M U N I

Design of PCR Primers

Hana Konečná
CEITEC Central European Institute of Technology
NCBR National Centre for Biomolecular Research

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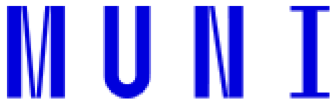
OLIGONUCLEOTIDES

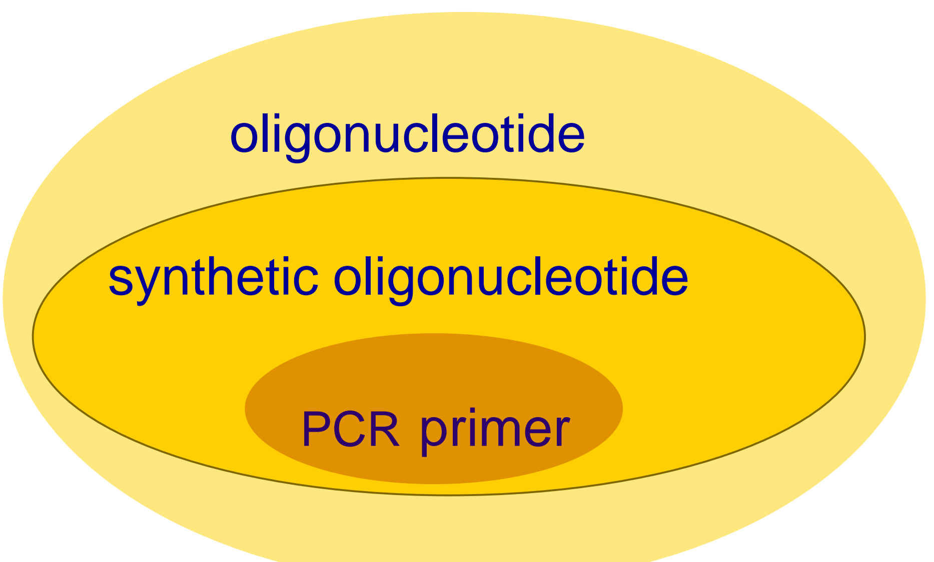
- definition
- aplications
- modifications
- synthesis
- purification
- quality control

- design of sequence
- rules
- software OLIGO 7
- example

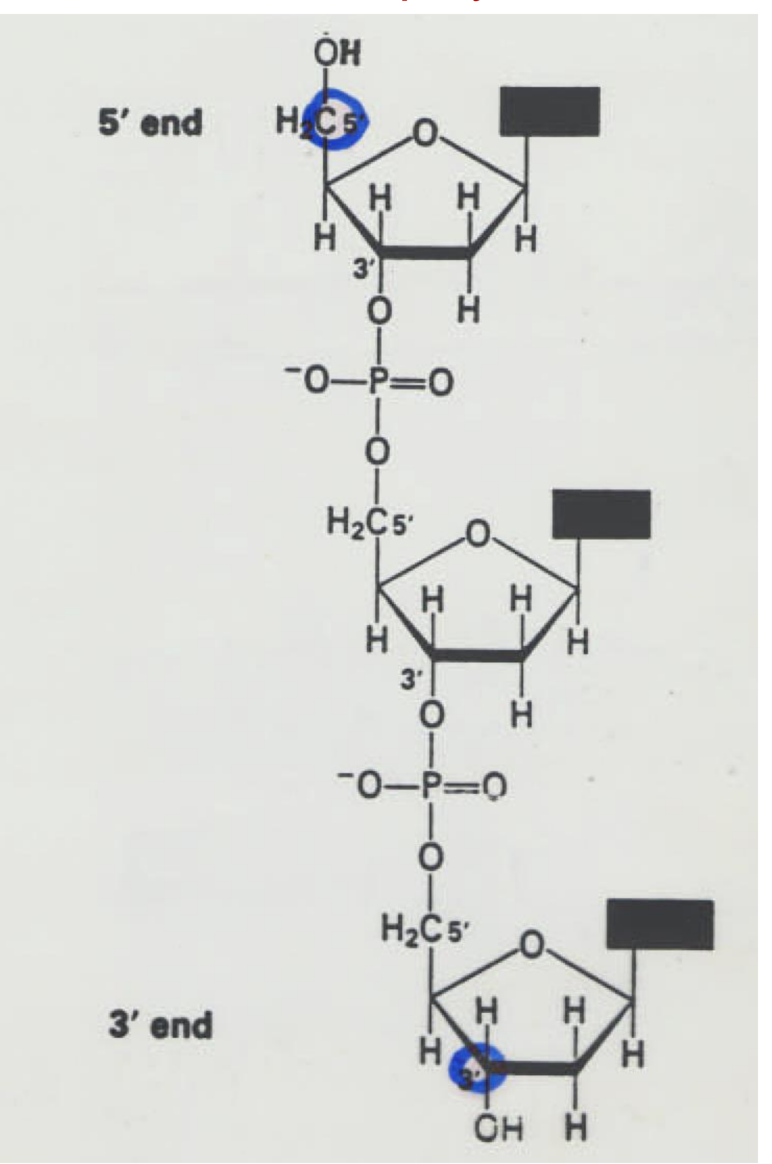


oligonucleotide

- short single stranded structure
- DNA or RNA (also PNA)
- **hydroxyl** on both ends
  (no phosphate on 5-end as usual)



orientation! polymerase!



