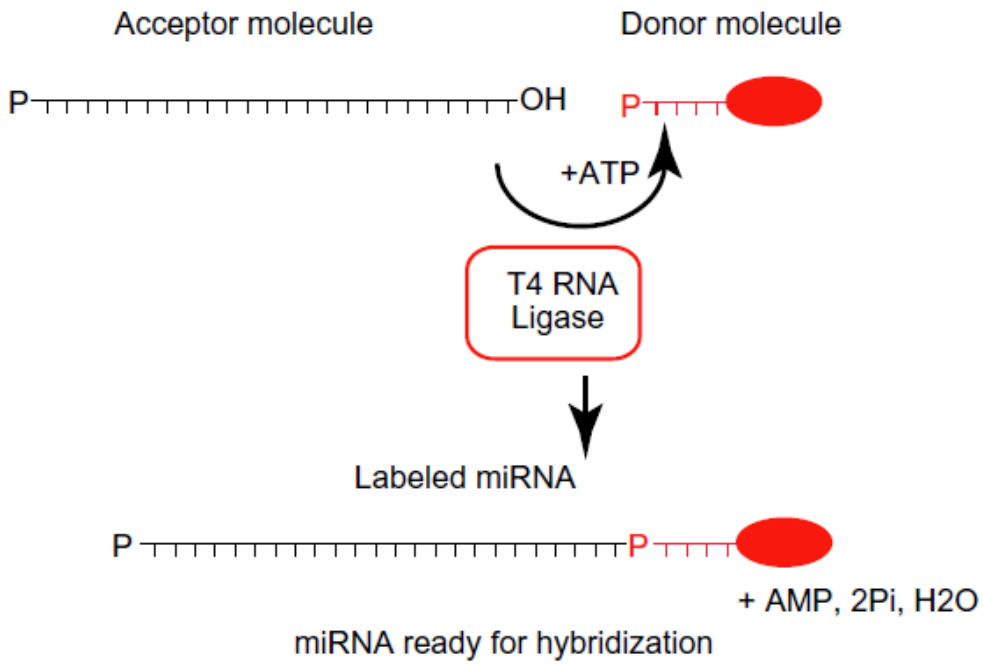


Fig. 2. Schematic representation of the miRNA labeling principle: a short Cy-dye labeled RNA-linker (donor molecule) is ligated to the single-stranded miRNA (acceptor molecule) by T4 RNA ligase in the presence of ATP.

Nepřímé značení:

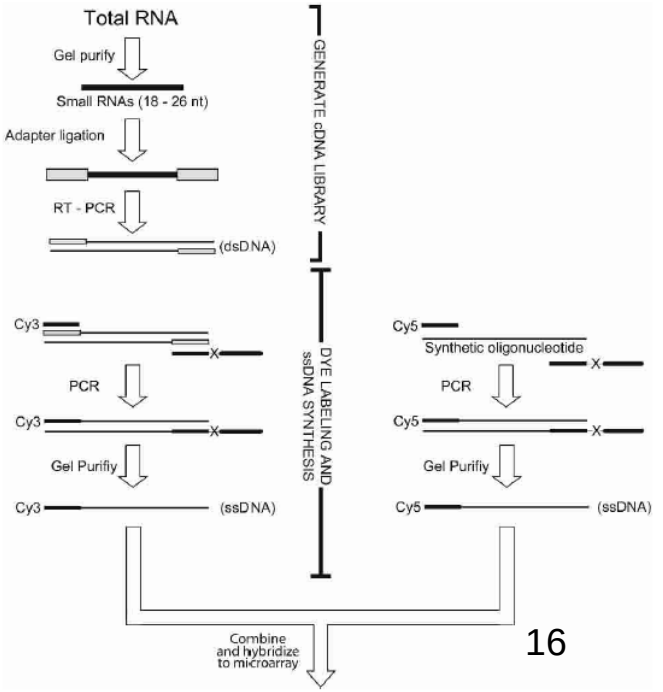
Značen je produkt reverzní transkripce či PCR
Výhody: cDNA je pak stabilní a lze uchovat, Pre-amplifikace a tím snadnější detekce méně exprimovaných miRNA

1/ značení revezního transkriptu miRNA

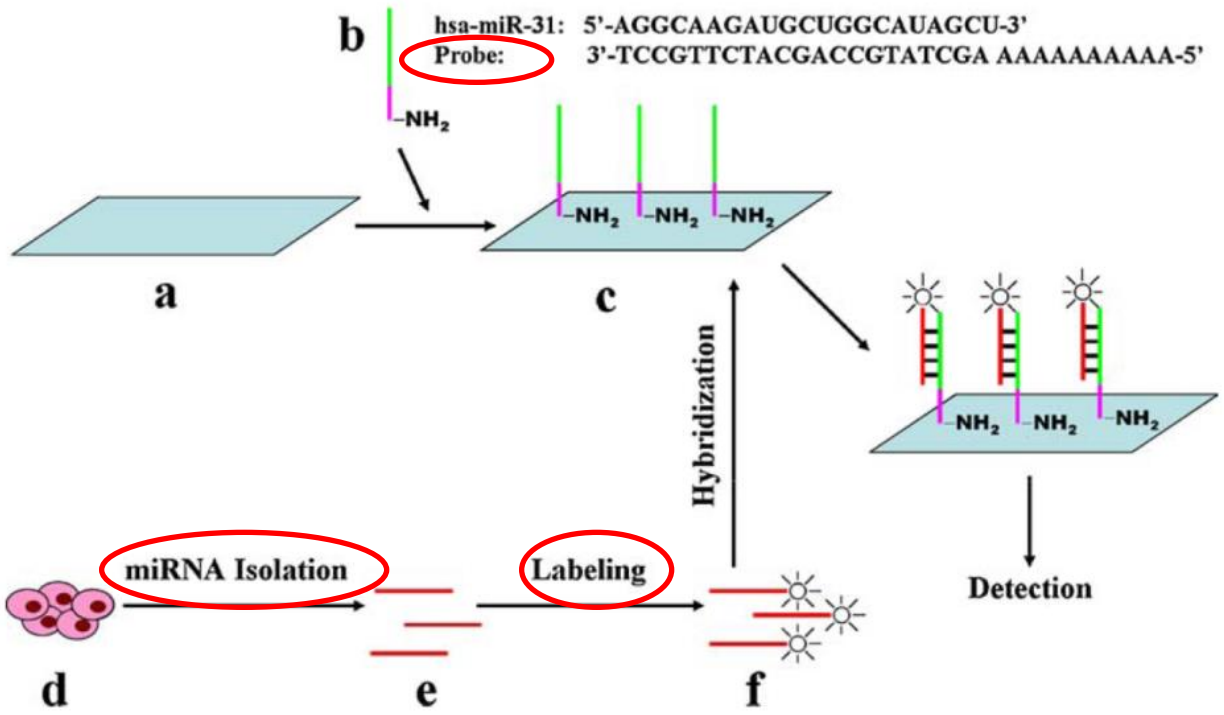
Reverzní transkripce pomocí náhodných 8-merů značených 2 biotiny (3‘-(N)8 – (A)12-biotin-(A)12-biotin-5‘ (Liu et al., 2004)
Reverzní transkripce pomocí náhodných neznačených 7-merů, následně označeny s pomocí terminální transferázy a biotin-dideoxy-UTP (Sun et al., 2004)
Nebezpečí chyb z nespecifické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

```
let-7b : TGAGGTAGTAGGTTGTGTGGTT : 22
let-7e : TGAGGTAGGAGGTTGTATAGT- : 21
let-7d : AGAGGTAGTAGGTTGCATAGT- : 21
let-7a : TGAGGTAGTAGGTTGTATAGTT : 22
let-7f : TGAGGTAGTAGATTGTATAGTT : 22
let-7i : TGAGGTAGTAGTTTGTGCT--- : 19
let-7g : TGAGGTAGTAGTTTGTACAGT- : 21
         tGAGGTAGtAG TTGt   gt
```

ÚPRAVA DÉLKY

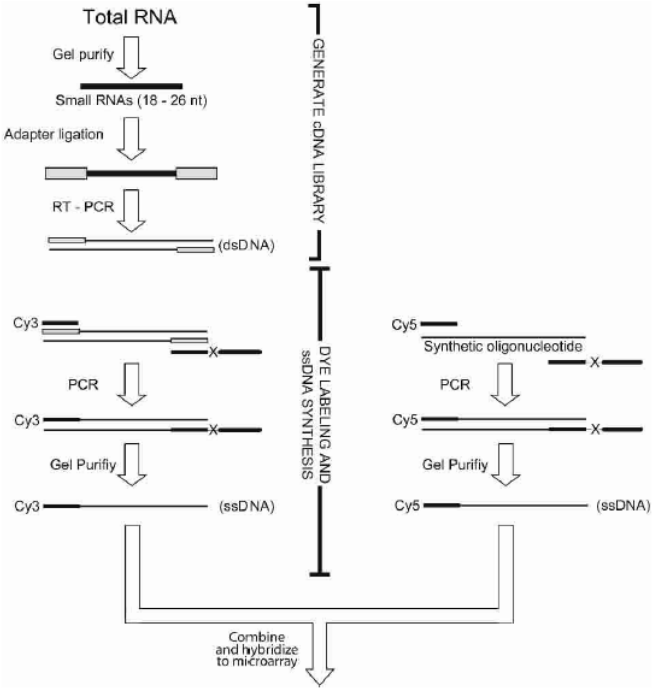
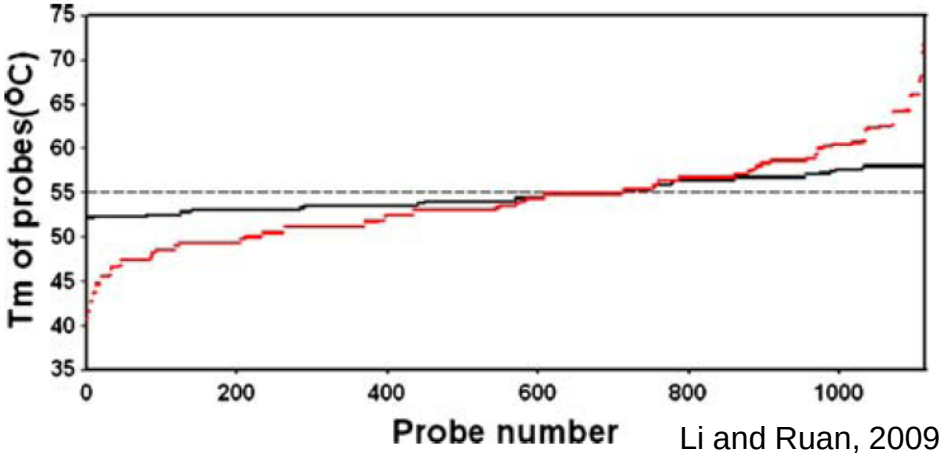


FIGURE 1. Microarray sample preparation and reference oligonucleotide synthesis. Small RNAs were fractionated on a polyacrylamide gel, and oligonucleotide primers were then ligated to the 5′ and 3′ ends of the small RNA library (Lau et al. 2001). A cDNA library was generated through reverse transcription, 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

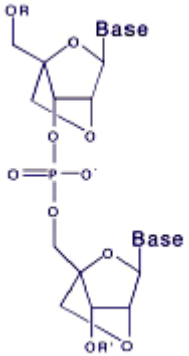


Li and Ruan, 2009

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)

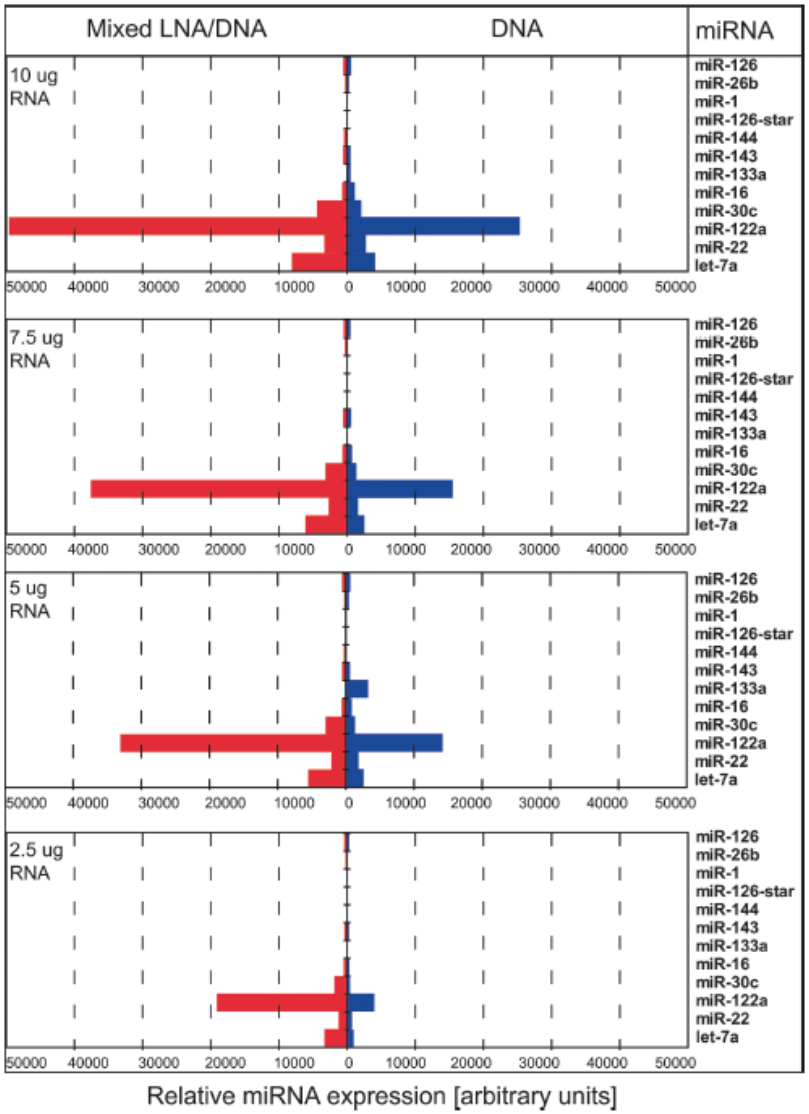
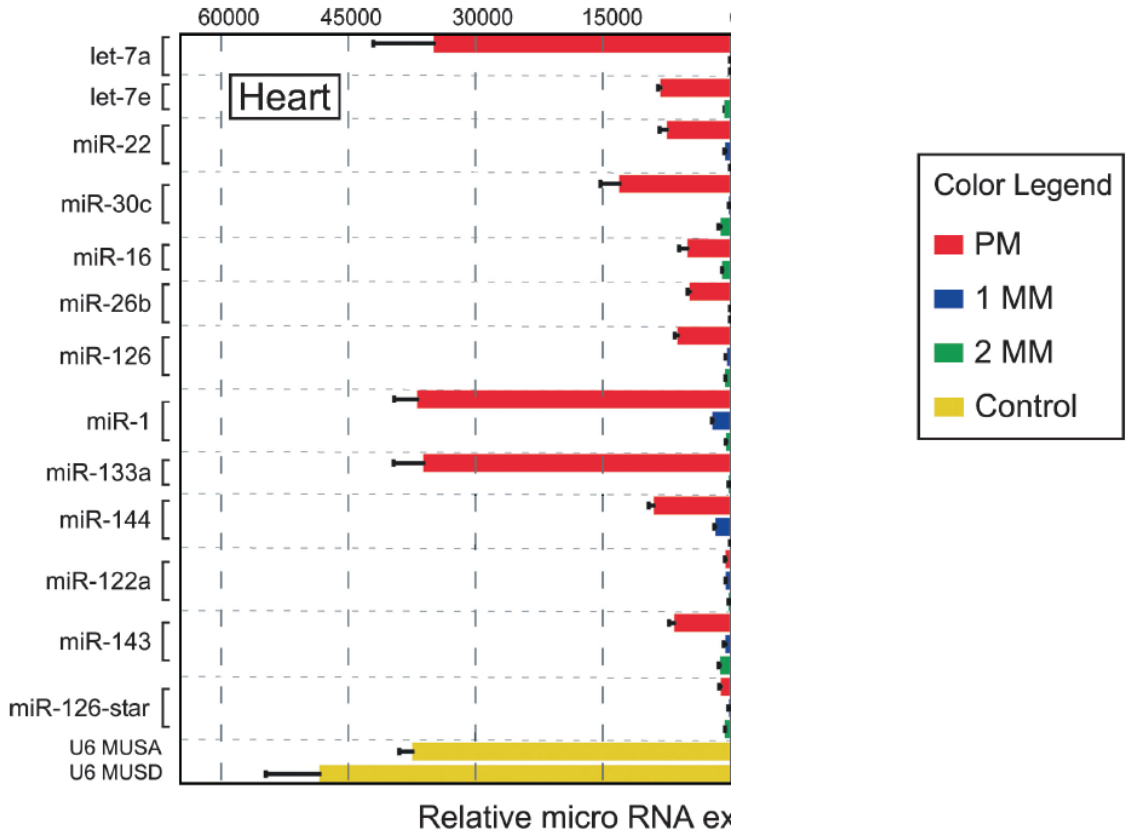


