



# MASARYKOVA UNIVERZITA

## Design sekvence PCR primerů

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Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ



# MASARYKOVA UNIVERZITA

- definice
- aplikace
- modifikace
- syntéza
- purifikace
- kontrola kvality

## OLIGONUKLEOTIDY

- design sekvence
- zásady navrhování
- software OLIGO 7
- praktická ukázka

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

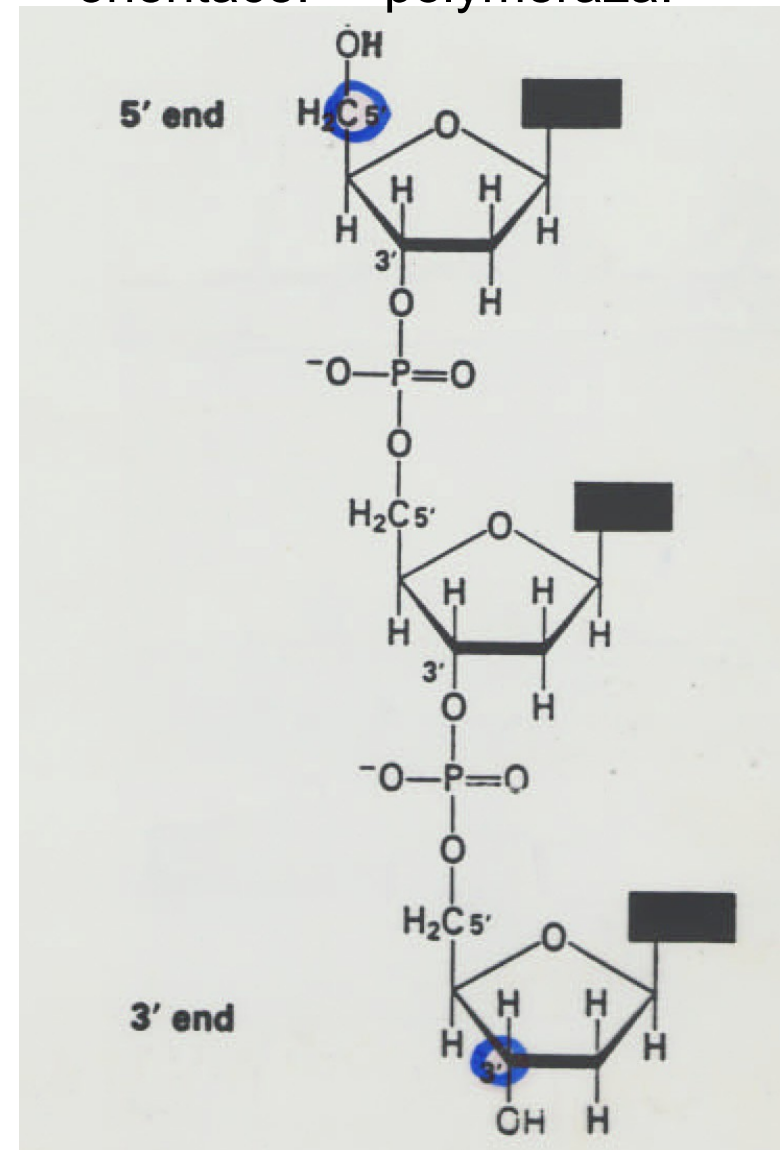
# oligonukleotid

- krátká jednořetězcová struktura
- DNA nebo RNA (event. PNA)
- **hydroxyl** na obou koncích  
(normálně na 5 - konci fosfát)

oligonukleotid

syntetický oligonukleotid

orientace! polymeráza!



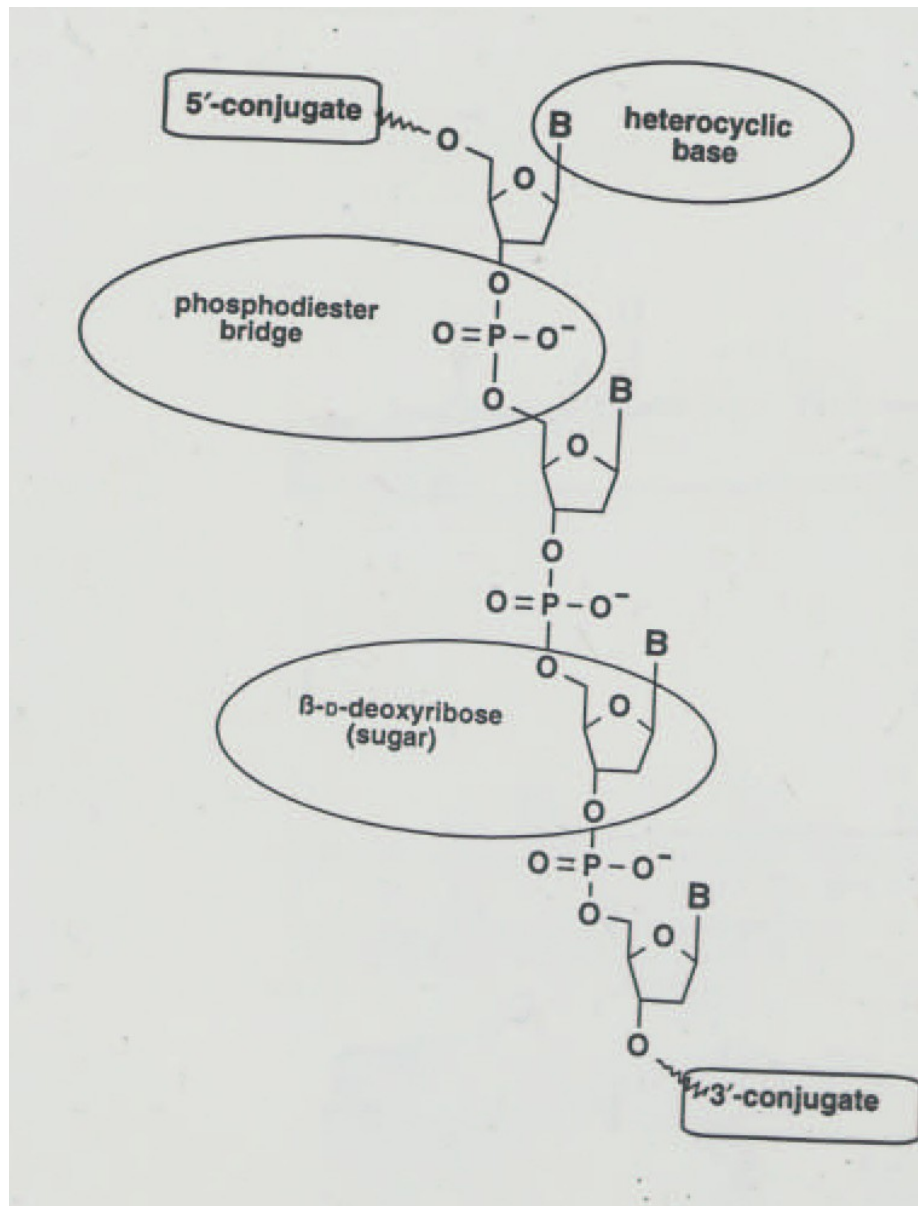
# Aplikace syntetických oligonukleotidů

- primery pro syntézu komplementární DNA  
*PCR, Real-Time PCR*
- syntéza genů a rekombinantní proteiny
- hybridizační sondy pro klonování
- místně cílená mutageneza
- sekvenování a genetické profilování
- diagnostika – testy a biosensory
- gene arrays
- blokace genové exprese *antisense oligo*
- potenciální léčiva a DNA vakcíny
- NMR studia interakcí DNA-protein
- strukturální rentgenová analýza NA



# Modifikace

- degenerace
- konce řetězce
- báze
- fosfát
- cukr
- PNA



## Degenerované oligonukleotidy

Příklady:

ACG TAC GTA CGT ACG TAC

nedegenerovaný

ACG TAM GTA CGT ACG TAC

M = A/C

ACG TAC GTA CDT ACG TAC

D = A/G/T

ACG TAC GTA CGT ACG NAC

N = A/C/G/T



# Degenerované oligonukleotidy

2-deoxyinosin

<b>M</b>	A or C
<b>R</b>	A or G
<b>W</b>	A or T
<b>S</b>	C or G
<b>Y</b>	C or T
<b>K</b>	G or T
<b>V</b>	A or C or G
<b>H</b>	A or C or T
<b>D</b>	A or G or T
<b>B</b>	C or G or T
<b>N</b>	G or A or T or C
<b>X</b>	G or A or T or C

## Modifikace na 5 - konci

postsyntetické modifikace →



sekvenování →  
fragmentační analýza  
gene arrays  
Real-Time PCR

5'

fosforylace

aminoskupina

thioskupina

digoxigenin

biotin

enzymy

psoralen

akridin

cholesterol

fluoresc. barviva

zhášedla

2,4-dinitrofenyl

TBR-chelát

spacer

větvení

blokáda



# Modifikace na 3' - konci

derivatizovaná matrice



3'

fosfát

thioskupina

aminoskupina

spacer

akridin



biotin



fluoresc.barviva



zhášedla

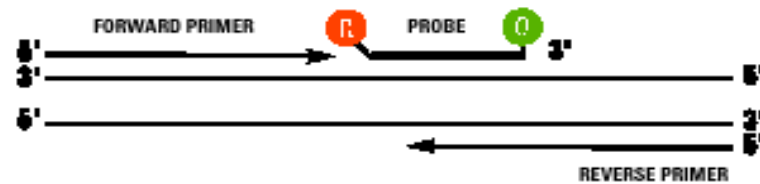
cholesterol

2,4-dinitrofenyl

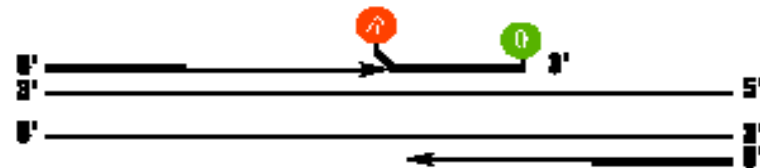


# Real-Time PCR

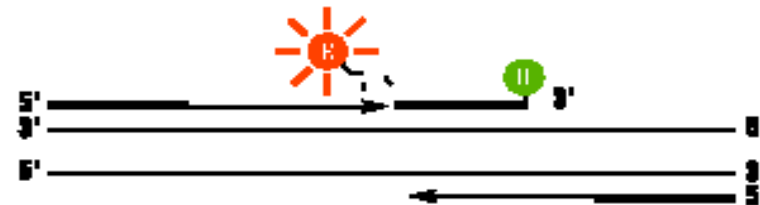
- 2x značená sonda
- REPORTER
- QUENCHER



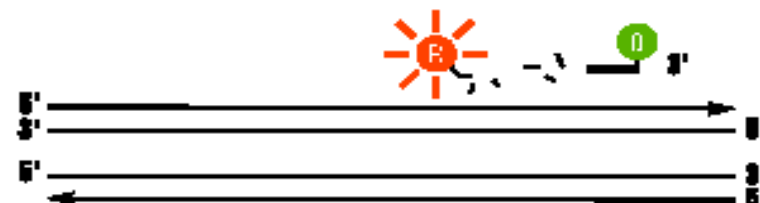
2. Strand displacement: When the probe is intact, the reporter dye emission is quenched.