Aplications of synthetic oligonucleotides

- primers for synthesis of complementary DNA
  PCR, Real-Time PCR
- gene synthesis and recombinant proteins
- hybridisation probes for cloning
- site directed mutagenesis
- sequencing ang genetic profiling
- diagnostics – tests and biosensors
- gene arrays
- blocation of gene expression - *antisense oligo*
- prospective therapeutics and DNA vaccines
- NMR monitoring of DNA - protein interactions
- structural X-ray analysis of NA

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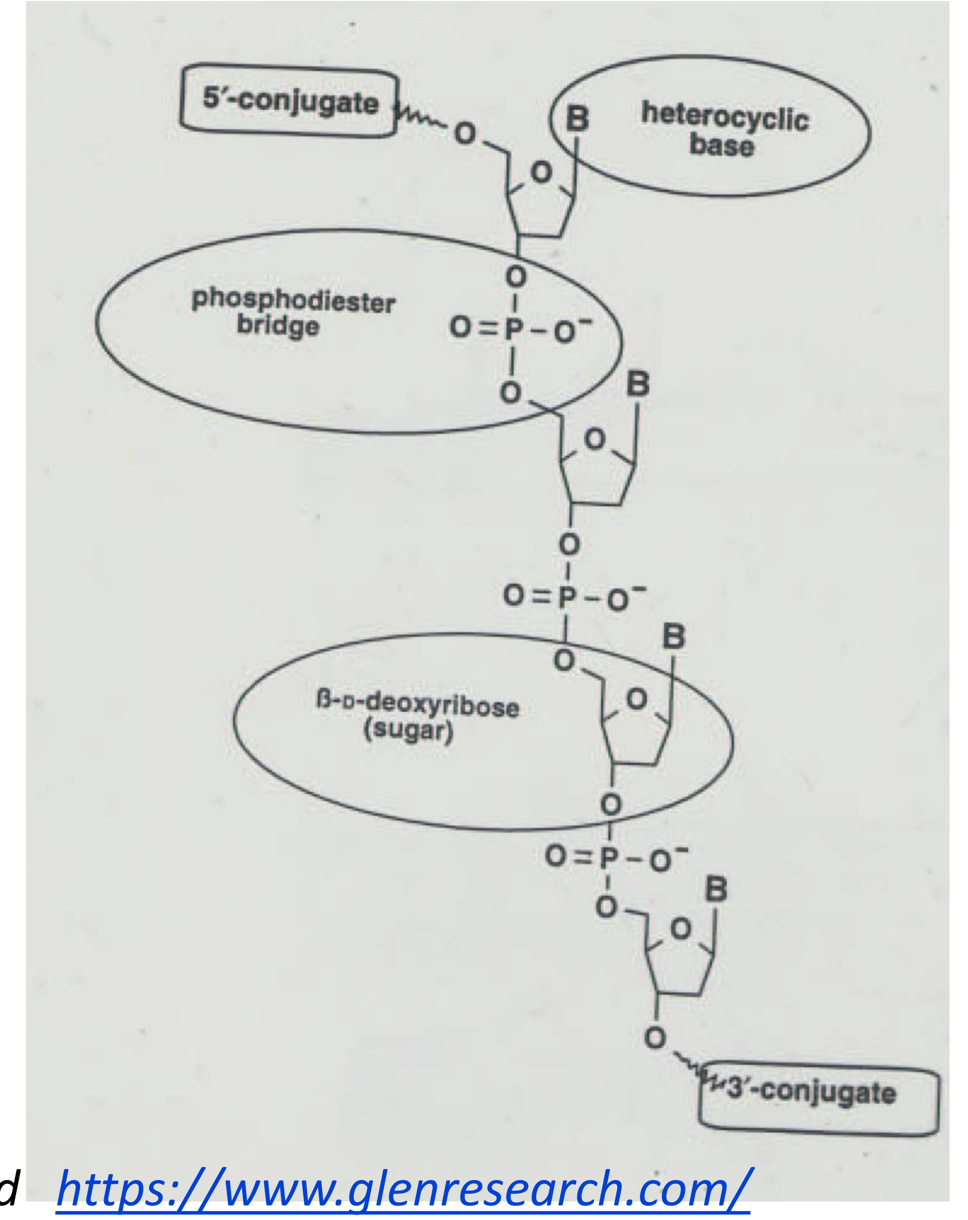
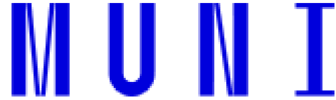
Modifications

