



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



# Analýza exprese microRNA

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Group leader CEITEC MU

- Nic si nepište...vše bude online
- Další hodina příští týden

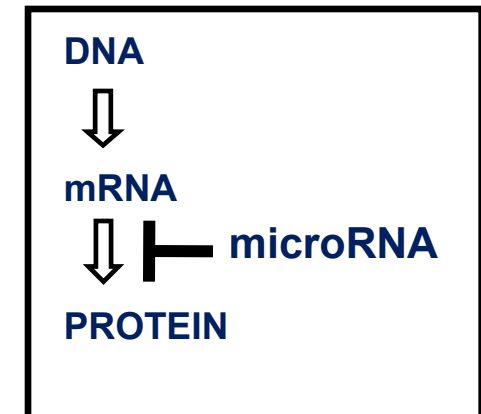
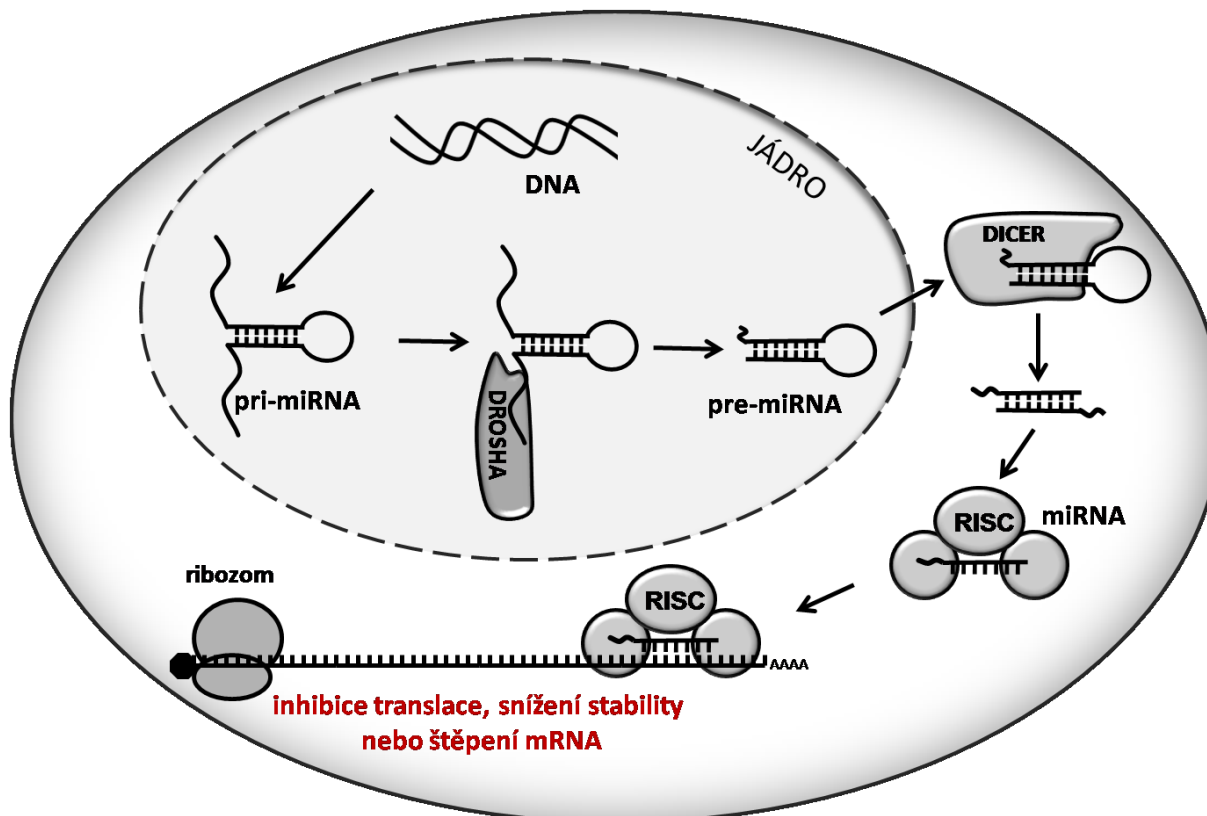
- Lidské miRNA geny: cca 2000

## microRNA (miRNA)

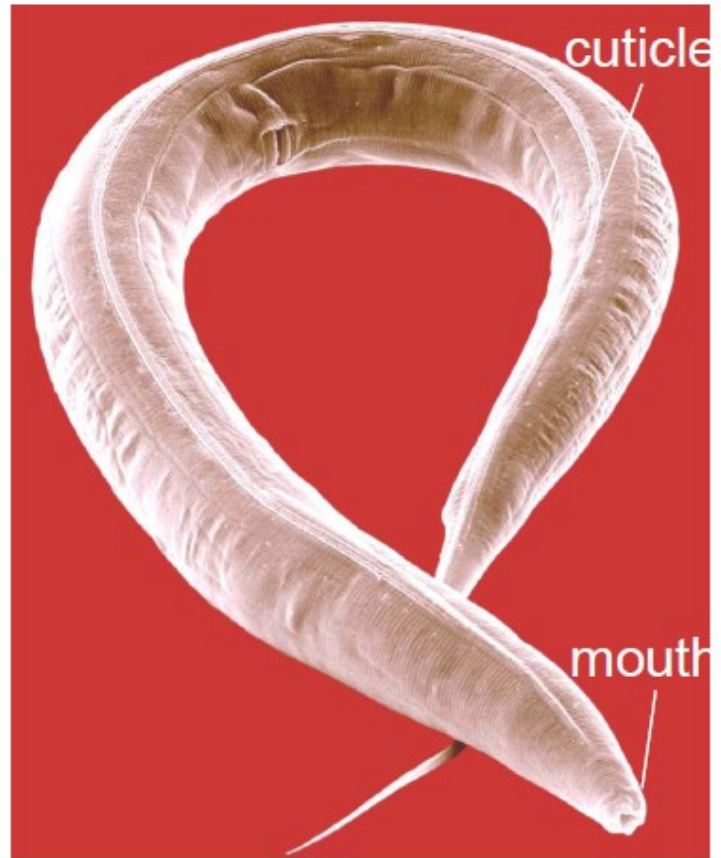
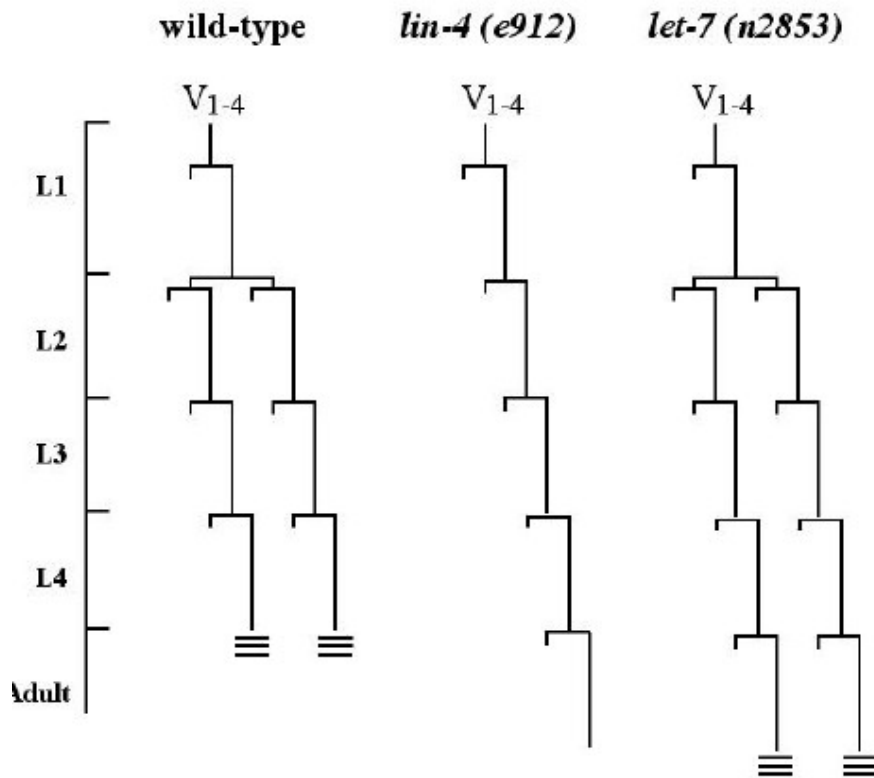
- ❑ krátké RNA molekuly  
~22 nukleotidů
- ❑ komplementární vazba k  
cílové mRNA
- ❑ inhibují translaci a snižují  
stabilitu mRNA



Stovky evolučně  
konzervovaných microRNA



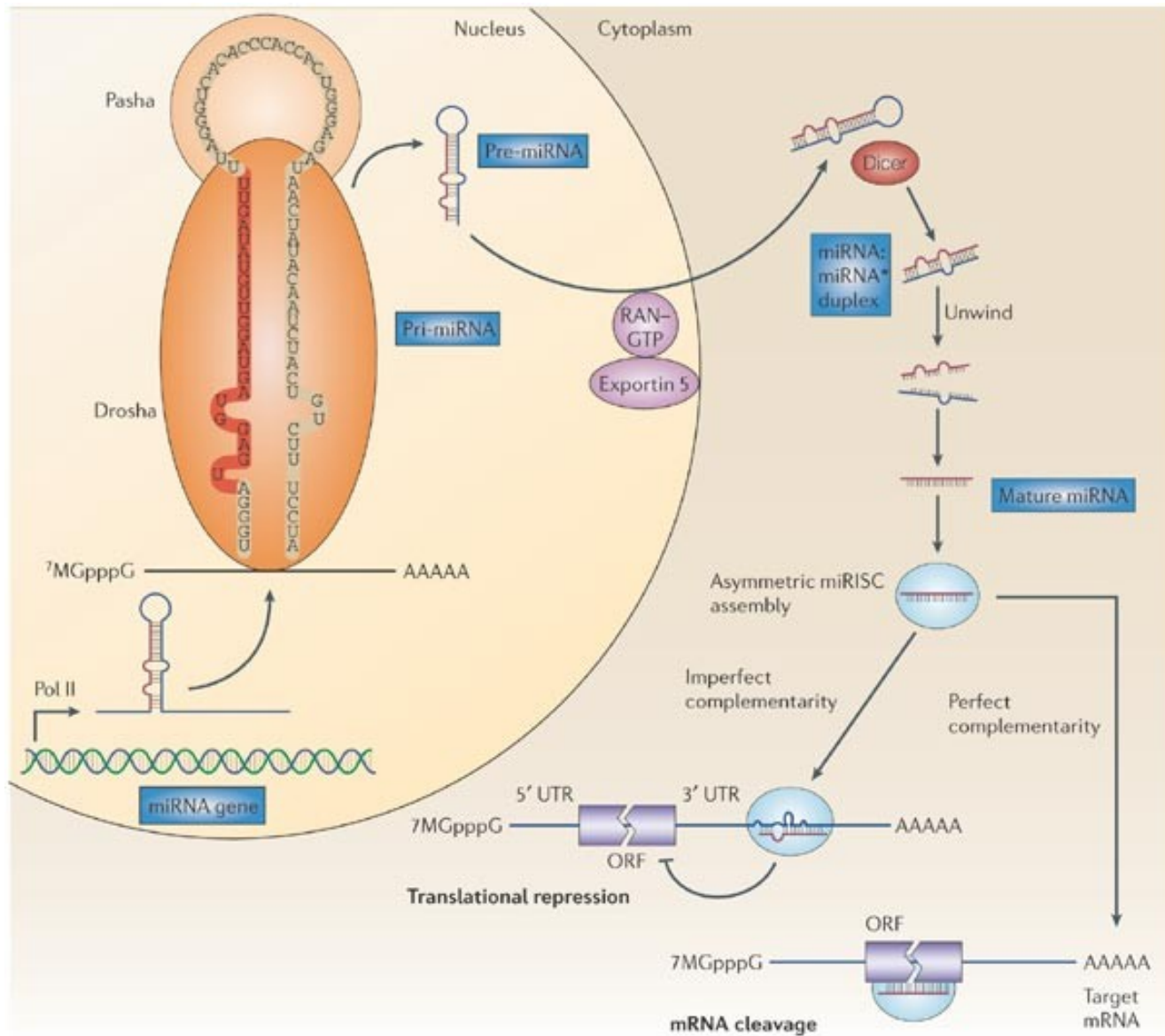
# MicroRNAs were discovered by V. Ambros and G. Ruvkun in *C. elegans*

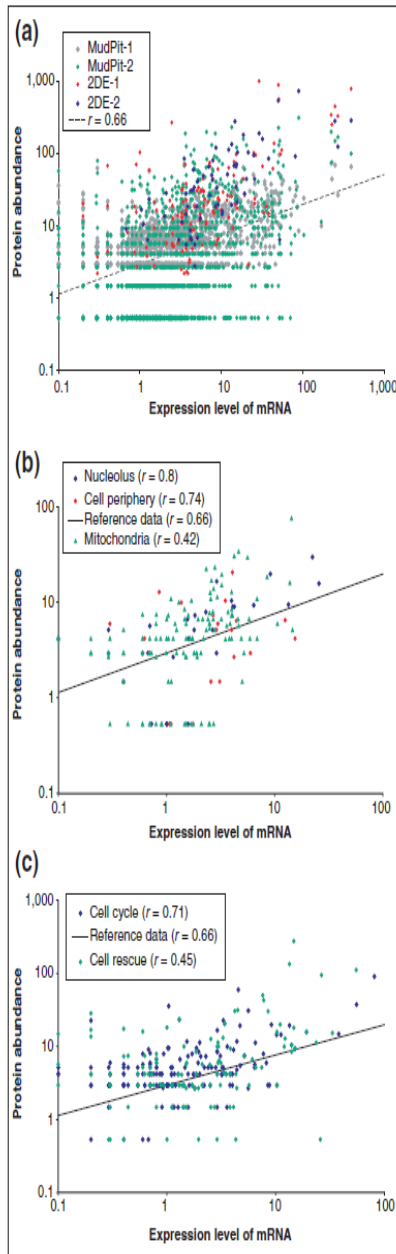


Lee et al. 1993 *Cell*

Reinhart et al. 2000 *Nature*

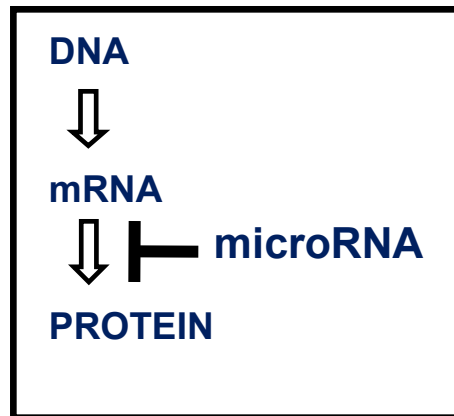






mRNA neznamená, že v buňce bude i protein

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



## Specifika analýzy exprese microRNAs:

- velmi malé molekuly – 22nt – specifikum izolace, specifické značení i design sond
- malé zastoupení ve vzorku – separace microRNA
- v lidském genomu 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 (cloning a Northern blot)

Real-Time PCR

NGS

## 1/ Izolace a stabilita microRNA

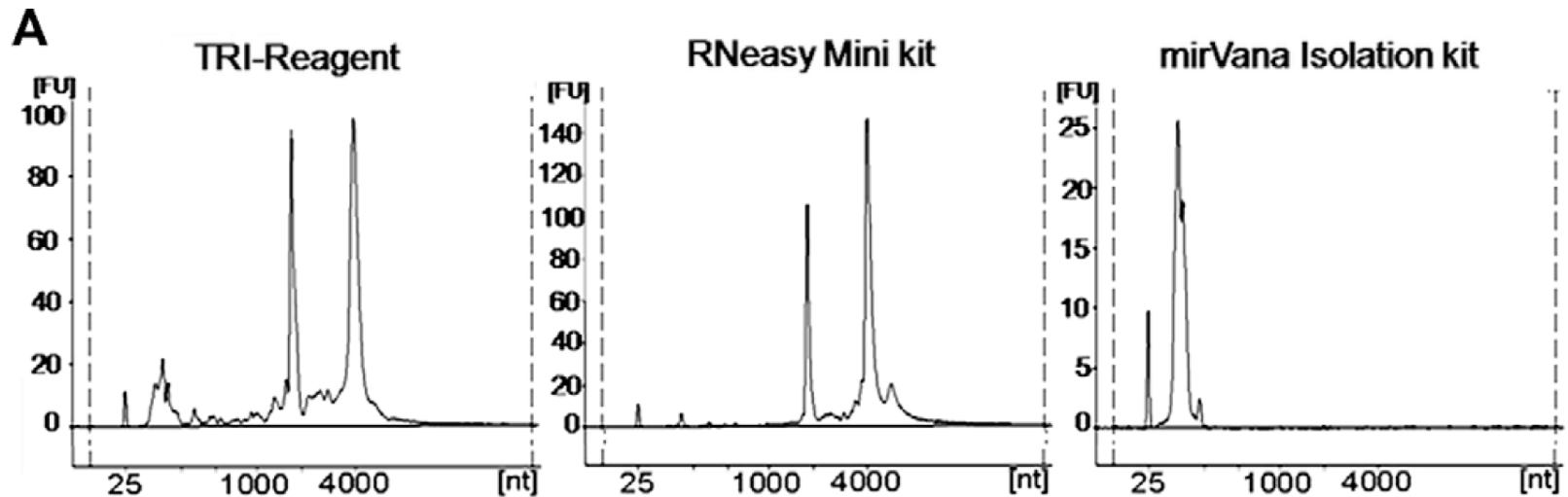
Problémy: velikost 22nt, celkově cca 0,01% z celkové RNA

### Izolace:

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

### Obohacení:

PAGE  
FlashPAGE Fractionator  
(Ambion)



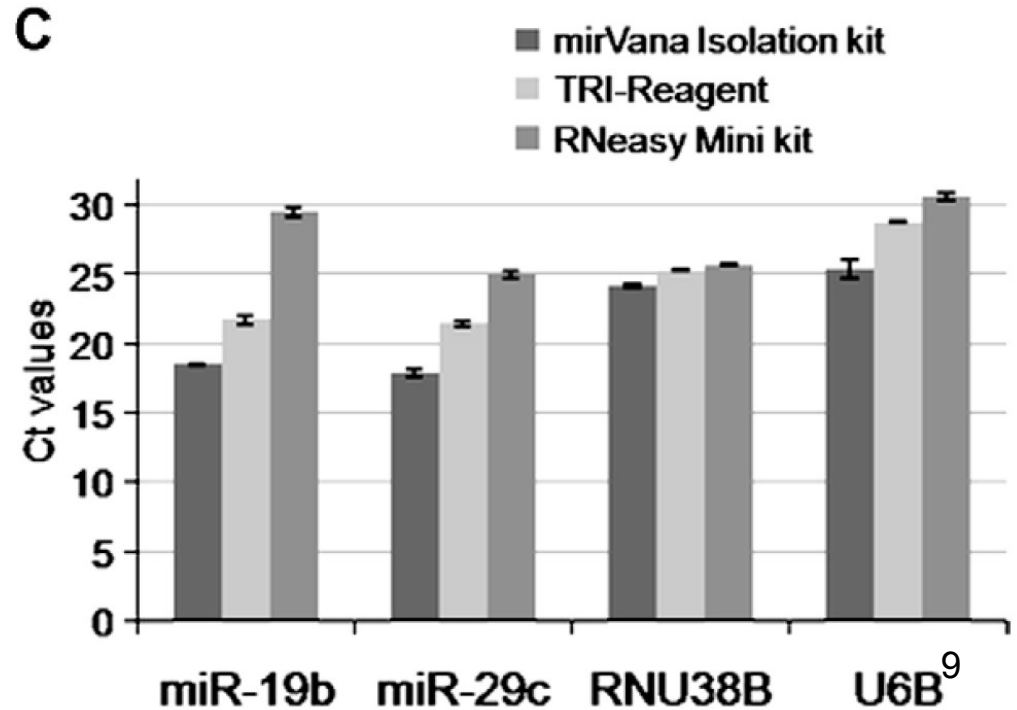
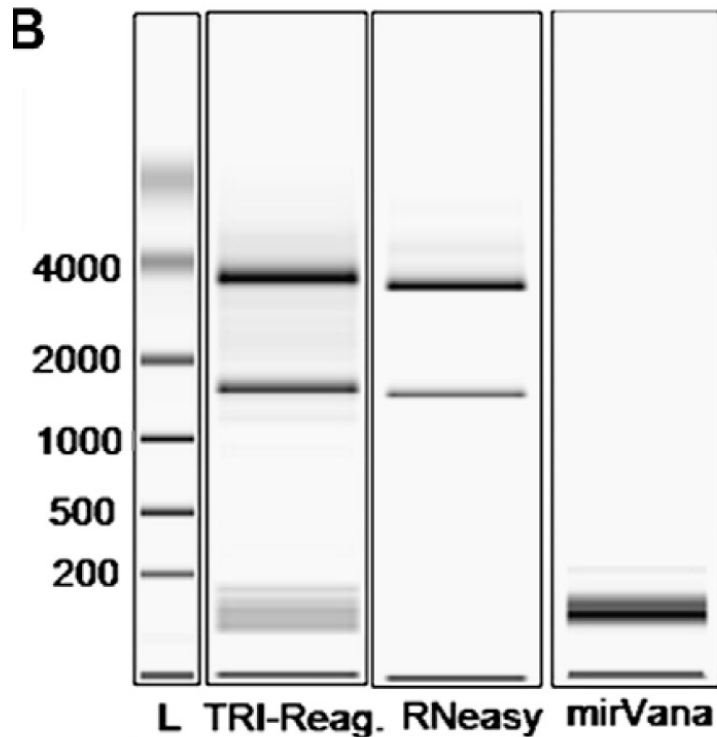
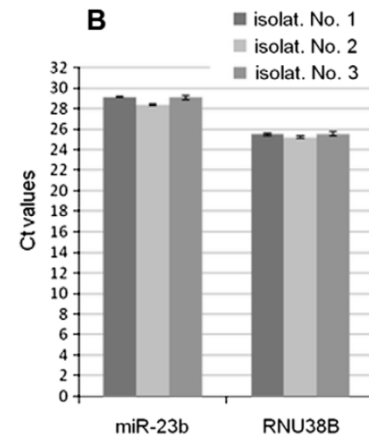
Mraz et al., 2009

## Izolace:

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

## Izolace:

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



# Obohaceni:

## PAGE

### FlashPAGE Fractionator (Ambion)



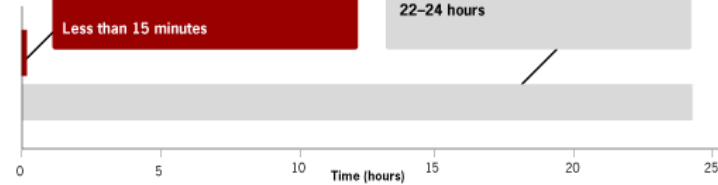
#### flashPAGE™ Protocol

1. Pipet flashPAGE™ Lower Running Buffer into the lower buffer chamber of the apparatus  
<30 seconds
2. Insert a "ready-to-use," pre-cast flashPAGE™ Gel Cartridge  
<5 seconds
3. Add flashPAGE™ Upper Running Buffer to the upper buffer chamber of the gel cartridge  
<30 seconds
4. Add your RNA or DNA sample (with flashPAGE™ A40 Dye Marker)  
<1 minute
5. Run gel at 70 V on any standard power supply  
~12 minutes
6. Collect PAGE-purified nucleic acid from lower buffer chamber\*  
<30 seconds