3. Cleavage: During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe.



4. Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence.



## Další modifikace

fosforothioáty  
fosforodithioáty  
H-fosfonáty  
metylfosfonáty

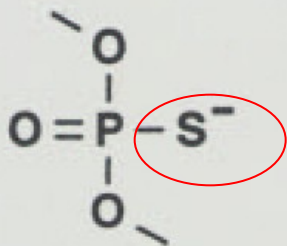
← páteř

cukr →

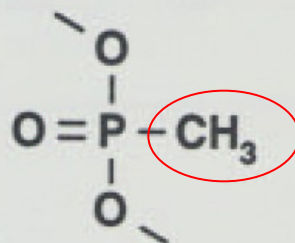
modifikace v 2' pozici  
modifikace ribózové jednotky

# Terapeutika

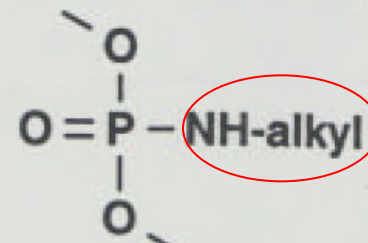
→ nedegradována nukleázami!  
modifikace fosfodiesterové vazby



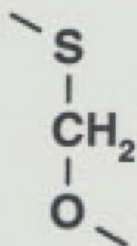
phosphorothioate



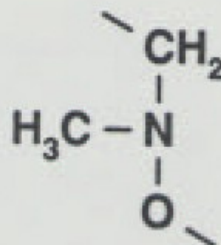
methylphosphonate



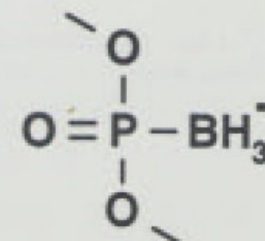
phosphoramidate



3'-thioformacetal



methylene(methyliminio)

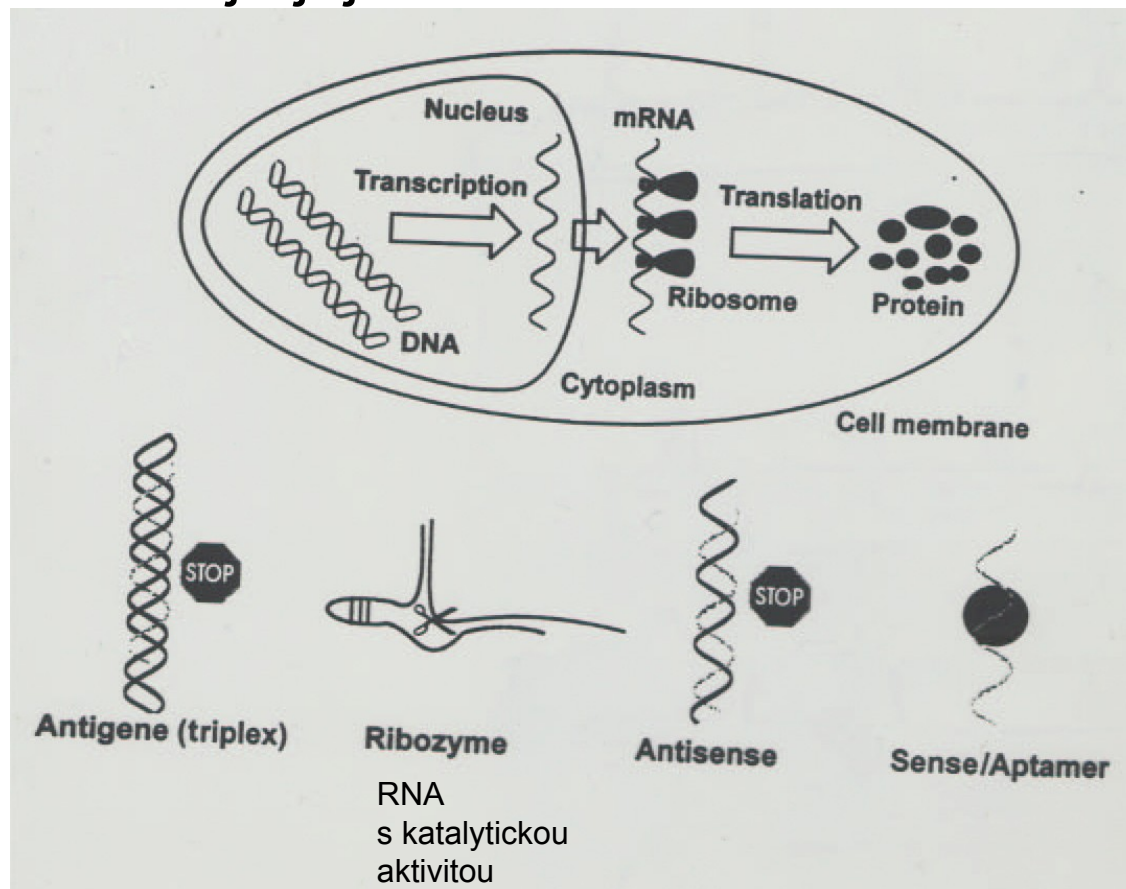


boranophosphate



## ANTISENSE oligonukleotid

- oligonukleotid nebo analog
- komplementární k segmentu RNA nebo DNA
- vazbou inhibuje jejich normální funkci