- degeneration
- end of sequence
- bases
- phosphate
- carbohydrate
- PNA



* *Additional resources for interested* https://www.glenresearch.com/



Degenerated oligonucleotides

ACG TAC GTA CGT ACG TAC	non-degenerated
ACG TA**M** GTA CGT ACG TAC	M = A/C
ACG TAC GTA C**D**T ACG TAC	D = A/G/T
ACG TAC GTA CGT ACG **N**AC	N = A/C/G/T

Degenerated oligonucleotides

2-deoxyinosin

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

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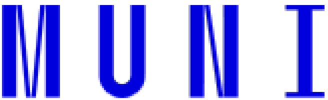
Modification on 5´- end

postsynthetic modifications

- Phosphorylation
- Amino group
- Thio group
- Digoxigenin
- Biotin
- Enzymes
- Psoralen
- Acridine
- Cholesterole
- Fluorescent dyes
- Quenchers
- 2,4- dinitrophenyl
- Spacer
- Branching
- Blocation

sequencing
fragmenation analysis
gene arrays
Real-Time PCR

** Additional resources for interested* https://www.glenresearch.com/



Modifications on 3´- end

derivatized matrix

- Phosphate
- Thio group
- → Amino group
- Spacer
- Acridine
- → Biotin
- → Fluorescent dyes
- → Quenchers
- Cholesterole
- 2,4 dinitrophenyl

** Additional resources for interested* *https://www.glenresearch.com/*

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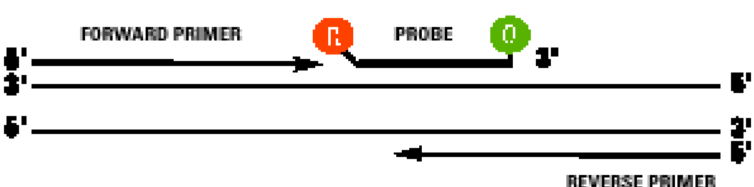
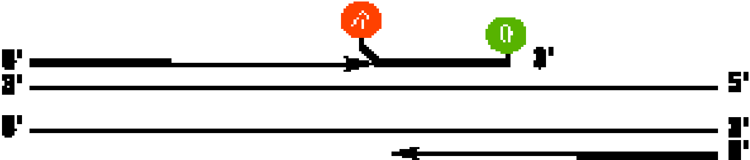
Real-Time PCR

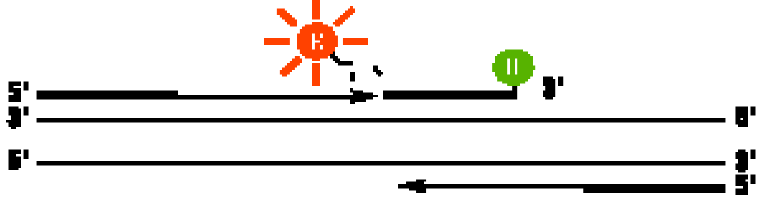
- 2x labeled probe
- REPORTER
- QUENCHER



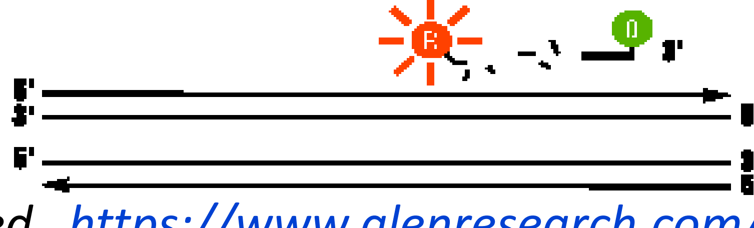
2. **Strand displacement:** When the probe is intact, the reporter dye emission is quenched.



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.



** Additional resources for interested* https://www.glenresearch.com/

Other modifications

Phosphorothioates
Phosphorodithioates
H-phosphonates
Methylphosphonates

← backbone

carbohydrate →

Modifications in 2´- position
Ribose modification

* Additional resources for interested https://www.glenresearch.com/

Therapeutics

→ Nondegradable by nucleases!