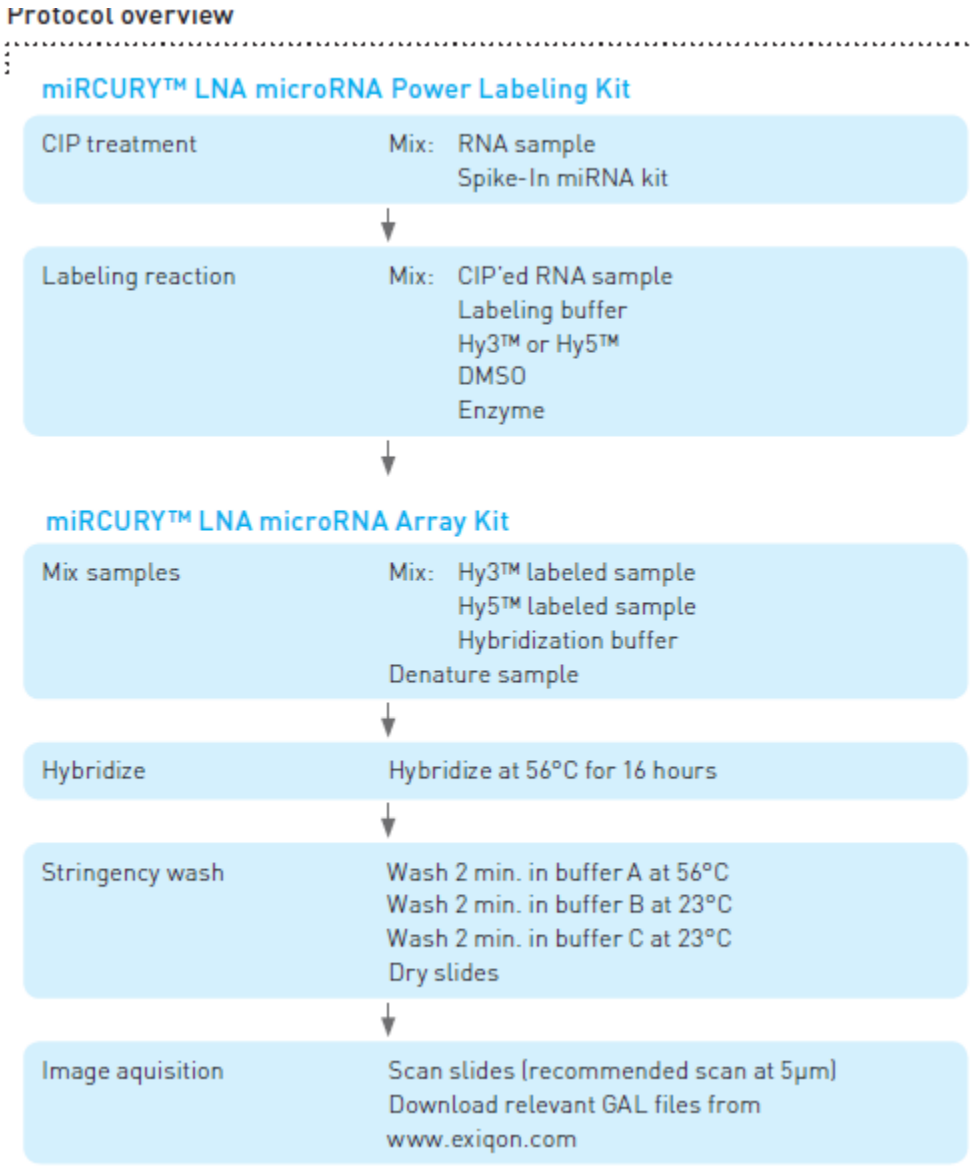
FIGURE 1. Mixed DNA/LNA capture probes display increased sensitivity for miRNA detection. miRNA expression was assessed in murine liver using a test set of LNA-modified (*left*) or unmodified DNA oligonucleotide capture probes (*right*). Decreasing amounts of total RNA were used as input material for 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)

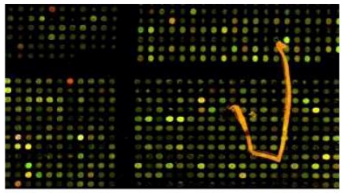


miRCURY LNA Array, Exiqon : 3 dny

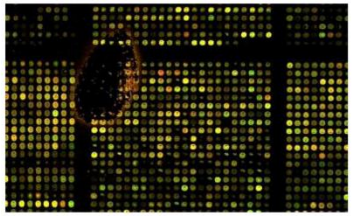


Co se nemusí podařit:

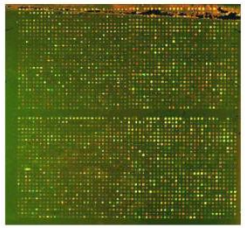
Nekvalitní RNA
Nepodaří se značení
Nepodaří se hybridizace
Nepodaří se promývání
Technická variabilita čipů je větší než ta biologická
Nepodaří se validace dat pomocí RT-PCR, atd



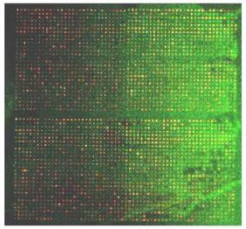
Fiber or scratch?



Bubble



Edge effect

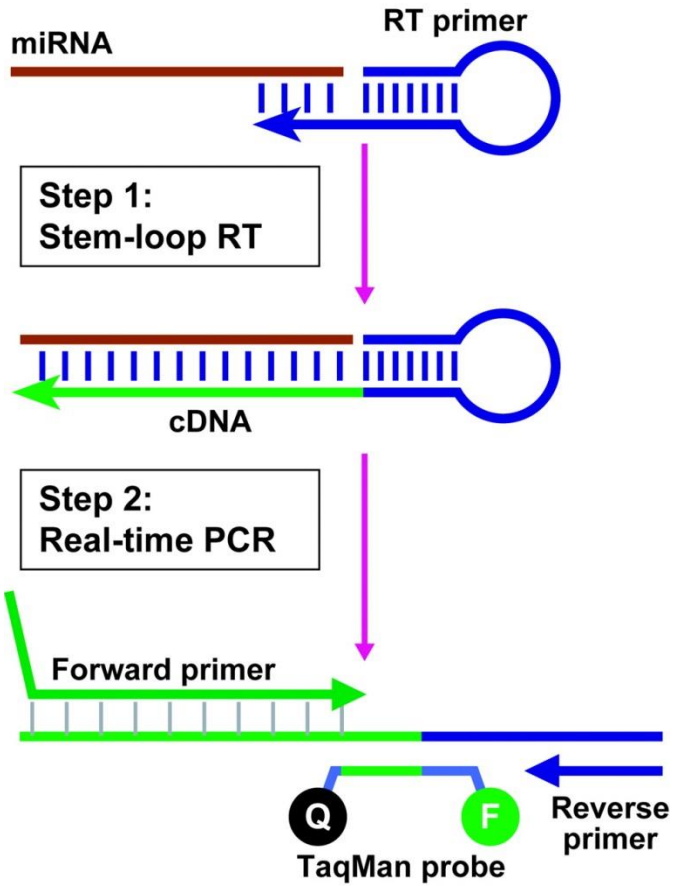


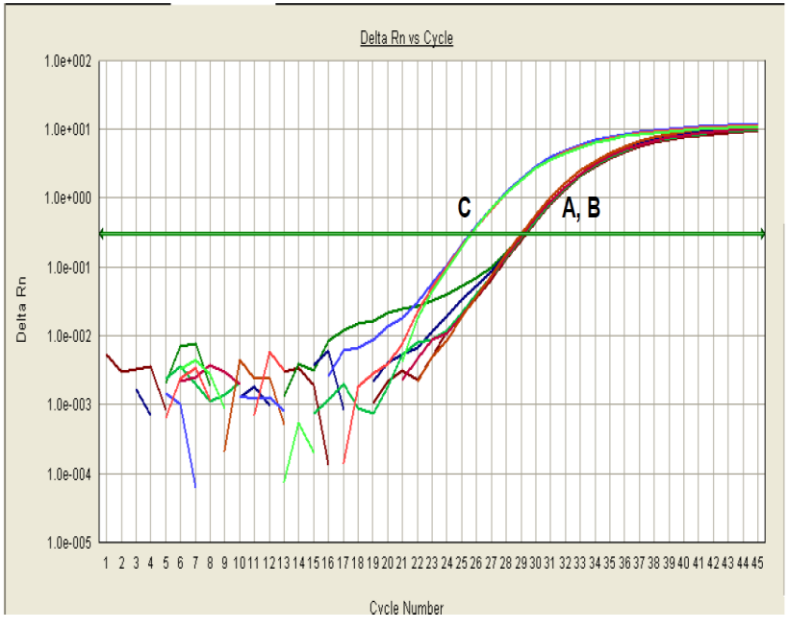
Background haze

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.)

RT-PCR

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

RNA Seq

Sanger vs NGS

‘Sanger sequencing’ has been the only DNA sequencing method for 30 years

……..

NGS has the ability to process millions of sequence reads in parallel rather than few at a time

MPS workflow

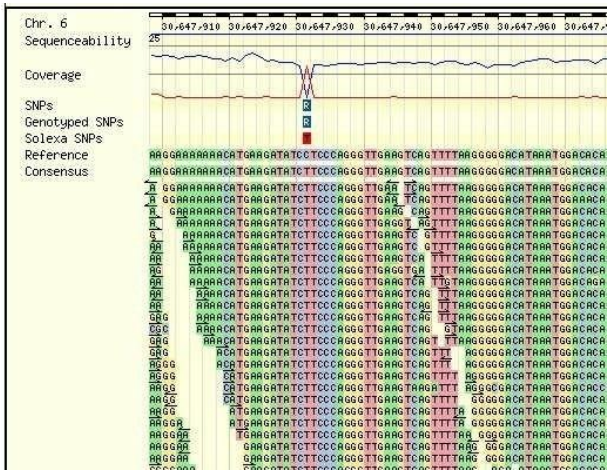
A SAMPLE

1) **Library preparation**

2) **Cluster generation on a flow cell**

3) **Sequencing & imaging**

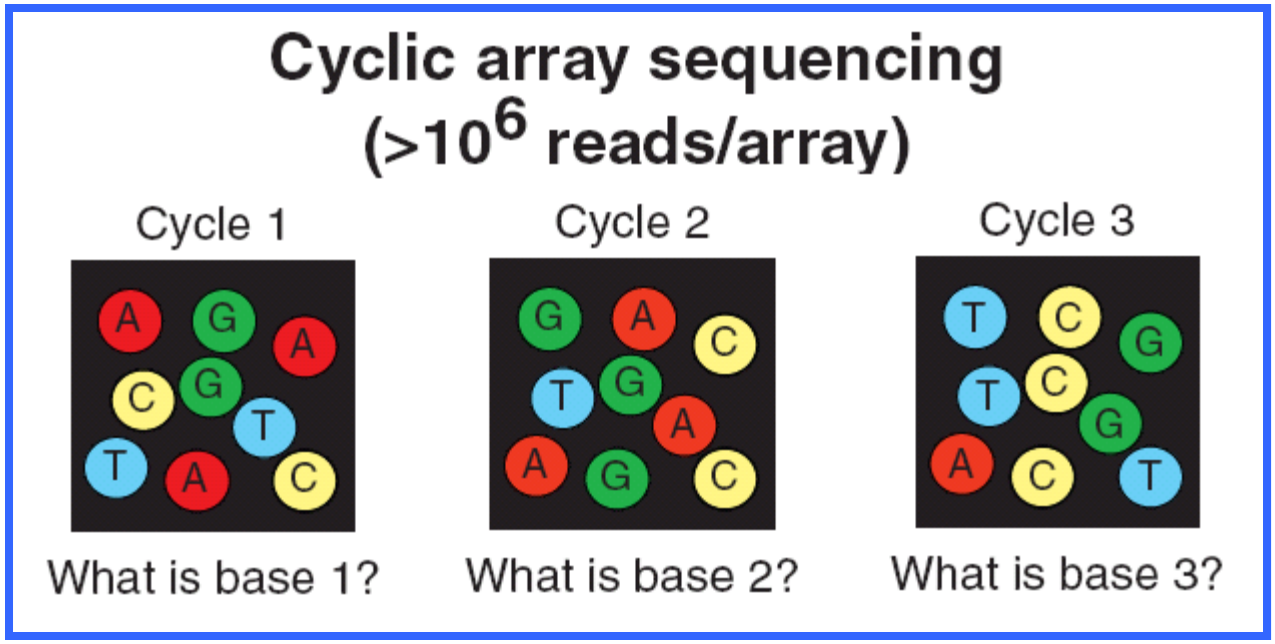
SE, PE reads, 50-250 bases (miseq)



4) **Data processing & analysis**

High Parallelism is Achieved in Polony Sequencing

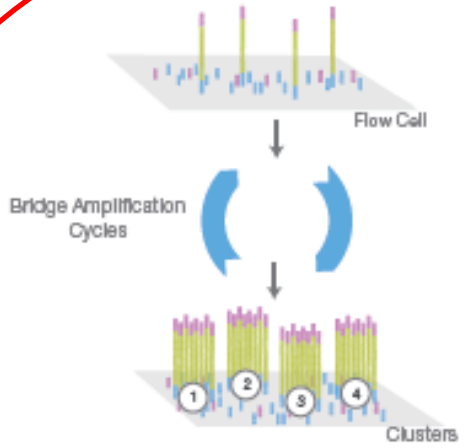
Sanger **Polony**



A. Library Preparation

NGS library is prepared by fragmenting a gDNA sample and ligating specialized adapters to both fragment ends.

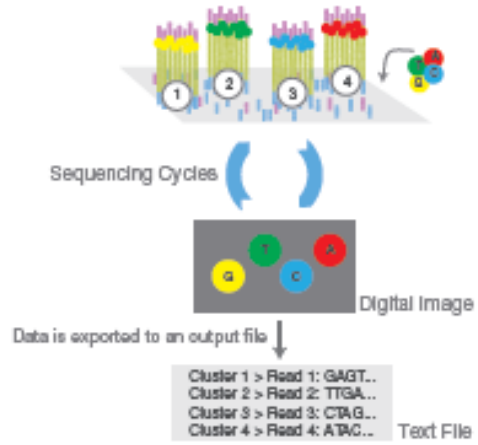
A. Cluster Amplification



Library is loaded into a flow cell and the fragments hybridize to the flow cell surface. Each bound fragment is amplified into a clonal cluster through bridge amplification.

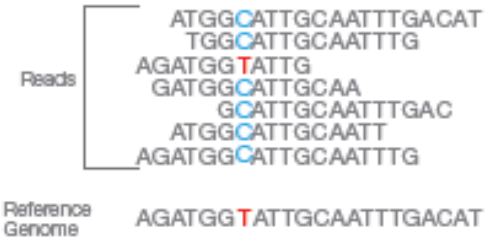
This is the trick

C. Sequencing

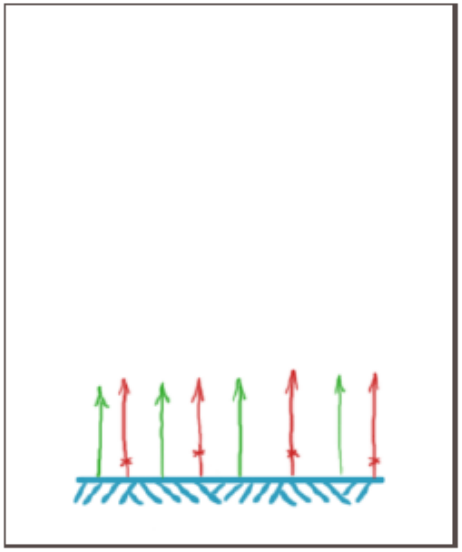


Sequencing reagents, including fluorescently labeled nucleotides, are added and the first base is incorporated. The flow cell is imaged and the emission from each cluster is recorded. The emission wavelength and intensity are used to identify the base. This cycle is repeated "n" times to create a read length of "n" bases.

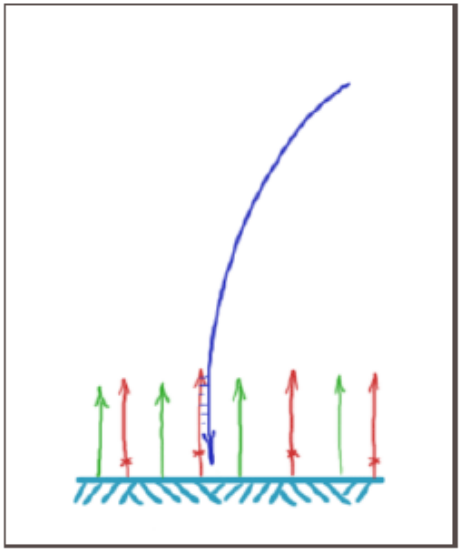
D. Alignment & Data Anaylsis



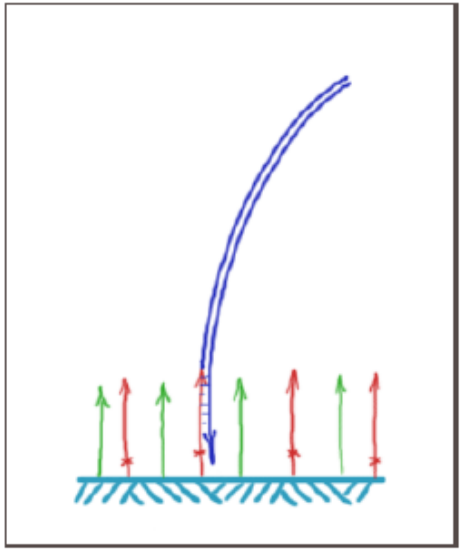
Reads are aligned to a reference sequence with bioinformatics software. After alignment, differences between the reference genome and the newly sequenced reads can be identified.