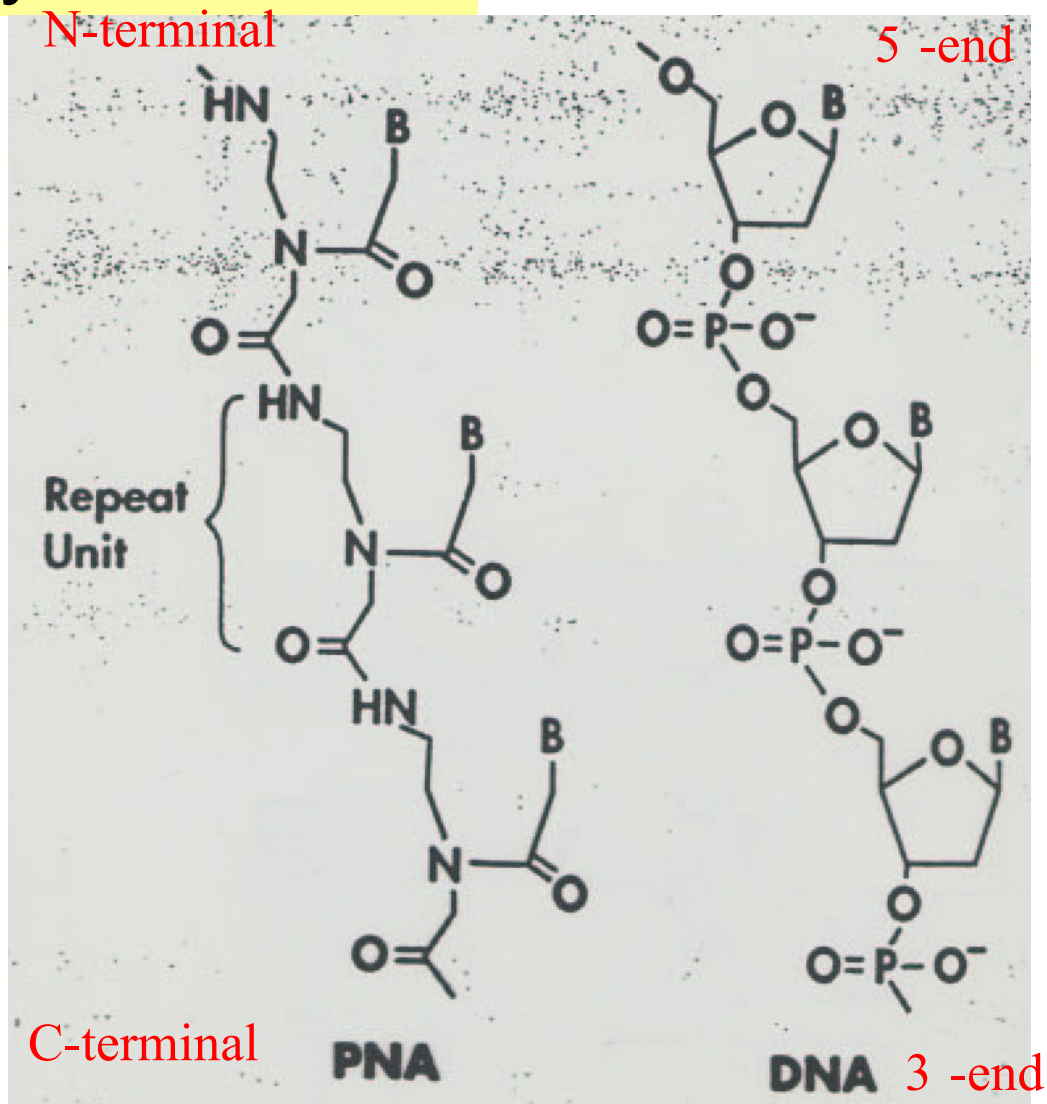


# Peptidonukleová kyselina **PNA**

## DNA

- nenabitá molekula
- vazba k DNA/RNA

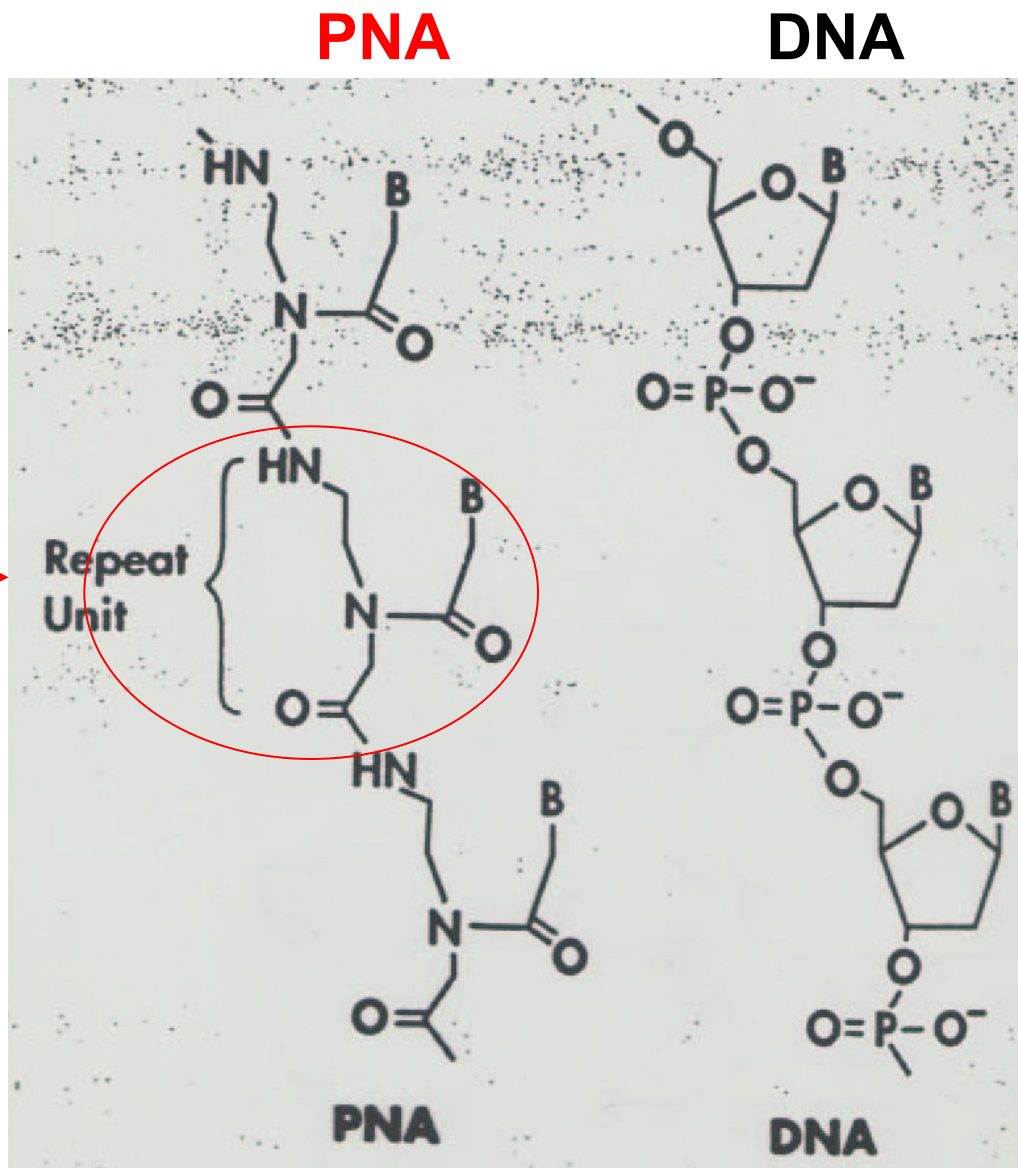
N-(2-aminoethyl)-glycin →



# Peptidonukleová kyselina

- nenabitá molekula
- vazba k DNA/RNA

N-(2-aminoethyl)-glycin →



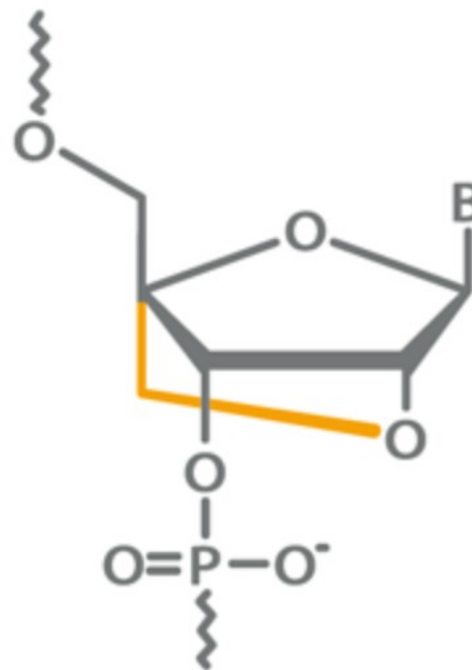
# Vlastnosti PNA

- vysoká termostabilita
- $T_m$  nezávisí na obsahu solí
- vyšší specifita
- vyšší afinita
- rezistentní k enzymům...

# LNA

## Locked Nucleic Acid

2'-O, 4'-C methylenový můstek  
potlačená flexibilita ribofuranózového kruhu  
struktura je **zamčena** do rigidní C3-endo konformace  
zlepšená hybridizace  
výjimečná biostabilita