Modification of phosphodiester

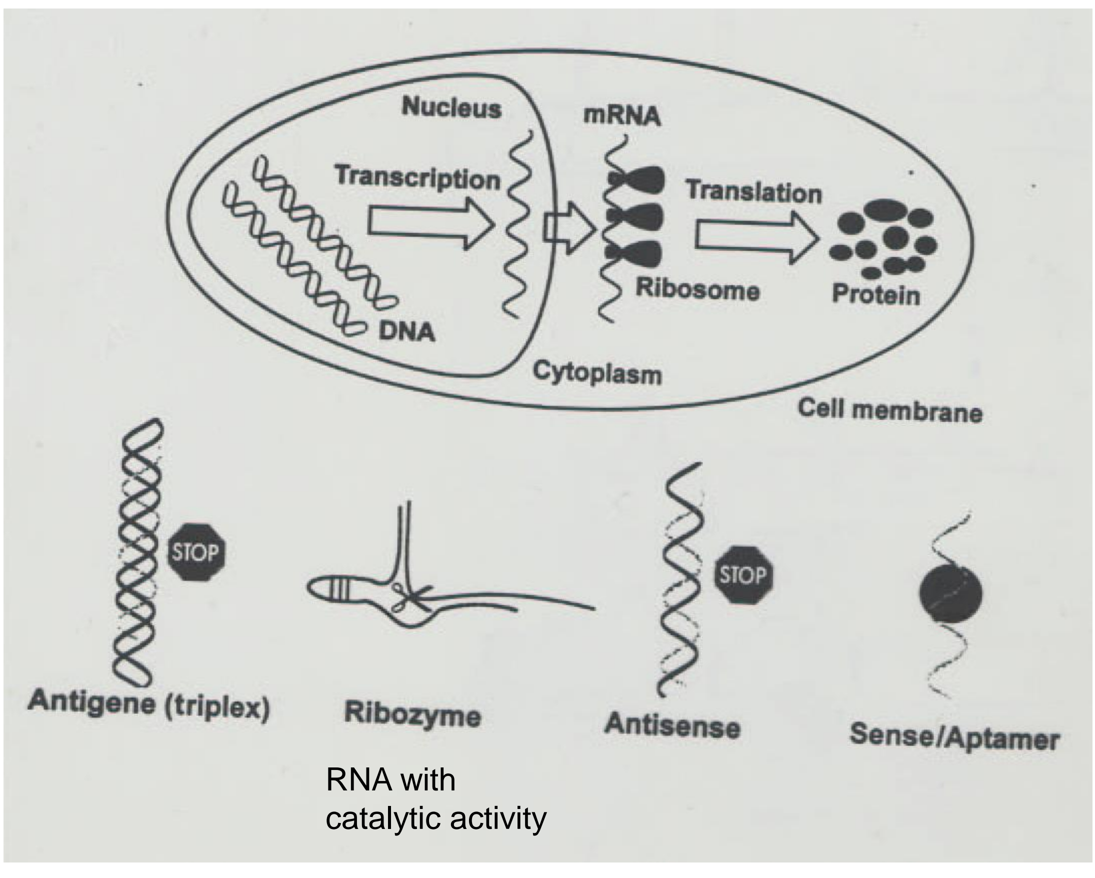
phosphorothioate	methylphosphonate	phosphoramidate
$O{=}P(-O-)(-O-)-S^-$	$O{=}P(-O-)(-O-)-CH_3$	$O{=}P(-O-)(-O-)-NH\text{-alkyl}$

3′-thioformacetal	methylene(methyliminio)	boranophosphate
$-S-CH_2-O-$	$-CH_2-N(CH_3)-O-$	$O{=}P(-O-)(-O-)-BH_3^-$

** Additional resources for interested* https://www.glenresearch.com/

ANTISENSE oligonucleotide

- oligonucleotide or analogue
- complementary to segment of RNA or DNA
- inhibition of normal function due to coupling



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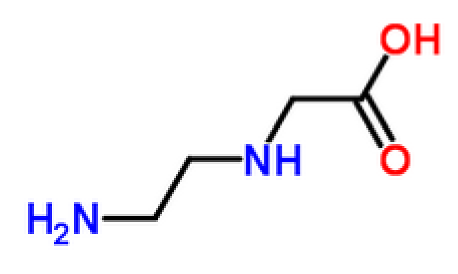
Peptidonucleic acid