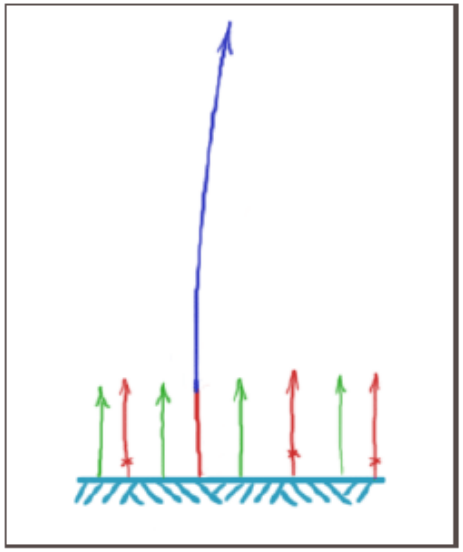
Two PCR primers are attached to the surface of flowcell. One of the primers has a cleavable site (cross on red primer);



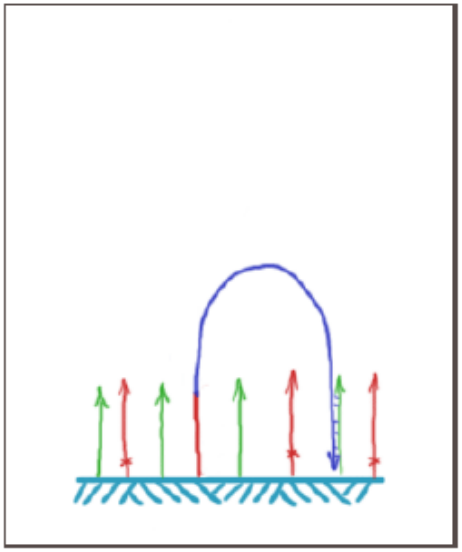
Preamplified library is denaturated in NaOH, then hybridization buffer is added to shift pH to neutral value. Library is loaded into the channel in neutral aquatious solution. DNA molecules can hybridize to the PCR primers. Red primer hybridize with a library molecule on the picture(complementary strand of this molecule have a chance to form a hybrid with a green primer).



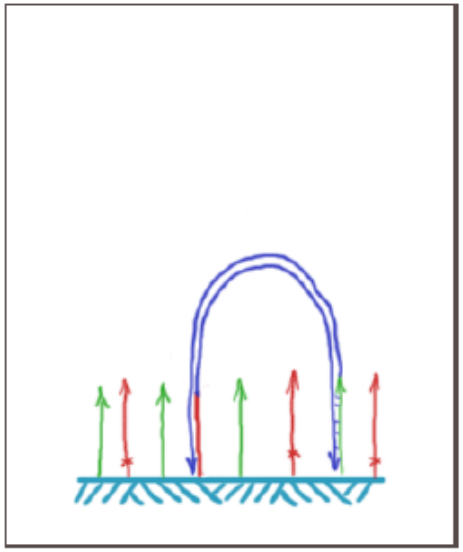
Elongation reaction: extension mix (buffer, dNTP's, Taq polymerase) is pumped into a channel. Hybridized primer extended on library molecule.



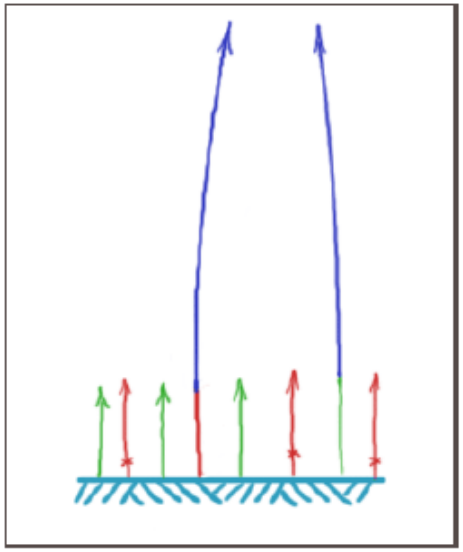
Formamide
Original library molecule is denatured and washed away.



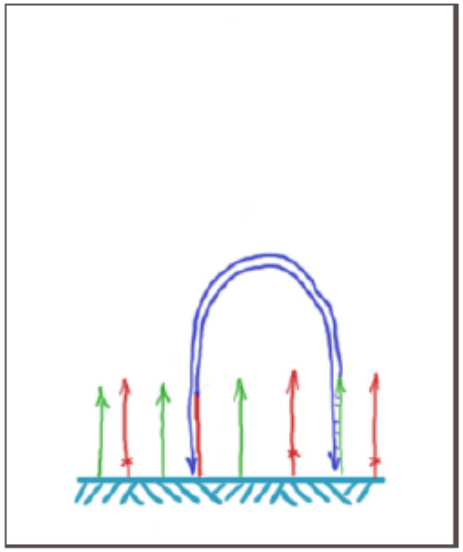
Extension buffer
Extended molecule bends and hybridizes to a second PCR primer (forms a bridge).



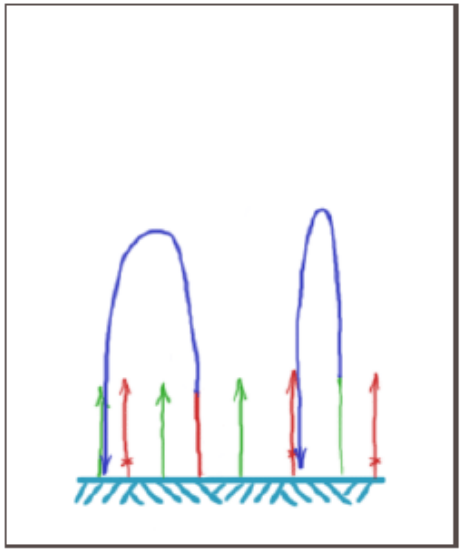
Extension mixture
Extension of hybridized primer.



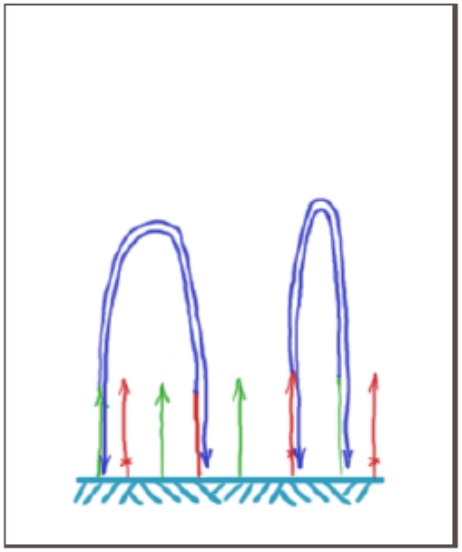
Formamide washing
Two DNA strands are denatured.



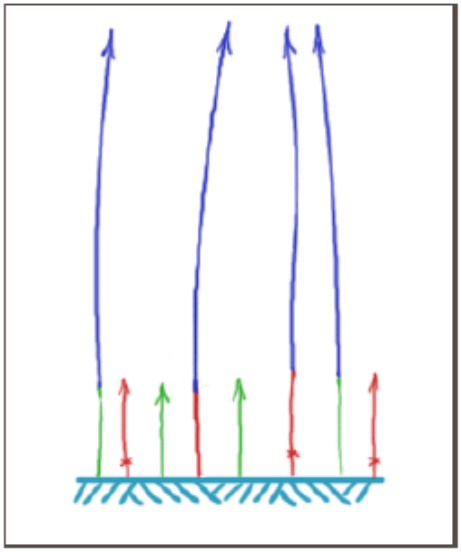
Extension buffer
Extended molecules may hybridize to each other again or find other PCR primers.



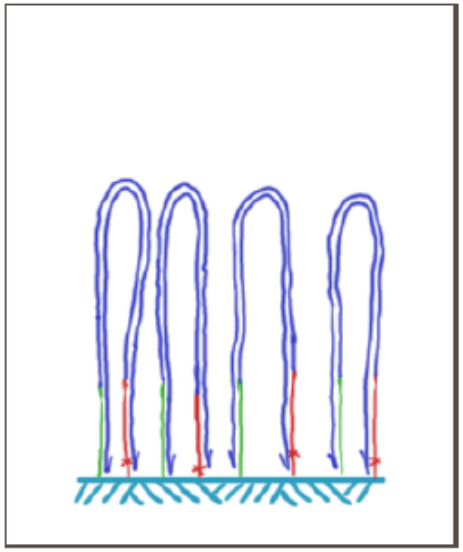
Extension buffer
Extended molecules may hybridize to each other again or find other PCR primers.



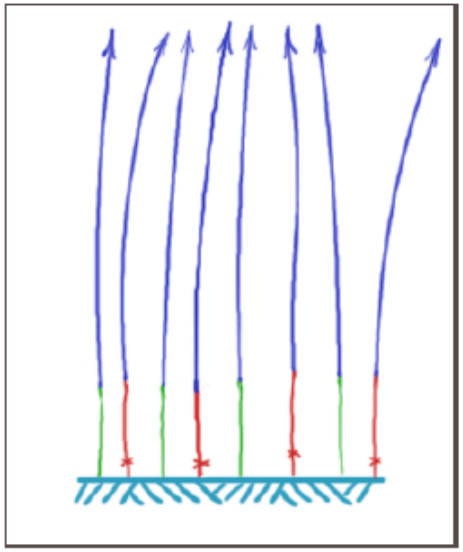
Extension mixture
In the second case they will duplicate again.



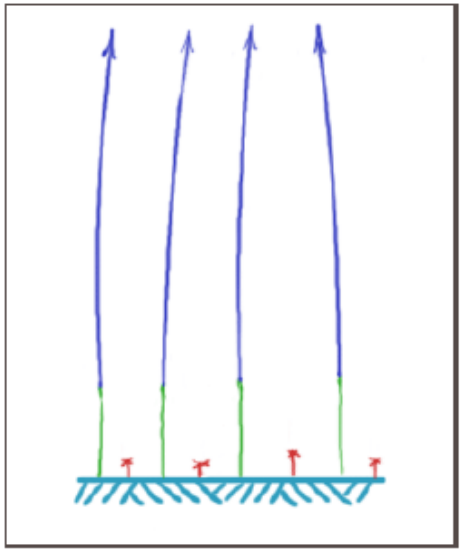
Formamide washing
DNA strands are denatured.



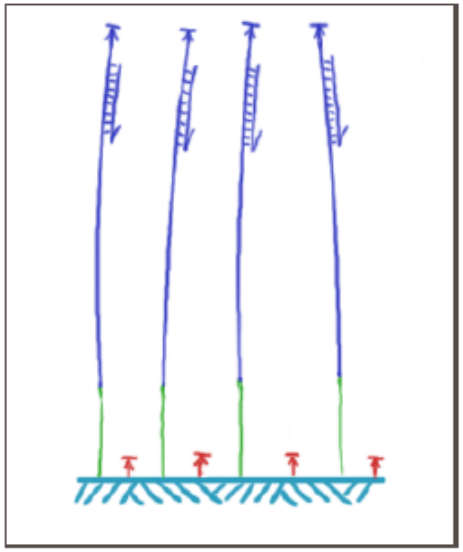
After 35 cycles cluster consists on a number of double-stranded bridges.



DNA is denaturated.



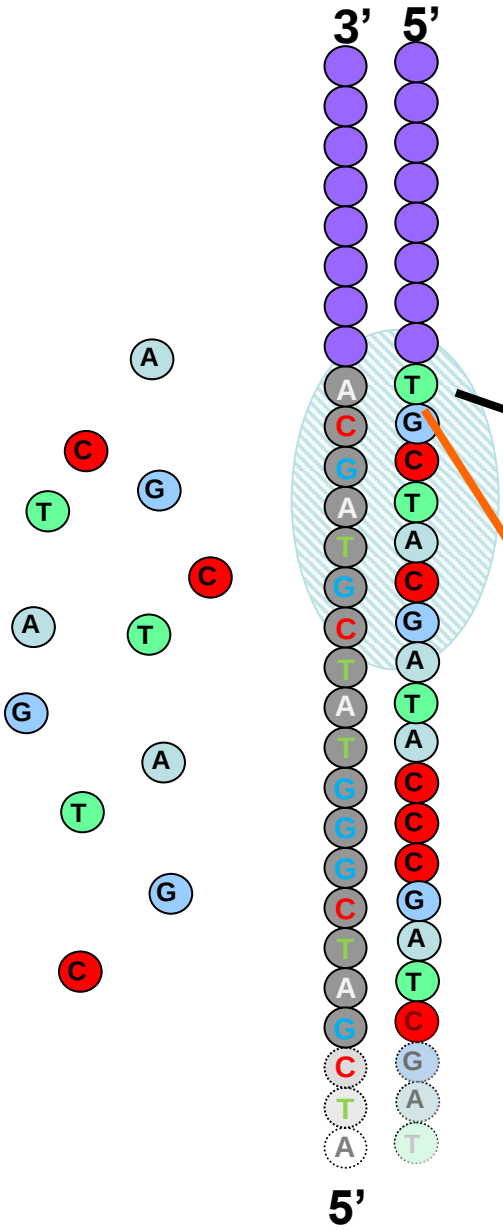
One primer is cut off and washed out.



the last operations, which are done on Cluster station are:

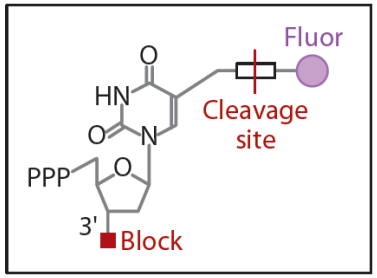
* * blocking of all 3' ends (ddNTP's and terminal transferase) to prevent extension of DNA molecules on each other;
* annealing of sequencing primer.

Sequencing by synthesis



Cycle 1: Add sequencing reagents

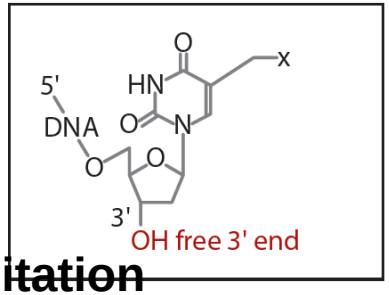
First base incorporated



Emission

Detect signal

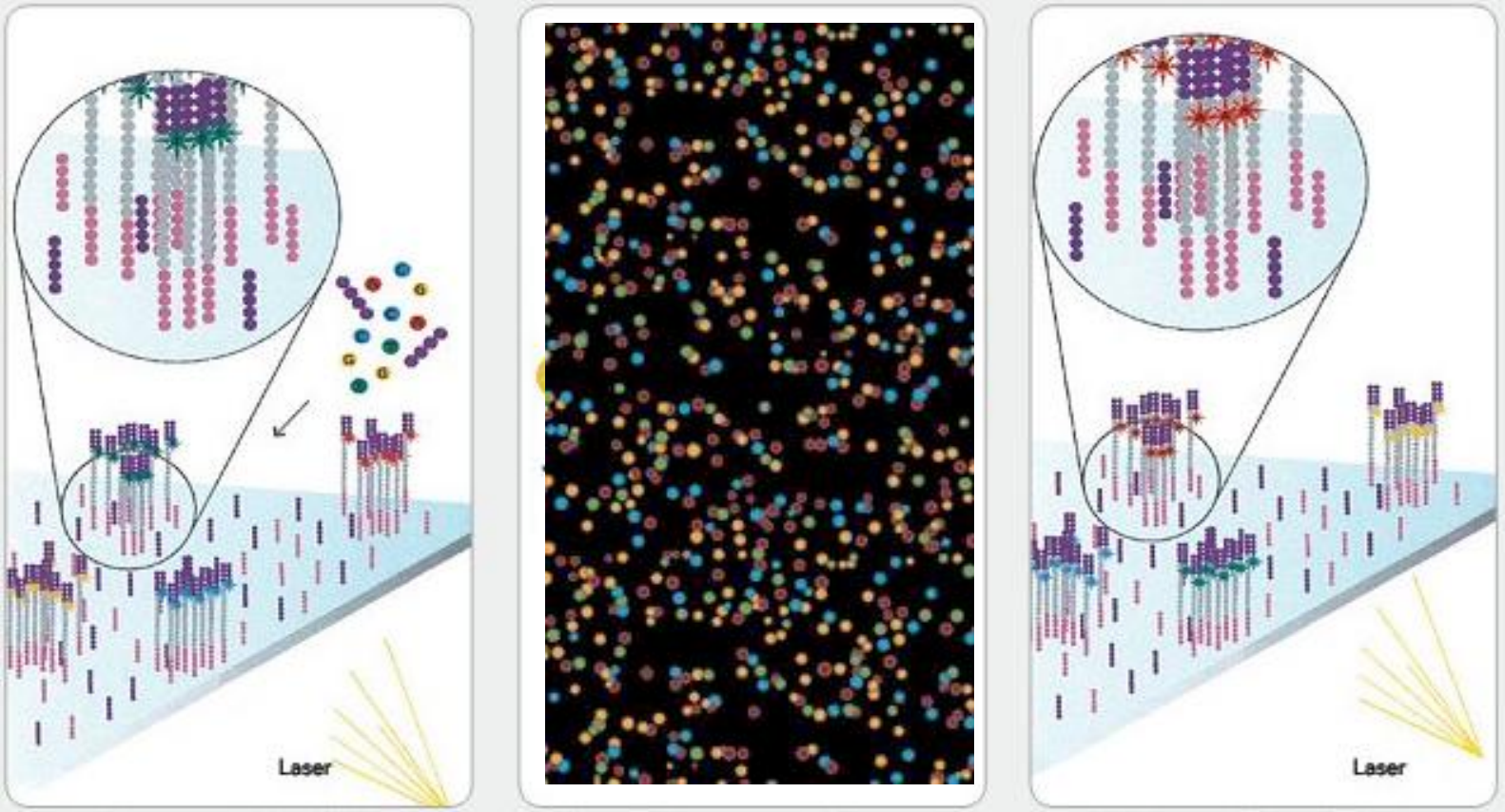
Cleave terminator and dye



Excitation

Cycle 2-n: Add sequencing reagents and repeat

Sequencing by Synthesis - Fluorescently labeled Nucleotides (Illumina)