## OLIGONUKLEOTIDY

design

syntéza

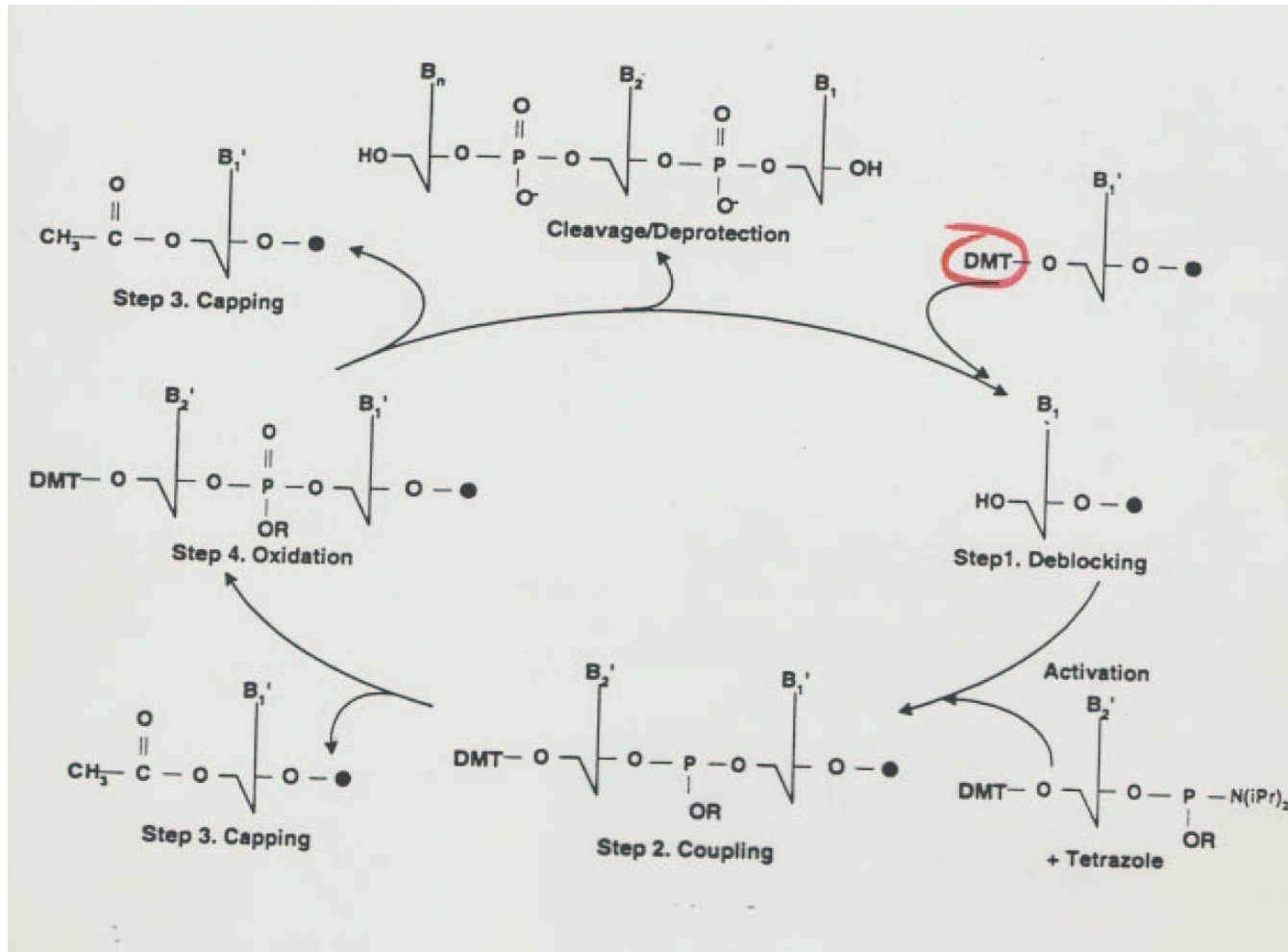
purifikace



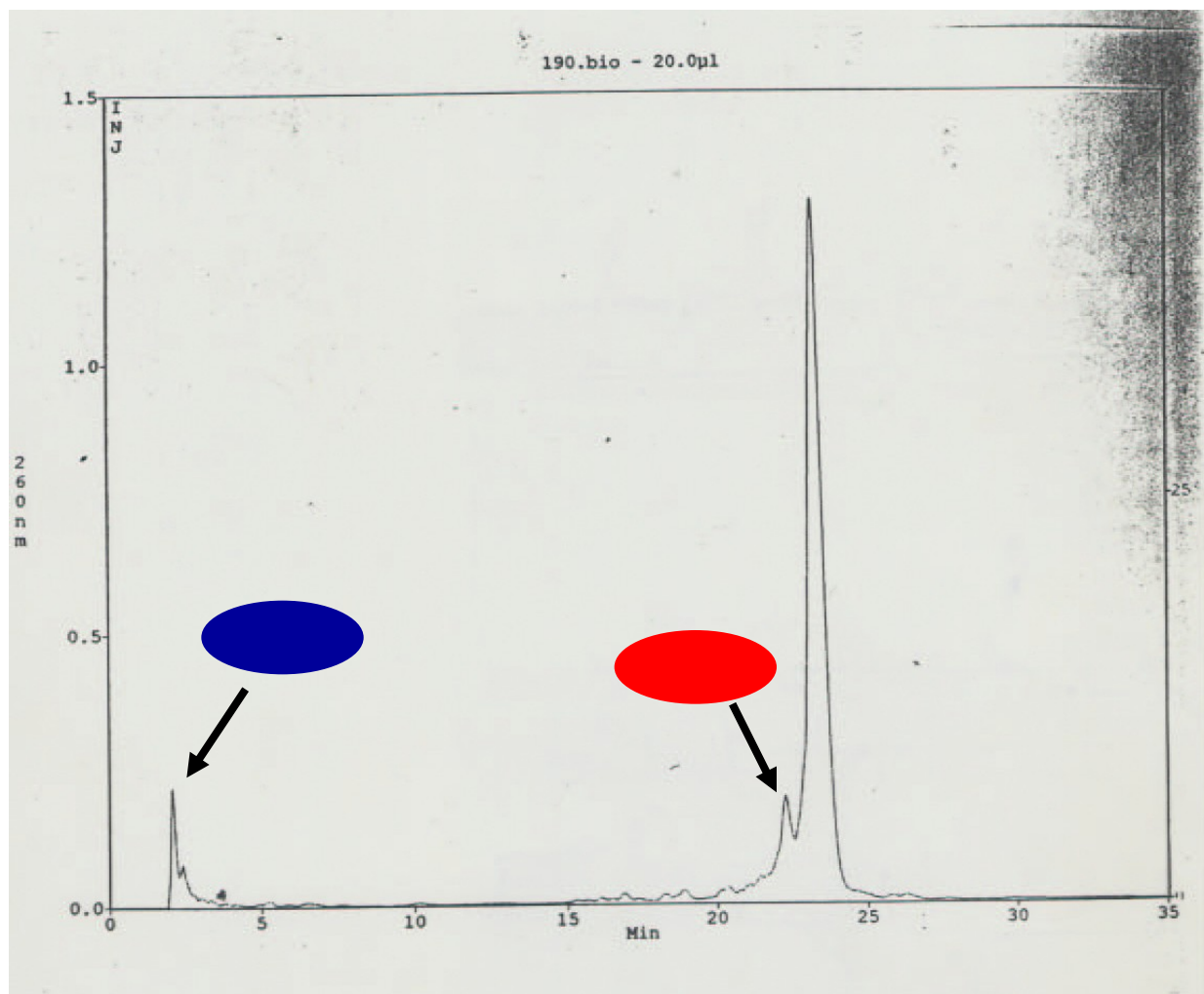
**EXPEDITE 8909**

# Syntéza oligonukleotidu

- syntéza na pevné fázi
- od 3 -konce k 5 -konci
- bezvodé prostředí



# Kontrola kvality



- HPLC
- Perfúzní chromatografie

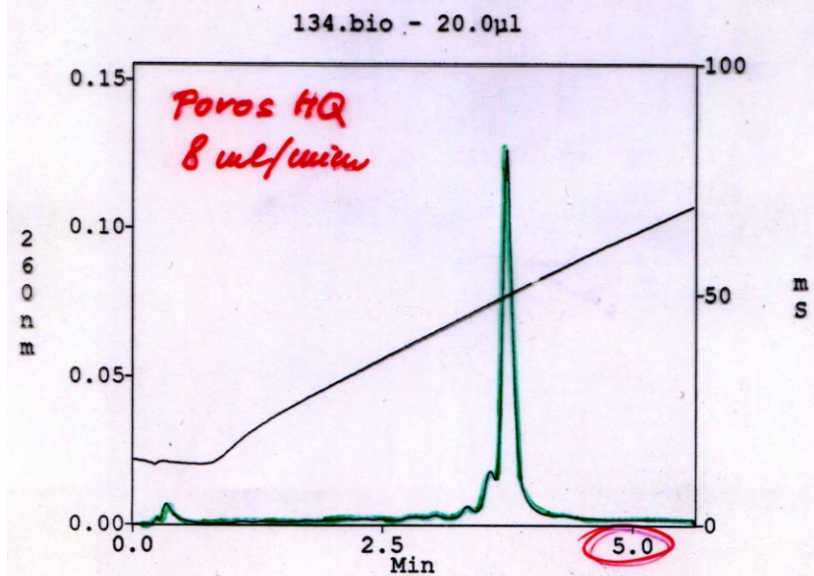
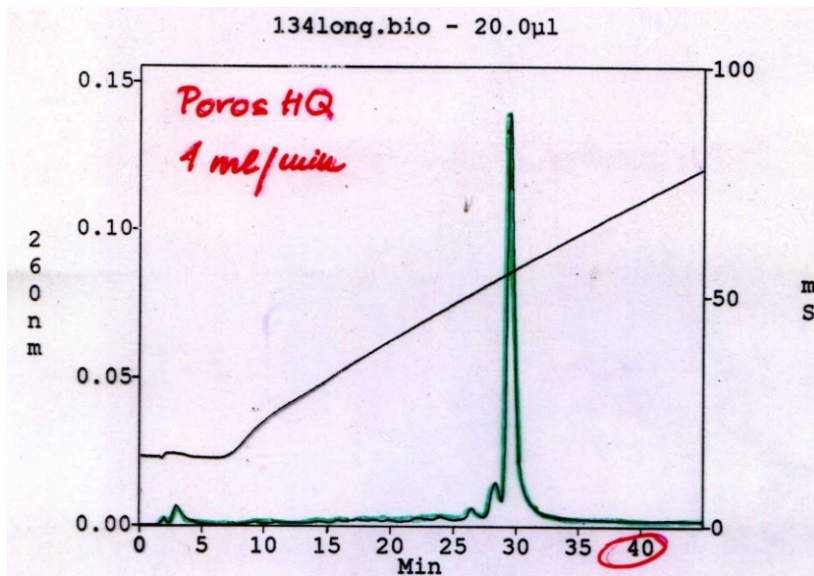
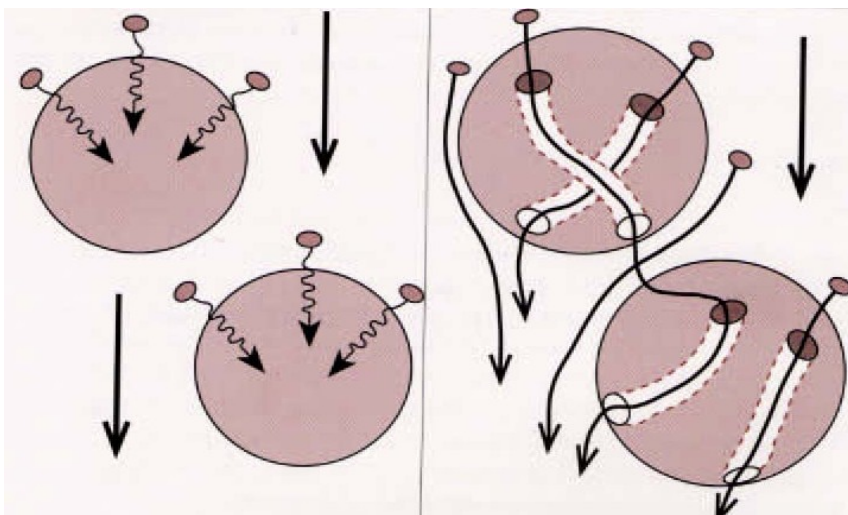
- anex
- RP