Less than 15 minutes

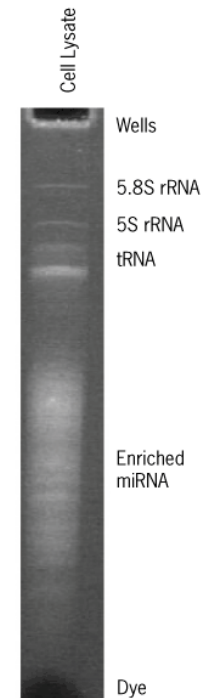
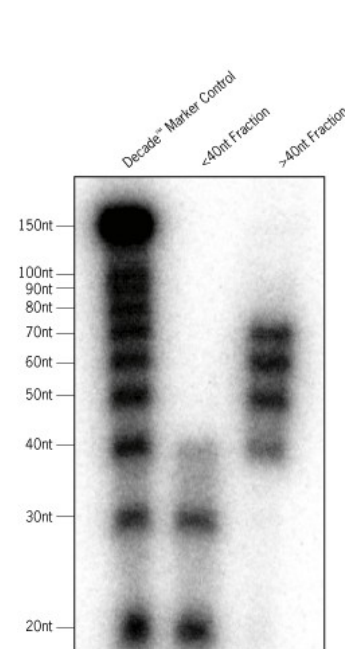
#### Traditional PAGE Purification

1. Prepare gel solutions  
30 minutes
  2. Cast gel  
2 hours
  3. Pre-run the gel  
30 minutes
  4. Load sample  
1 minute
  5. Electrophorese  
30-60 minutes
  6. Stain gel to visualize region of interest  
10 minutes
  7. Excise desired size fraction  
5 minutes
  8. Soak crushed gel with elution buffer  
overnight
  9. Collect first elution and elute again  
2 hours
- 22-24 hours



15min

20hours

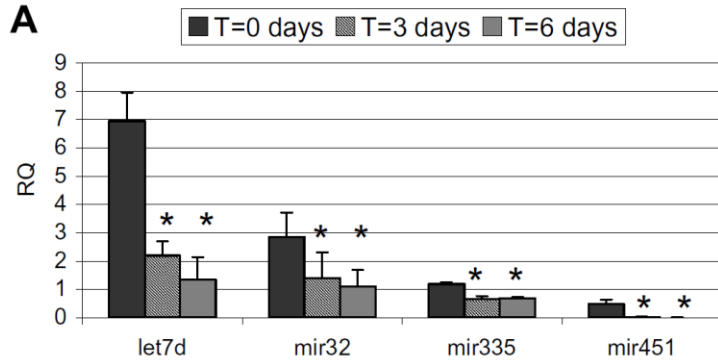


# Stabilita microRNA :

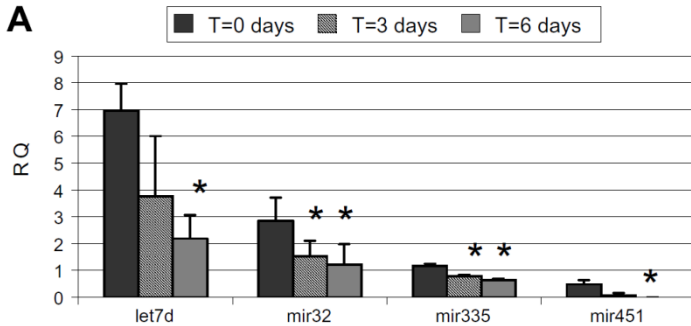
## Stabilita po izolaci

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

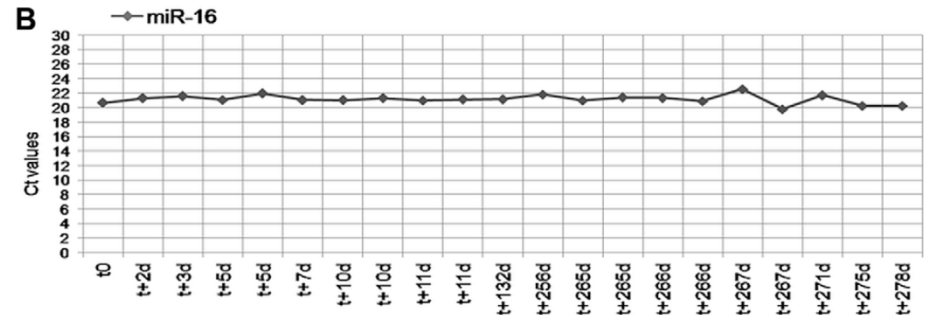
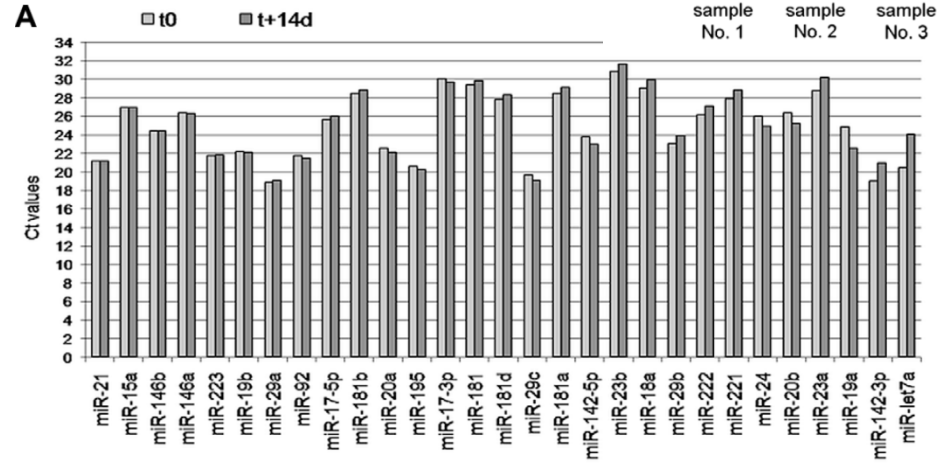
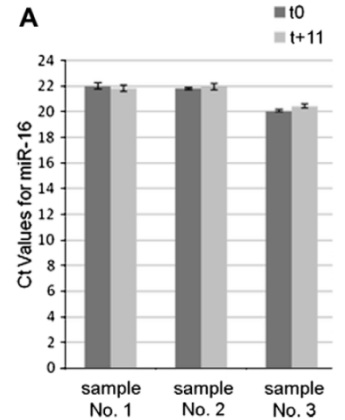
### RNA



### cDNA



Bravo et al., 2007



Mráz et al., 2009

# Stabilita microRNA :

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

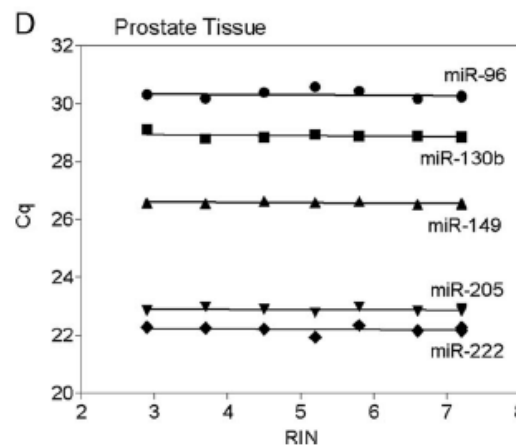
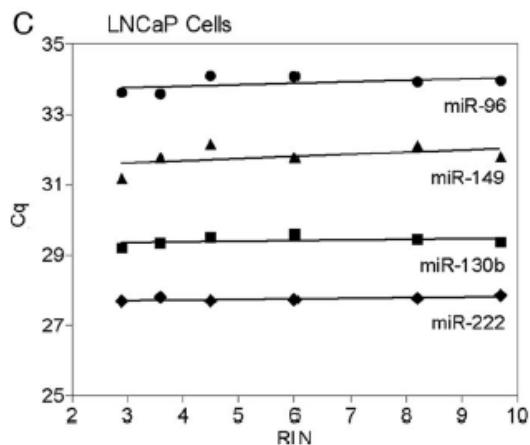
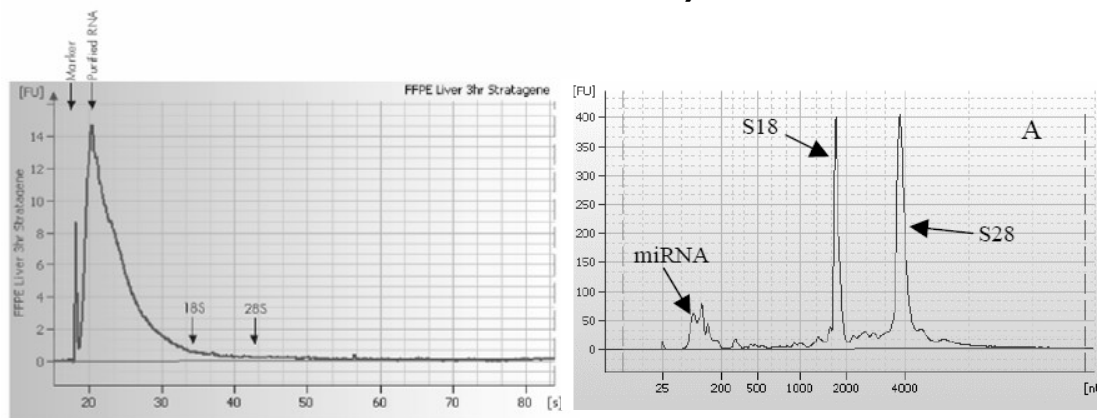
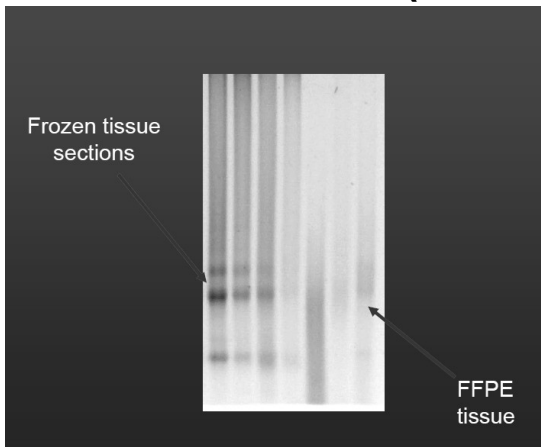


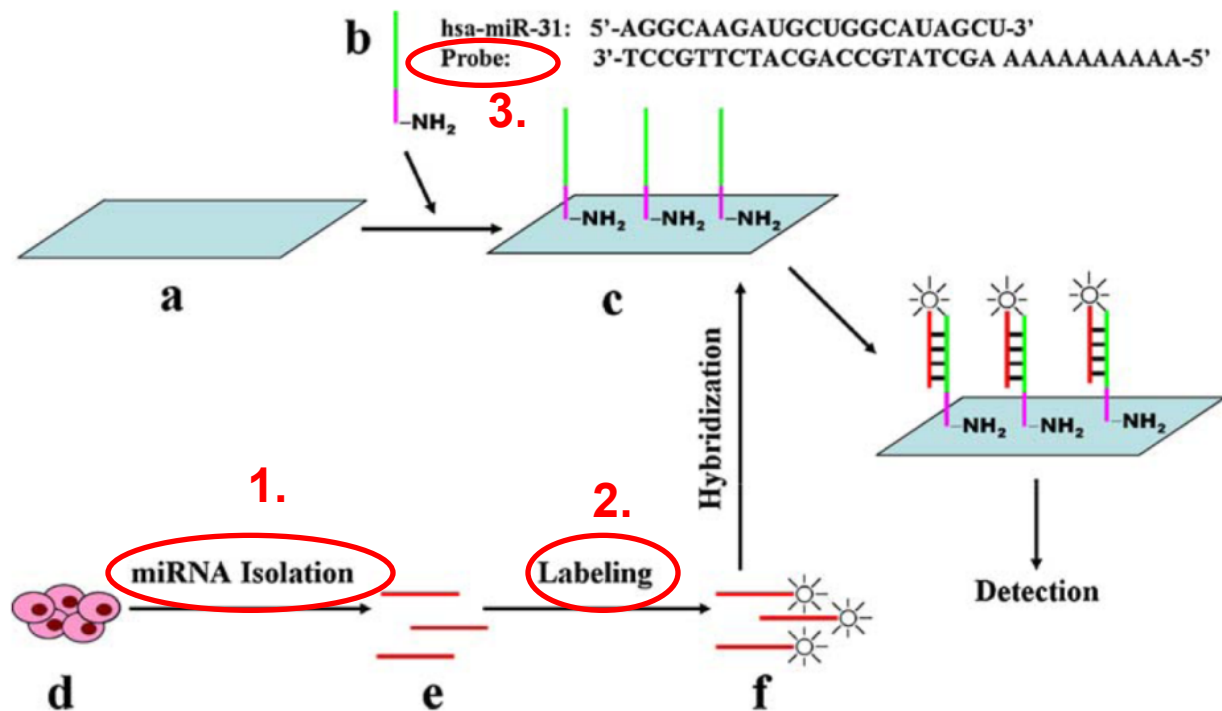
Fig. 2. Influence of RNA integrity on miRNA gene expression.

(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.



## Expression microarrays pro microRNAs:

- velmi malé molekuly – 22nt – specifika izolace, specifické značení i design sond
- malé zastoupení ve vzorku – separace microRNA
- v lidském genomu cca 2000 genů – menší počet sond na čipu
- 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



### 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

Fluochromem 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)

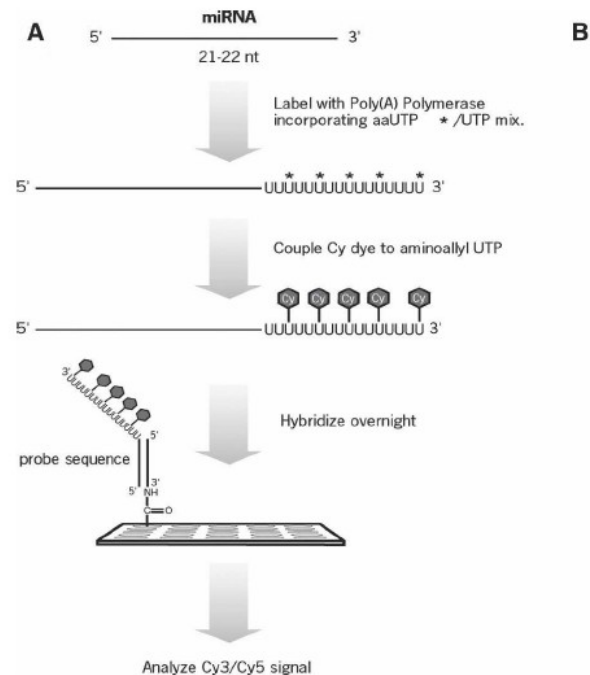
(Babak et al., 2004)

#### 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)

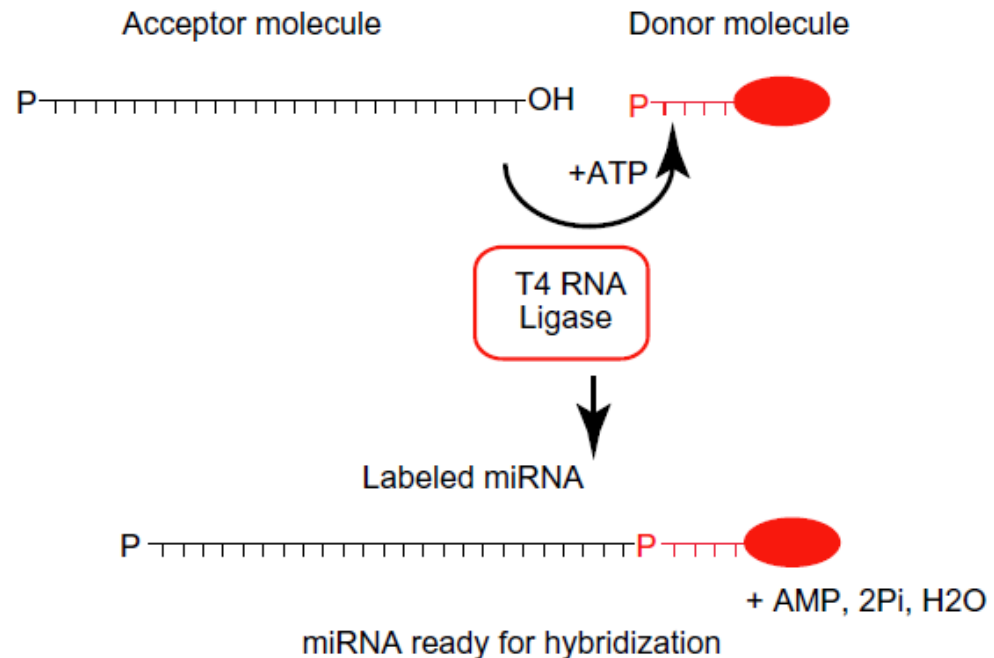


Fig. 2. Schematic representation of the miRNA labeling principle: a short Cy-dye labeled RNA-linker (donor molecule) is ligated to the single-stranded miRNA (acceptor molecule) by T4 RNA ligase in the presence of ATP.

## 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 reverzního 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

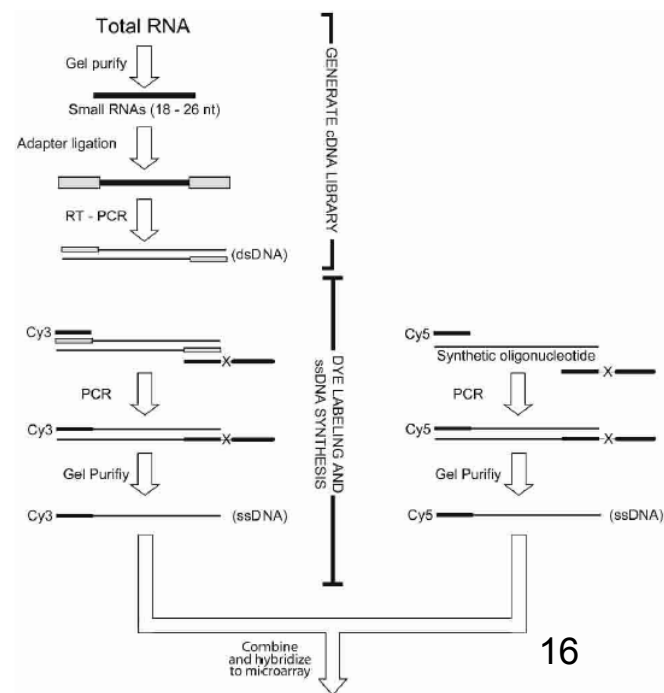
fluorescenčně-značený primer (k adaptoru)

(Miska et al., 2004)

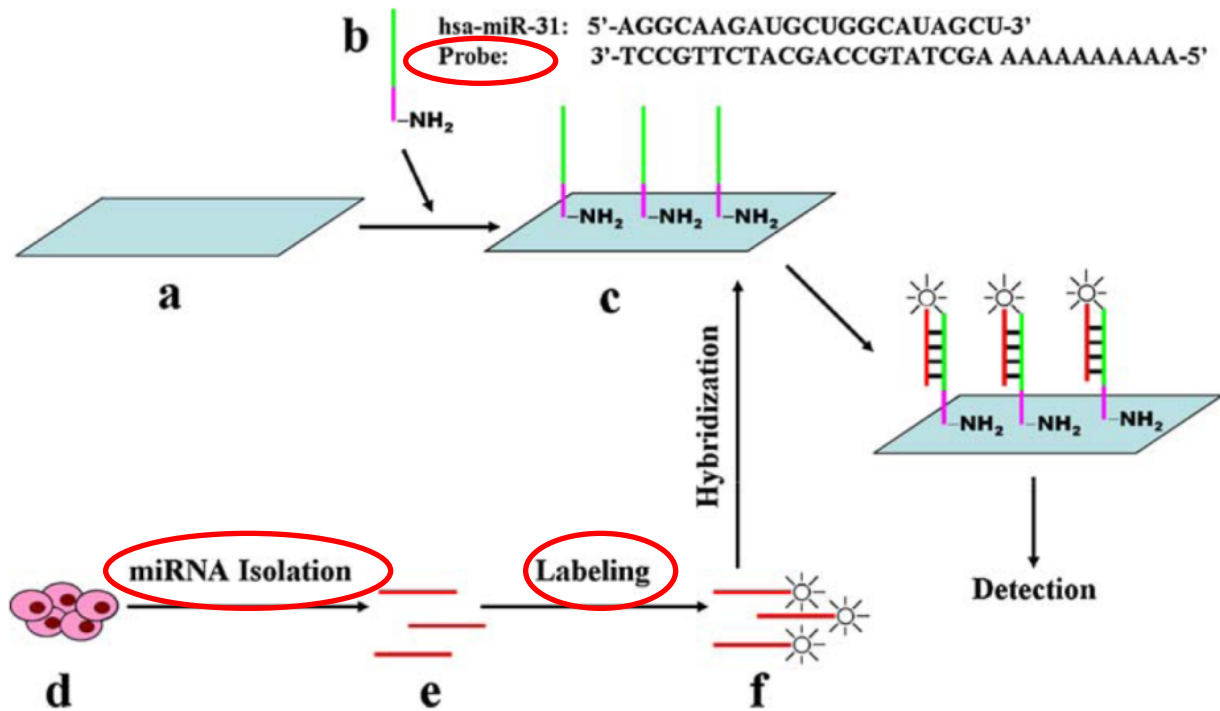
Nevýhoda: antisense strand přiromen při hybridizaci

Rešením je různá délka sense a antisense ->PAGE

(Baskerville, 2005)



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



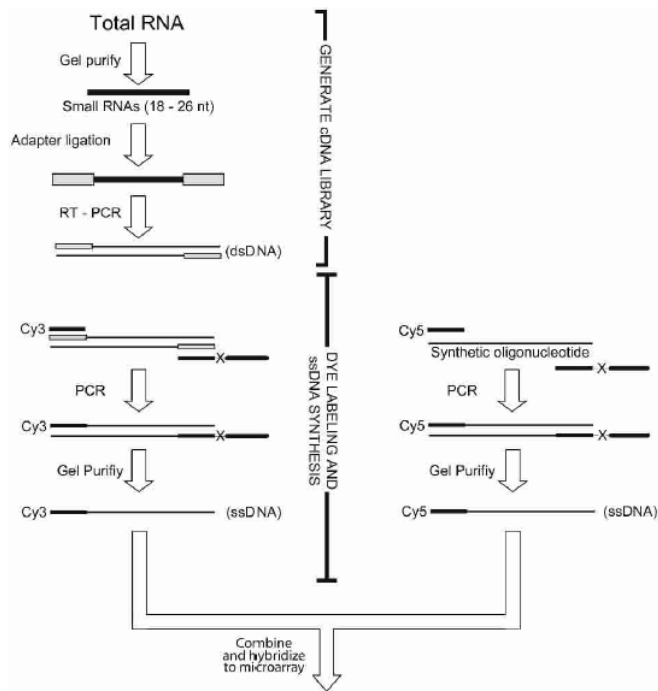
$T_m$  – melting temperature určité próby  
T – hybridizační teplota

$T_m < T$  .....nižší efektivita vazby miRNA  
 $T_m > T$  .....vyšší efektivita vazby miRNA