- noncharged molecule
- binding with DNA/RNA

N-(2-aminoethyl)-glycine →



PNA **DNA**

Repeat Unit

PNA

DNA

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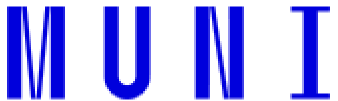
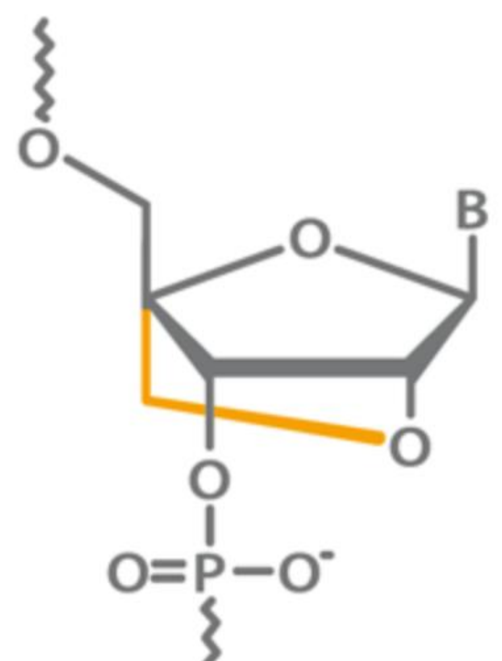
Why PNA

- thermostable
- Tm not depending on salts
- high specificity
- high affinity
- resistant towards enzymes

** Additional resources for interested* Gambari R. *Expert Opin Ther Pat*. 2014, 24(3):267-94.
Peptide nucleic acids: a review on recent patents and technology transfer.





Locked Nucleic Acid

- 2'-O, 4'-C methylene bridge
- supressed flexibility of ribofuranose ring
- structure is locked into rigid C3-endo conformation
- enhanced hybridisation
- outstanding biostability

** Additional resources for interested* https://www.glenresearch.com/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3412486/
LNA-based Oligonucleotide Electrotransfer for miRNA Inhibition. Molecular Therapy (2012); ***20*** *8, 1590–1598*

OLIGONUCLEOTIDES

design

synthesis

purification

EXPEDITE 8909

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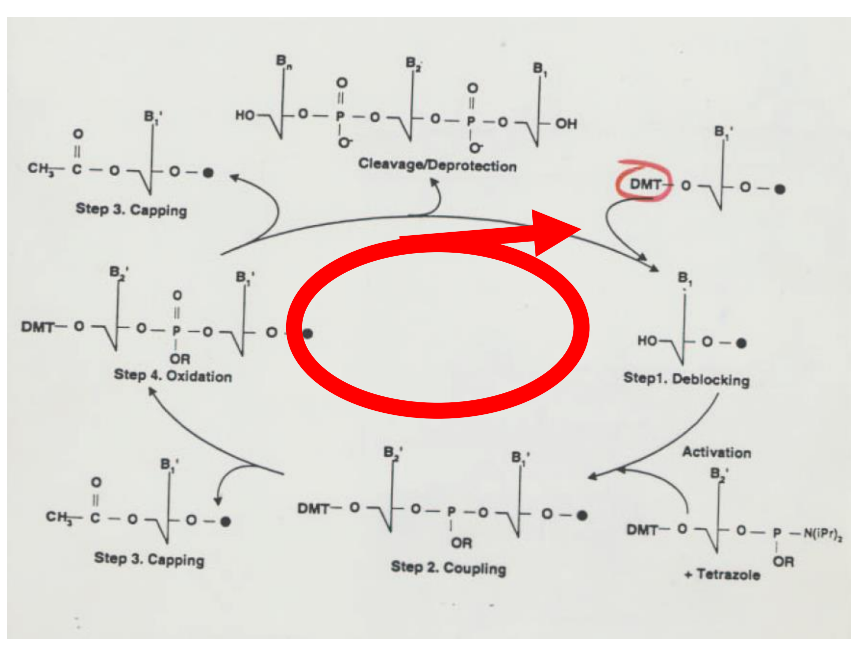
Oligonucleotide Synthesis

- no enzyme
- organic synthesis on solid matrix
- adds new base in cycles
- from 3´-end to 5´-end
- in anhydrous environment