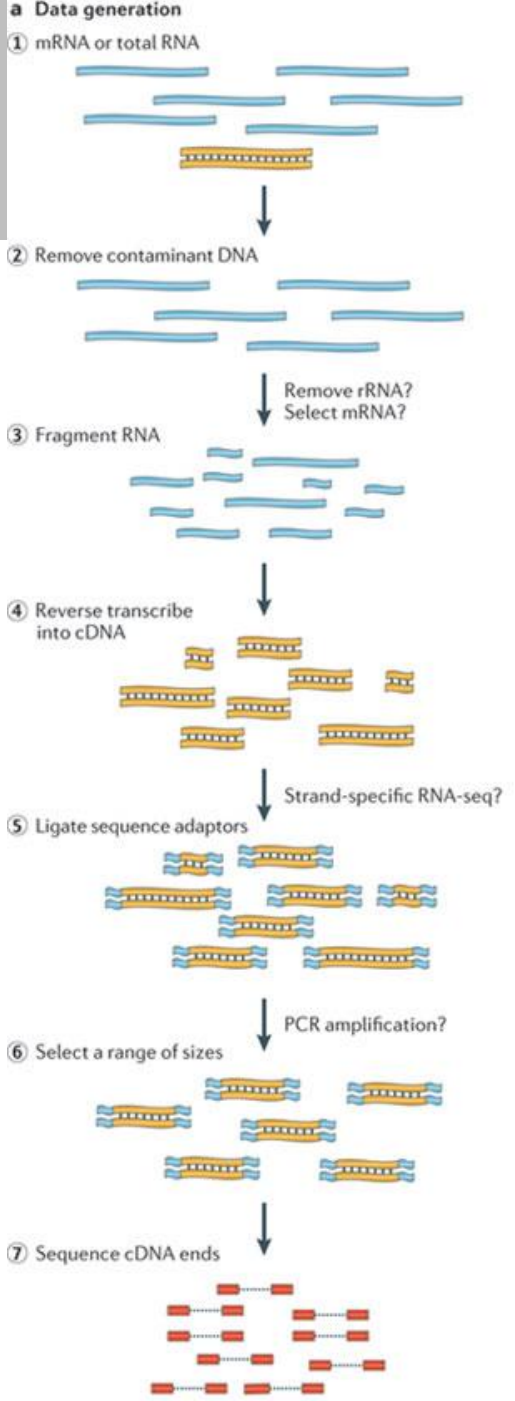
Complementary strand elongation: DNA Polymerase

video

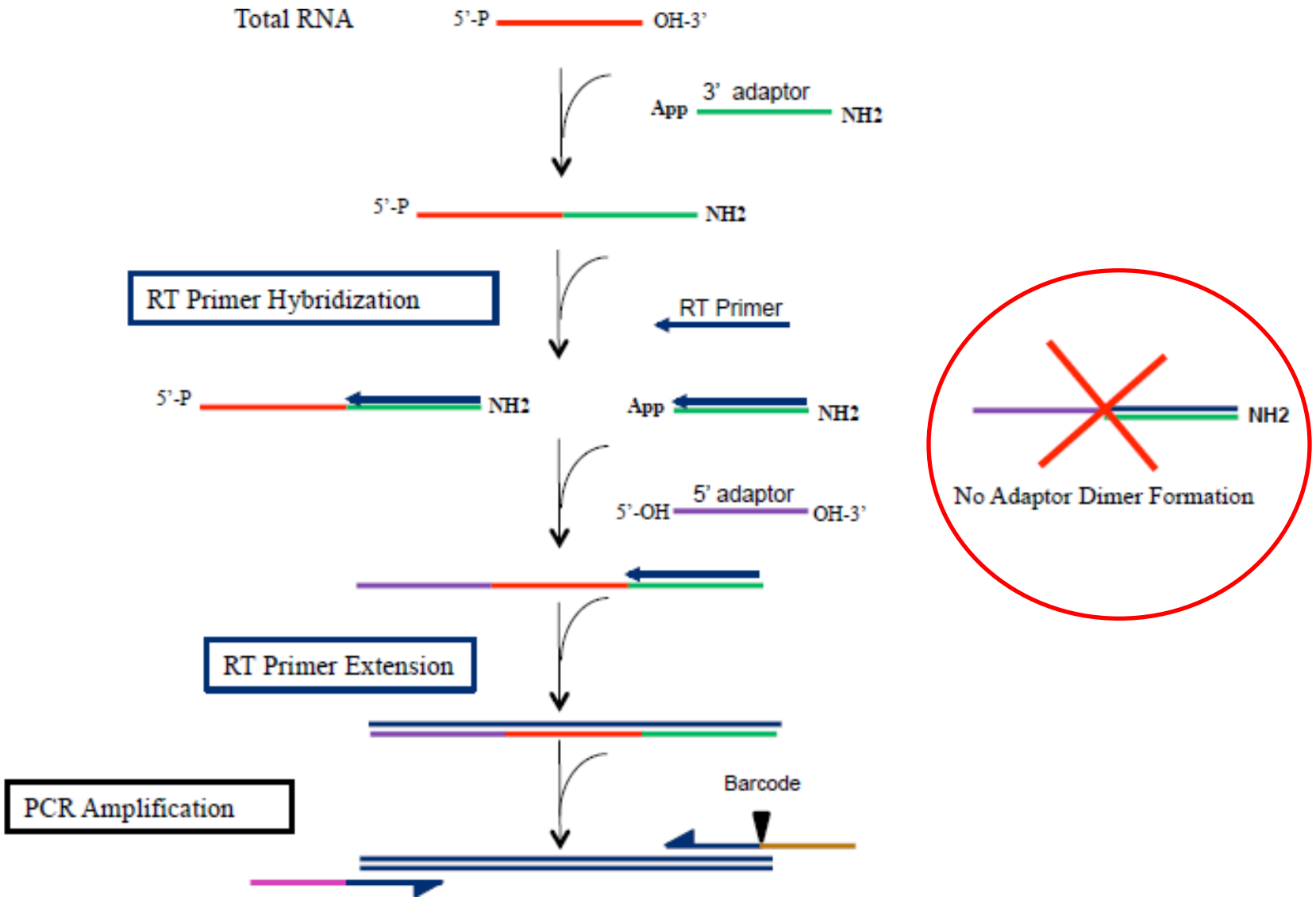
- https://www.youtube.com/watch?v=womKfikWlxM

The general experimental procedure for RNA

Transcriptom = sum of all RNA (mRNA, rRNA, tRNA and noncoding RNA)



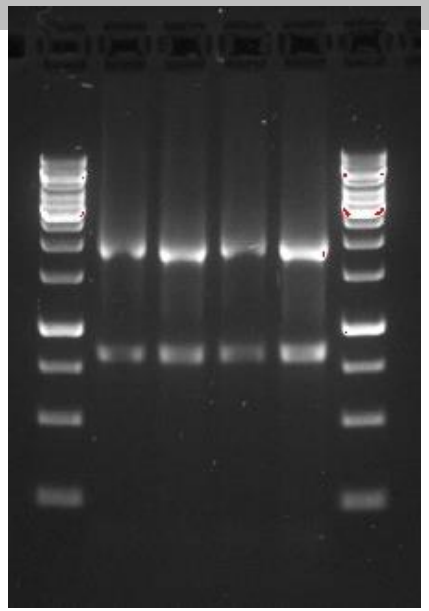
The general experimental procedure for miRNA

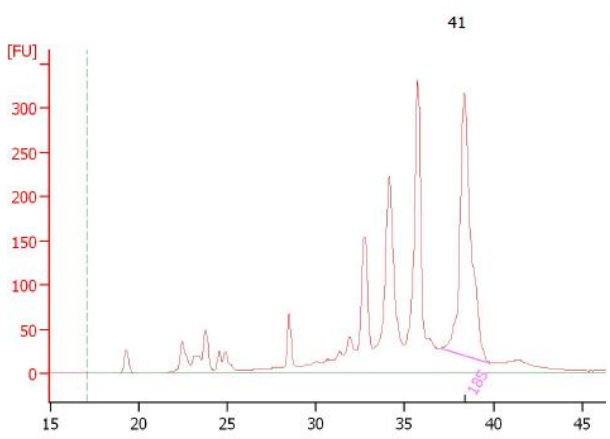


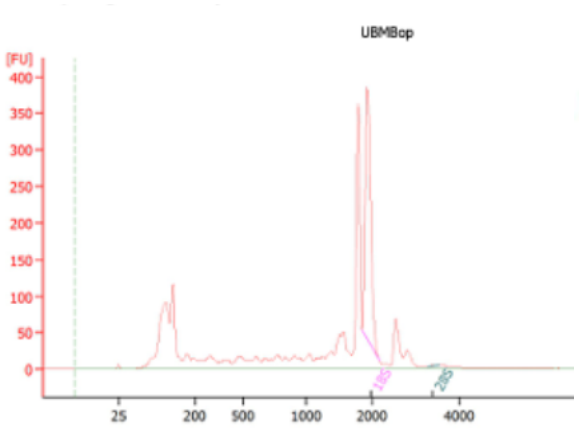
Library preparation

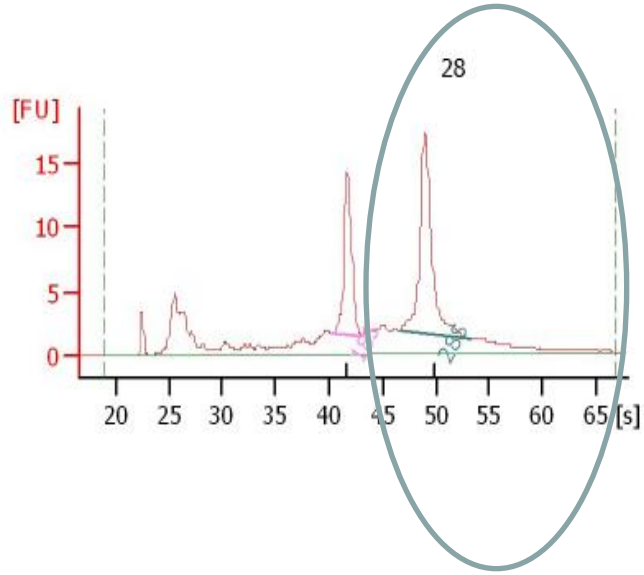
Strict QC of starting material

- appropriate quantification
- gel images, bioanalyzer traces
- which carrier was used – salmon sperm DNA, yeast RNA ☹, linear acrylamide ☺
- How to get rid of rRNA…



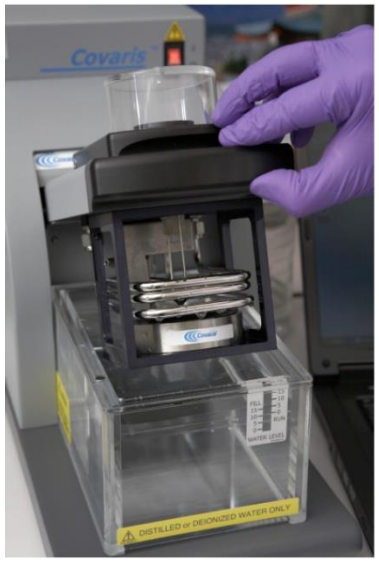






Library preparation

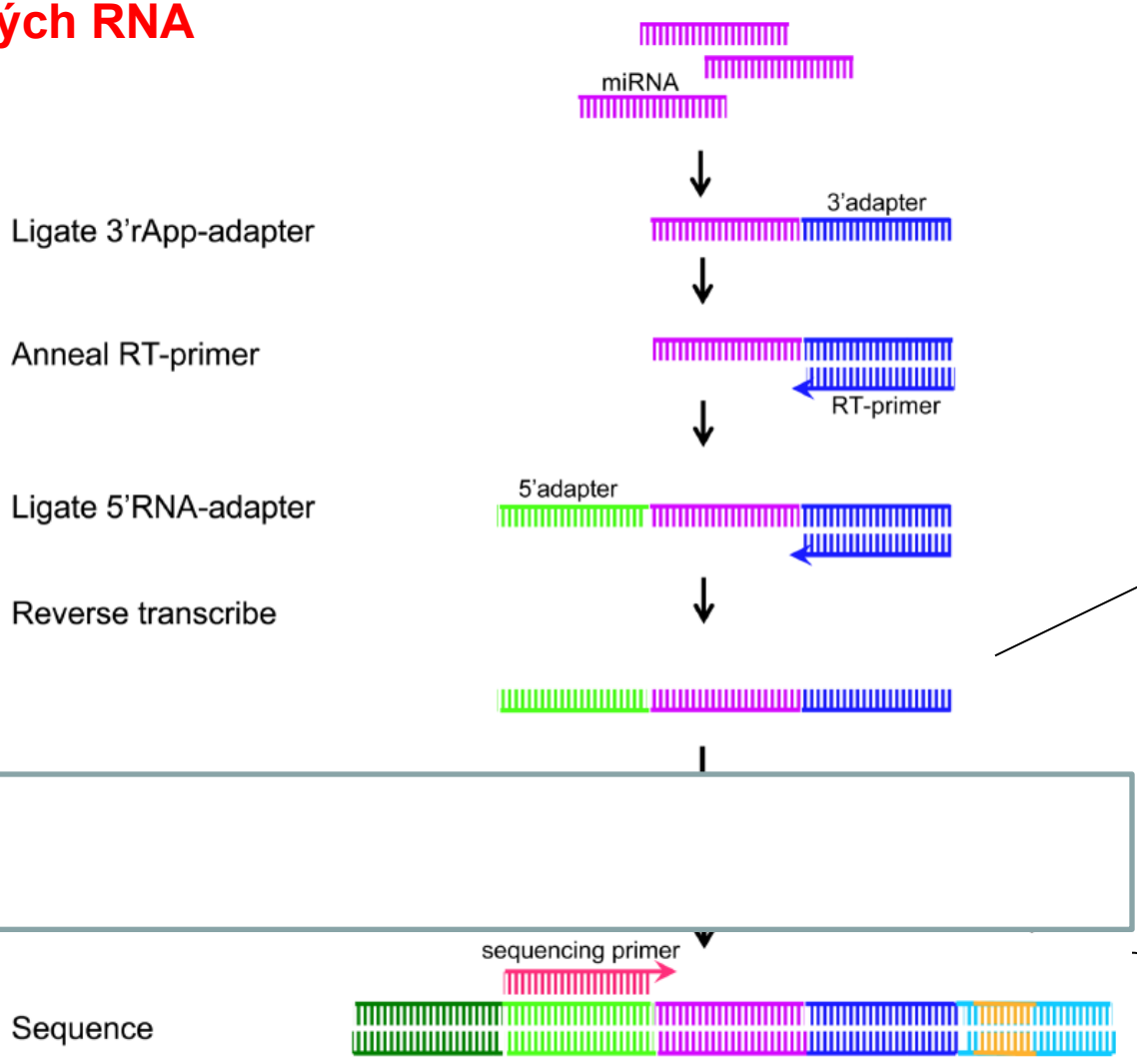
- Fragmentation: Covaris, enzymes, for RNA ions+heat
- Size selection: gel vs beads



Covaris

E-gel

Konstrukce cDNA knihovny malých RNA



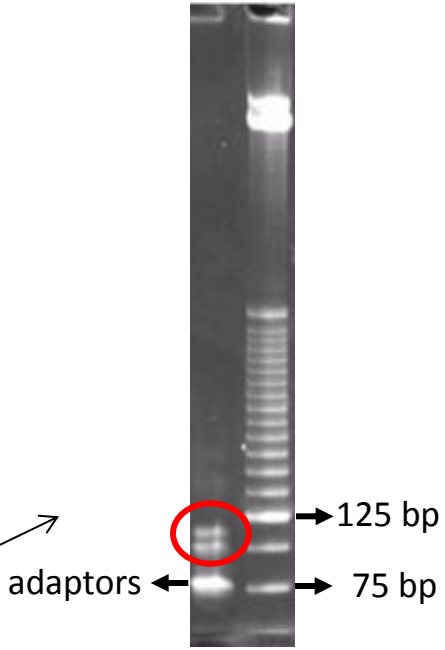
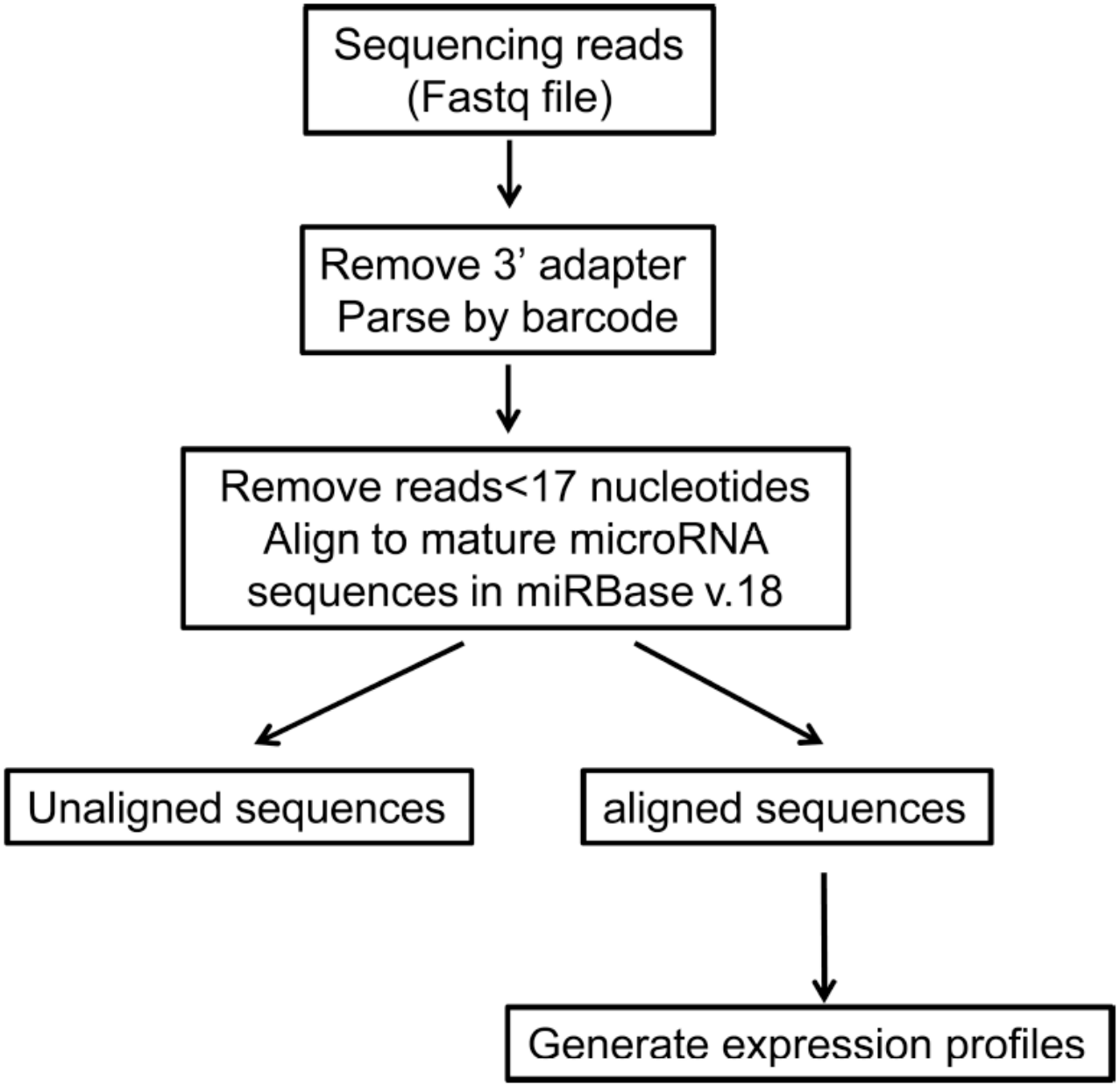