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

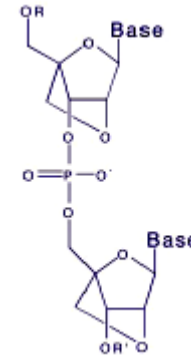


**FIGURE 1.** Microarray sample preparation and reference oligonucleotide synthesis. Small RNAs were fractionated on a polyacrylamide gel, and oligonucleotide primers were then ligated to the 5' and 3' ends of the small RNA library (Lau et al. 2001). A cDNA library was generated through reverse transcription,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

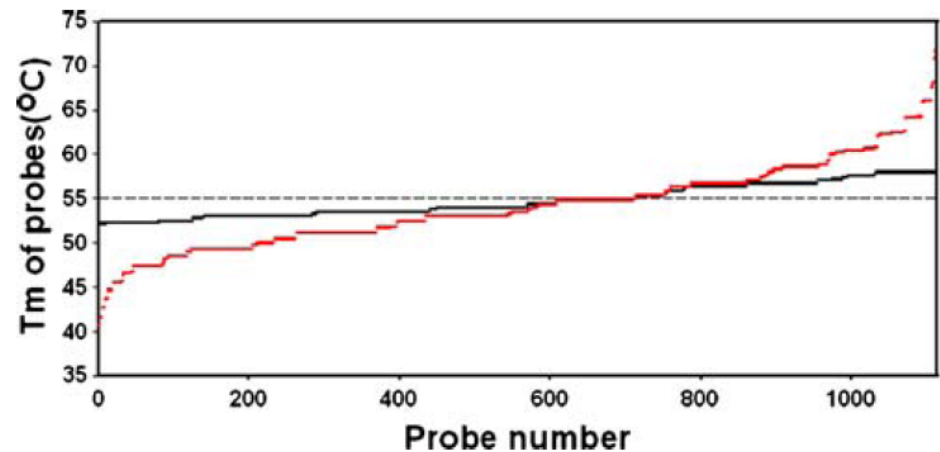
# ÚPRAVA SÍLY VAZBY NUKLEOTIDŮ

LNA próby (Locked Nucleic Acid)

ribózový kruh je „uzamčen“ methylenovým můstkem mezi atomy 2'-O a 4'-C

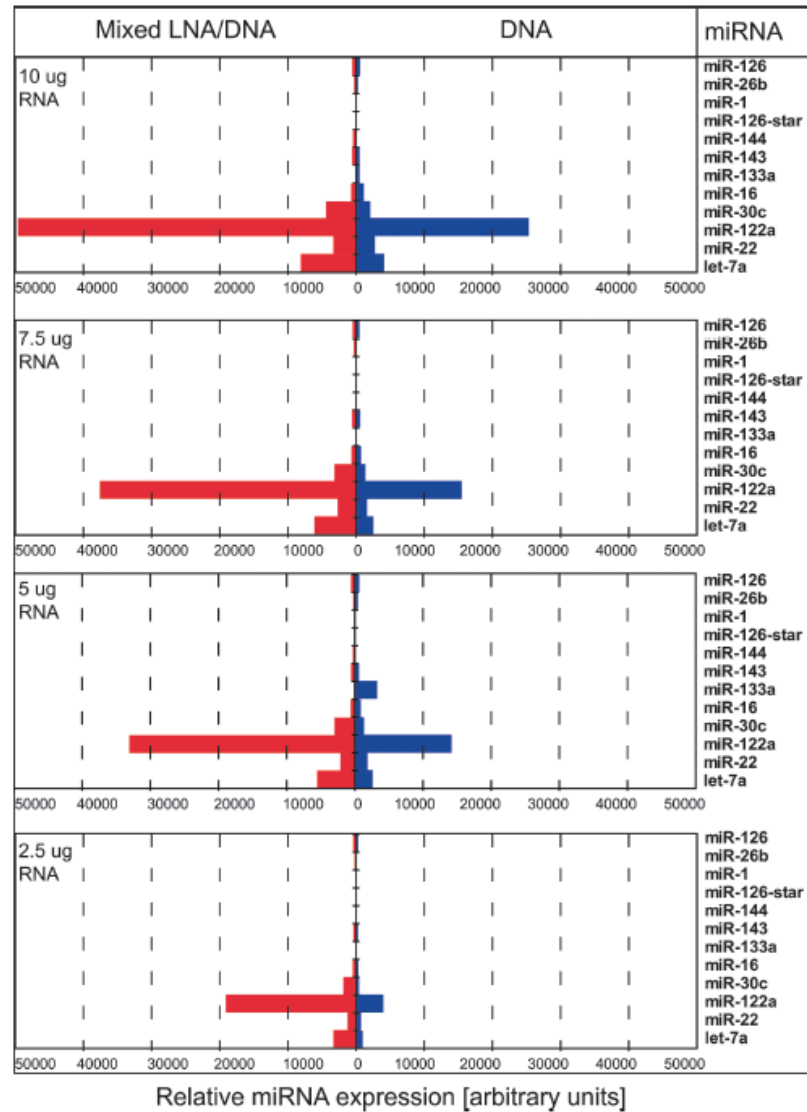


Použití LNA pro některé báze v próbě



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

**SÍLA VAZBY:  
LNA vs DNA próba  
Tm až 72°C  
(Castoldi et al., 2006)**

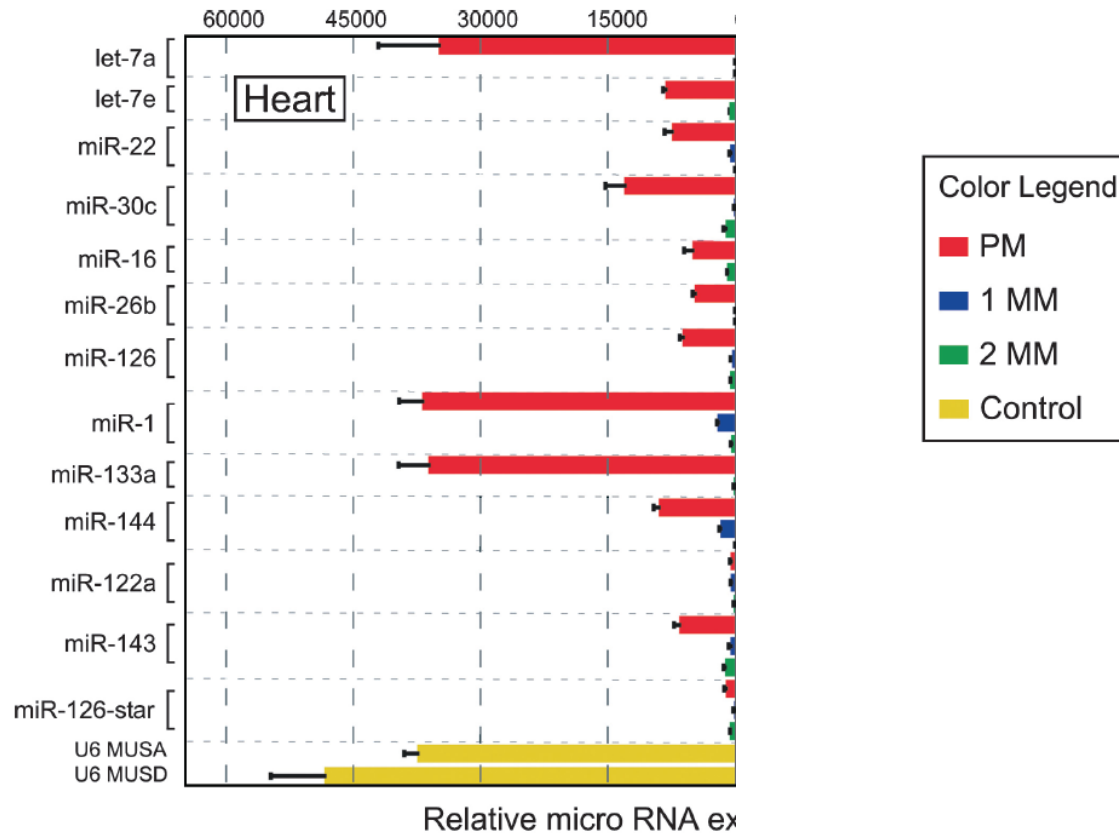


**FIGURE 1.** Mixed DNA/LNA capture probes display increased sensitivity for miRNA detection. miRNA expression was assessed in murine liver using a test set of LNA-modified (*left*) or unmodified DNA oligonucleotide capture probes (*right*). Decreasing amounts of total RNA were used as input material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

## Protocol overview

### miRCURY™ LNA microRNA Power Labeling Kit

CIP treatment

Mix: RNA sample  
Spike-In miRNA kit



Labeling reaction

Mix: CIP'ed RNA sample  
Labeling buffer  
Hy3™ or Hy5™  
DMSO  
Enzyme



### miRCURY™ LNA microRNA Array Kit

Mix samples

Mix: Hy3™ labeled sample  
Hy5™ labeled sample  
Hybridization buffer  
Denature sample



Hybridize

Hybridize at 56°C for 16 hours



Stringency wash

Wash 2 min. in buffer A at 56°C  
Wash 2 min. in buffer B at 23°C  
Wash 2 min. in buffer C at 23°C  
Dry slides



Image acquisition

Scan slides (recommended scan at 5µm)  
Download relevant GAL files from  
[www.exiqon.com](http://www.exiqon.com)

# Co se nemusí podařit:

**Nekvalitní RNA**

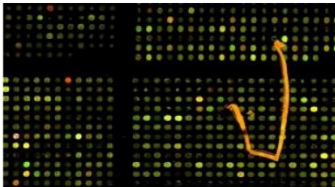
**Nepodaří se značení**

**Nepodaří se hybridizace**

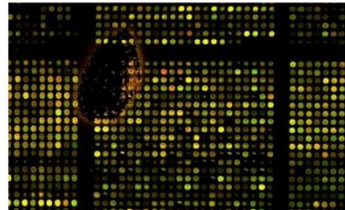
**Nepodaří se promývání**

**Technická variabilita čipů je větší než ta biologická**

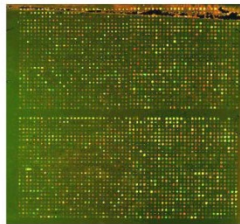
**Nepodaří se validace dat pomocí RT-PCR, atd**



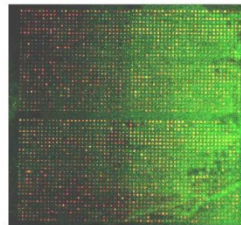
Fiber or scratch?



Bubble



Edge effect

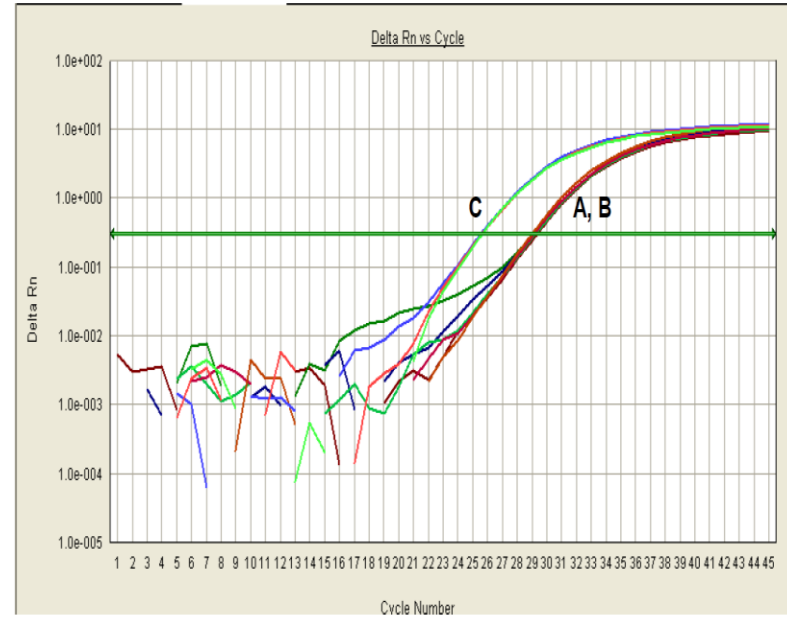
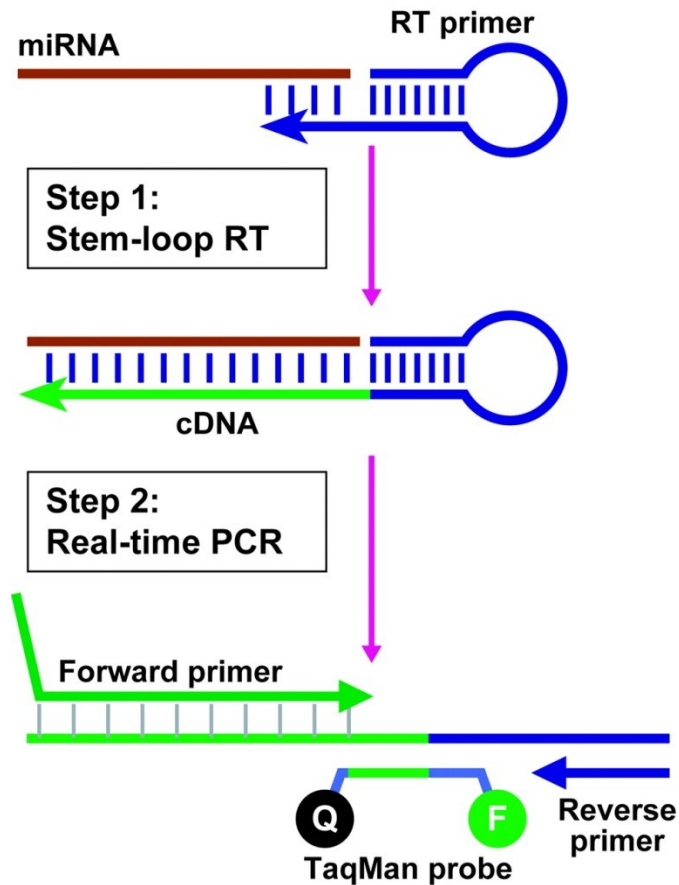


Background haze

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



# RNA Seq

# Sanger vs NGS

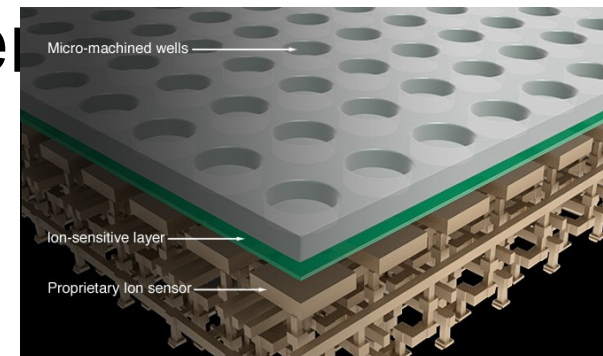
‘Sanger sequencing’ has been the only DNA sequencing method for 30 years

.....

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

# Next Generation Sequencing

- Takes advantage of miniaturization to engage in massively parallel analysis
  - Essentially carrying out millions of sequencing reactions simultaneously in each of 10 million tiny wells
- Sophisticated computer analysis of huge amounts of information allows “assembly” of a given sequence



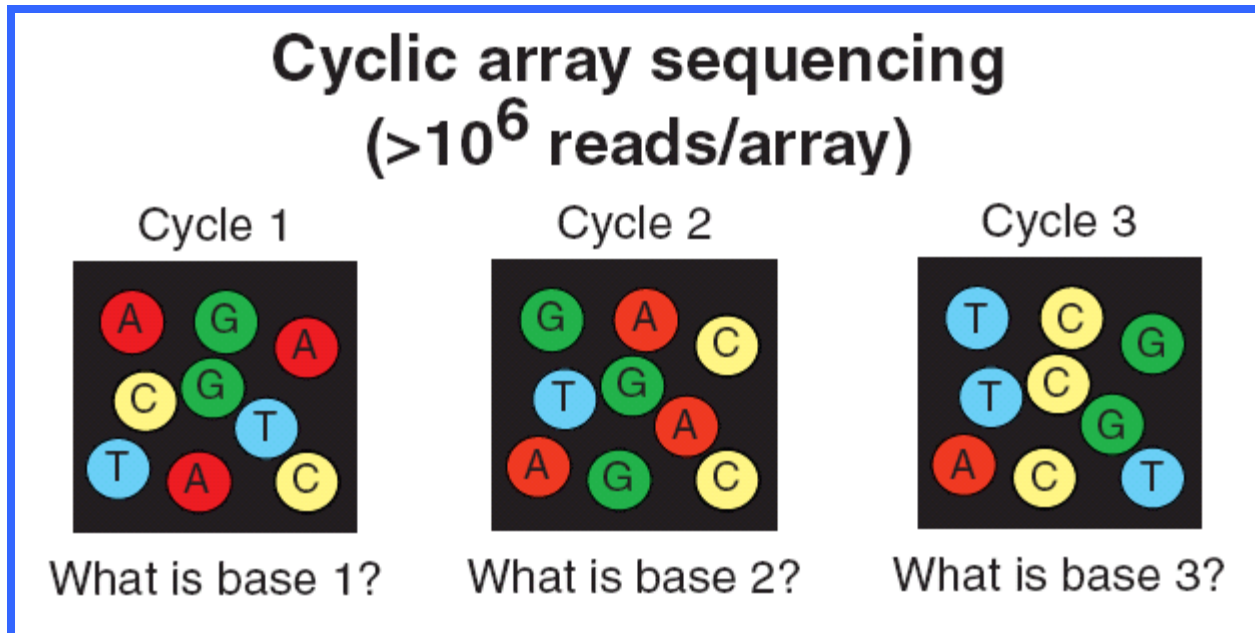




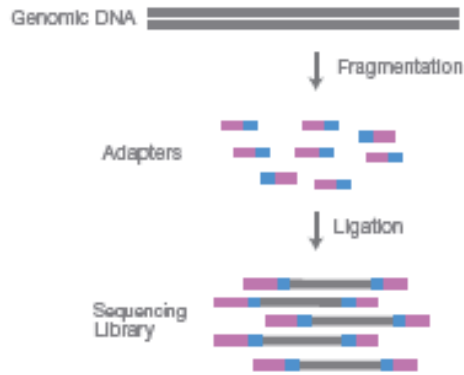
# High Parallelism is Achieved in Polony Sequencing

**Sanger**

**Polony**

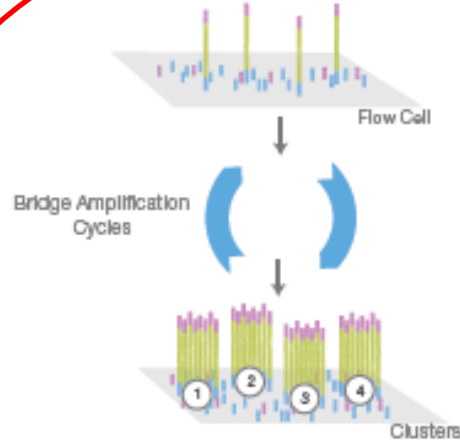


### A. Library Preparation



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

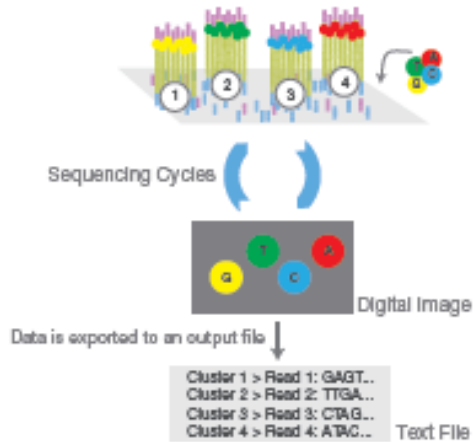
### A. Cluster Amplification



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.

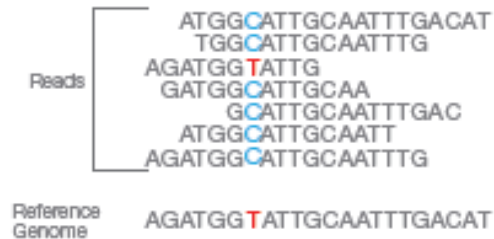
This is the trick

### 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 Analysis

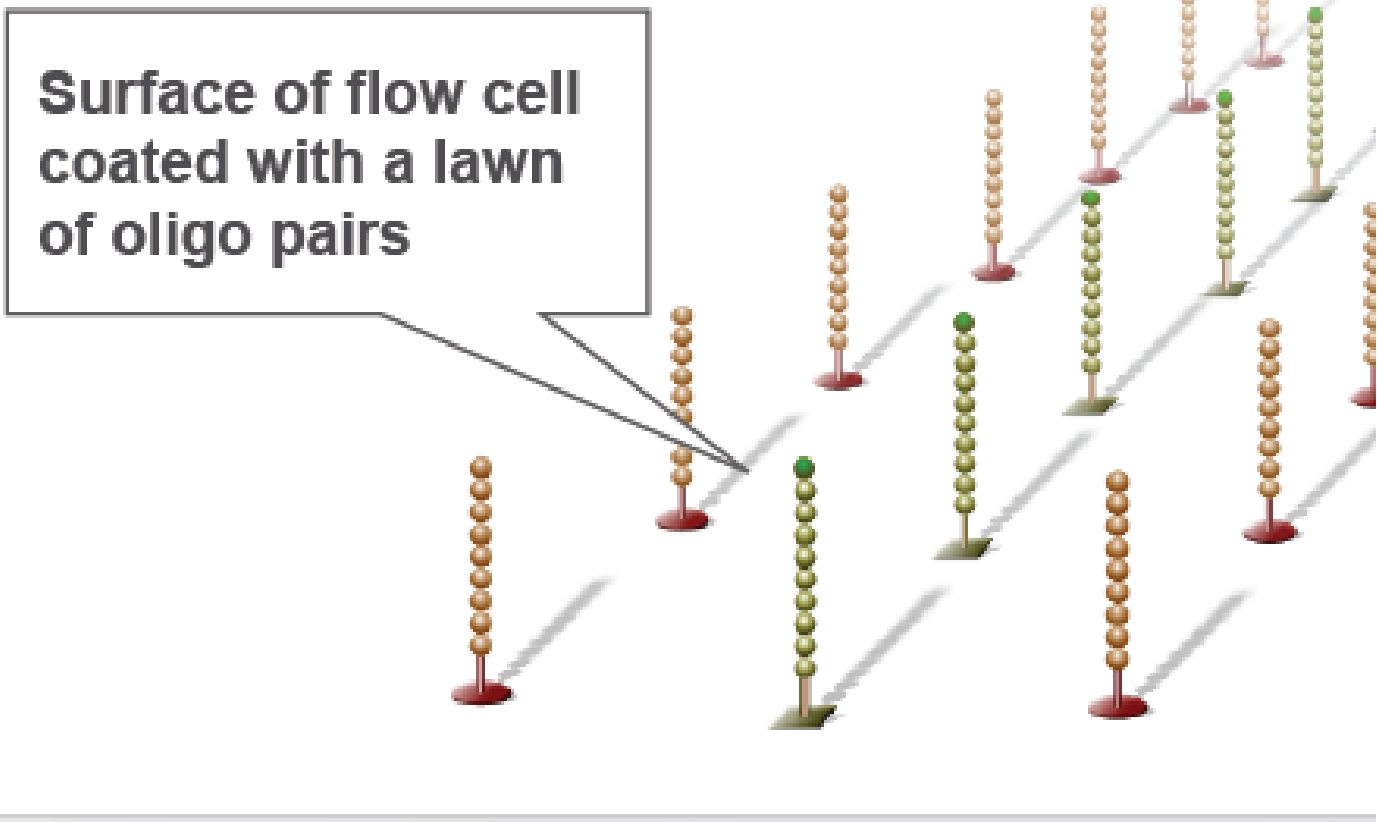


Reads are aligned to a reference sequence with bioinformatics software. After alignment, differences between the reference genome and the newly sequenced reads can be identified.