# Perfúzní chromatografie

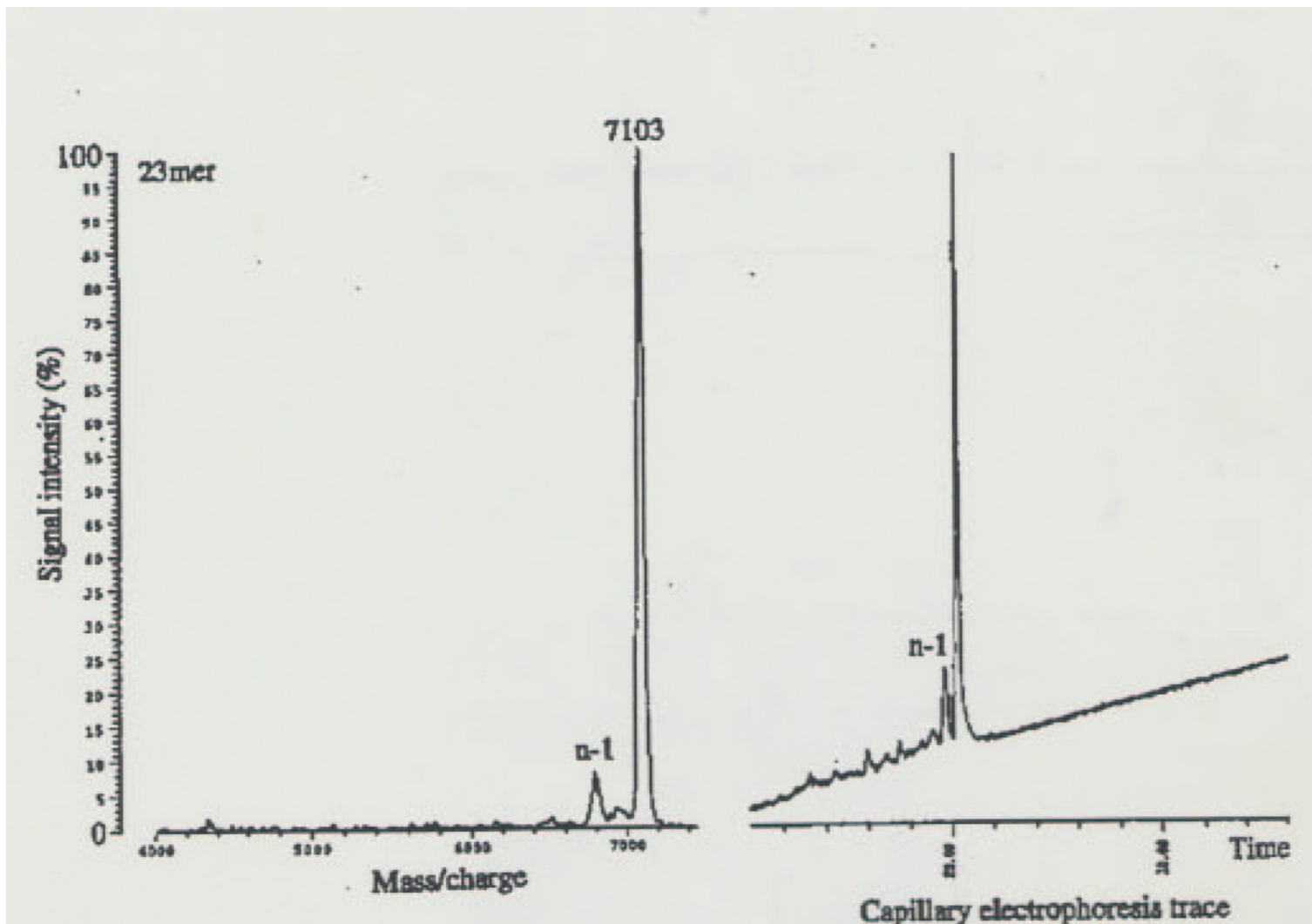
klasický sorbent

**POROS**

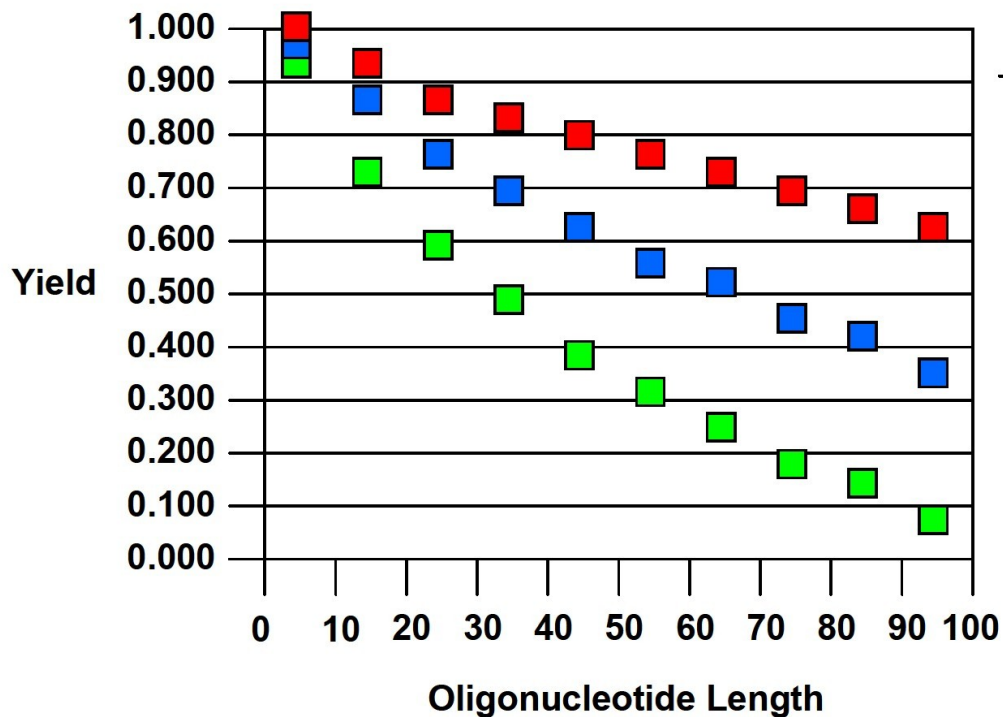


Maldi-TOF MS

CE



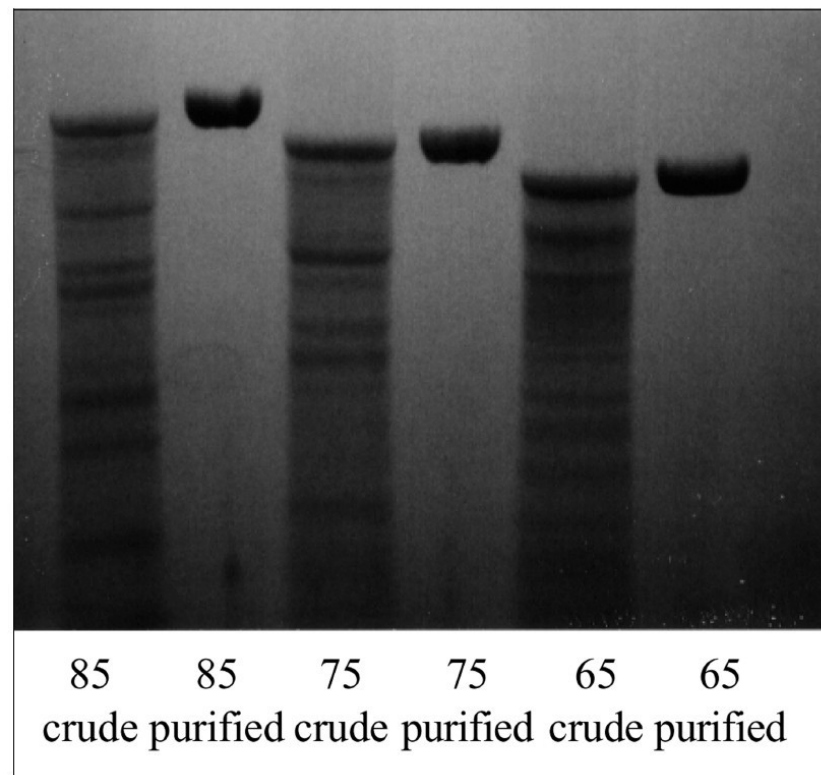
# VÝTĚŽEK



Efficiency

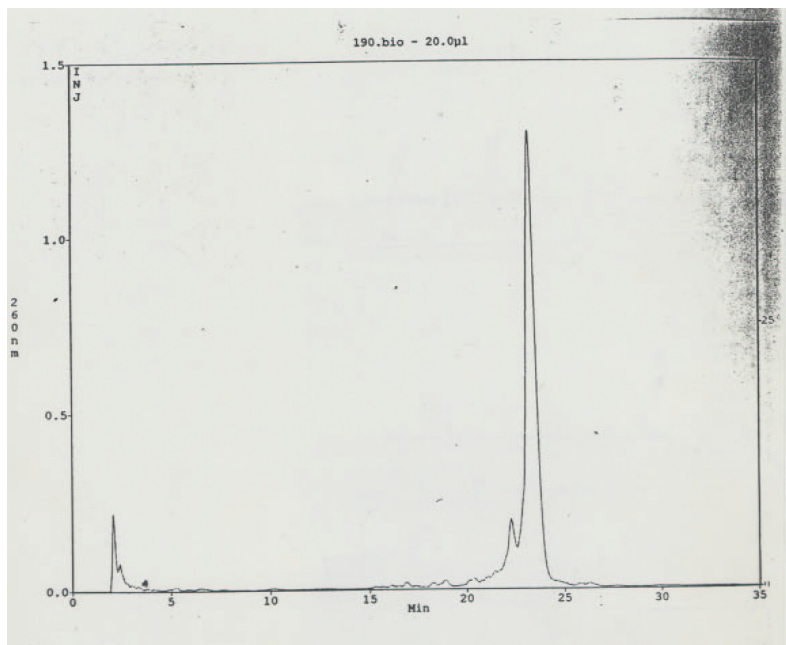
- 0.995
- 0.990
- 0.980

# PAGE



# PURIFIKACE

- Sephadex
- RP cartridge
- HPLC



## DESIGN OLIGONUKLEOTIDU

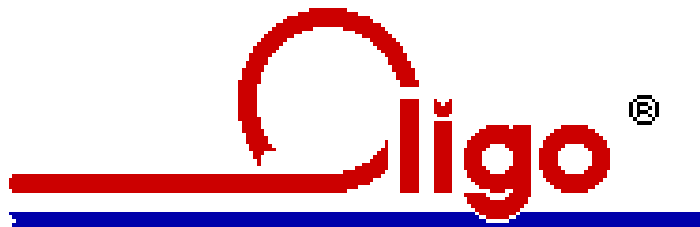
- manuální
- počítačový

[www.protocol-online.org/prot/Research\\_Tools/Online\\_Tools/Oligo\\_Design/index.html](http://www.protocol-online.org/prot/Research_Tools/Online_Tools/Oligo_Design/index.html)

## Hlavní kritéria pro sekvenci PCR primeru

- vysoce specifické
- netvoří dimery a vlásenky
- stabilní duplexy s aktivní sekvencí
- nepřiliš stabilní 3'-konec