Figure 1.
Construction of multiplex miRNA libraries. Small RNAs are first captured by ligating a 3rApp adapter, annealing an RT primer, and then ligating a 5 RNA adapter. The resulting ligation product is reverse transcribed to obtain cDNA, which is amplified using a PCR1 forward primer and PCR2 barcoded reverse primers to generate an ~135-bp product containing miRNAs (~22 bp). The final product is purified prior to sequencing. Adapted from Vigneault et al. (2012).

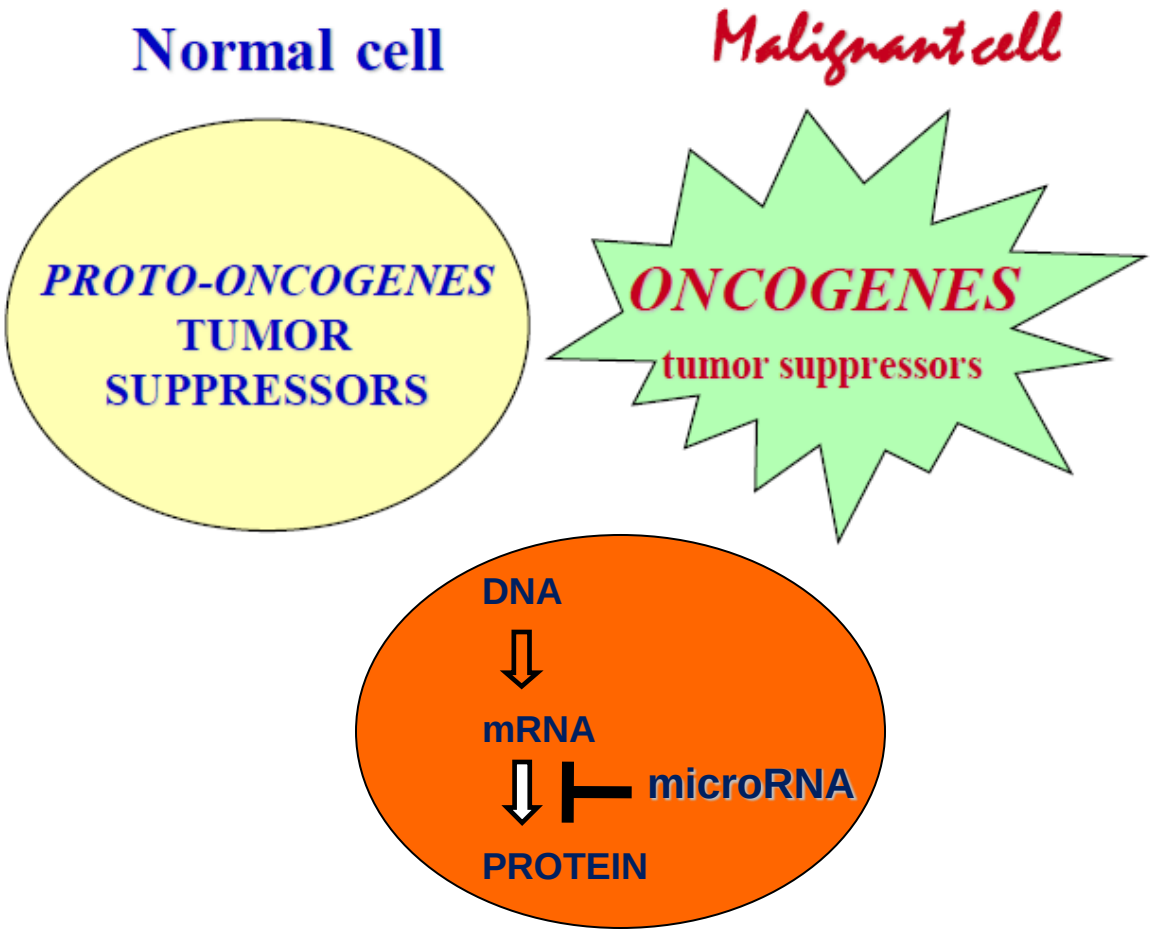

Sequencing reads
(Fastq file)
Remove 3' adapter
Parse by barcode
Remove reads<17 nucleotides
Align to mature microRNA
sequences in miRBase v.18
Unaligned sequences
aligned sequences
Generate expression profiles

ODPOČINEK

Year 2K "Central dogma" of molecular oncology

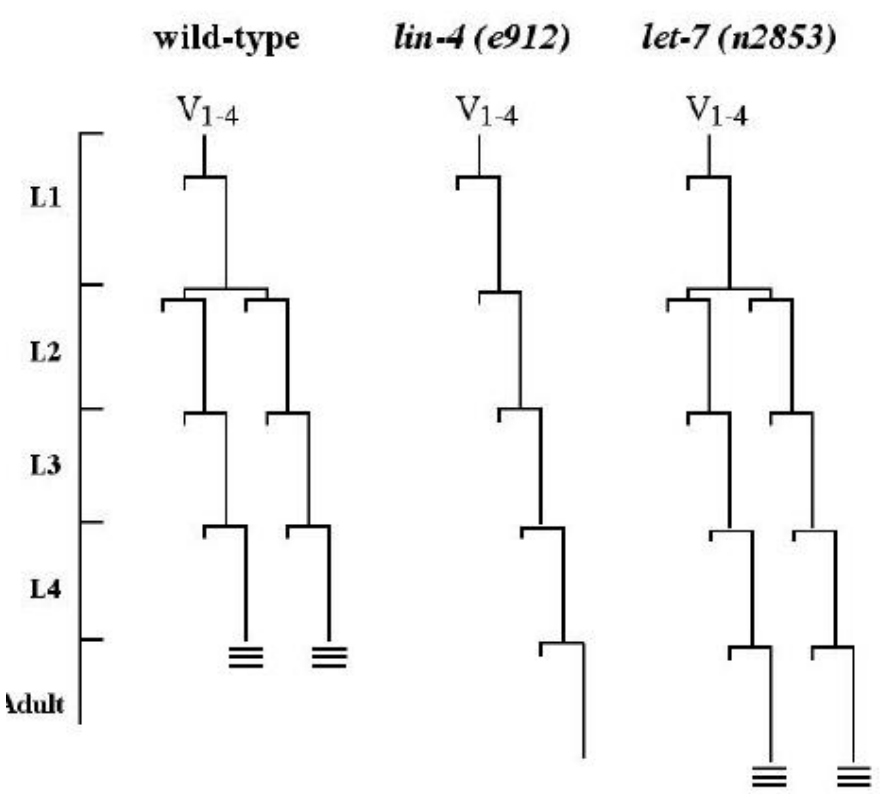
Cancer is the GENETIC DISEASE with the most complex mechanism.

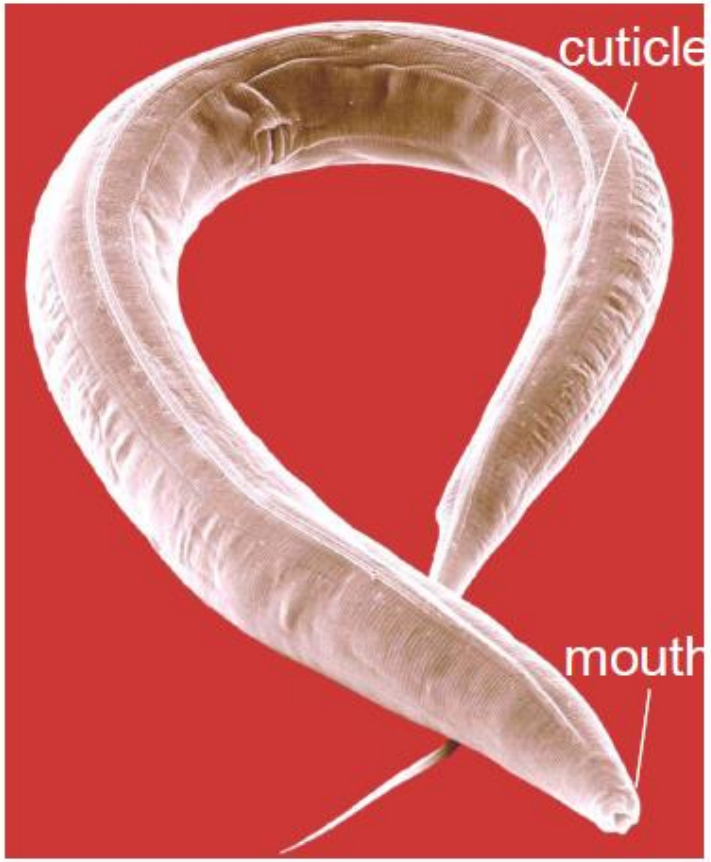
Oncogenes and Tumor-suppressors are the two types of PROTEINS deregulated in cancer cells.



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

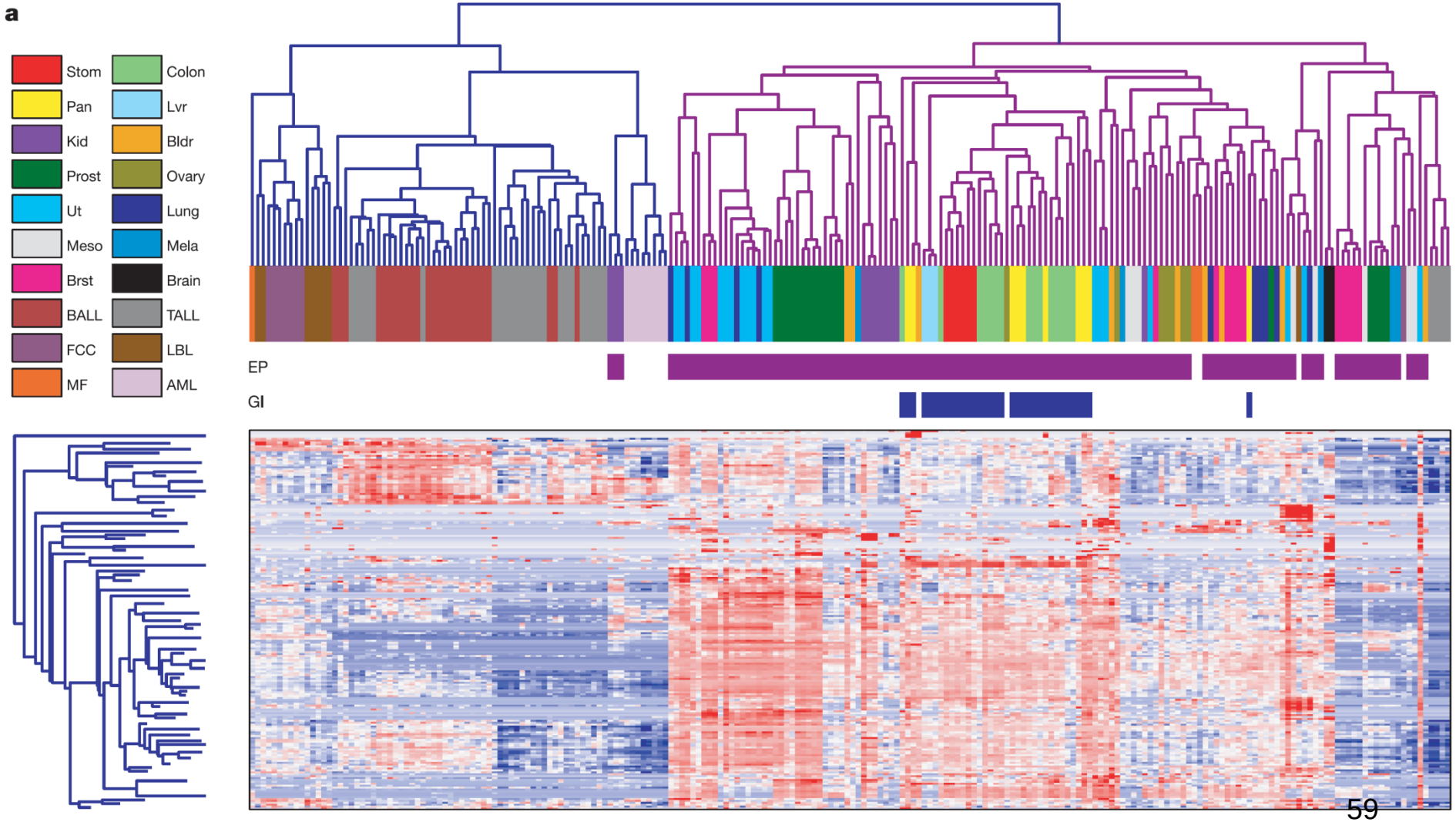
Table 2. Examples of miRs located in minimal deleted regions, minimal amplified regions, and breakpoint regions involved in human cancers