E



Two PCR primers are attached to the surface of flowcell. One of the primers has a cleavable site

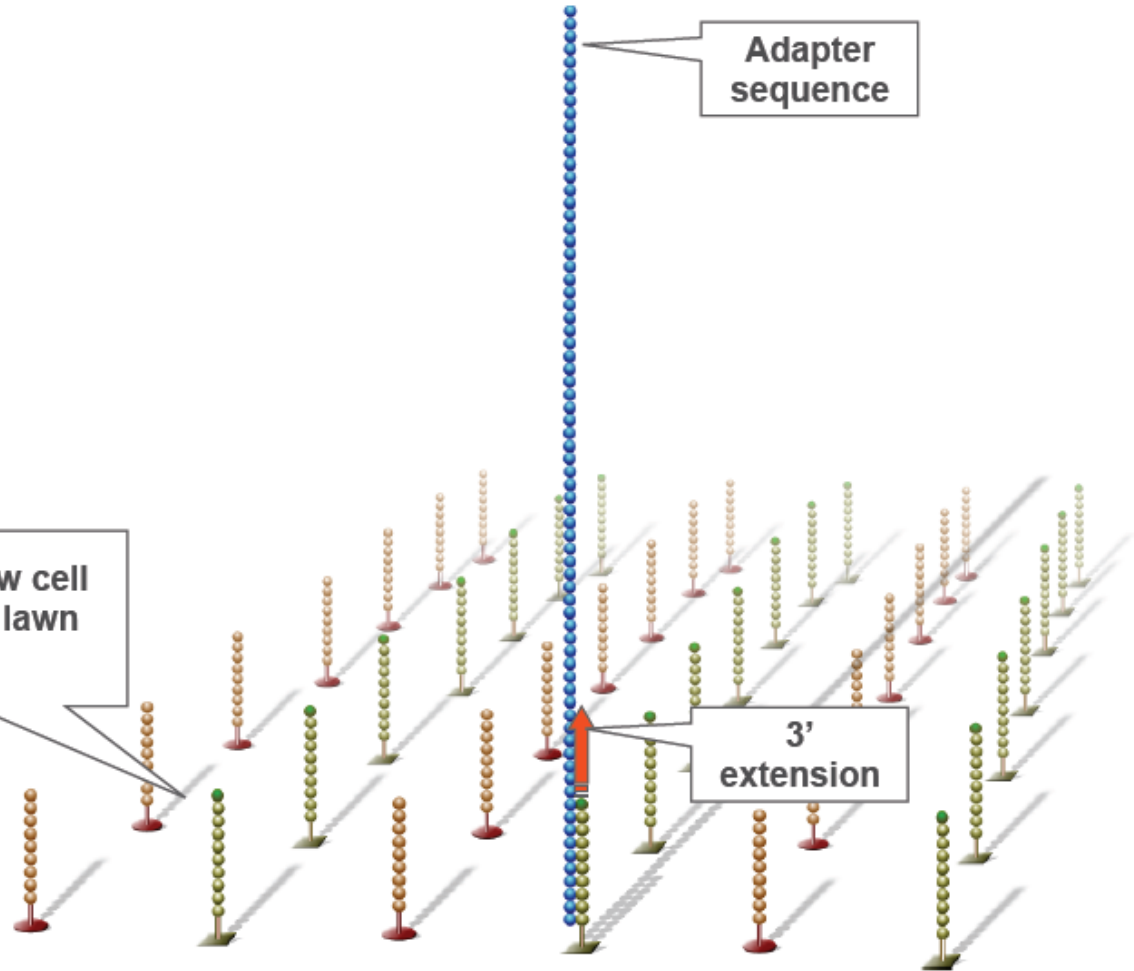


# Hybridize Fragment & Extend

Single DNA libraries are hybridized to primer lawn

Bound libraries are then extended by polymerases

Surface of flow cell coated with a lawn of oligo pairs

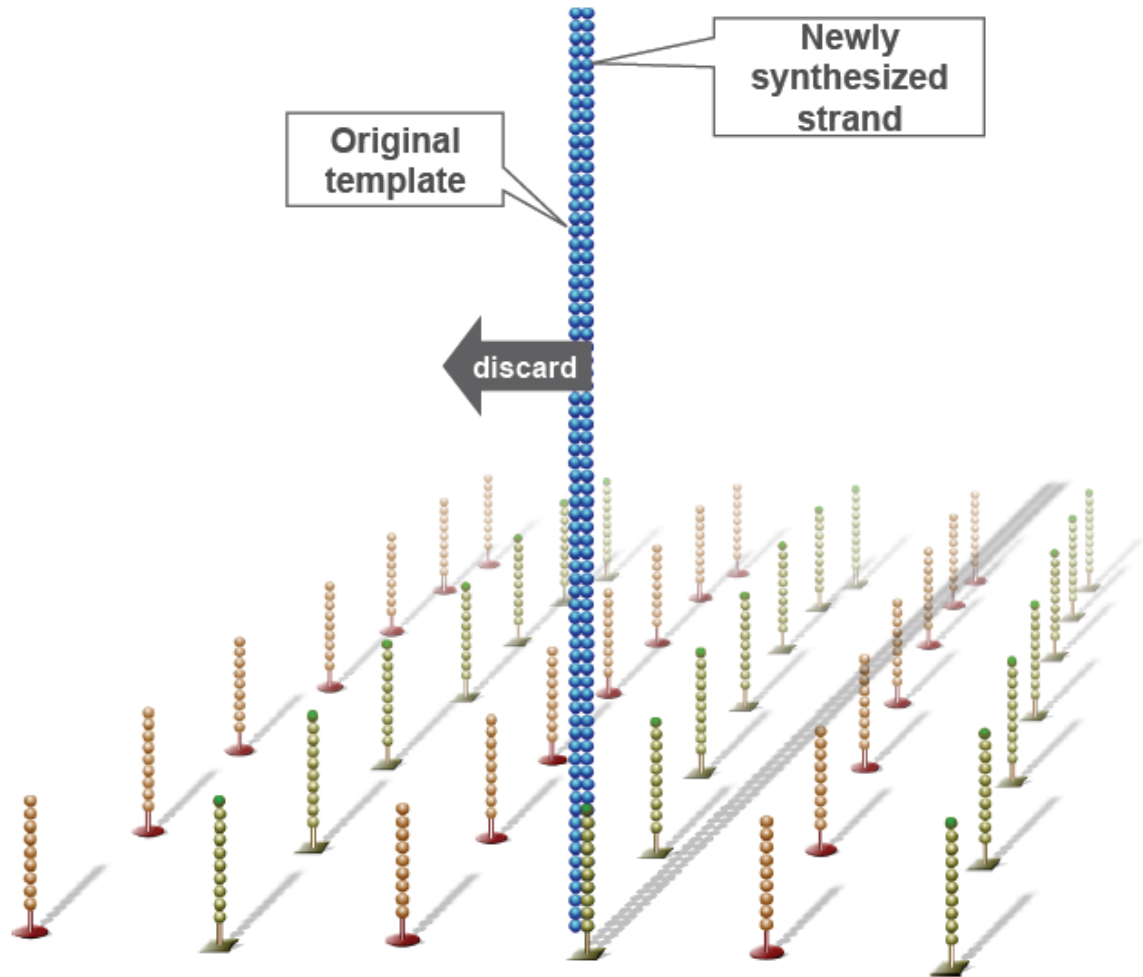


# Denature Double-Stranded DNA

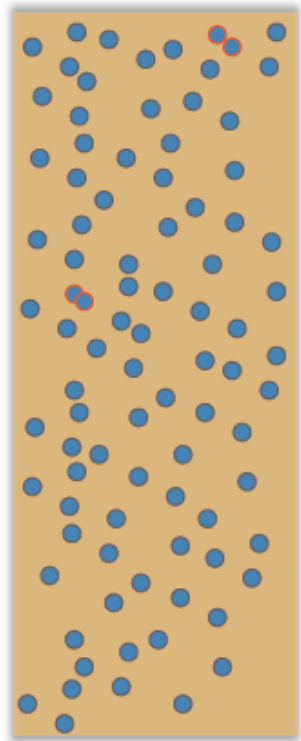
Double-stranded molecule is denatured

Original template washed away

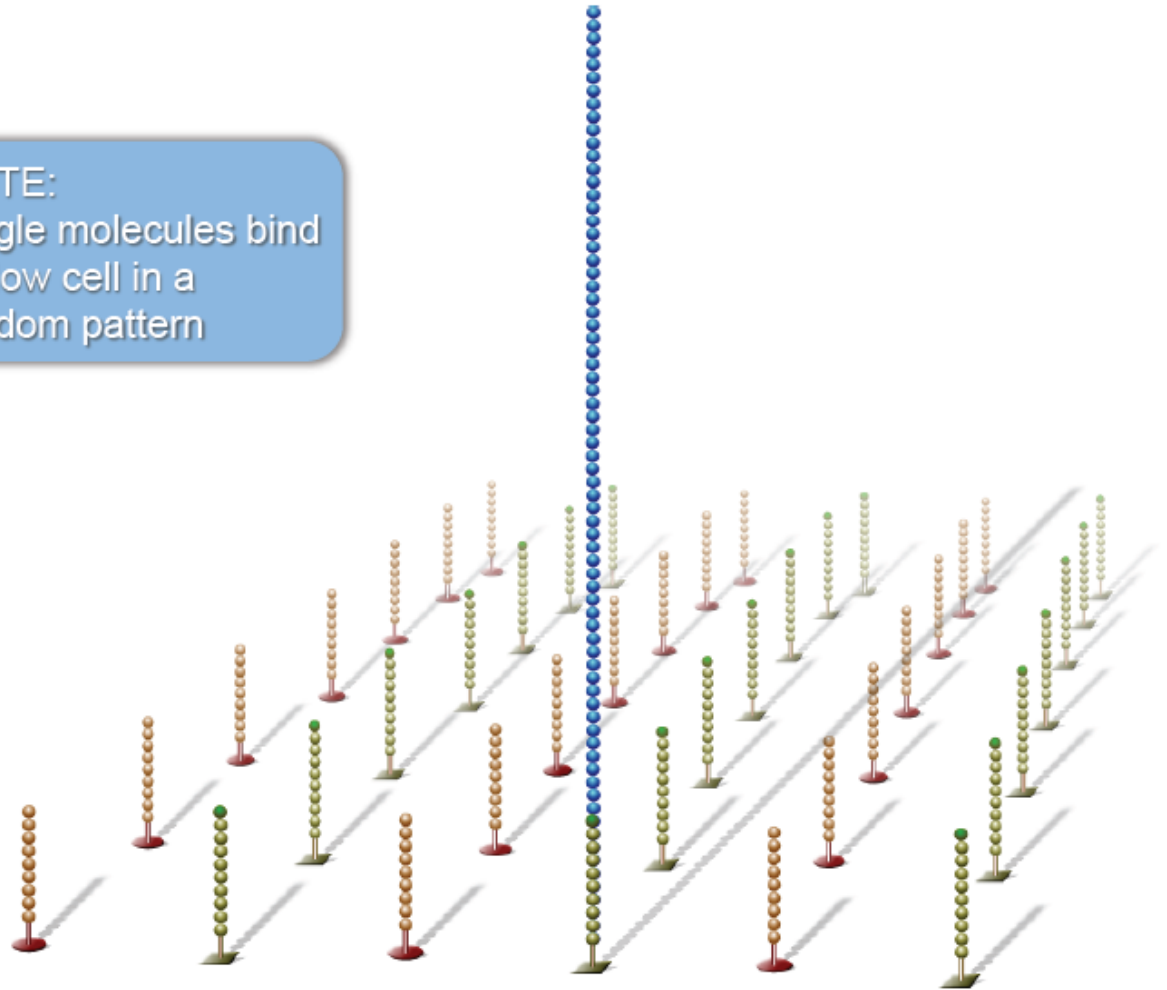
Newly synthesized strand is covalently attached to flow cell surface



# Single-Stranded DNA



NOTE:  
Single molecules bind  
to flow cell in a  
random pattern

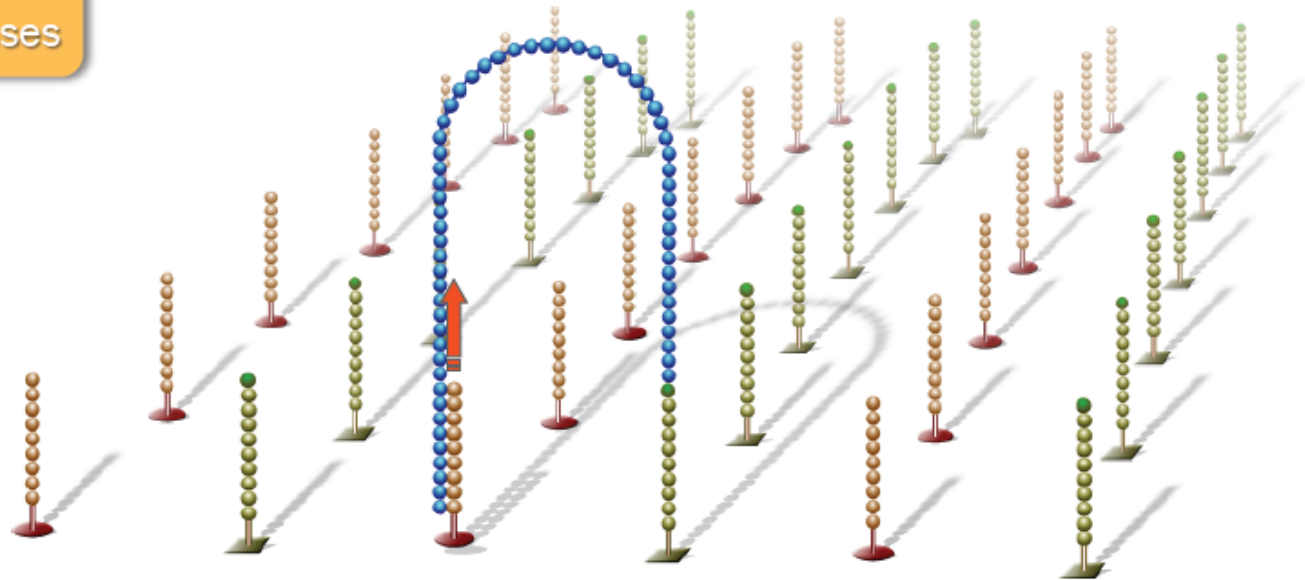




# Bridge Amplification

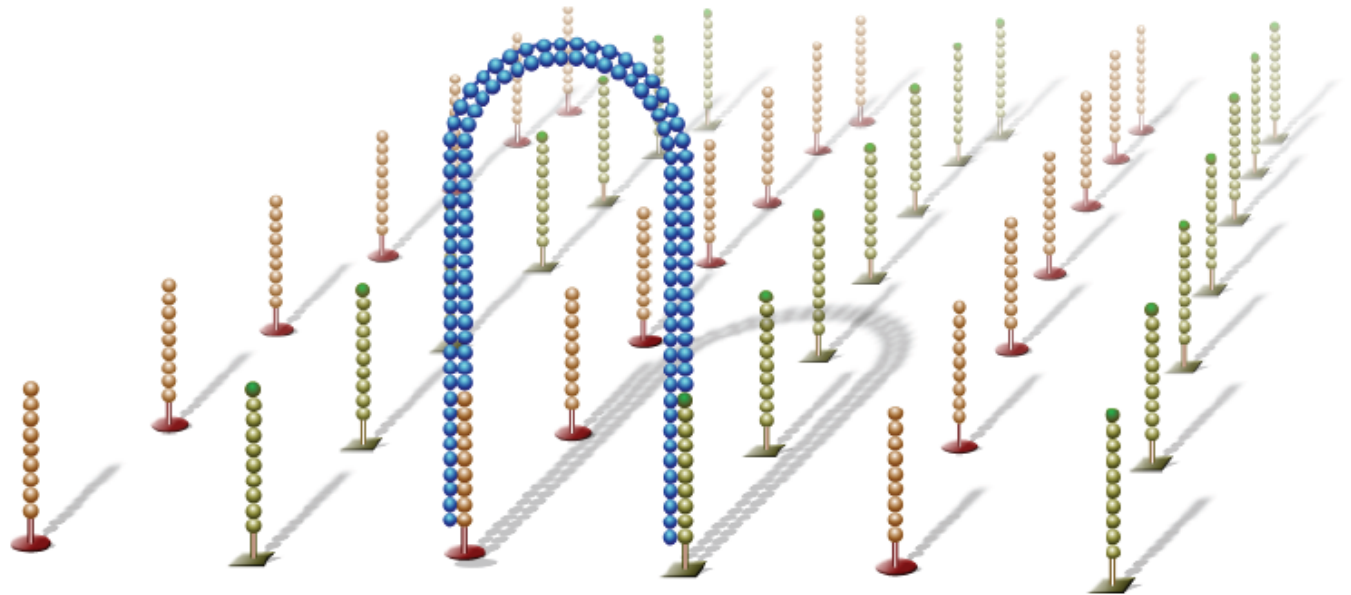
Single-stranded molecule flips over and forms a bridge by hybridizing to adjacent, complementary primer

Hybridized primer is extended by polymerases



# Bridge Amplification

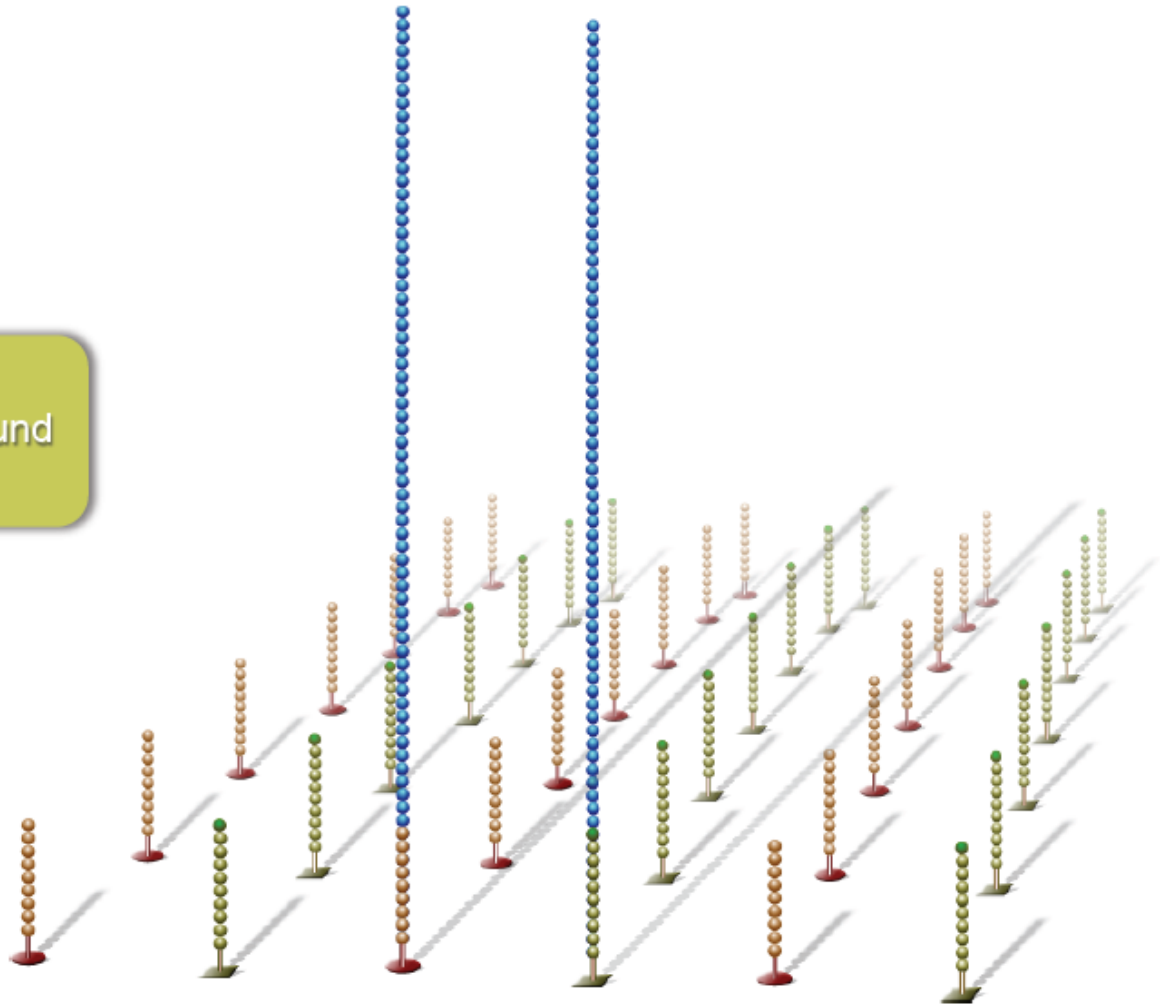
Double-stranded bridge is formed



# Denature Double-Stranded Bridge

Double-stranded bridge is denatured

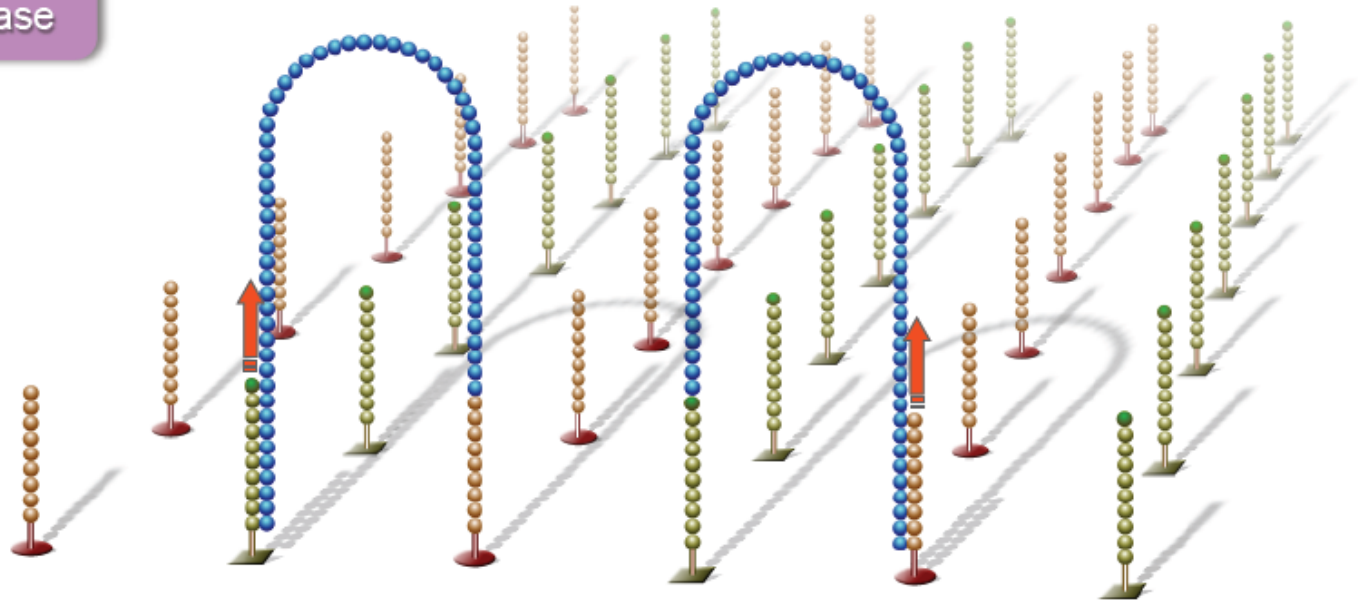
Result:  
Two copies of covalently bound single-stranded templates