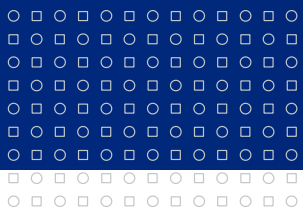


## OLIGO 6

- PCR primery,
- hybridizační sondy
- sekvenační primery

## OLIGO 7 (od roku 2008)

- TaqMan sondy
- primery pro *nested PCR*
- *molecular beacons*
- siRNA



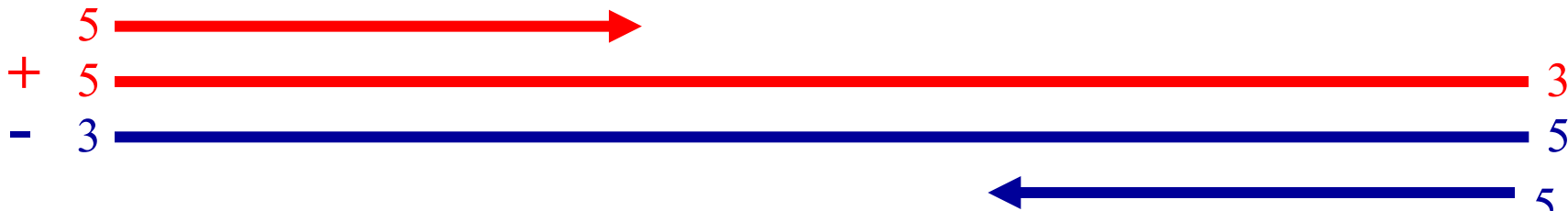
# Terminologie PCR primerů

forward primer... část sekvence + vlákna

reverse primer... část sekvence - vlákna



UPPER - FORWARD - LEFT



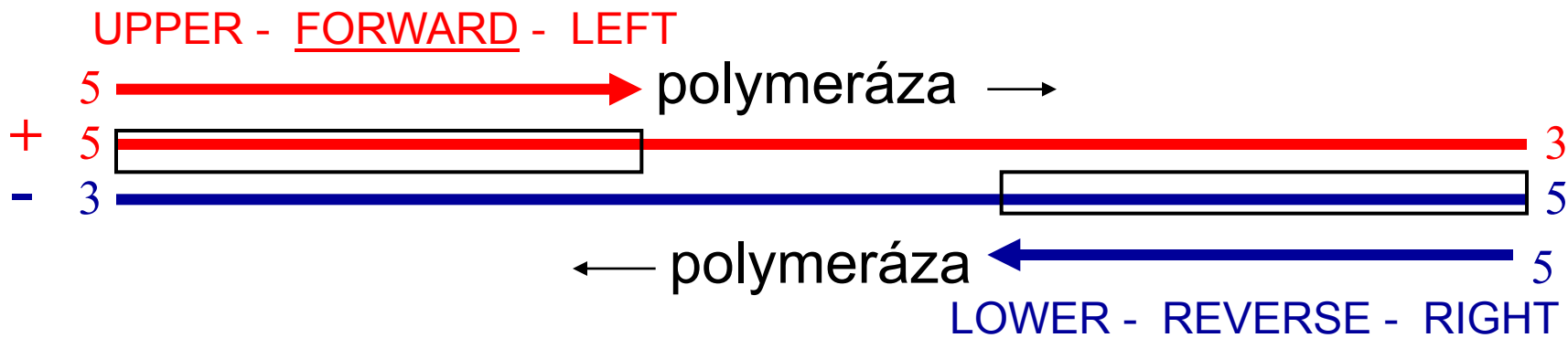
LOWER - REVERSE - RIGHT



# Terminologie

forward primer... část sekvence + vlákna

reverse primer... část sekvence - vlákna

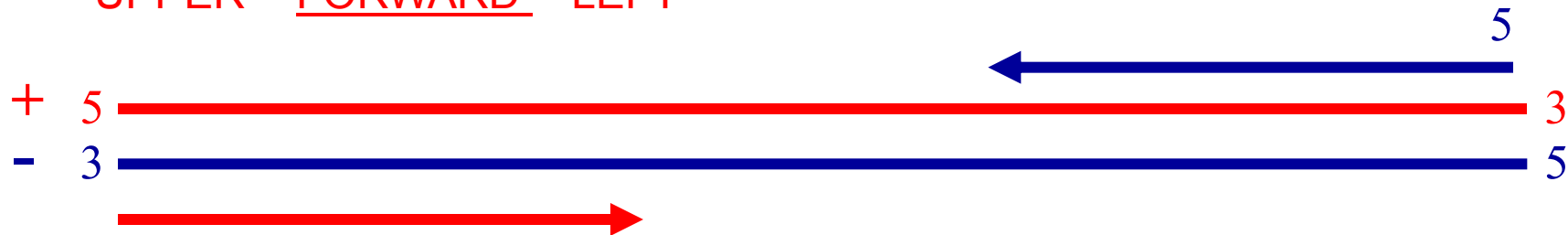




# Nasedání PCR primerů



UPPER - FORWARD - LEFT



LOWER - REVERSE - RIGHT



5 CTT CTG CTC AAT CTT TCT AC 3 FORWARD

+ 5

1 ATGGCTTCTG CTCAATCTTT CTACAAACCAA AGCTCTGTCT TGAAAATCAA  
 51 TGTCATGGTT GGGACGAIG ATCATGTTTT CCTTGATATC ATGTCACGCA  
 101 TGCTTCAACA CTCCAAATAC AGAGGTAATT AAATATTATT ATCATATTAT  
 151 ATATAATATG TTATTGATTT TTTGTTTGTG ATTTCATTTA GATTTTTATT  
 201 TCTATGATTT CTTAGCATGA AATACAATTT TTGGAGAAAC AACTAGCAGT  
 251 TTTAAAAACA AAACCTGAAT TTTGAGAAAT TCAAAGATGT TATATATATA  
 301 TGTCAAAATT TAACAATTAT TCTTCTAAAT CATCCGGATT CCGTTTACAT  
 351 GTACACATCT ACAATTTTCA ATTGAGGTAT TCTTGTTTTG ATGCCTTTGA  
 401 GACGAATAGT TTGATTGATA AAAAAAATTC TAACCAATAT GATATATAAA  
 451 GTTTTTTTTT TTTTGTCAA ACCACTTTT ATACTATGTA ACTTTTTTAA  
 501 GAGATTATTG AAAATAGTTT ATTTATAAAA TAGTAACCTA TTGTTGAATT  
 551 AAAAAAAAAA AAAAAATTGT AAATCGTGTT TGCAAACGAC ATGTGATTTA  
 601 TCTTAGTTTA AACTAGCTG ATATTCTTCA AATCGACTGT TCTTATAAGT  
 651 AATCAACCAA ~~TTAGCATCAA TCACAATAAA~~ TTGTAACAC TTCAATGAAA  
 701 ATGGTGATTT TAAAGAATAT GTTTTACTTA TGTTATGAAC TATCTCAAAT  
 751 TTGTGAAATA TTTCATAACT AATGTGGAAA ACTATATAAC CCCTCCATAC  
 801 AAAACGTAAG TAAAATTTAT GAAATCCTAT CTTTTTAAA GGTTAAACCA  
 851 ATCAAAAAGT AATAATTCTT GGTACTTGCA ATATTTTTGT CATTATATTT  
 901 TAGTTTATTA ATTTTATTTT GATTAAATGG TTTTAGATCC ATCAGTTATG  
 951 GAGATCGCAG TTATAGCTGT AGACGATCCG AAGAAAGCAT TATCTACTCT  
 1001 AAAAATTCOA CGAGACAATA TAGATCTCAT AATCACAGAT TATTATATGC  
 1051 CTGGTATGAA CGGTTTACAA CTCAAAAAAC AAATCACTCA GGAATTTGGA  
 1101 AATTTACCGG TCTTAGGTAA CTTTTTTTGT TCTTTACAAC TTAAATTTAA

3'

5 TGAAGAATA TCA GCT AGT TT 3 REVERSE

Sequence

File: Human 4E.seq

DNA Sequence		Selected Oligo	Position	Length	#	Feature	Location
Sequence Length:	1868 nt	Forward Primer	259	18	1	source	-18..1850
Reading Frame:	+1	Reverse Primer	328	18	2	CDS	1..651
Current Oligo Length:	21 nt	Upper Oligo	---	---			
Position:	356	Lower Oligo	294	22			
$t_m$ :	59.3°C	PCR Product	87 nt				

pos: 350 tm: 57.1

CCTGGCTGTGACTACTCA >

TTAATGCCTGGCTGTGACTACTCACTTTTAAAGGATGGTATTGAGCCTATGTGGGAAGATGAGAAAAACAACGGGGAGGACGATGGCTAATTACATTGAACAAACAGCAGAGACGAAGTGACCTC  
AATTACGGACCGACTGATGAGTGAAAAATTCCTACCATAACTCGGATACACCCCTCTACTCTTTTGTGGCCCTCTGCTACCGATTAATGTAACTTGTGTGCTCTCTGCTTCACTGGAG  
ACTCGGATACACCCCTCTACTC  
CCTCCTGCTACCGATTAA

L M P G C D Y S L F K D G I E P M W E D E K N K R G G R W L I T L N K Q Q R R S D L



Search for Primers & Probes

Search Options    Subsearches

Search in:     + Strand     - Strand  
Search Mode:     Select     Verify

Complex Substrate

---

PCR Primers  
Compatible with the     Forward Primer     Reverse Primer

---

TaqMan Probes & PCR Pairs  
Compatible with the     Upper Probe     Lower Probe

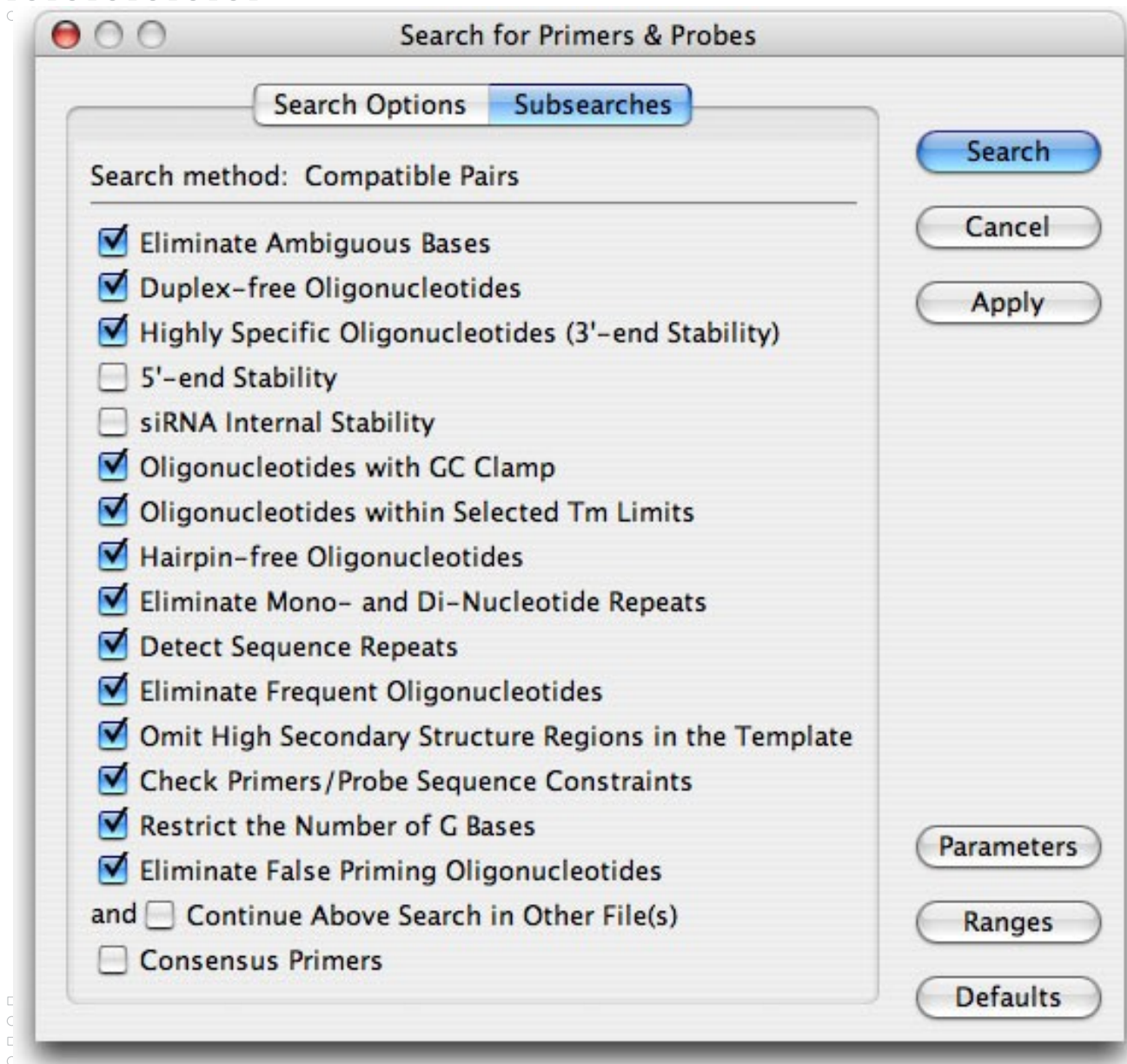
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Molecular Beacons & PCR Pairs  
 Nested Primers  
 Sequencing Primers  
 Hybridization Probes  
 siRNA Probes

---

After successfull search show:    All Results    ▾

Search  
Cancel  
Apply  
Parameters  
Ranges  
Defaults



**PCR**

File: Human 4E.seq

Optimal Annealing Temperature: 50.8 °C (Max: 66.3 °C)

	Position and Length		T <sub>m</sub> [°C]	GC [%]	P.E.#	Score
Product	862		78.9	29.6	n/a	697
Forward Primer	918	22	56.9	45.5	471 / 471	840
Reverse Primer	1753	27	55.3	29.6	489 / 489	834
Upper Oligo	979	24	56.5	33.3	479 / 479	917
Lower Oligo	1694	23	55.4	39.1	457 / 457	841

Product T<sub>m</sub> - Reverse Primer T<sub>m</sub> : 23.6 °C  
 Primers T<sub>m</sub> difference: 1.6 °C      Comments:

	Concentration	
Forward Primer	200.0	nM
Reverse Primer	200.0	nM
Upper Oligo	200.0	nM
Lower Oligo	200.0	nM
Monovalent Cation	50.0	mM
Free Mg[2+]	0.7	mM