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

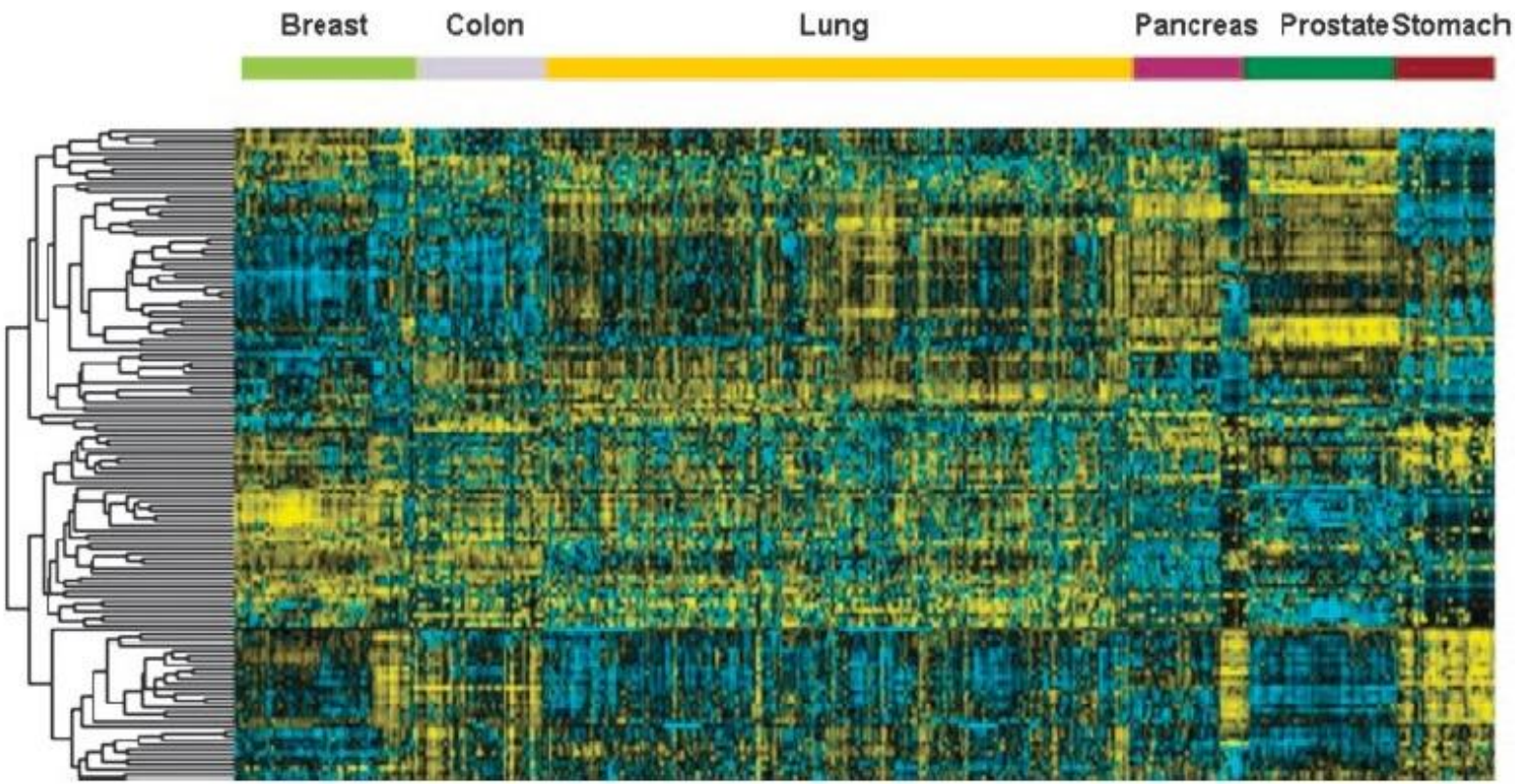
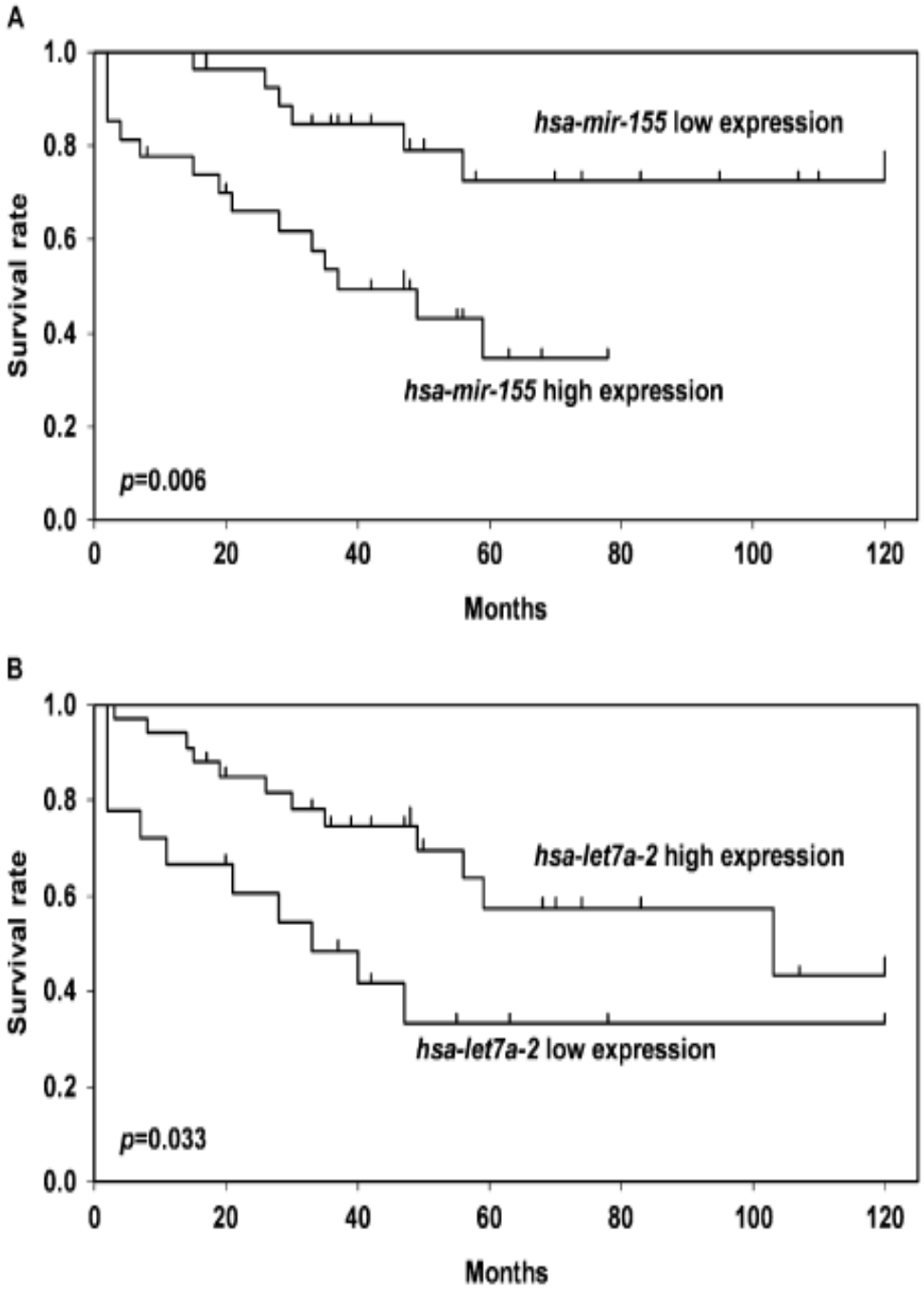


Table 2. The miRNAs shared by the signatures of the six solid cancers

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 = 2.5×10^{-3}).


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

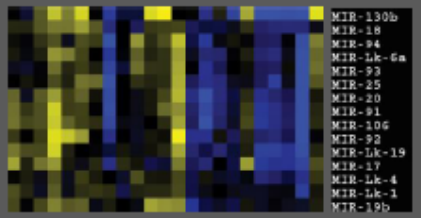
Table 5. Postoperative survival of patients with lung adenocarcinoma in relation to clinicopathological characteristics and miRNA expression analyzed by microarray analysis

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)

A polycistronic cluster of microRNAs are overexpressed in cancer



Proliferation

Angiogenesis

Apoptosis

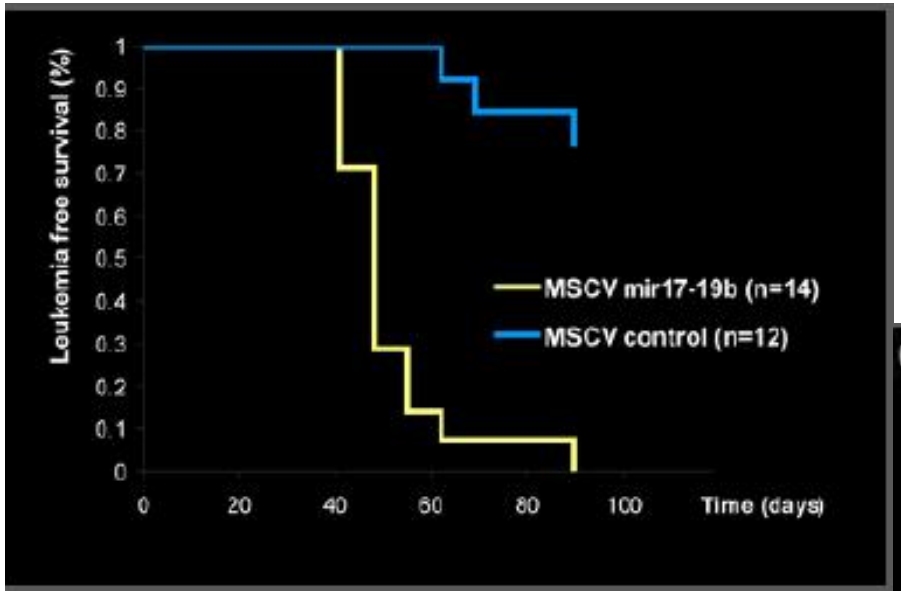
Invasiveness

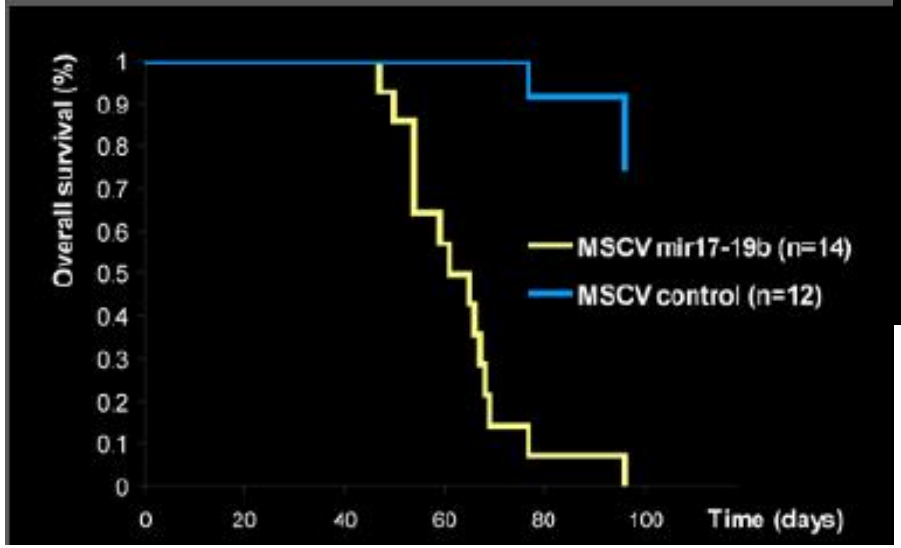
miR-17 miR-18 miR-19a miR-20 miR-19b miR-92

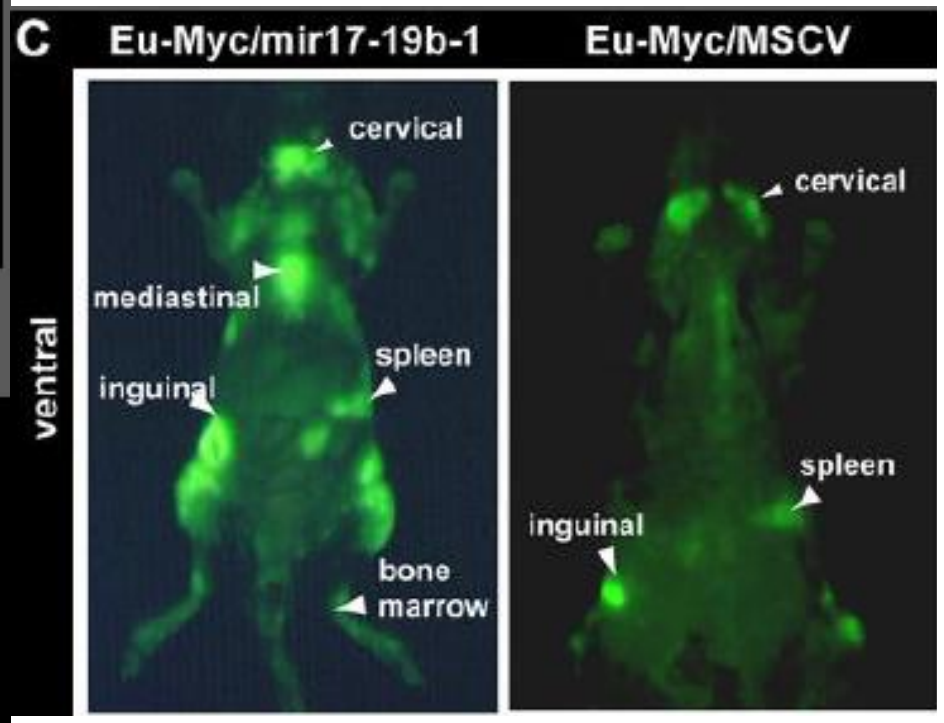
Ch13-ORF25

OncomiR-1 (Oncogenic microRNA-1)

courtesy of S. Hammond





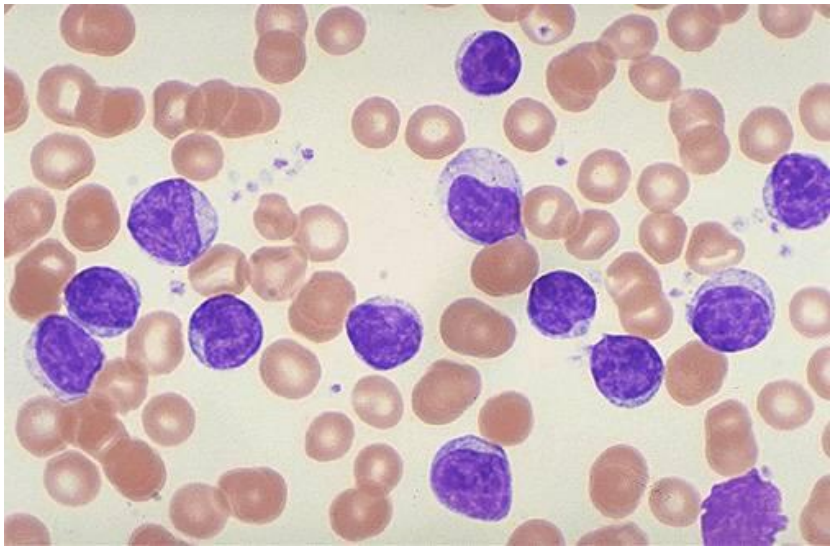


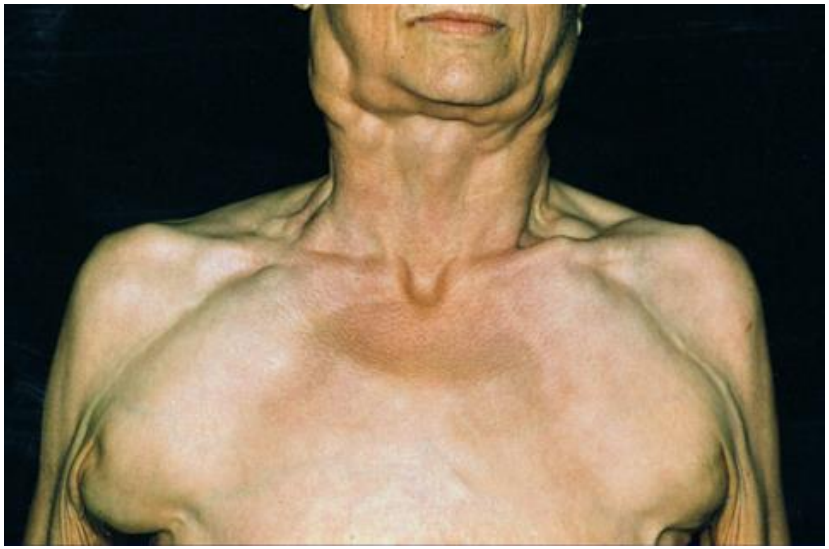
courtesy of S. Hammond

Chronická lymfatické leukémie

- Z maturovaných B lymfocytů
- Nejčastější leukémie dospělých

- Extrémně variabilní prognóza
- Nejčastější aberace del13q14 – obsahuje 2 miRNA (miR-15a, miR-16)