# Bridge Amplification

Single-stranded molecules flip over to hybridize to adjacent primers

Hybridized primer is extended by polymerase

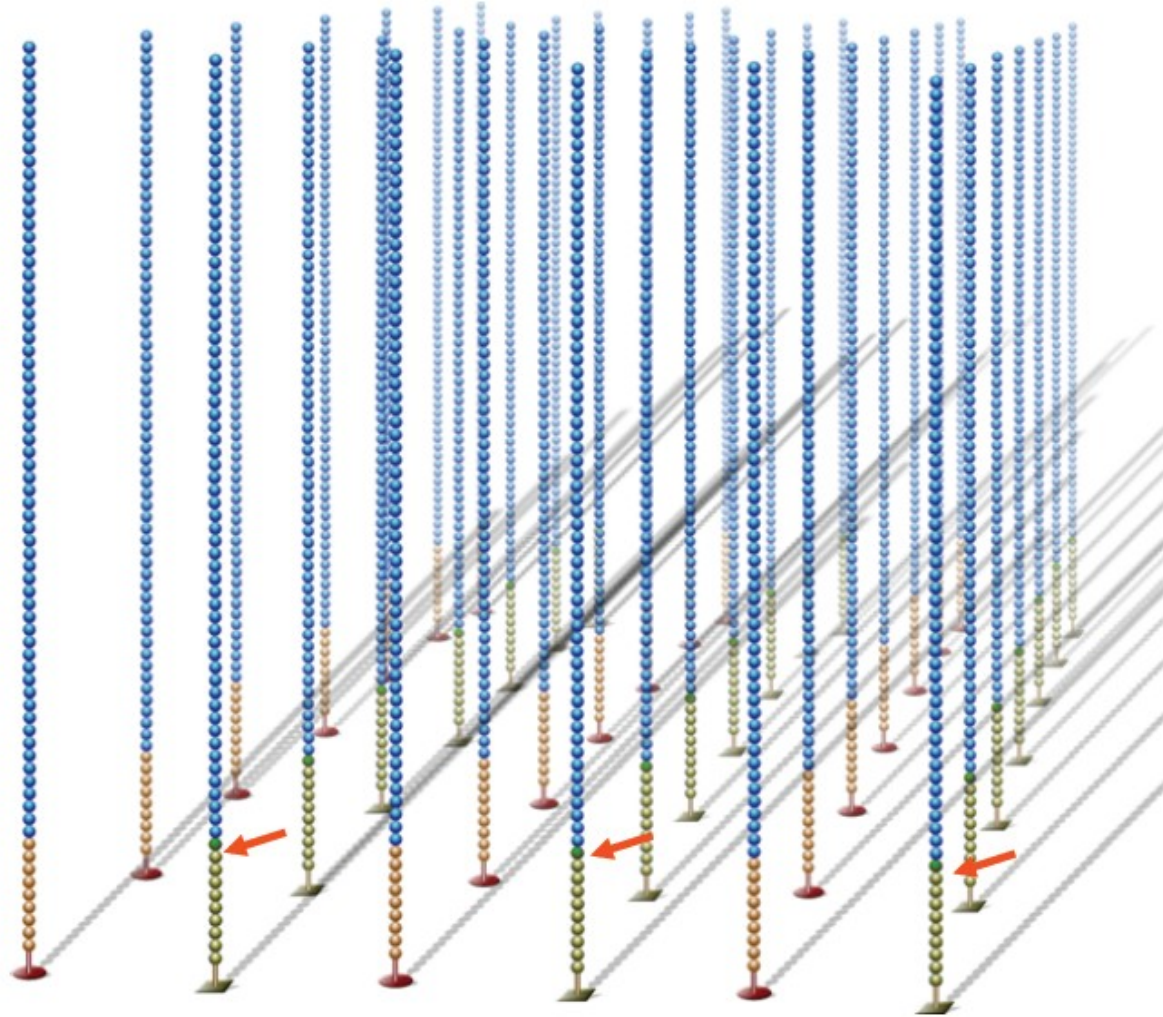






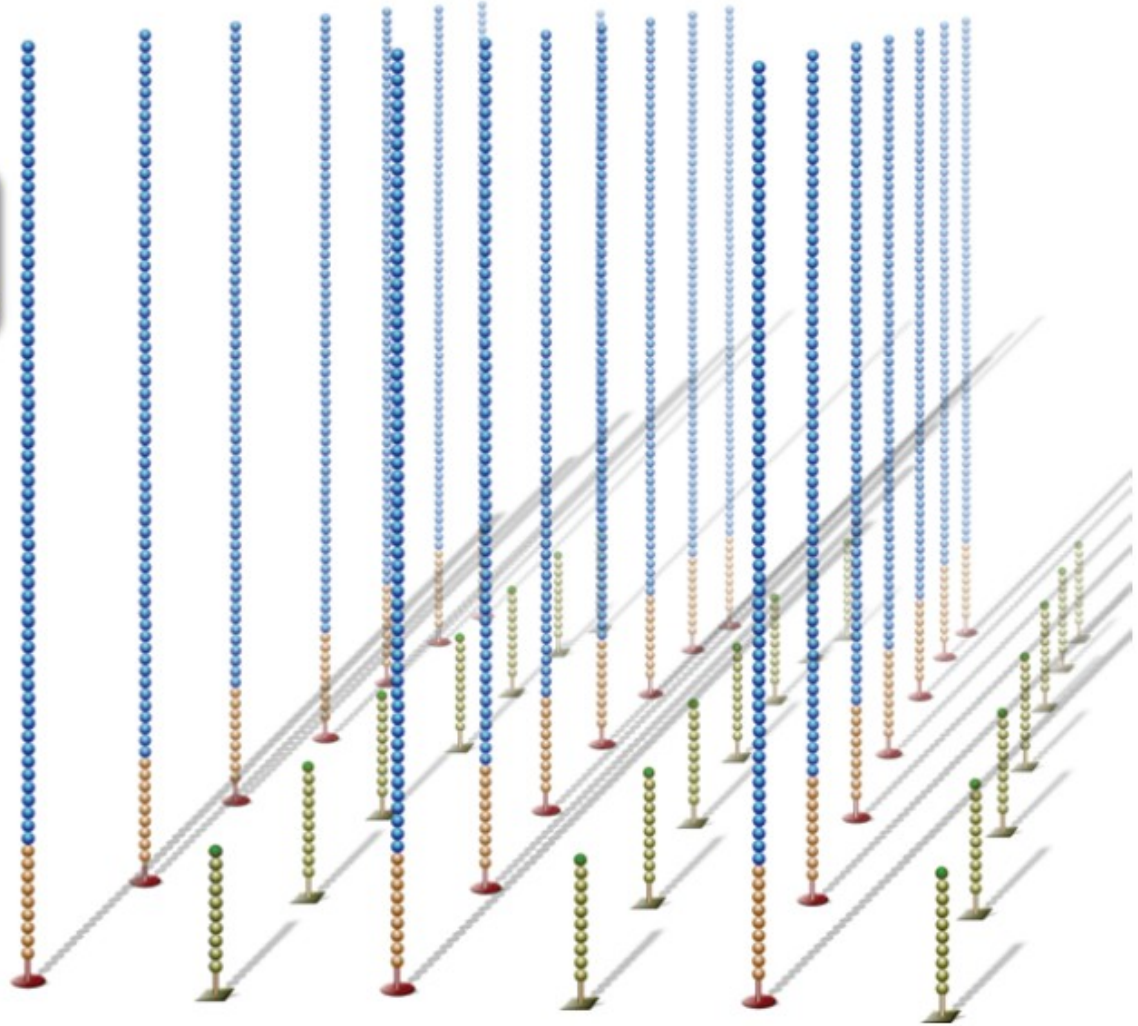
# Linearization

dsDNA bridges are denatured



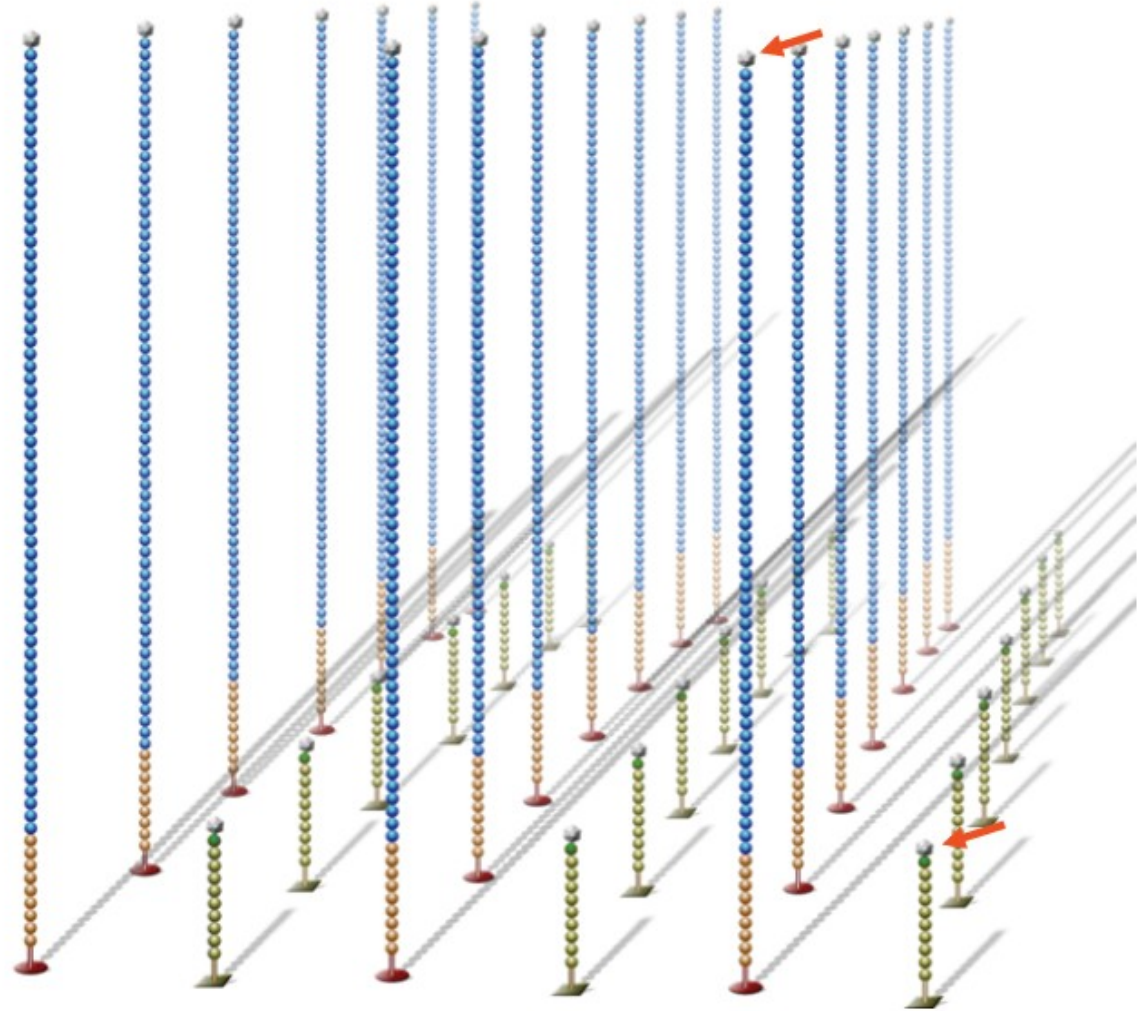
# Reverse Strand Cleavage

Reverse strands are cleaved and washed away, leaving a cluster with forward strands only



# Blocking

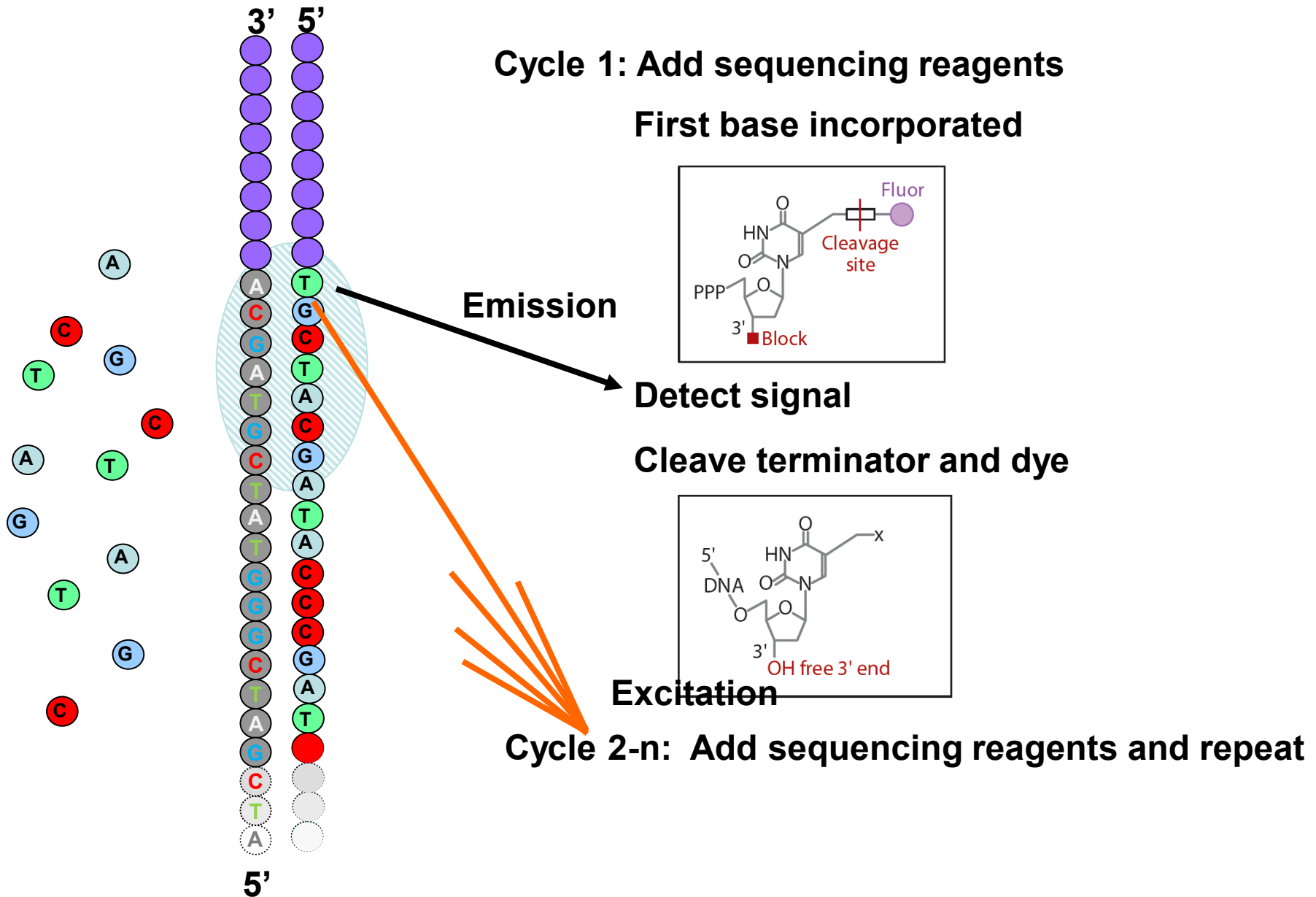
Free 3' ends are blocked to prevent unwanted DNA priming



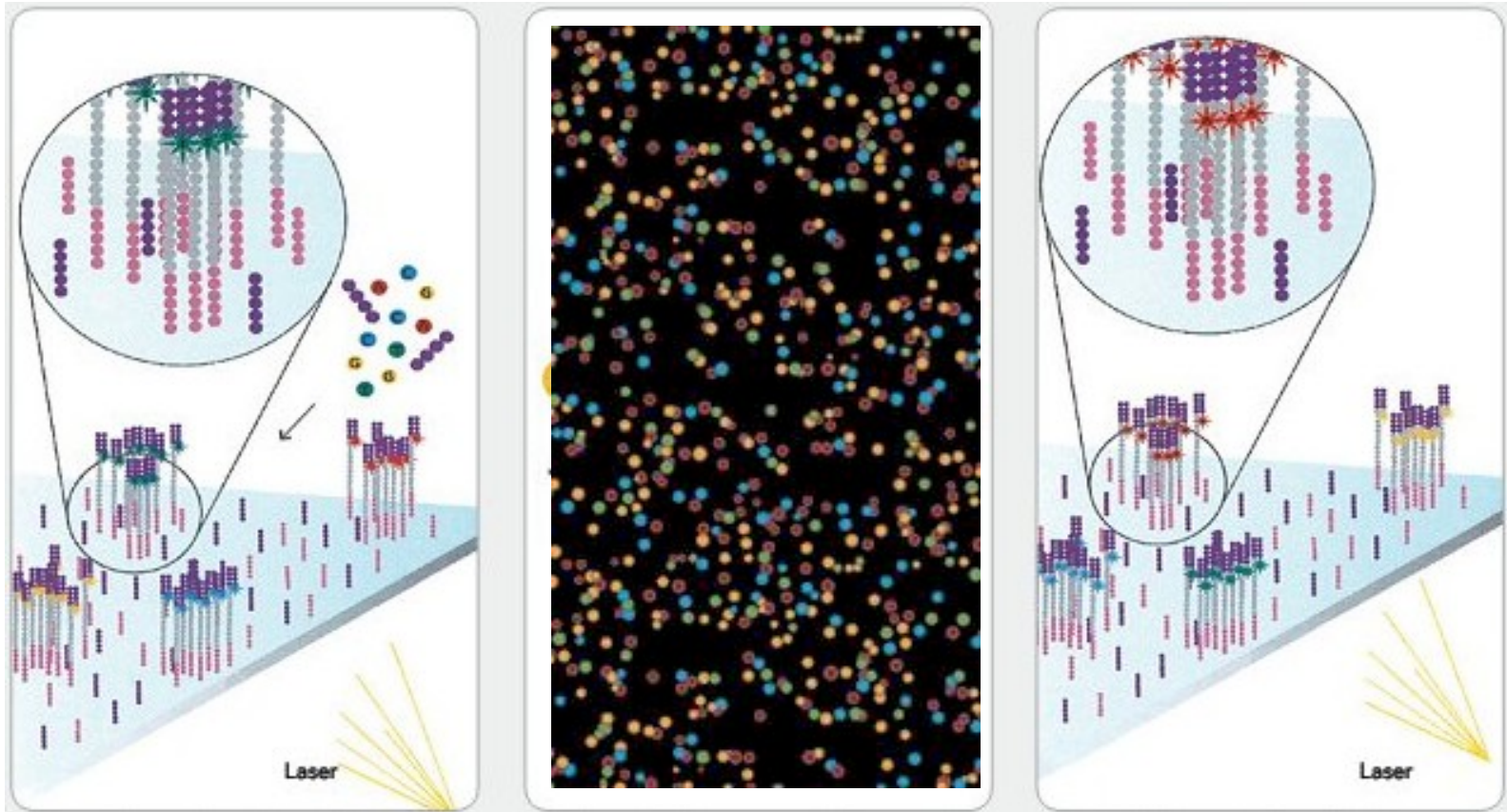




# Sequencing by synthesis



# Sequencing by Synthesis - Fluorescently labeled Nucleotides (Illumina)



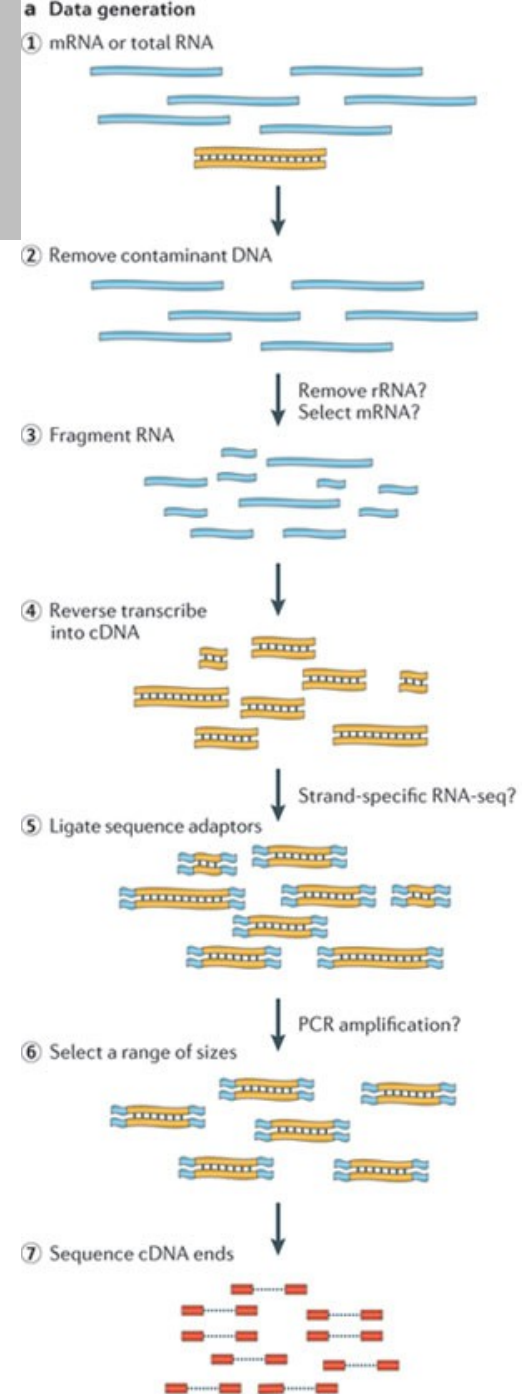
**Complementary strand elongation: DNA Polymerase**