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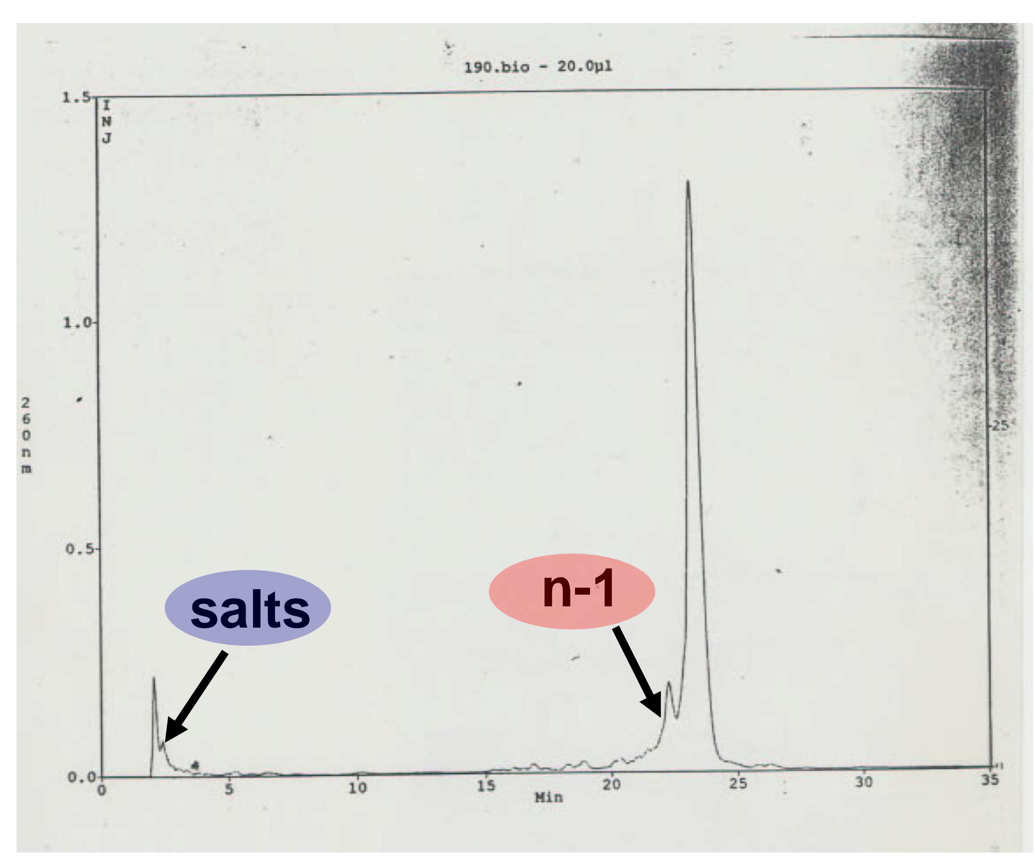
Quality Control



Liquid Chromatography

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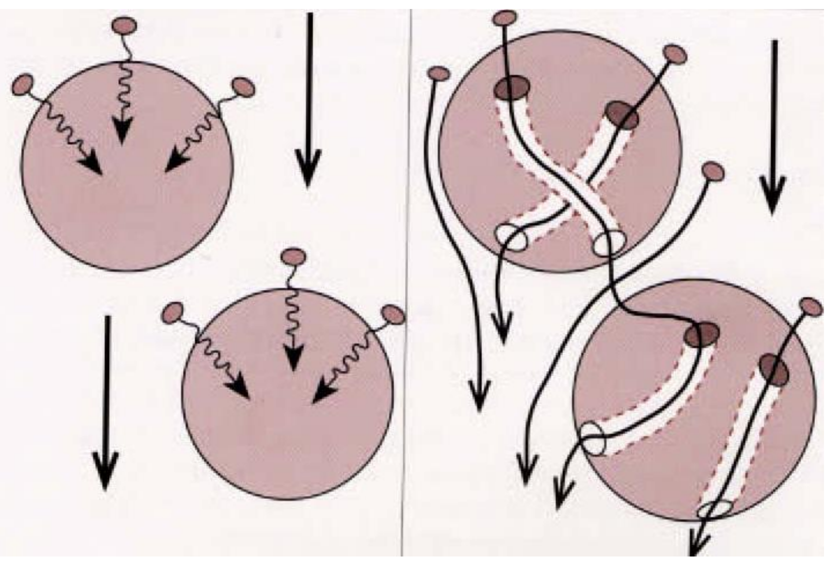
LC option:
Perfusion Chromatography

classical sorbent POROS



134long.bio - 20.0µl

Poros HQ
1 ml/min

0.15 0.10 0.05 0.00

260 nm

100 50 0

mS

0 5 10 15 20 25 30 35 40
Min

134.bio - 20.0µl

Poros HQ
8 ml/min

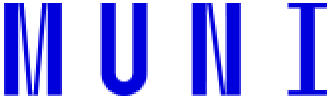
0.15 0.10 0.05 0.00

260 nm

100 50 0

mS

0.0 2.5 5.0
Min



Other tools for **Q**uality **C**ontrol

LC-MS **additional literature for interested*

An automated compliance-ready LC-MS workflow for mass confirmation of both modified and unmodified oligonucleotides, Sep 2020 https://www.waters.com/webassets/cms/library/docs/720006820en.pdf

PAGE

Maldi-TOF MS

CE

7103

23mer

n-1

Signal intensity (%)