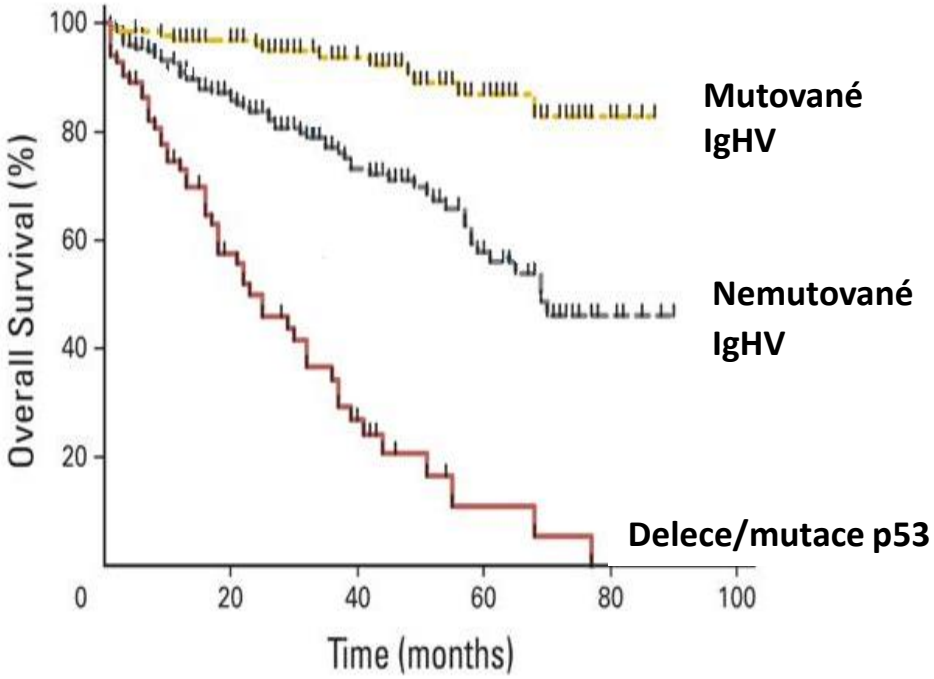




Exprese miRNA asociuje s prognostickými subtypy CLL

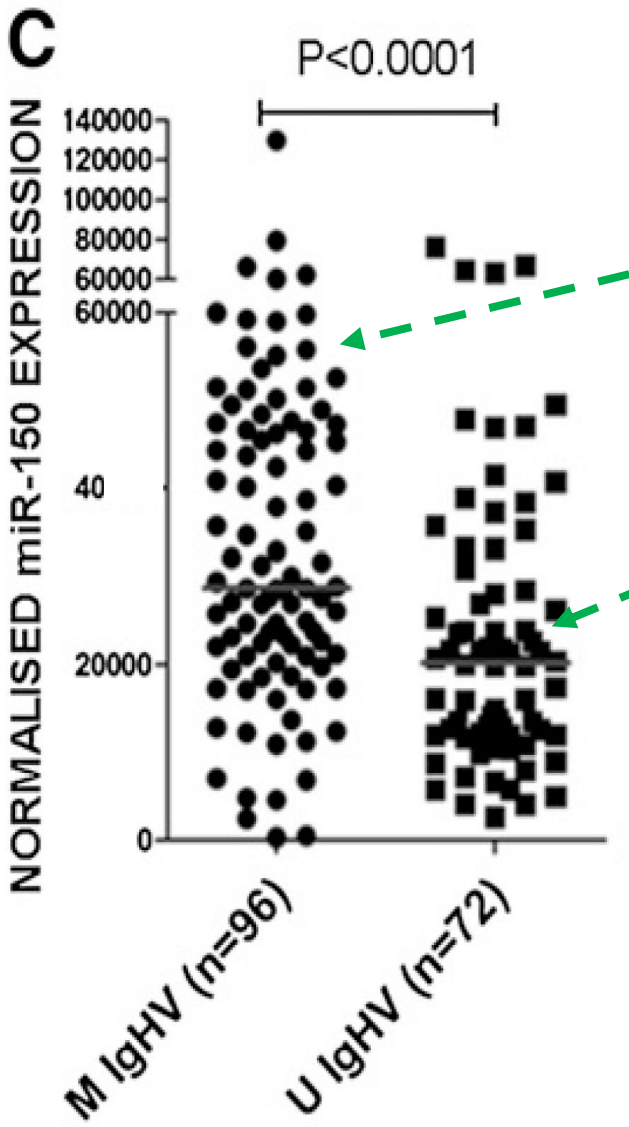
~ 20 miRNAs

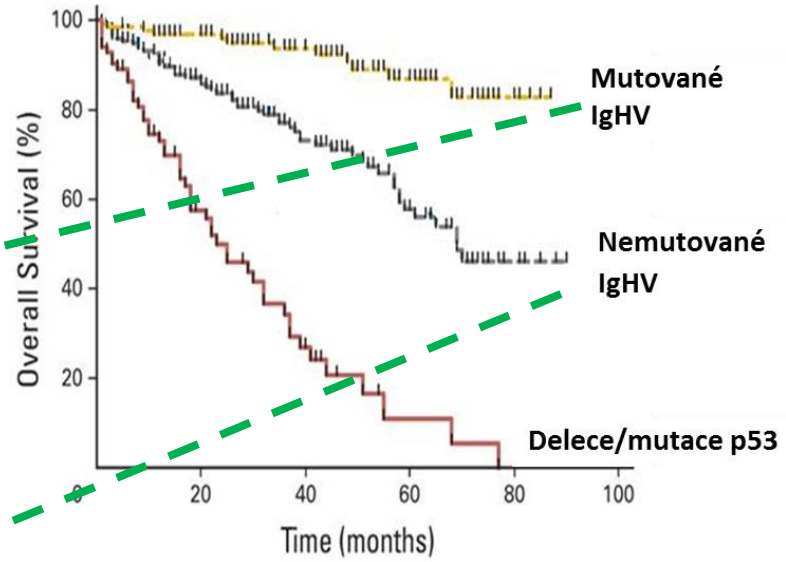
Calin et al., 2005
Fulci et al, 2007
Zenz et al., 2009
Stamatopoulos et al., 2009
Mraz et al., 2009a, 2009b, 2012, 2014

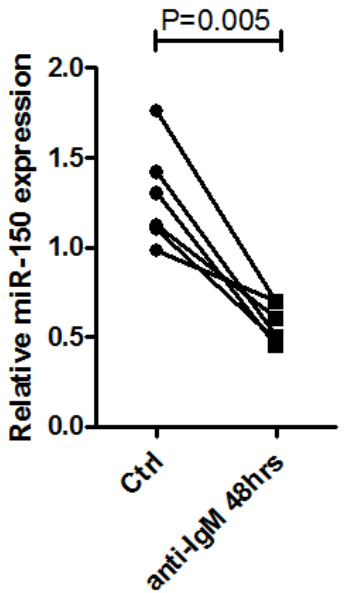


What is the functional significance of these observations?

Pacienti s nemutovaným IgHV mají nižší expresi miR-150

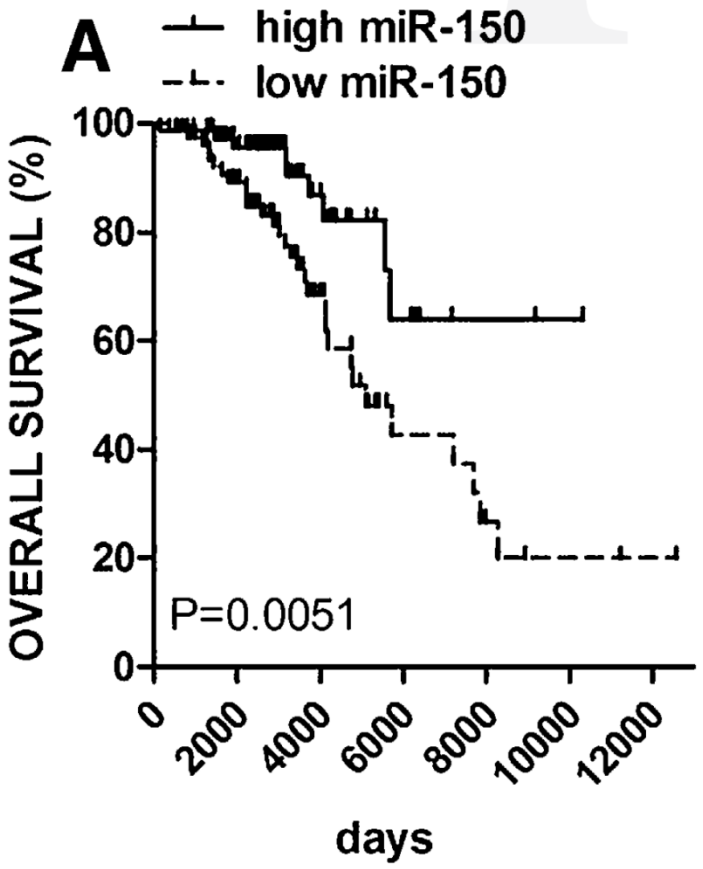






Nižší hladiny miR-150 asociují s kratším celkovým přežitím a časem do první léčby

n =168

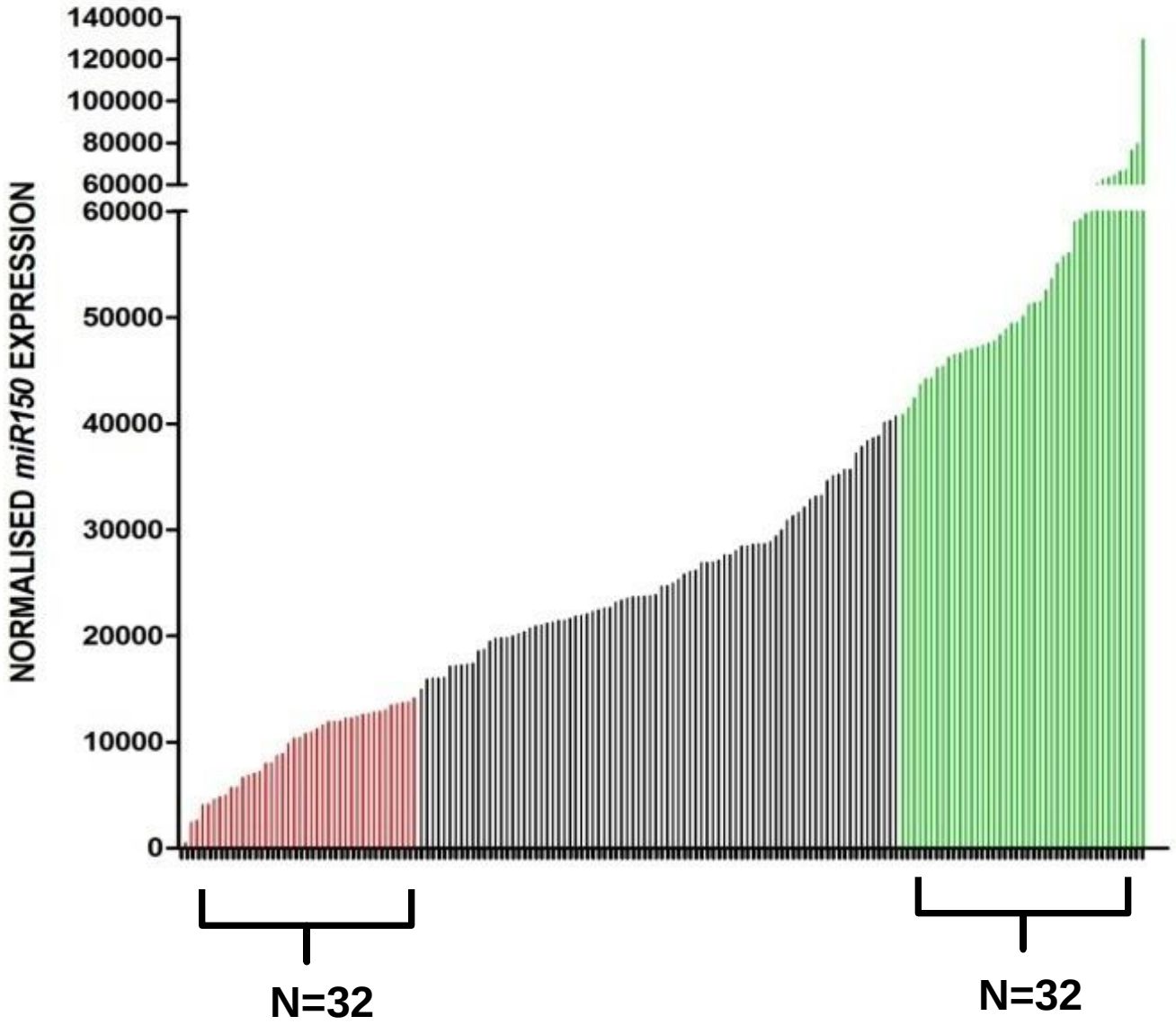


Variable[&]		HR[#]	CI[#]	*P-val*[#]
miR-150 (≤ vs. > median)		**5.6**	2.1-14.9	***0.001***
IGHV (unmut. vs. mut.)		2.8	0.9-9.1	*0.08*
ZAP-70 (pos. vs. neg.)		5.6	1.7-17.9	*0.004*
CD38 (pos. vs. neg.)		1.4	0.7-2.9	*0.37*
Gender (male vs. female)		2.9	1.3-6.5	*0.008*
Rai stage	I vs. 0	4.9	1.4-17	*0.01*
	II vs. 0	6.6	1.7-25.8	*0.01*
	≥III vs. 0	3.6	1-13.1	*0.05*
Age (> vs. ≤ median)		3.0	1.4-6.8	*0.01*

Mraz et al, Blood, 2014

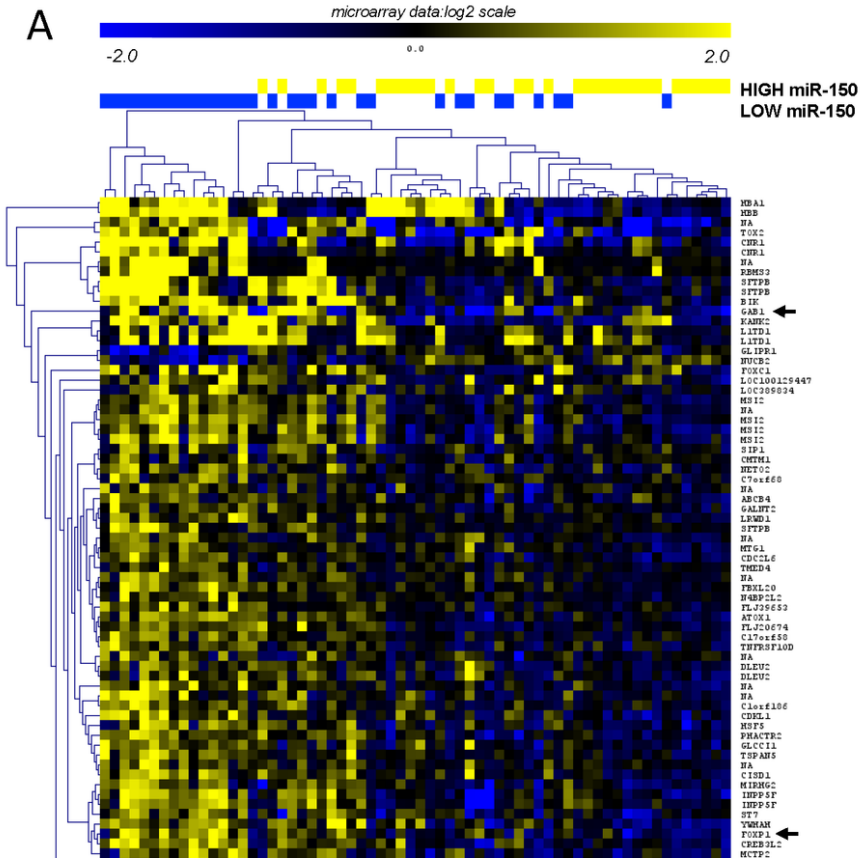
Jak identifikovat cíle miR-150 u CLL?

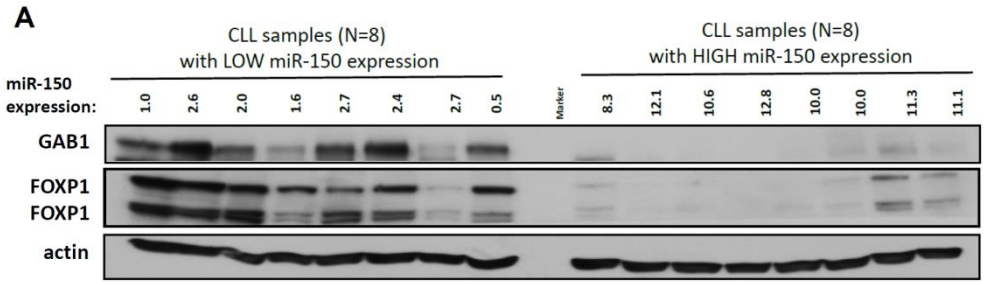
(HG-U133 Plus 2.0, Affymetrix)

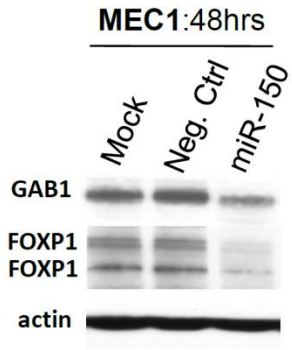


Genové expresní čipy pro CLL s nízkou vs vysokou hladinou miR-150

- 58 rozdílně exprimovaných genů
- 2 geny s evolučně konzervovanými vazebnými místy pro miR-150– **GAB1 a FOXP1**