# video

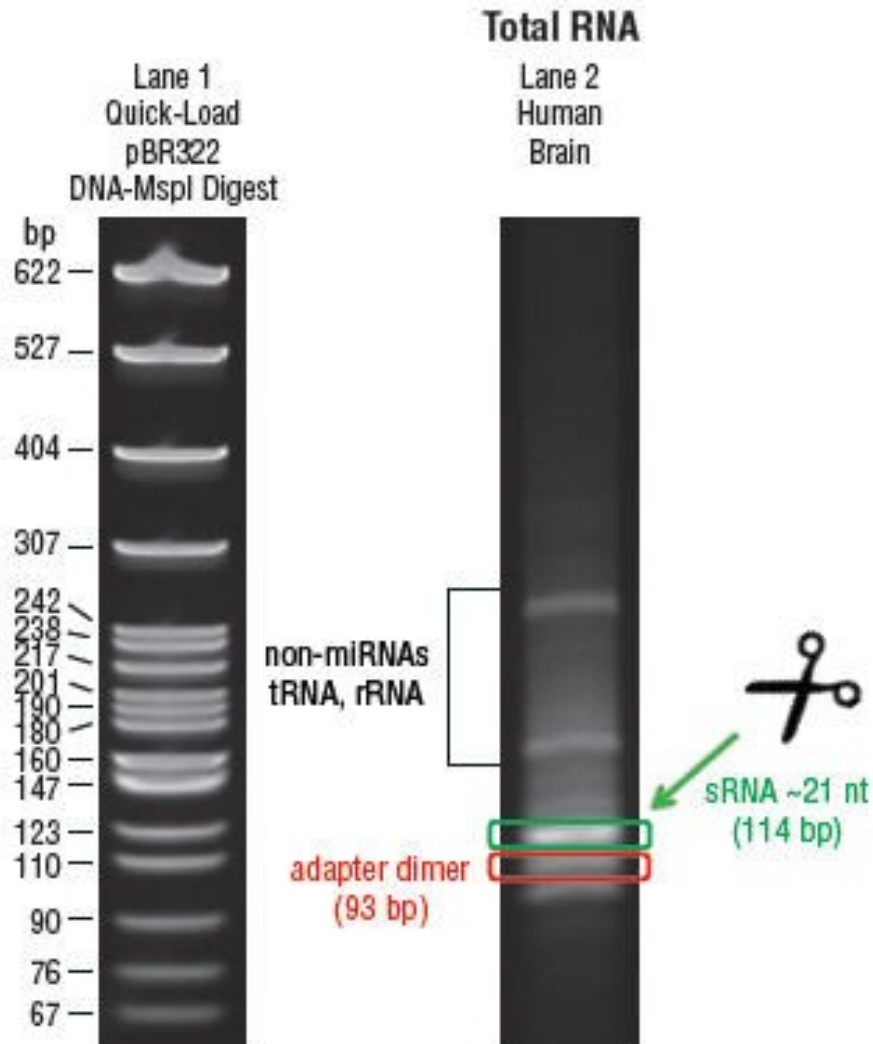
- <https://www.youtube.com/watch?v=womKfikWlxM>

# The general experimental procedure for RNA

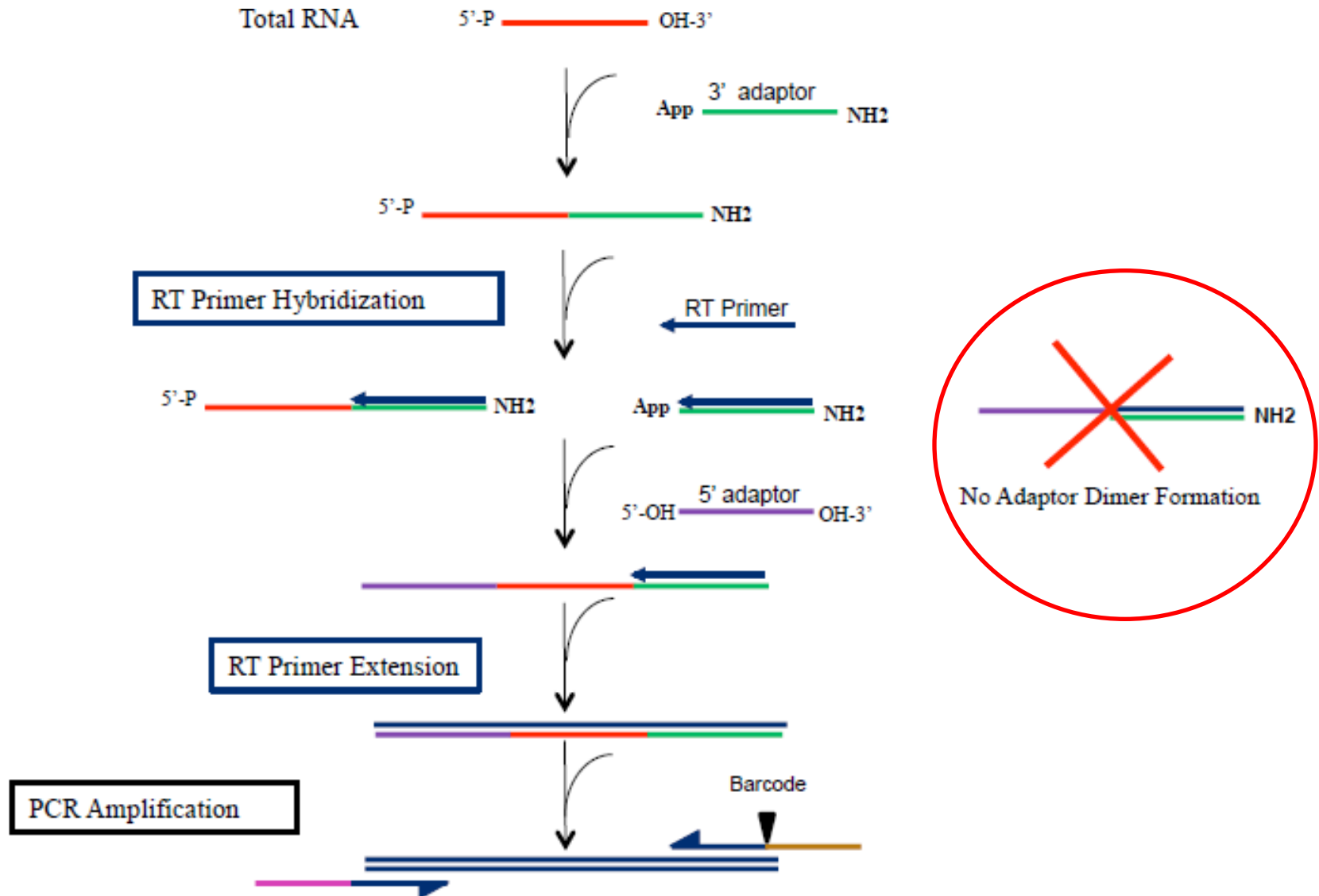
**Transcriptom** =  
sum of all RNA  
(mRNA, rRNA,  
tRNA and  
noncoding RNA)

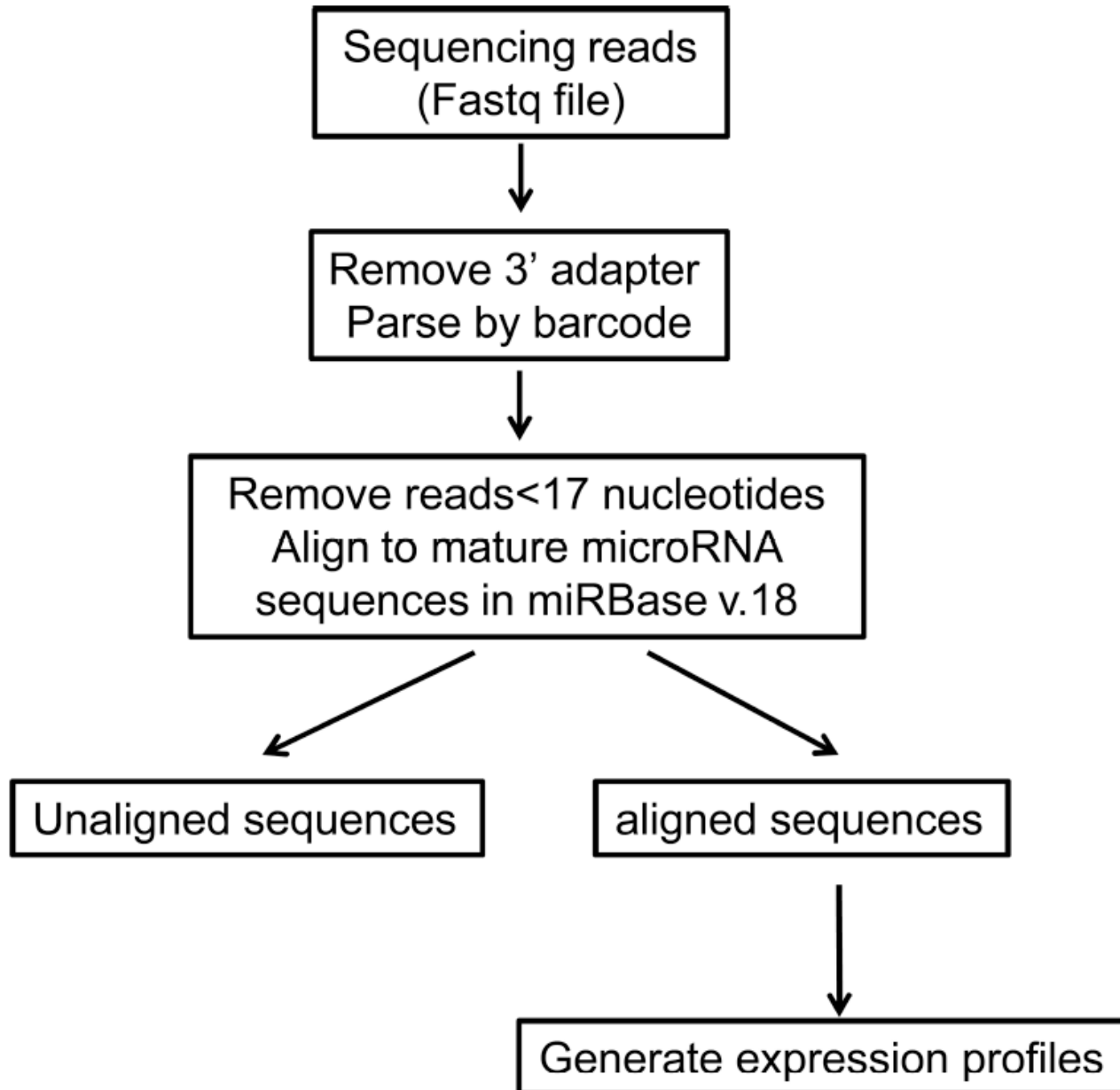


# The general experimental procedure for miRNA



# The general experimental procedure for miRNA



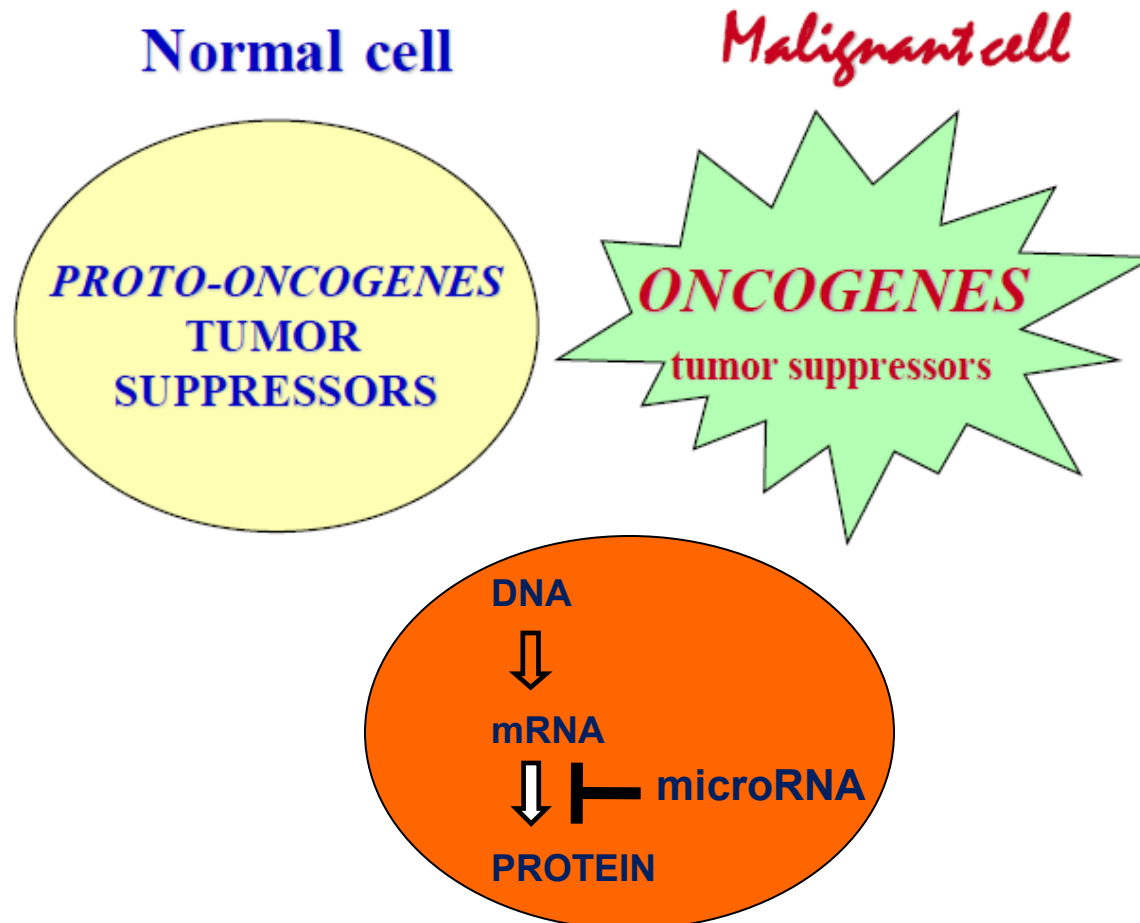




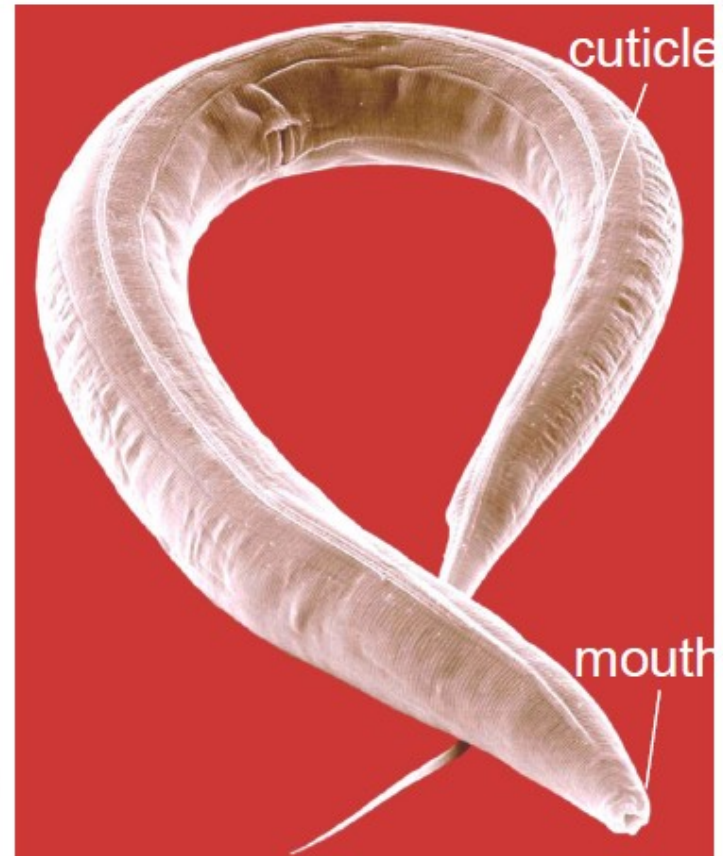
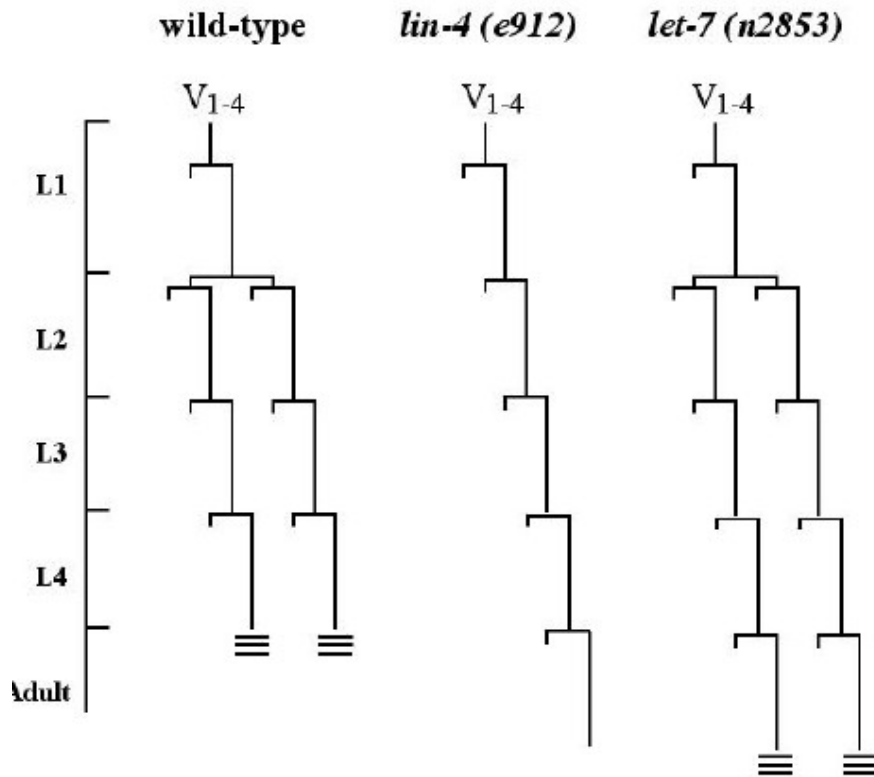
## **ODPOČINEK**

# 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.



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

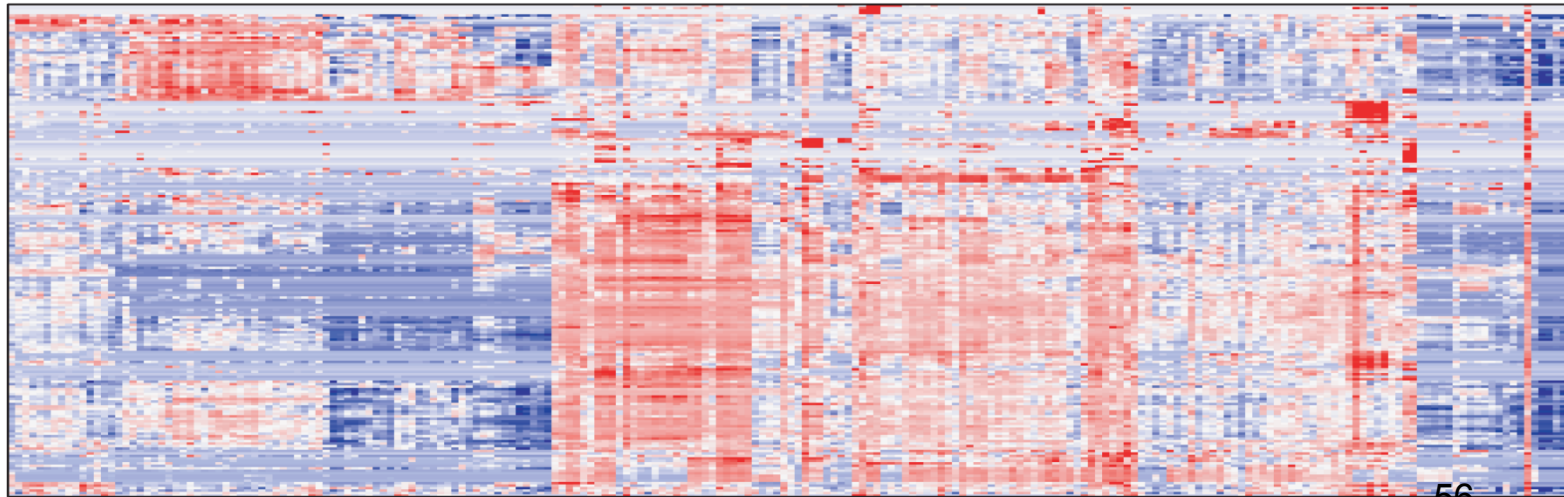
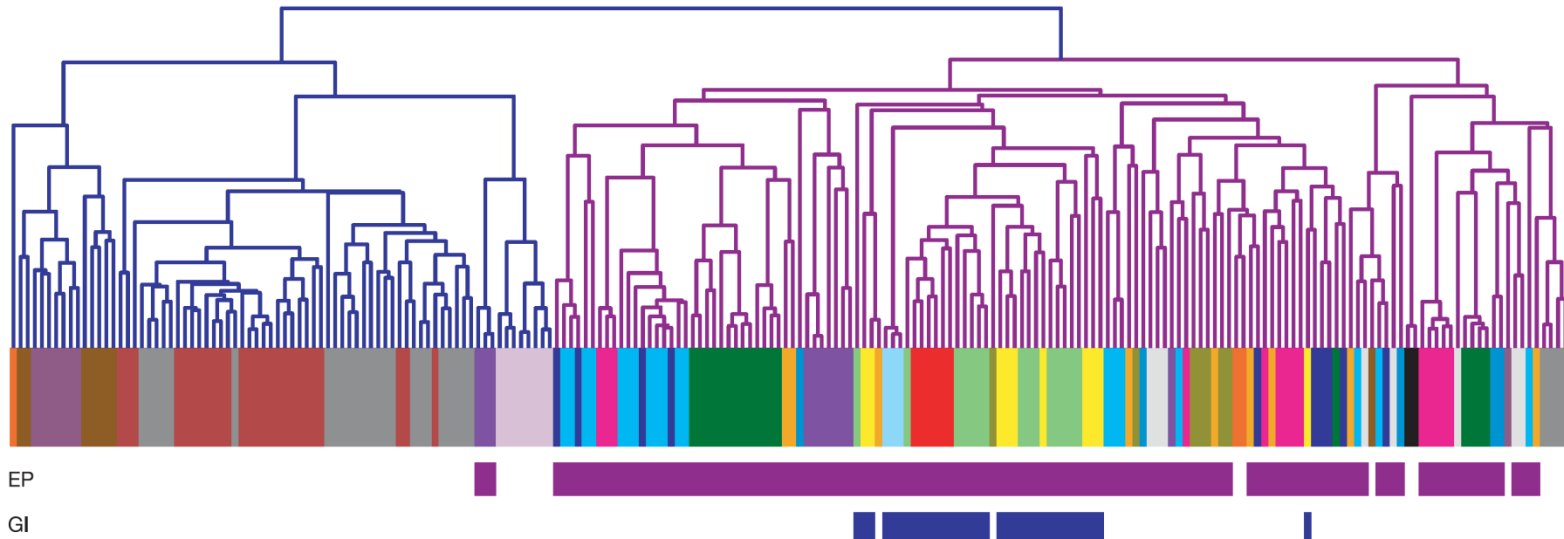