Mass/charge

n-1

Time

Capillary electrophoresis trace

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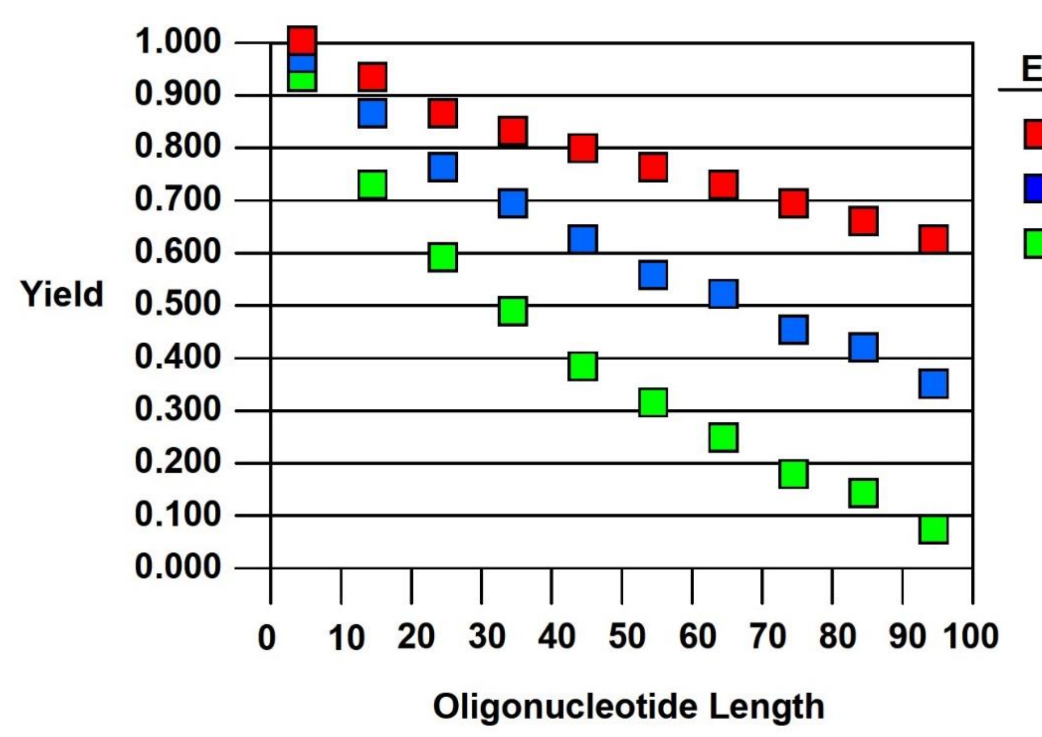
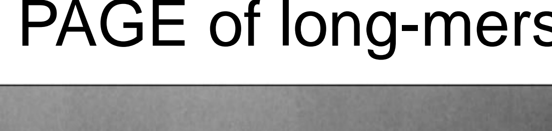
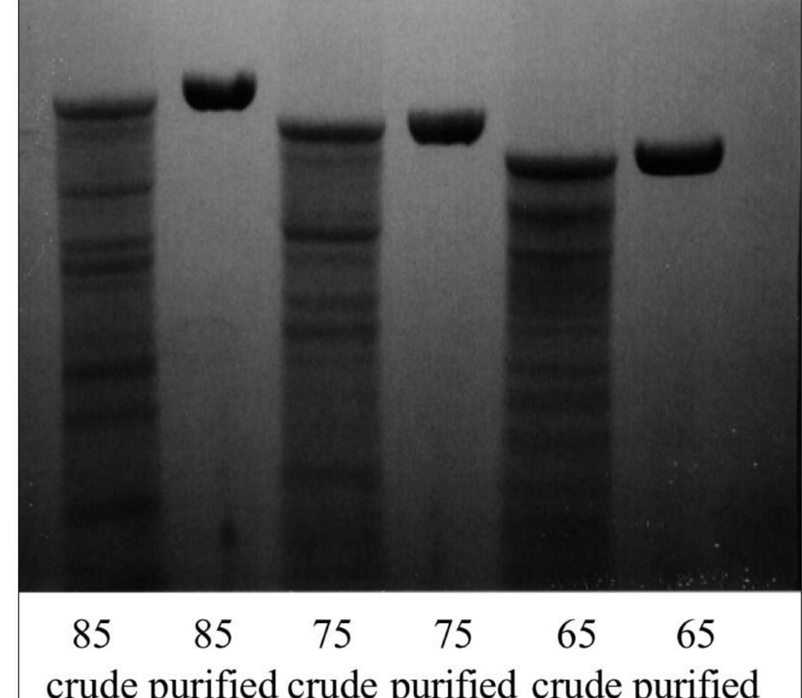
YIELD