Total Na[+] Equivalent: 155.8 mM

Selected Primers			
File: BRCA2 gene.seq			
AY436640:15438F22		AY436640:15917R20	
5' CAATATATACCGTAGTCCCCTA 3'		5' CAGCTACATATTACGCCAGA 3'	
Length:	22-mer	Length:	20-mer
Score:	802 points	Score:	914 points
5' Position:	15438	3' Position:	15917
$T_m/t_m$ :	53.4	$T_m/t_m$ :	53.1
	52.6 °C		53.8 °C
$\Delta G/\Delta g$ (25 °C):	-30.5	$\Delta G/\Delta g$ (25 °C):	-28.6
	-29.2 kcal/mol		-28.5 kcal/mol
$\Delta S/\Delta s$ :	-472.1	$\Delta S/\Delta s$ :	-430.5
	-449.5 cal/°K * mol		-419.6 cal/°K * mol
$\Delta H/\Delta h$ :	-171.3	$\Delta H/\Delta h$ :	-157.0
	-163.2 kcal/mol		-153.6 kcal/mol
3' $\Delta G$ :	-6.5 kcal/mol	3' $\Delta G$ :	-6.9 kcal/mol
Degeneracy:	1	Degeneracy:	1
P.E.#:	443/443	P.E.#:	477/477
1/E:	4.63 nmol/A <sub>260</sub>	1/E:	5.05 nmol/A <sub>260</sub>
	31.1 µg/A <sub>260</sub>		31.0 µg/A <sub>260</sub>

Priming Efficiency PE  
Score



- HAIRPIN intramolekulární
- DIMER intermolekulární

File: BRCA2 gene.seq

Current Oligo 21-mer [5042]

[Current+ Oligo] - The most stable 3'-dimer: # of hydrogen bonds = 10;  $\Delta G = -0.7$  kcal/mol

5' GAATTAGATAAAATTCAAATTA 3'  
3' ATTAAACTTAAATAGATTAAG 5'

[Current- Oligo] - The most stable 3'-dimer: # of hydrogen bonds = 10;  $\Delta G = -7.3$  kcal/mol;  $T_m = 2.9^\circ\text{C}$

5' TAATTTGAATTTATCTAATTC 3'  
3' CTTAATCTATTTAAGTTTAAT 5'

The most stable dimer overall: # of hydrogen bonds = 10;  $\Delta G = -7.4$  kcal/mol;  $T_m = 2.2^\circ\text{C}$

5' GAATTAGATAAAATTCAAATTA 3'  
3' ATTAAACTTAAATAGATTAAG 5'

Hairpin: loop = 5 nt;  $\Delta G = -3.0$  kcal/mol;  $T_m = 54.6^\circ\text{C}$

5' GAATTAG  
3' ATTAAACTTAAAT





## Current Oligo Hairpin Stems

File: BRCA2 gene.seq

Current Oligo 21-mer [5042]

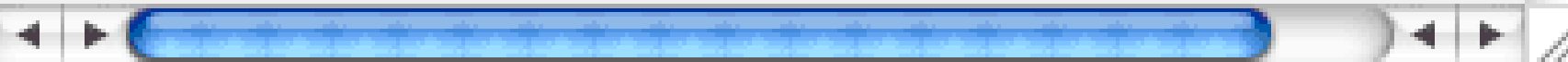
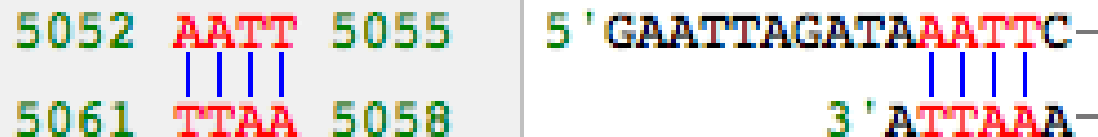
1. # of paired bases = 5; loop = 5 nt;  $\Delta G = -3.0$  kcal/mol;  $T_m = 54.6$  °C



2. # of paired bases = 6; loop = 5 nt;  $\Delta G = 0.2$  kcal/mol;  $T_m = 21.7$  °C



3. # of paired bases = 4; loop = 2 nt;  $\Delta G = 0.9$  kcal/mol;  $T_m = 8.7$  °C



Reverse Primer False Priming Sites

File: M13MP18

Reverse Primer M13MP18:6310R19 (positive strand)  
 Priming efficiency of the perfect match is 482 (above the threshold)

Priming efficiency: 482 (above the threshold)

```

5' (6328) GGTTTTCCCAGTCACGACG (6310)3'
          |||
3' (6328) ccaaaagggtcagtgctgc (6310)5'
    
```

Priming efficiency: 244 (above the threshold)

```

5' (6328) GGTTTTCCCAGTCACGACG (6310)3'
          |||
3' (626)  agcaaatggtc--tgctgc (610)5'
    
```

Priming efficiency: 193 (above the threshold)

```

5' (6328) GGTTTTCCCAGTCACGACG (6310)3'
          |||
3' (5125) tctaaagtggtcagtg-tgc (5108)5'
    
```



**Forward Primer Composition**

File: BRCA2 gene.seq

**Forward Primer AY436640:6275F19**

T <sub>d</sub>	64.2°	[nearest neighbor method]
T <sub>m</sub>	56.5°	[nearest neighbor method]
T <sub>m</sub>	70.8°	[%GC method]
T <sub>m</sub>	56°	[2(A+T) <sup>o</sup> + 4(G+C) <sup>o</sup> method]
T <sub>m</sub> (RNA)[1M Na]	81°	[%GC method]
T <sub>m</sub> (DNA:RNA)[1M Na]	74.7°	[%GC method]
A <sub>260</sub> /A <sub>280</sub>	1.59	[single strand]
Molecular Weight	5.8K	[one strand]
Molecular Weight	11.7K	[two strands]
µg/OD	47.4	[dsDNA]

Base	Number	%
A	2	[10.5%]
C	5	[26.3%]
G	4	[21.1%]
T	8	[42.1%]
A + T	10	[52.6%]
G + C	9	[47.4%]

Oligonucleotide Database

File: NewDatabase.odb

# of Records: 29

#	Date	ID Number	Sequence	3'-Dim. ΔG	P.E. / p.e.	Tm / t <sub>m</sub>
<input type="checkbox"/> 21	12/02/06	AY436640:5916R19	AATGCCTGCCTTTAGTCTG	- SC	430 430	54.1 54.5
<input type="checkbox"/> 22	12/02/06	AY436640:5916R20	CAATGCCTGCCTCTAGTCTG	0.3 SC	366 450	50.9 57.2
<input type="checkbox"/> 23	12/02/06	AY436640:5937R21	TCAATTTCTTTAGCTTGCCAT	0.3 SC	449 449	54.7 53.1
<input checked="" type="checkbox"/> 24	12/02/06	AY436640:5937R22	TTCAATTTCTTTAGCTTGCCAT	0.3 SC	458 458	55.9 53.8
<input type="checkbox"/> 25	12/02/06	AY436640:4695U22	TGCCTTAACAAAAGTAATCCAT	0.3 SC	432 432	54.5 53.0
<input type="checkbox"/> 26	12/02/06	AY436640:5325U22	AATTACGTCTTTCTTATGCCAA	0.3 SC	453 453	53.3 53.0
<input type="checkbox"/> 27	12/02/06	AY436640:5786L23	CTCTGCCTAGAACATTATCACTC	-0.3 SC	451 451	54.8 55.0
<input type="checkbox"/> 28	12/02/06	AY436640:5860L19	AACAACCAAAGCCAACCTG	-0.9 SC	444 444	55.3 55.9

Oligonucleotide Sets (64)

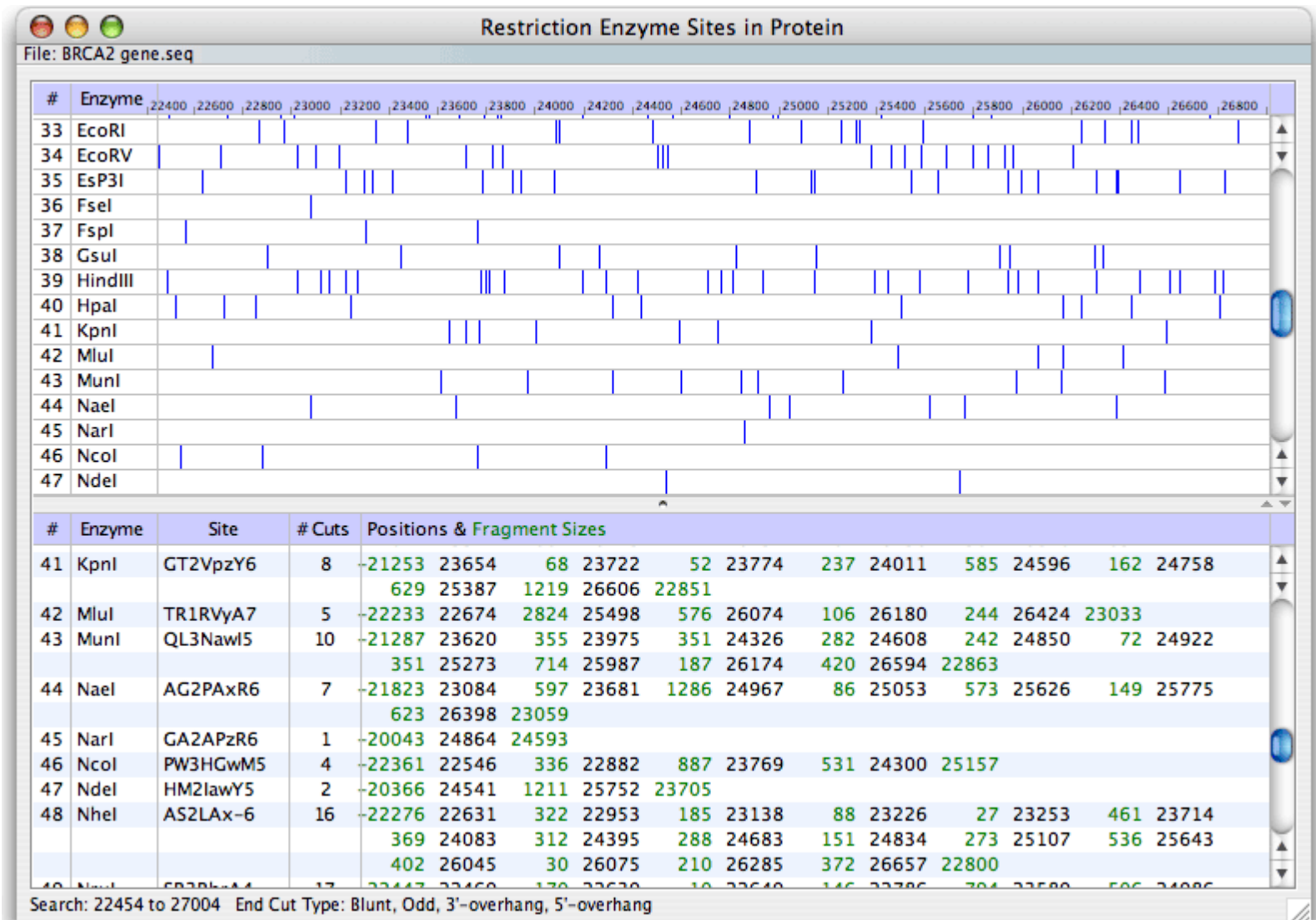
#	Forward Primer	Reverse Primer	Upper Oligo	Lower Oligo
1	2	3	4	
<input type="checkbox"/> 36	8	23	25	28
<input type="checkbox"/> 42	8	24	25	28
<input checked="" type="checkbox"/> 47	9	14	25	27
<input type="checkbox"/> 39	9	15	25	27
<input type="checkbox"/> 33	9	16	25	27
<input type="checkbox"/> 61	9	17	25	27
<input type="checkbox"/> 48	9	18	25	27