Lee et al. 1993 *Cell*

Reinhart et al. 2000 *Nature*

# microRNA exprese je schopná rozlišit původ nádoru

**a**

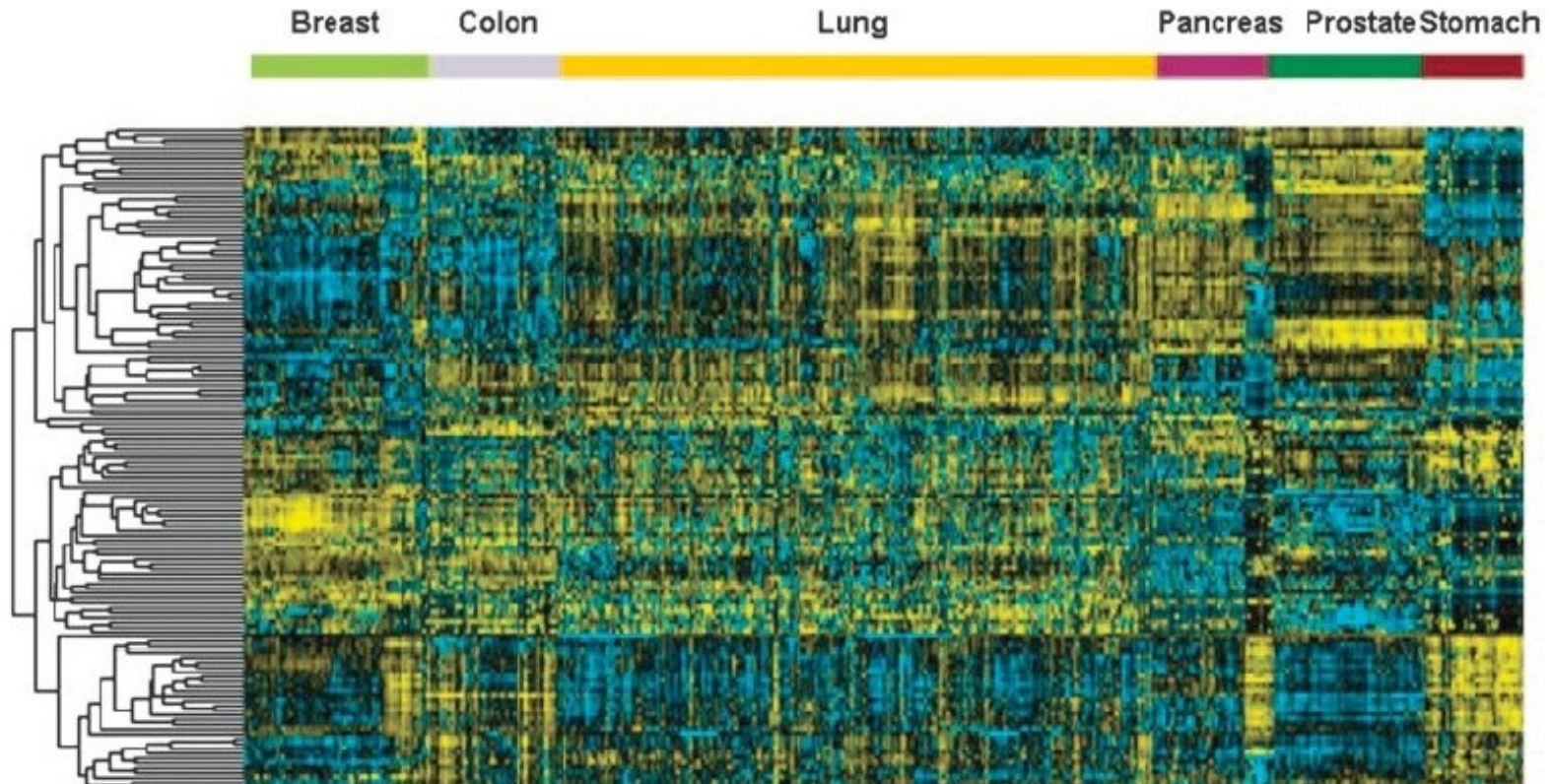




# A microRNA expression signature of human solid tumors defines cancer gene targets

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

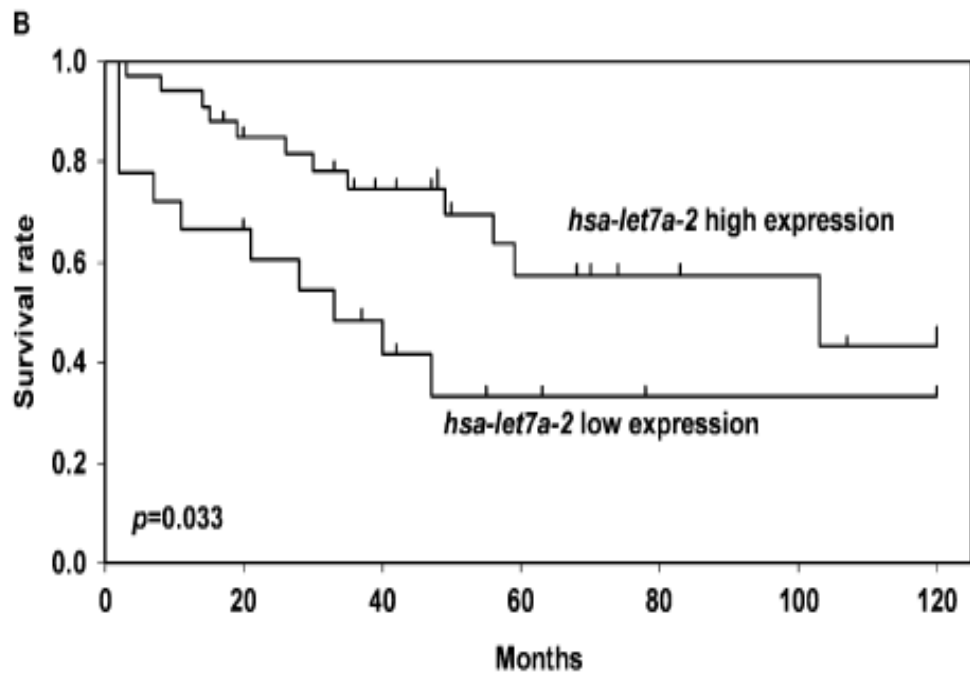
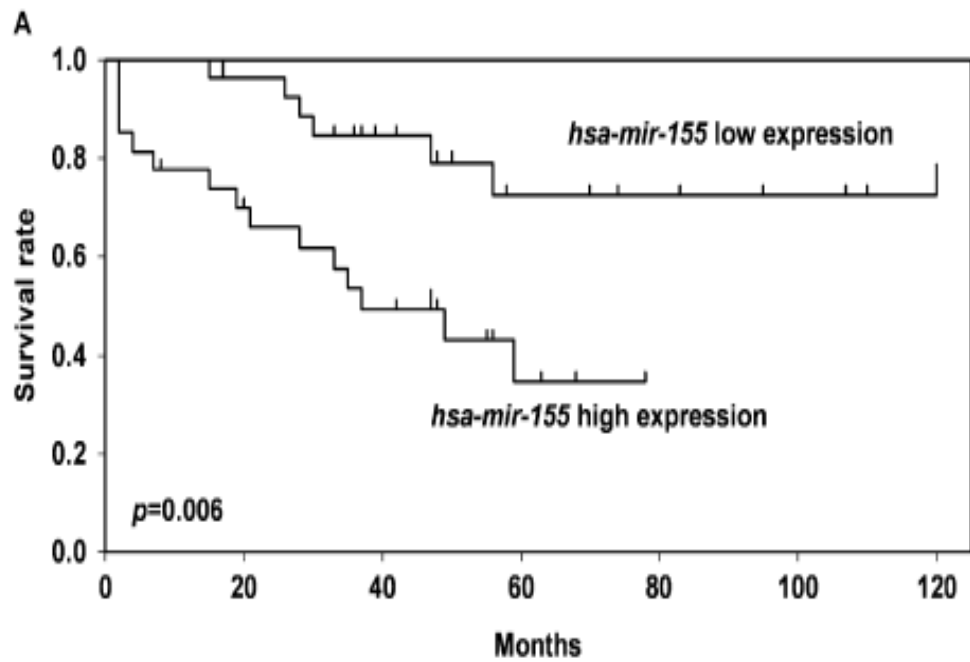
<sup>\*</sup>Department of Molecular Virology, Immunology, and Medical Genetics and Cancer Comprehensive Center, Ohio State University, Columbus, OH 43210; <sup>§</sup>Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; <sup>†</sup>Telethon Facility–Data Mining for Analysis of DNA Microarrays, Department of Morphology and Embryology, and <sup>¶</sup>Department of Experimental and Diagnostic Medicine and Interdepartmental Center for Cancer Research, University of Ferrara, 44100 Ferrara, Italy; <sup>||</sup>Department of Pathology, University of Verona, 37100 Verona, Italy; and <sup>\*\*</sup>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	<i>N</i>	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 =  $2.5 \times 10^{-3}$ ).



## *A unique miRNA signature is associated with lung cancer prognosis*

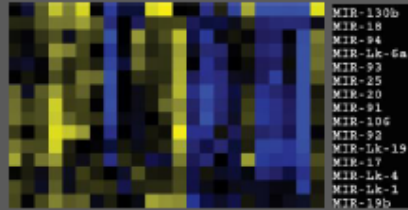
**Table 5.** Postoperative survival of patients with lung adenocarcinoma in relation to clinicopathological characteristics and miRNA expression analyzed by microarray analysis

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) <sup>a,b</sup>			
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

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



# A polycistronic cluster of microRNAs are overexpressed in cancer



Proliferation

Angiogenesis

Apoptosis

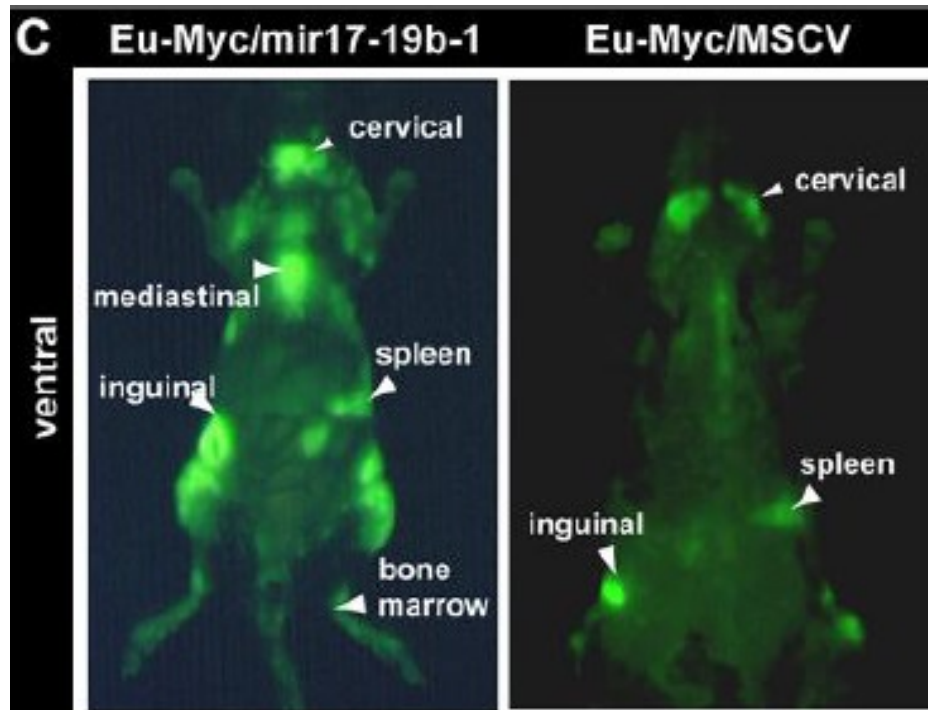
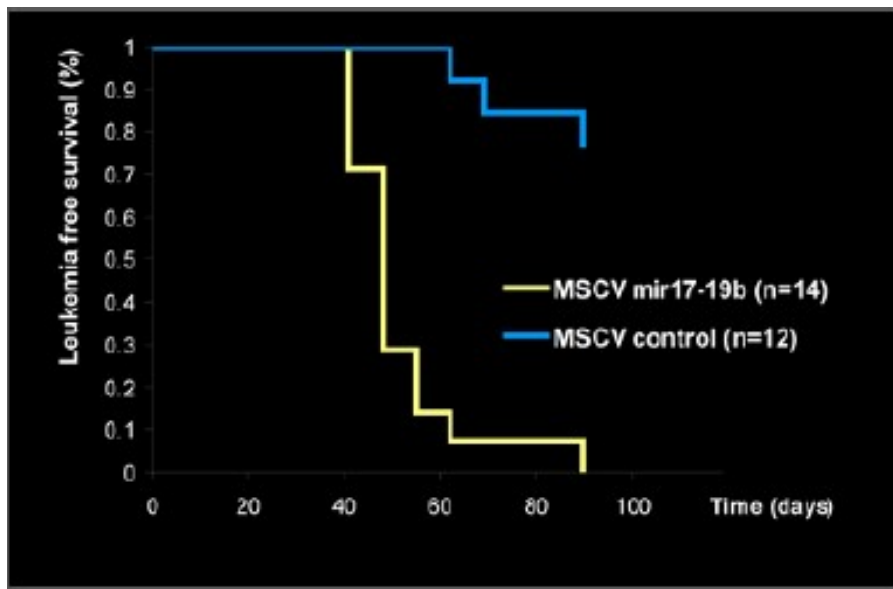
Invasiveness



Ch13-ORF25

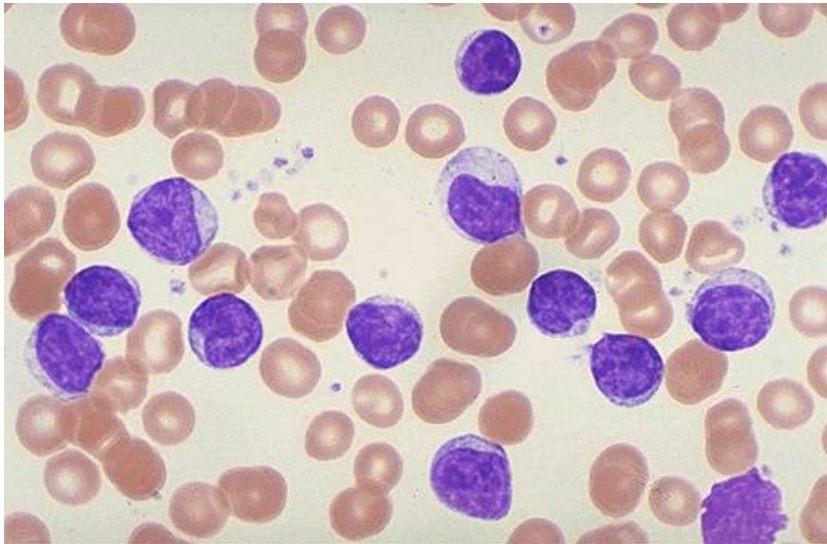


OncomiR-1 (Oncogenic microRNA-1)



# Chronická lymfatické leukémie

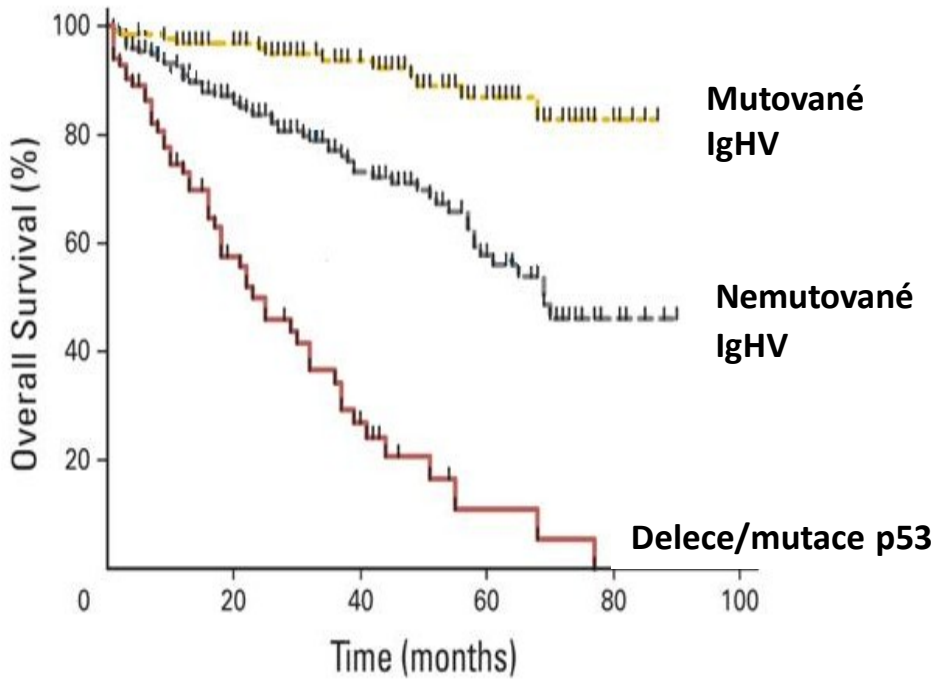
- ❑ Z maturovaných B lymfocytů
- ❑ Nejčastější leukémie dospělých
- ❑ Extrémně variabilní prognóza
- ❑ Nejčastější aberace del13q14 – obsahuje 2 miRNA (miR-15a, miR-16)



# Expresse miRNA asociuje s prognostickými subtypy CLL

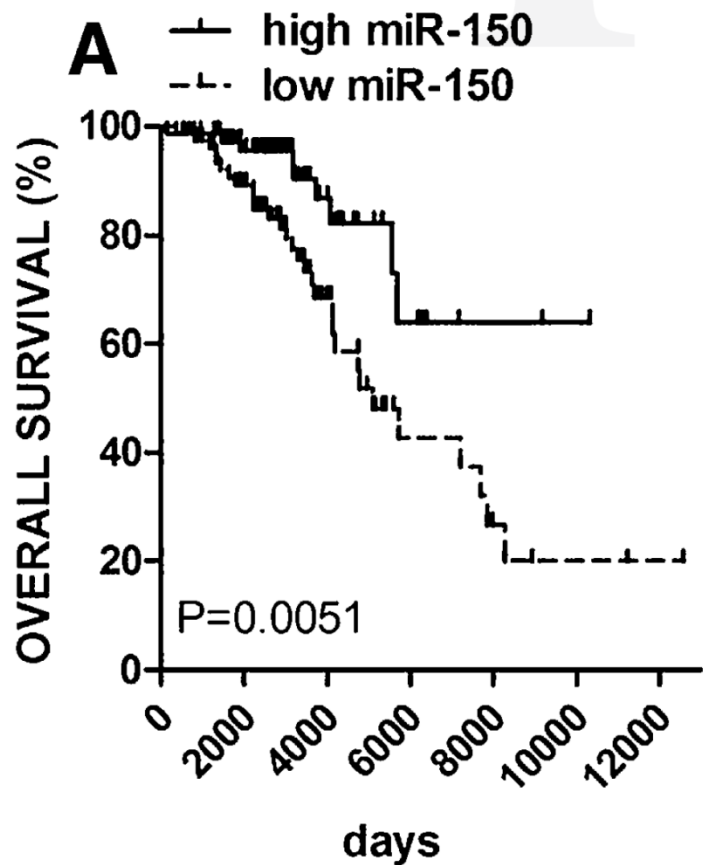
~ 20 miRNAs

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



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

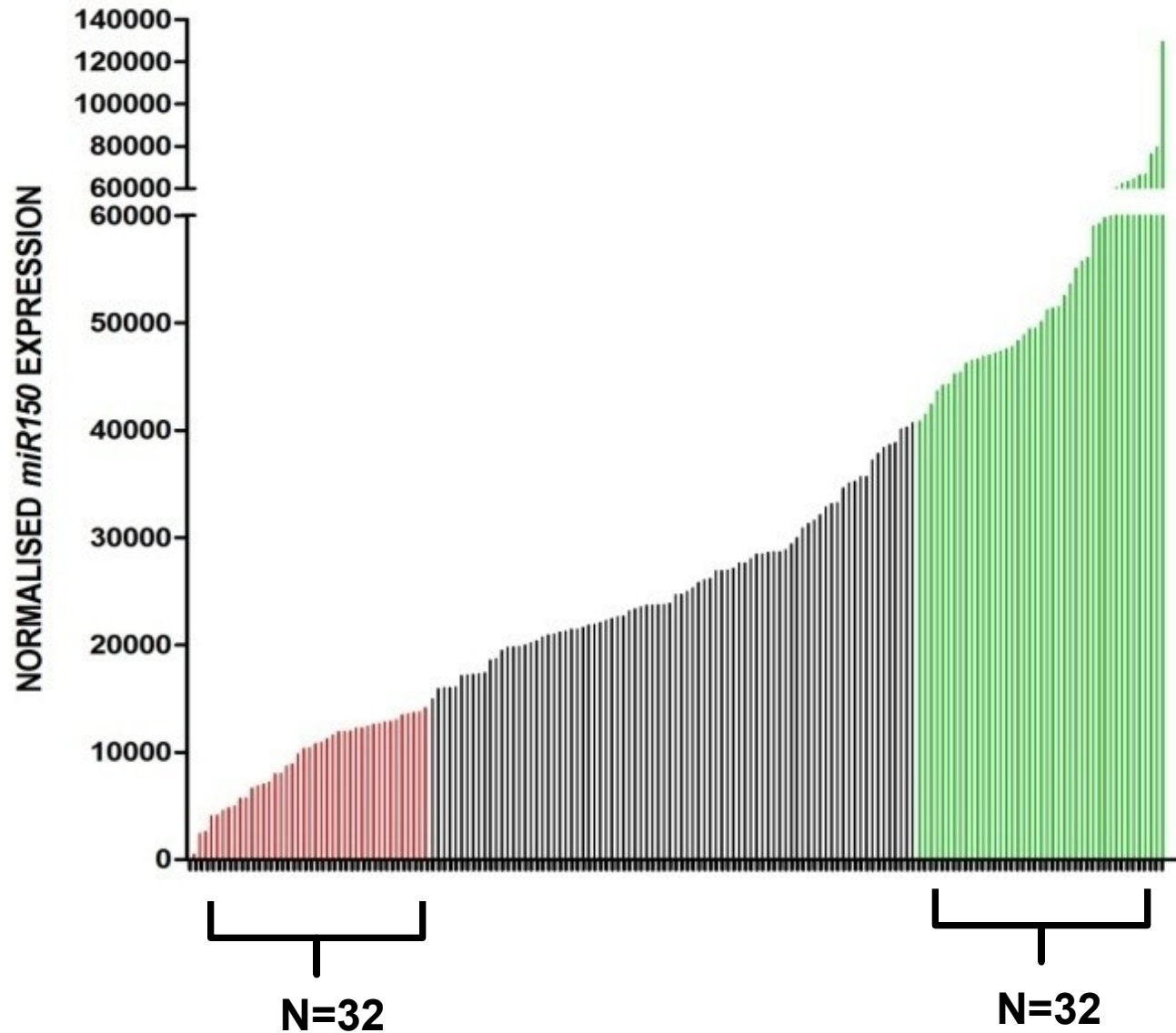
n = 168



Variable <sup>&amp;</sup>	HR <sup>#</sup>	CI <sup>#</sup>	P-val <sup>#</sup>	
miR-150 ( $\leq$ 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
	$\geq$ III vs. 0	3.6	1-13.1	0.05
Age ( $>$ vs. $\leq$ median)	3.0	1.4-6.8	0.01	

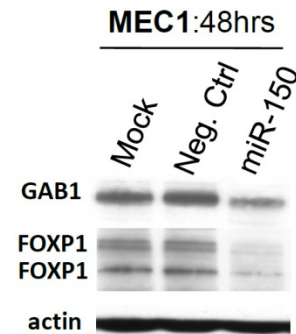
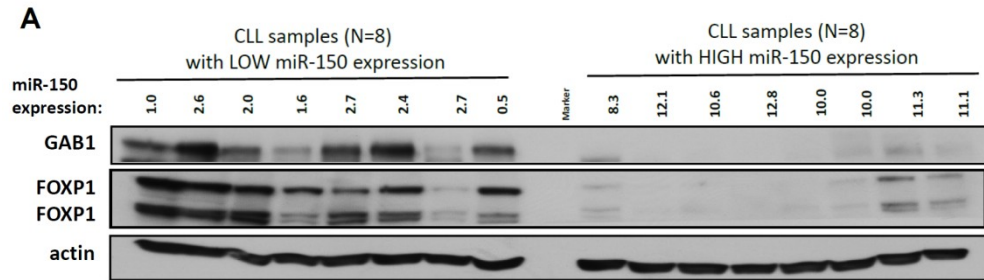
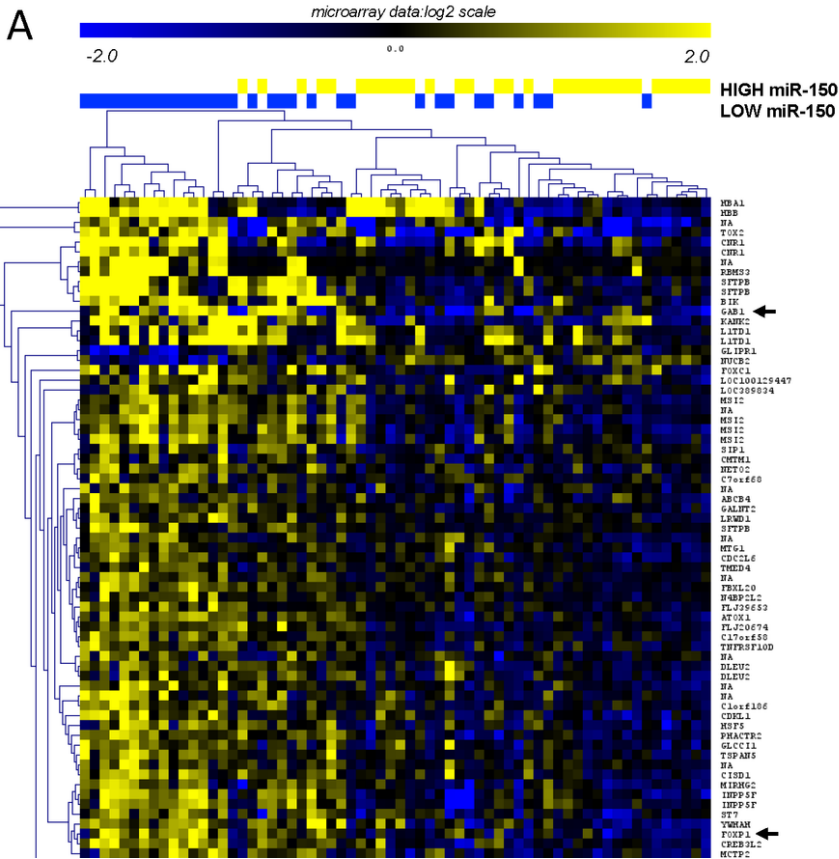
# Jak identifikovat cíle miR-150 u CLL?