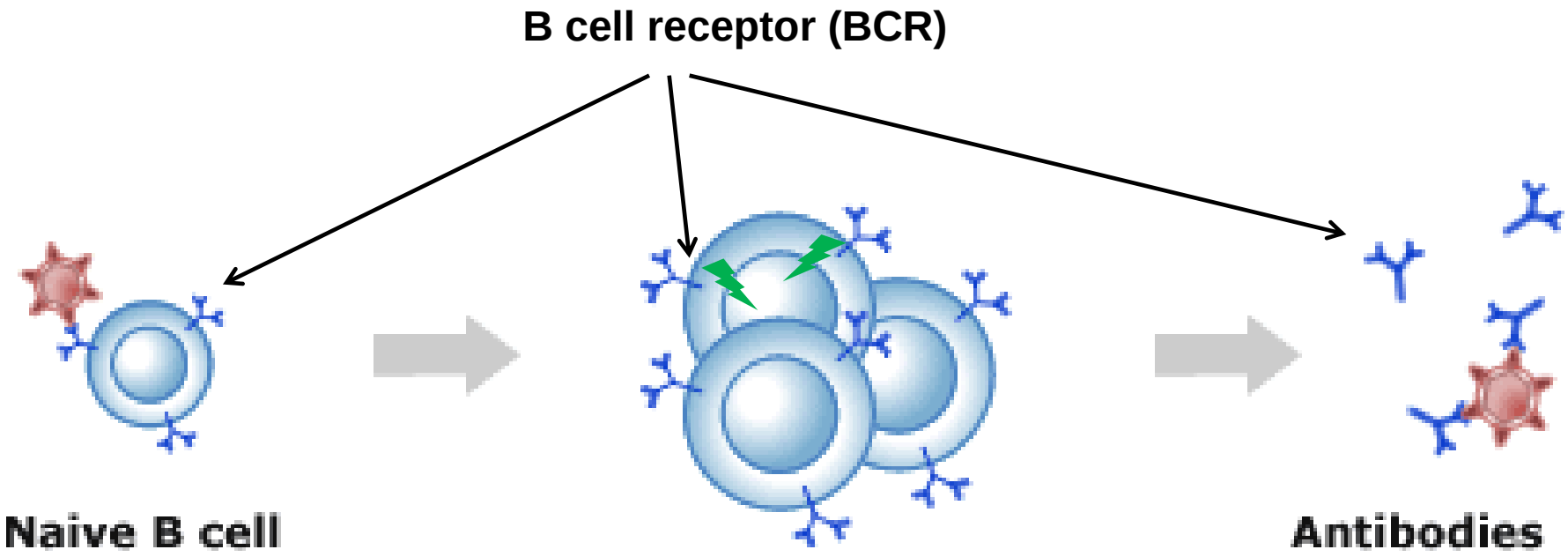


Mraz et al, Blood, 2014

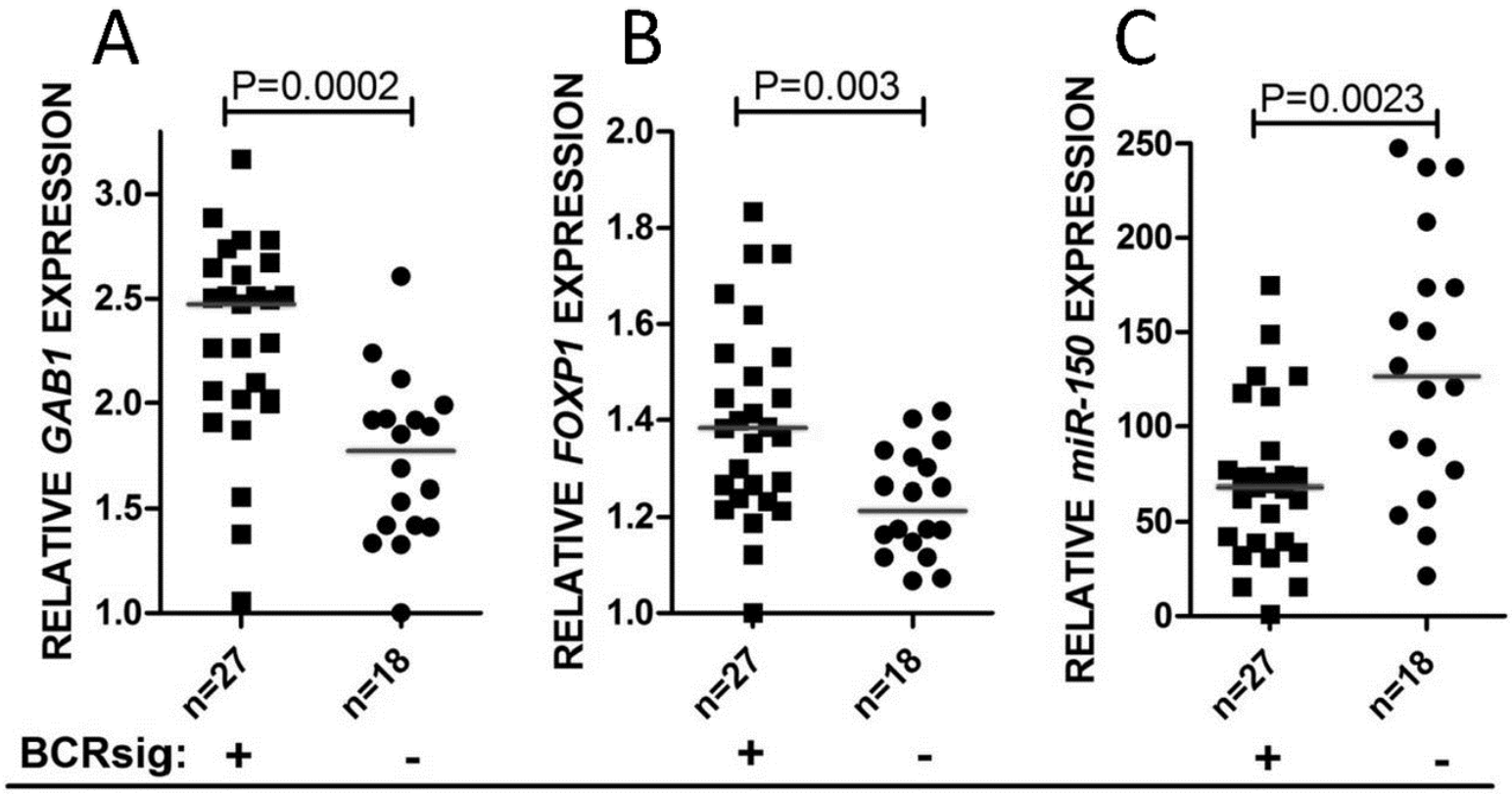
GAB1 je adaptorová molekula, která je nutná k vazbě PI3K na membránu a amplifikaci BCR signalizace (Ingham et al. JBC, 2001).

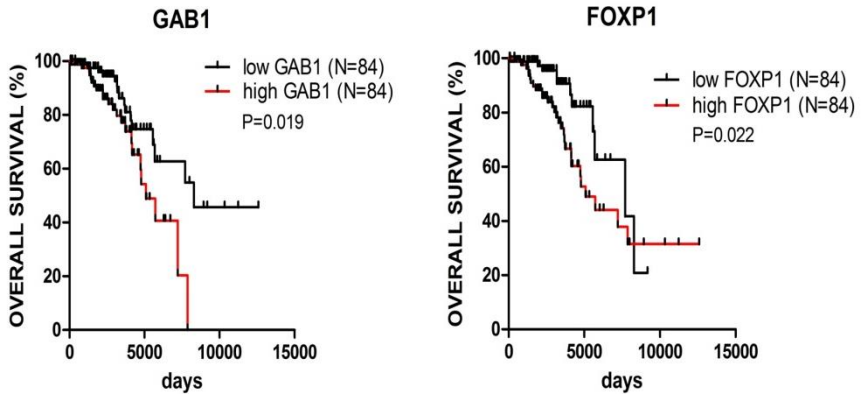
FOXP1 je transkripční faktor důležitý pro vývoj B lymfocytů a asociovaný s ABC DLBCL a progresí B buněčných lymfomů (Hu et al. Nat Immunol, 2006).

Adaptivní imunity- centrální dráha BCR

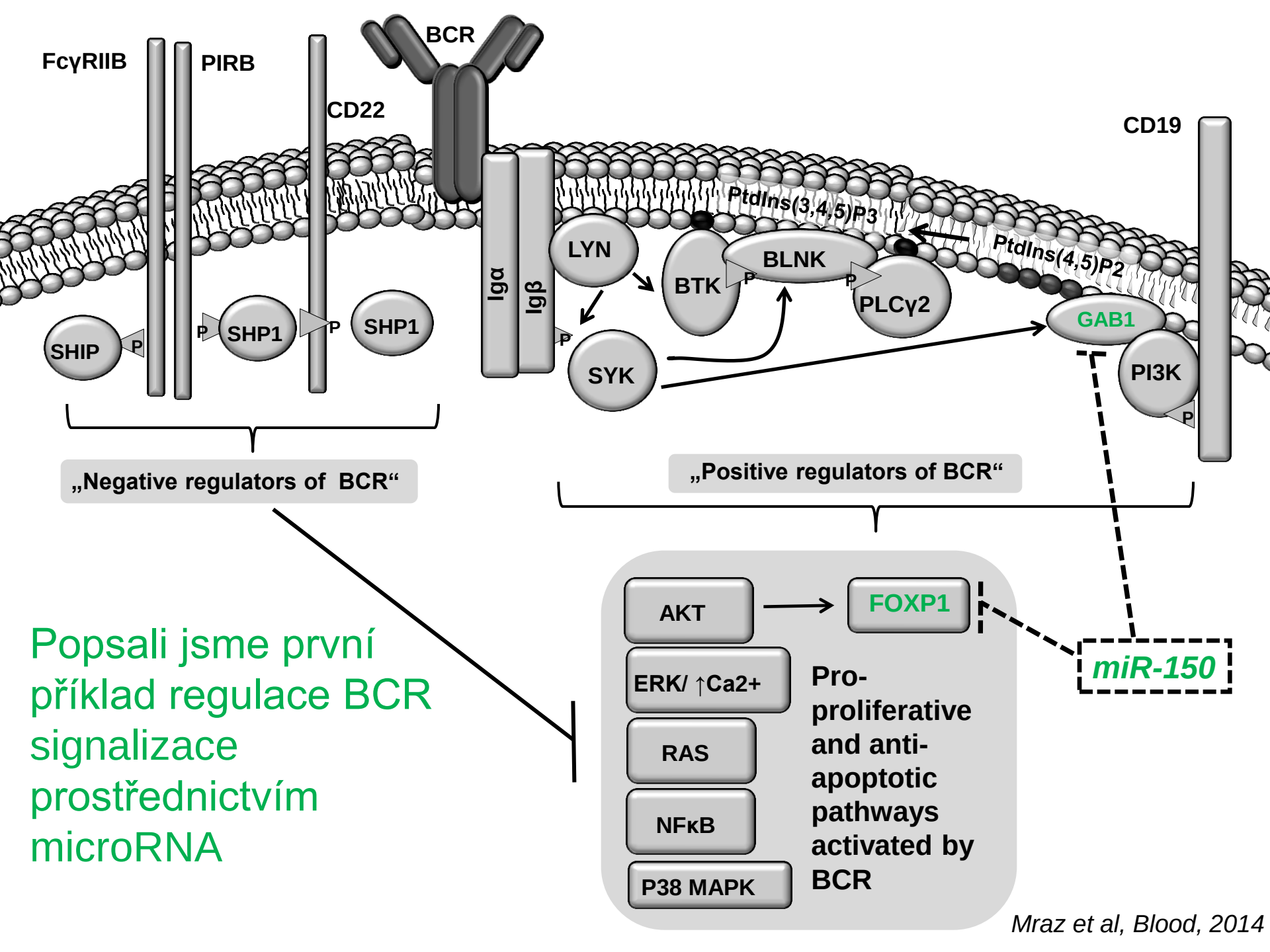


Vyšší hladiny GAB1 nebo FOXP1 znamenaní silnější BCR signalizaci





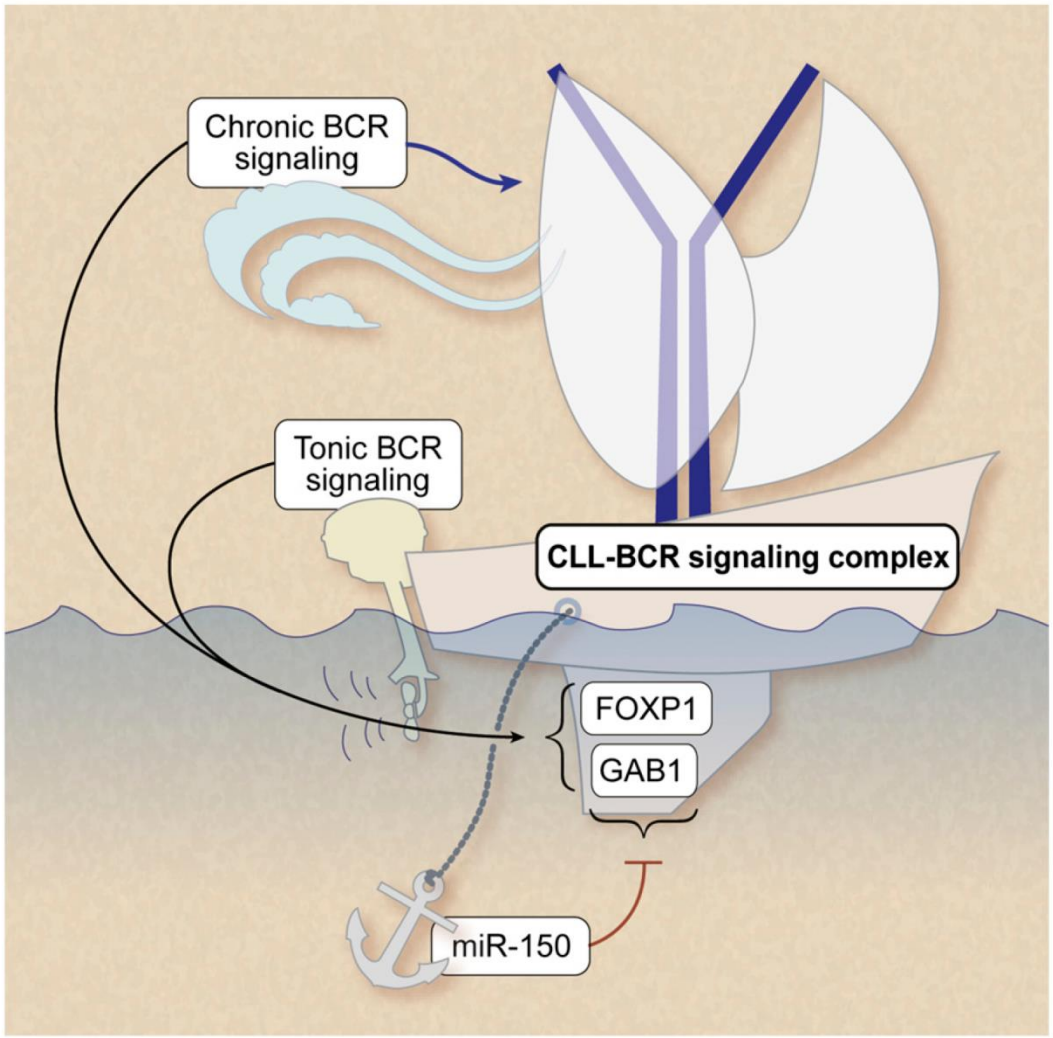
Mraz et al, Blood, 2014



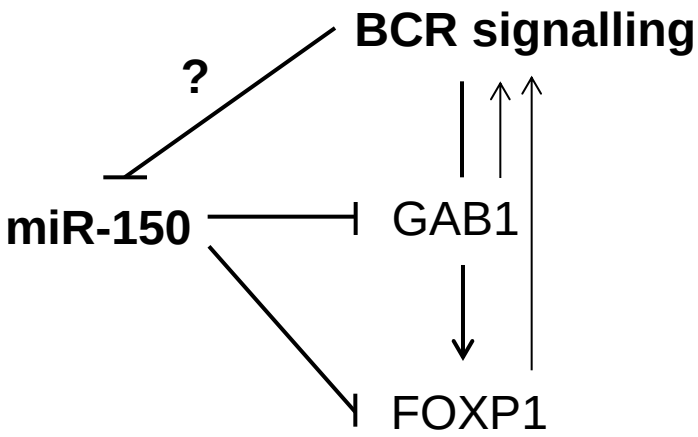
Popsali jsme první příklad regulace BCR signalizace prostřednictvím microRNA

Mraz et al, Blood, 2014

First description of miRNAs role in BCR signalling…not only in CLL



Ligand-independent ("tonic") and ligand-dependent ("chronic") BCR signaling play a pivotal role in CLL survival and growth. MiRNA-150 dampens the threshold for BCR signaling by repressing expression levels of GAB1 and FOXP1. Professional illustration by Debra T. Dartez.

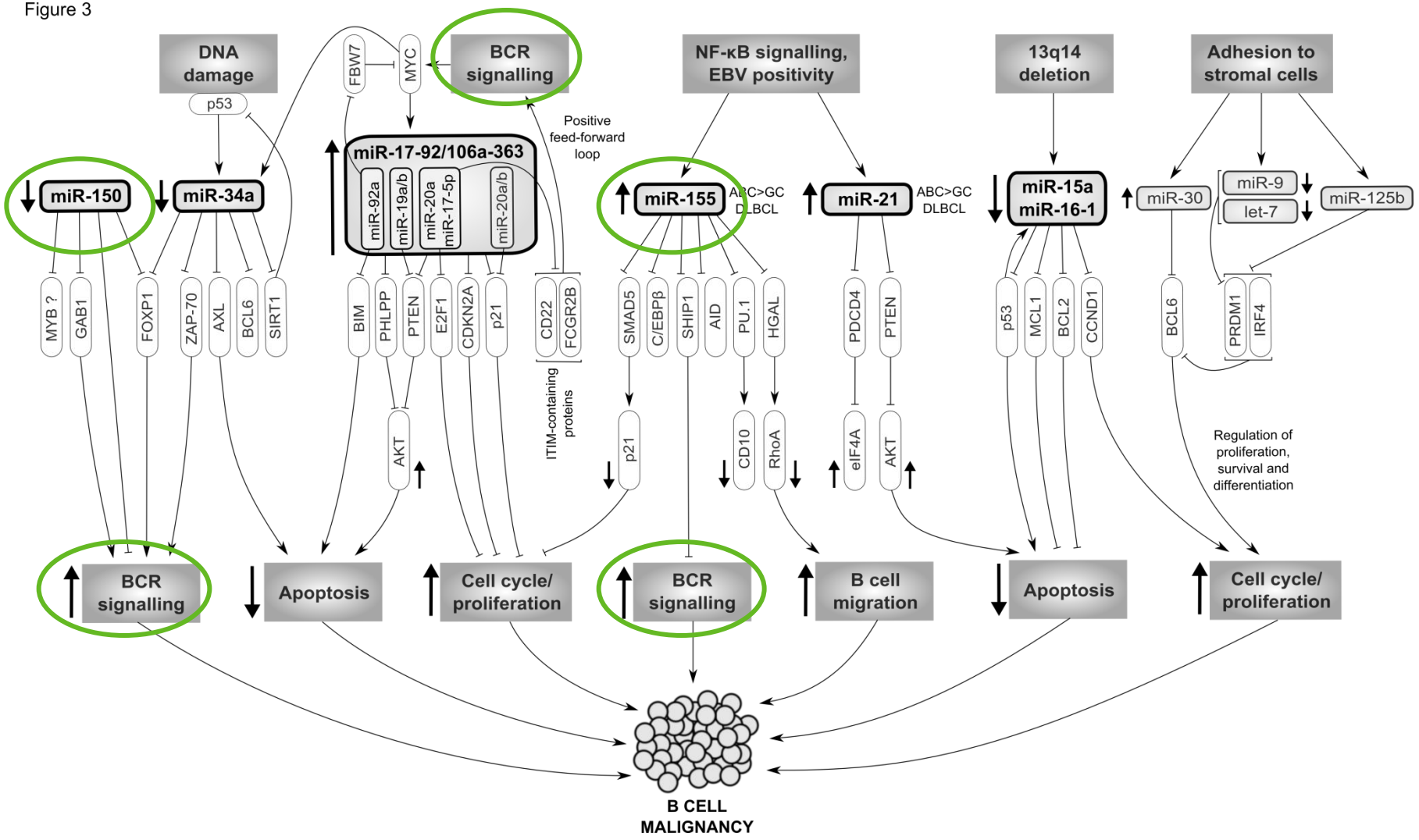


Double negative loop

Kater and Eldering, BLOOD, 3 JULY 2014 x VOLUME 124, NUMBER 1

miRNAs přispívají k regulaci mnoha drah

Figure 3



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