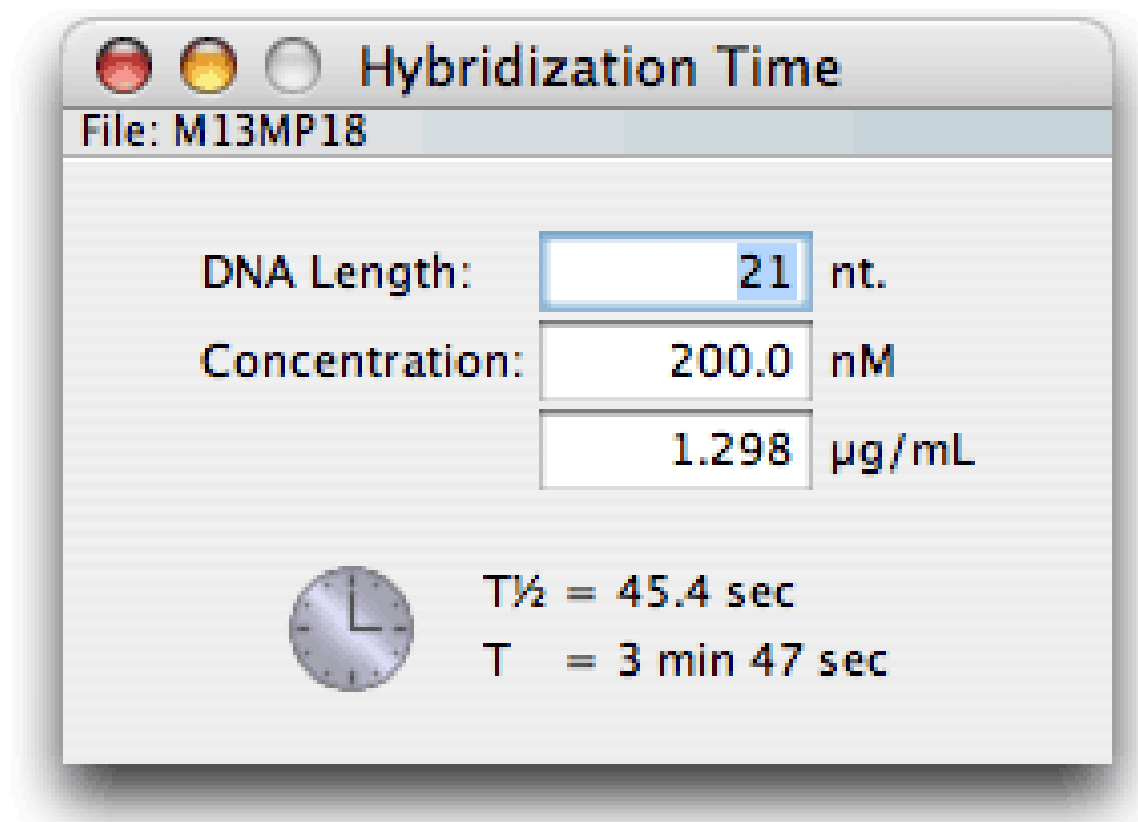
This database is linked to BRCA2 gene.seq

Selected oligo

Checked Set of nested primers







File: BRCA2 gene.seq

Constant Concentration     Constant Volume

<input checked="" type="radio"/> Current +Oligo:	5.08 nmol/OD, 32.5 µg/OD
<input type="radio"/> Current -Oligo:	4.67 nmol/OD, 30.9 µg/OD
<input type="radio"/> Entire Sequence (ds):	0.001 nmol/OD, 48.1 µg/OD
<input type="radio"/> Forward Primer:	5.98 nmol/OD, 35.0 µg/OD
<input type="radio"/> Reverse Primer:	5.31 nmol/OD, 34.0 µg/OD
<input type="radio"/> PCR Product (ds):	0.146 nmol/OD, 48.1 µg/OD
<input type="radio"/> Upper Oligo:	4.83 nmol/OD, 31.2 µg/OD
<input type="radio"/> Lower Oligo:	4.67 nmol/OD, 30.9 µg/OD

32.5 µg  
or 1.0 OD(260)  
or 5.084 nmol  
in 508.4 µL  
yields 10.0 µM



**AHP2 cDNA (TAIR database)**

**Sequence: AT3G29350.1** Date last modified 2007-04-17 Name AT3G29350.1 Tair  
Accession Sequence:4010737427 Sequence Length (bp) 827

1 ACAATTCGCG AGAAAGACAA AACACAAGTT TCTTCTTCTT GGGATTGGCT  
51 ATTTCCAGAA ATCCAAGTCA ATAATCAAAG TCCAAACAAA AAAATCCTCT  
101 CCCAATCTCC GCTTCACTCT TCTCATGGAC GCTCTCATTG CTCAGCTTCA  
151 GAGACAATTT CGTGATTACA CCATTTCTCT CTACCAACAG GGGTTTTTGG  
201 ATGATCAATT TACTGAGTTG AAAAAGCTAC AAGATGATGG AAGTCCTGAT  
251 TTTGTGTCTG AAGTGCTTTC ACTTTTCTTT GAAGATTGTG TGAAGCTTAT  
301 CAGTAACATG GCTAGAGCTT TGGACACGAC AGGAACTGTA GATTTTAGTC  
351 AGGTAGGTGC TAGTGTGCAT CAATTGAAGG GTAGTAGCTC AAGTGTTGGT  
401 GCCAAGAGGG TCAAAACTTT GTGTGTTAGC TTCAAGGAAT GTTGTGAAGC  
451 TAAGAACTAC GAAGGGTGTG TGAGATGTTT GCAGCAAGTG GATATTGAGT  
501 ACAAGGCGTT AAAGACAAAG CTTCAAGATA TGTTCAATCT TGAGAAACAG  
551 ATCATTCAAG CTGGTGGTAT AGTTCCTCAA GTGGATATTA ACTAAAGAGA  
601 CTAGTCCATA AGAAGAAAAA AGATGATGAC TTTCTTTCTT TAGTTTCTCT  
651 TCTAAATTAT TTTGGATTTG GTGTTTGCTC AAAAACTCAA TAAAATATGT  
701 GCAAAAAGAA ACAAAAACAA GTGATGGTTG TTTATAAATC AGTAGTATGT  
751 ATTGTTTGAT CTCATCCGAG AAAATTGAAA CCATTGGACT AATGAATGTG  
801 ATGATAATAT ATATTGGTTT GCTTCTG



101 CCCAATCTCC GCTTCACTCT TCTCATGGAC GCTCTCATTG CTCAGCTTCA  
 151 GAGACAATTT CGTGATTACA CCATTTCTCT CTACCAACAG GGGTTTTTGG  
 201 ATGATCAATT TACTGAGTTG AAAAAGCTAC AAGATGATGG AAGTCCTGAT  
 251 TTTGTGTCTG AAGTGCTTTC ACTTTTCTTT GAAGATTGTG TGAAGCTTAT  
 301 CAGTAACATG GCTAGAGCTT TGGACACGAC AGGAACTGTA GATTTTAGTC  
 351 AGGTAGGTGC TAGTGTGCAT CAATTGAAGG GTAGTAGCTC AAGTGTTGGT  
 401 GCCAAGAGGG TCAAAACTTT GTGTGTTAGC TTCAAGGAAT GTTGTGAAGC  
 451 TAAGAACTAC GAAGGGTGTG TGAGATGTTT GCAGCAAGTG GATATTGAGT  
 501 ACAAGGCGTT AAAGACAAAG CTTCAAGATA TGTTCAATCT TGAGAAACAG  
 551 ATCATTCAAG CTGGTGGTAT AGTTCCTCAA GTGGATATTA ACTAAAGAGA

### EcoRI restriction site

5'.....G|AATTC.....3'

3'.....CTTAA|G.....5'

|

### Design of primers

#### AHP2ex\_up

5'- CCG GAA TTC ATG GAC GCT CTC ATT GCT CAG – 3'

#### AHP2ex\_low

5'- CCG GAA TTC TTA GTT AAT ATC CAC TTG AGG – 3'

101 CCCAATCTCC GCTTCACTCT TCTC**ATGGAC GCTCTCATTG CTCAGCTTCA**  
 151 GAGACAATTT CGTGATTACA CCATTTCTCT CTACCAACAG GGGTTTTTGG  
 201 ATGATCAATT TACTGAGTTG AAAAAGCTAC AAGATGATGG AAGTCCTGAT  
 251 TTTGTGTCTG AAGTGCTTTC ACTTTTCTTT GAAGATTGTG TGAAGCTTAT  
 301 CAGTAACATG GCTAGAGCTT TGGACACGAC AGGAACTGTA GATTTTAGTC  
 351 AGGTAGGTGC TAGTGTGCAT CAATTGAAGG GTAGTAGCTC AAGTGTTGGT  
 401 GCCAAGAGGG TCAAAACTTT GTGTGTTAGC TTCAAGGAAT GTTGTGAAGC  
 451 TAAGAACTAC GAAGGGTGTG TGAGATGTTT GCAGCAAGTG GATATTGAGT  
 501 ACAAGGCGTT AAAGACAAAG CTTCAAGATA TGTTCAATCT TGAGAAACAG  
 551 ATCATTCAAG CTGGTGGTAT AGTT**CCTCAA GTGGATATTA ACTAA**AGAGA

### EcoRI restriction site

5'.....G|AATTC.....3'

3'.....CTTAA|G.....5'

|

### Design of primers

#### AHP2ex\_up

5'- CCG **GAA TTC** ATG GAC GCT CTC ATT GCT CAG – 3'

#### AHP2ex\_low

5'- CCG **GAA TTC** TTA GTT AAT ATC CAC TTG AGG – 3'

## LITERATURA

- Artificial DNA: Methods and Applications; Khudyakov, Y.E., Fields, W.A., Ed. (2003)
- PCR Primer: A Laboratory Manual (2003)
- OLIGO Primer analysis software, Version 7

Discovery is not in seeking new landscapes,  
but in having new eyes...

Marcel Proust

Tato prezentace vznikla s podporou projektu **OP VK** „Rozvoj týmu pro výuku, výzkum a aplikace v oblasti funkční genomiky a proteomiky“ (CZ.1.07/2.3.00/09.0132)

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