(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**

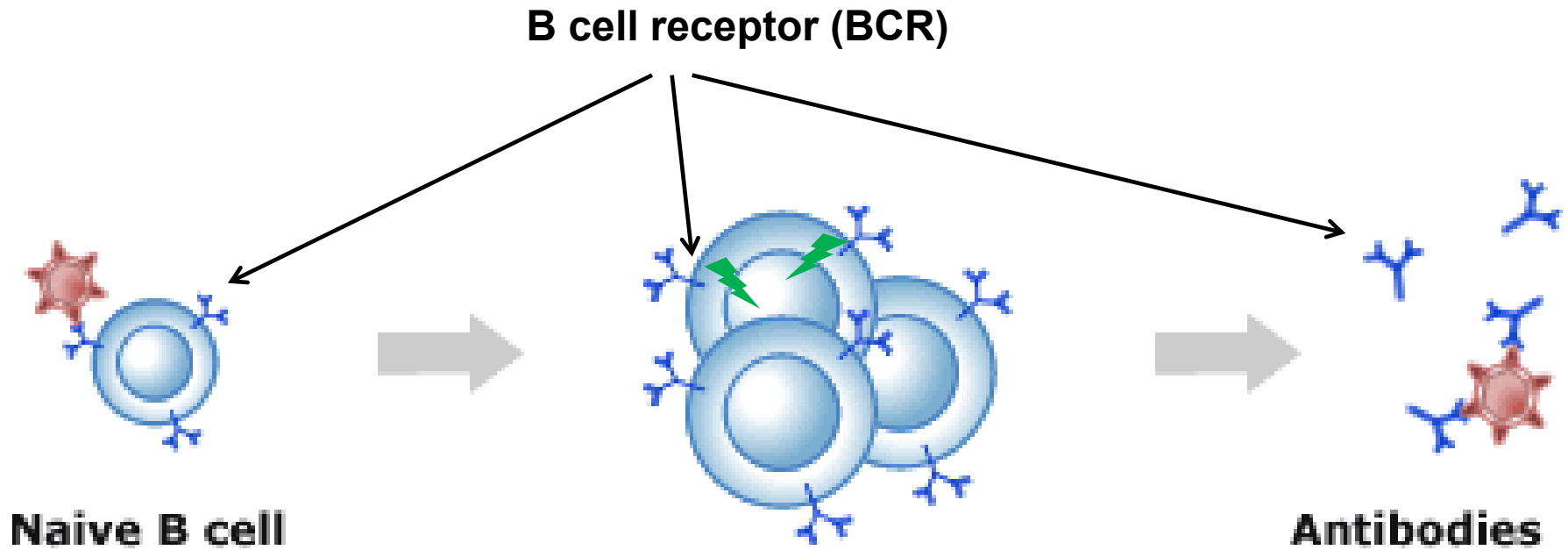


**GAB1** je adaptorová molekula, která je nutná k vazbě PI3K na membránu a amplifikaci BCR signalizace (Ingham et al. JBC, 2001).

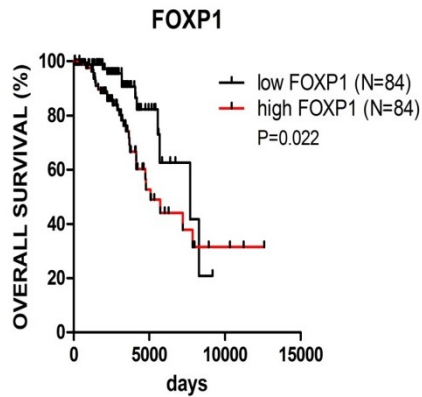
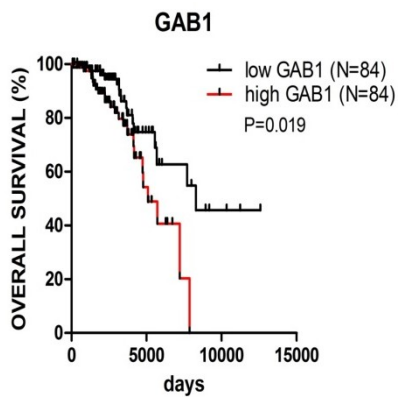
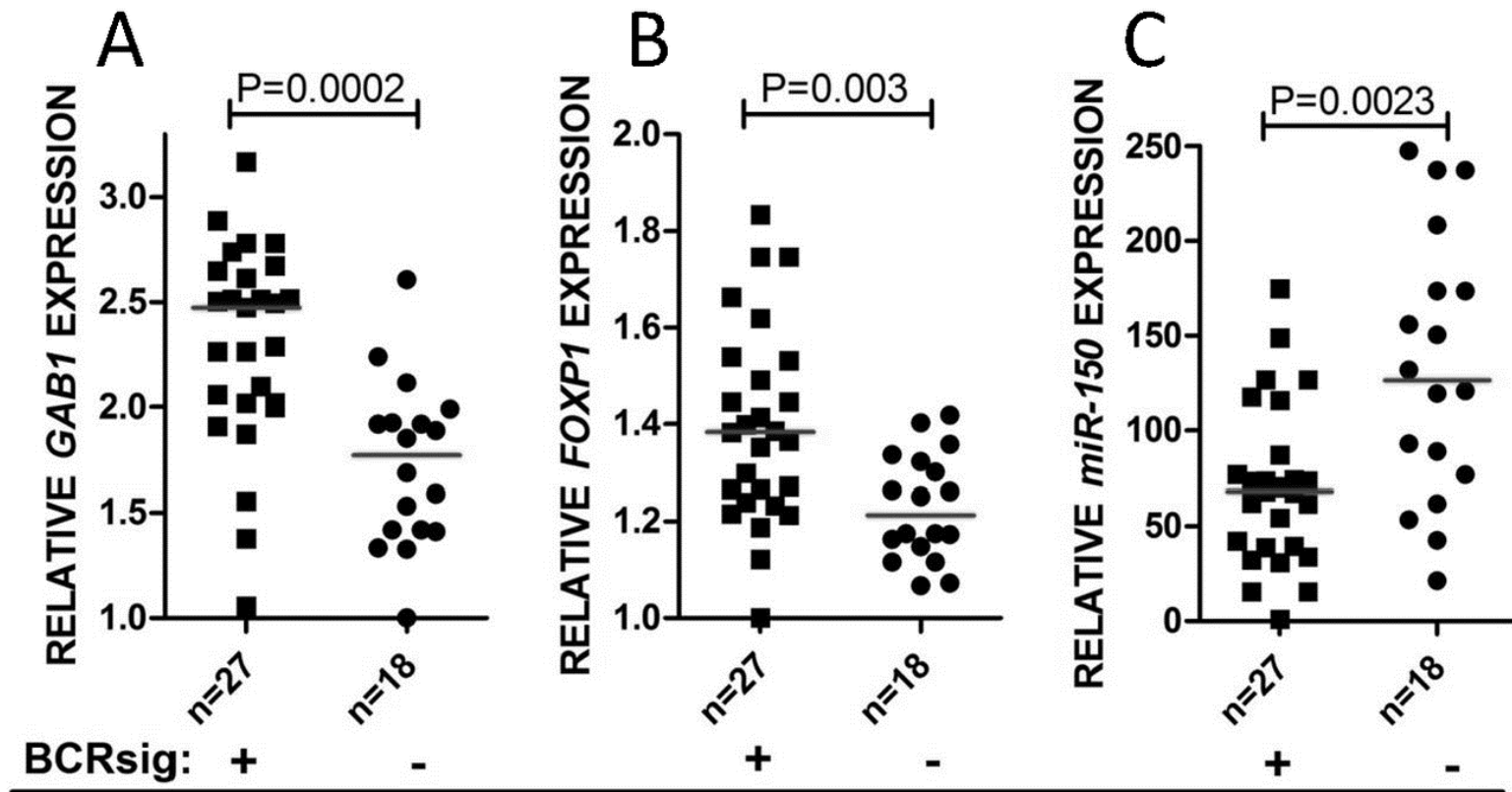
**FOXP1** je transkripční faktor důležitý pro vývoj B lymfocytů a asociovaný s ABC DLBCL a progresí B buněčných lymfomů (Hu et al. Nat Immunol, 2006).

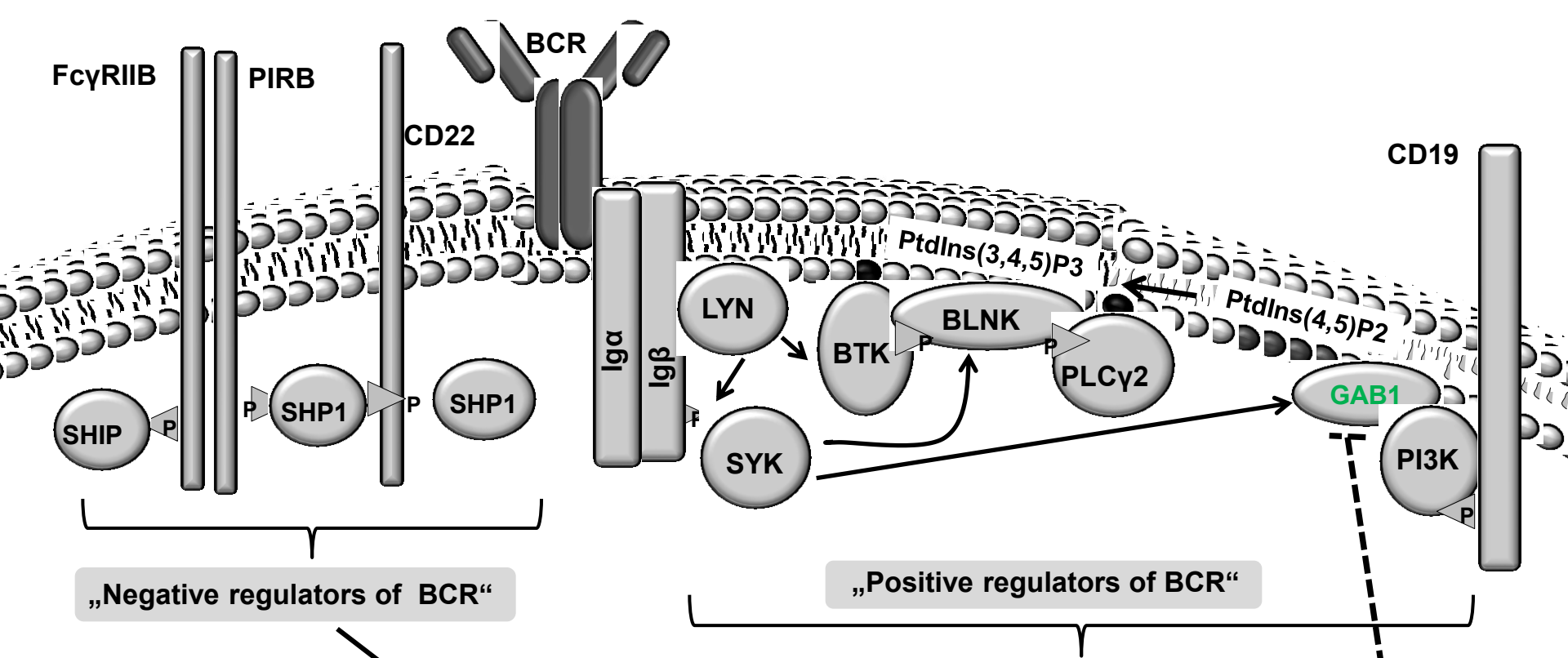


# Adaptivní imunity- centrální dráha BCR

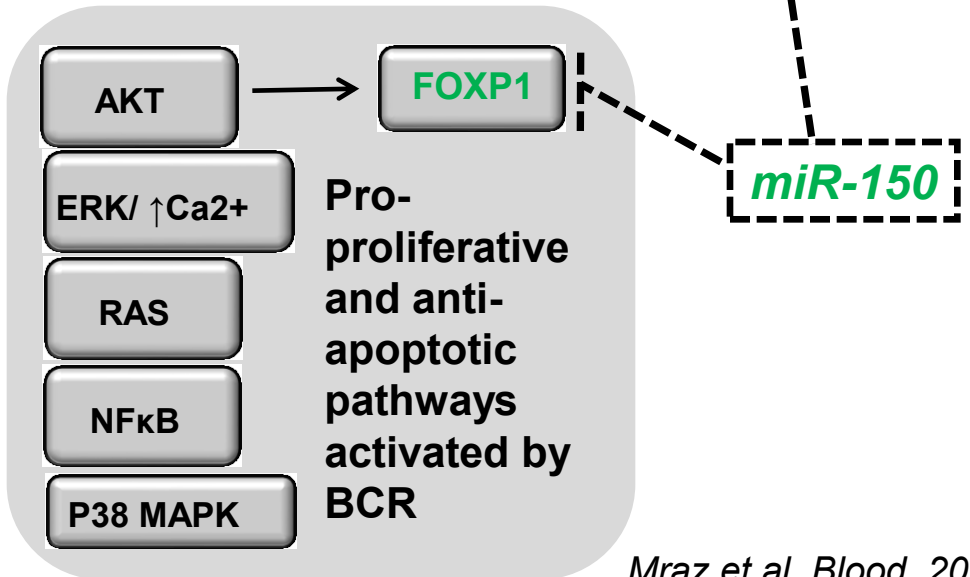


# Vyšší hladiny GAB1 nebo FOXP1 znamenají silnější BCR signalizaci

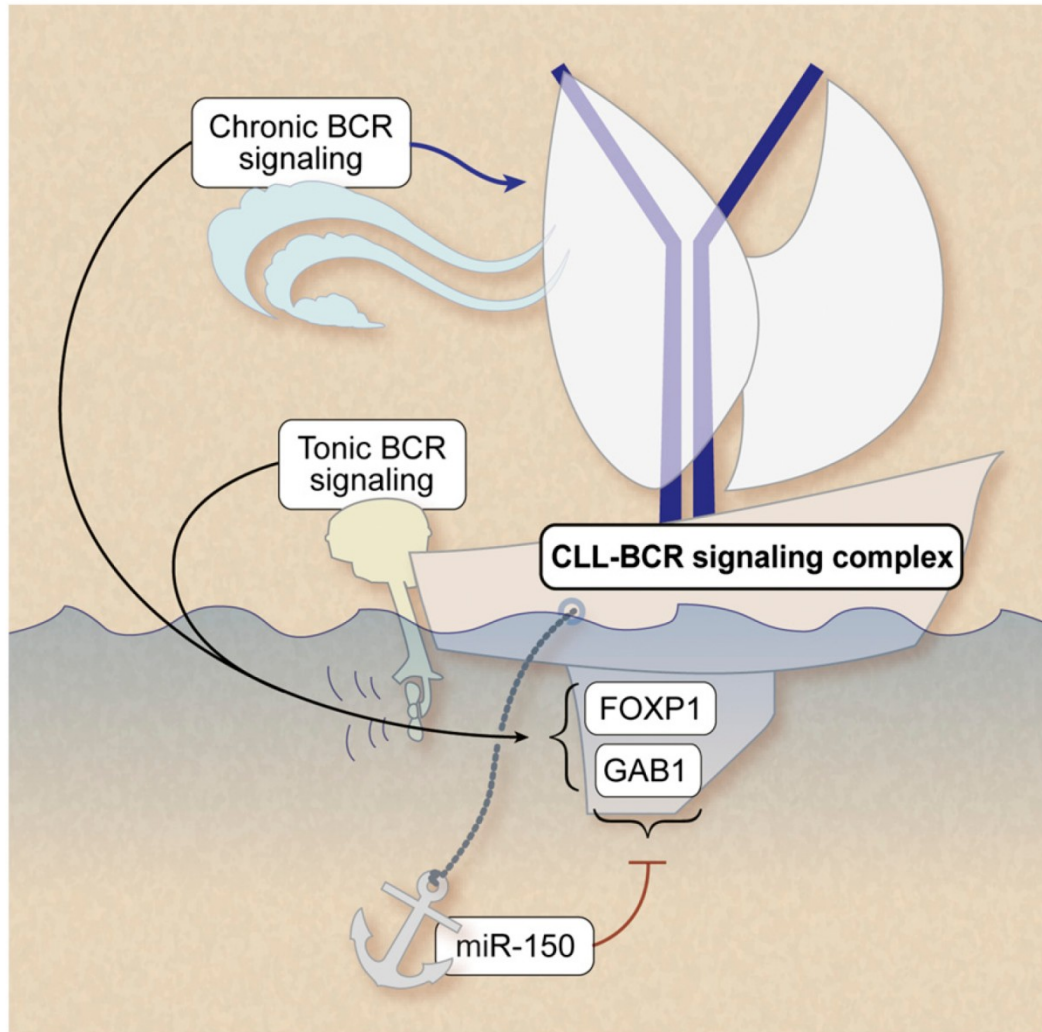




Popsali jsme první příklad regulace BCR signalizace prostřednictvím microRNA



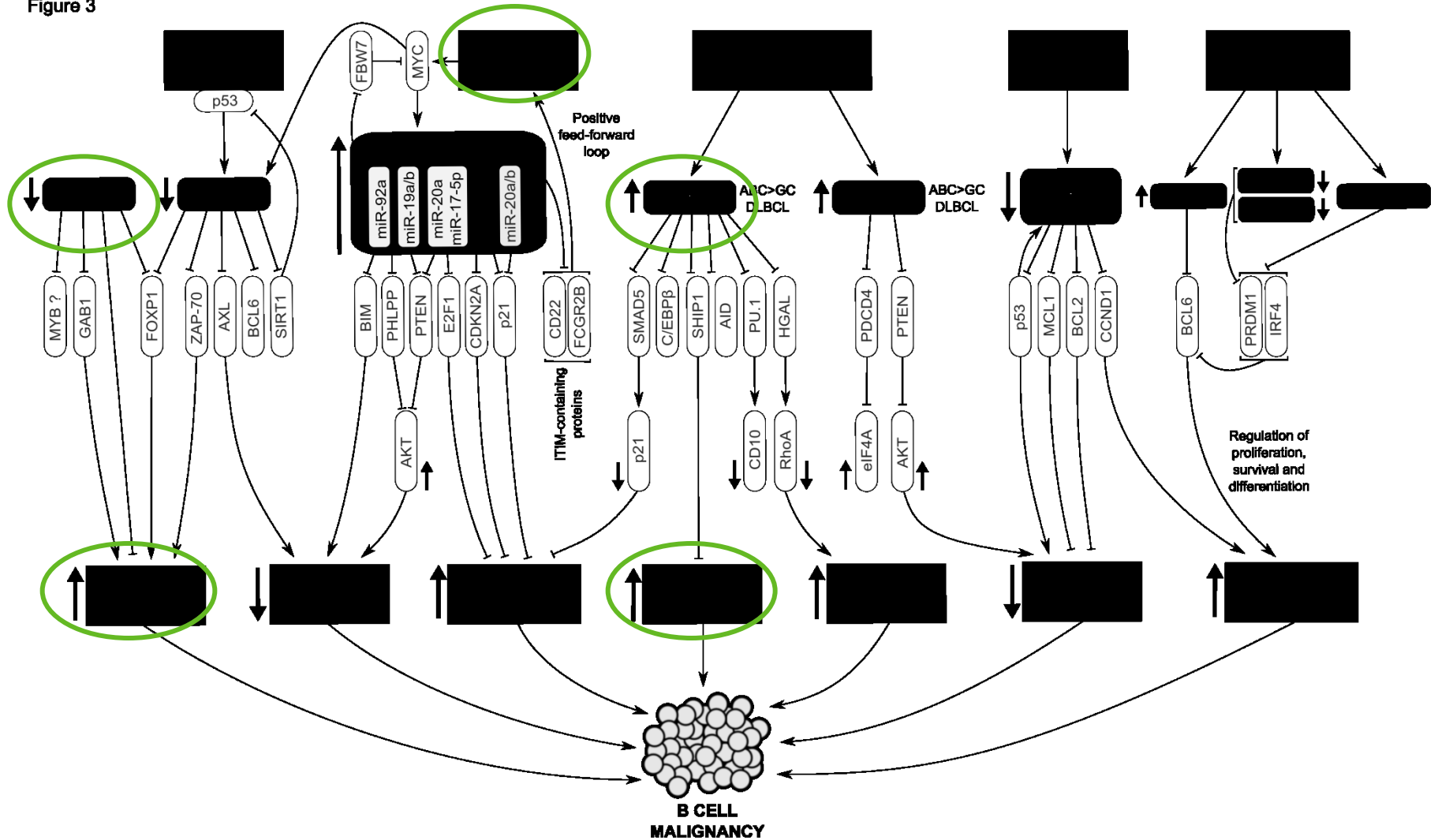
# First description of miRNAs role in BCR signalling...not only in CLL



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.

# miRNAs přispívají k regulaci mnoha drah

Figure 3



- potentially useful as **therapeutic targets**

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

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LETTERS

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**LNA-mediated microRNA silencing in non-human primates**

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

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

# Díky za pozornost

***CEITEC MU***

**Mraz Lab:** Katerina Cerna, Katerina Musilova, Vasek Seda,  
Gabriela Pavlasova, Veronika Svobodova, Sonali Sharma, Jan Oppelt

[Marek.Mraz@email.cz](mailto:Marek.Mraz@email.cz)

**Hledáme nadšené studenty (Bc, Mgr, PhD) a post-doky**