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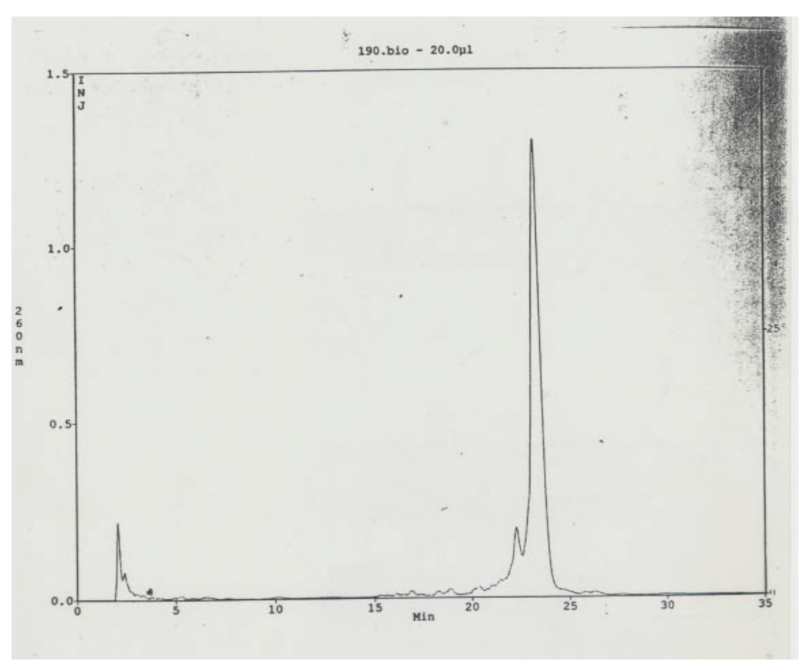
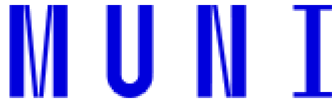
PURIFICATION

- Sephadex
- RP cartridge
- HPLC





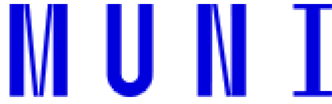
OLIGONUCLEOTIDE DESIGN

- manual
- computer assisted

**Additional literature for interested*
https://www.sciencedirect.com/science/article/pii/S2001037019300844
www.protocol-online.org/prot/Research_Tools/Online_Tools/Oligo_Design/index.html

Main features of good PCR primer sequence

- highly specific
- no dimers and hairpins
- stable duplexes with active sequence
- lightly unstable 3´-end



OLIGO 6

- PCR primers
- hybridisation probes
- sequencing primers

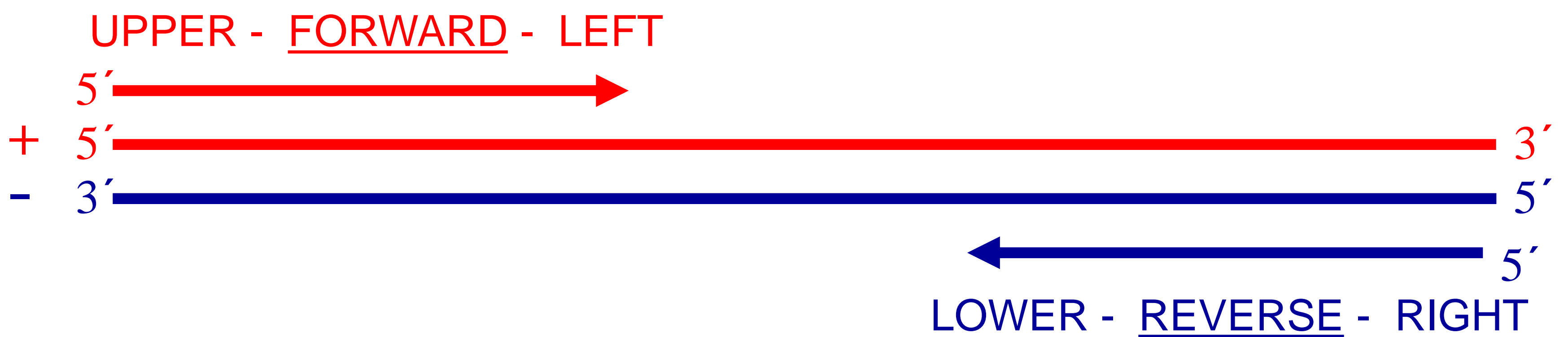
OLIGO 7 (from 2008)

- TaqMan probes
- primers for *nested PCR*
- *molecular beacons*
- siRNA

** Additional resources for interested* http://oligo.net/tutorials.html

Terminology of PCR primers

forward primer… part of the + string
reverse primer… part of the - string

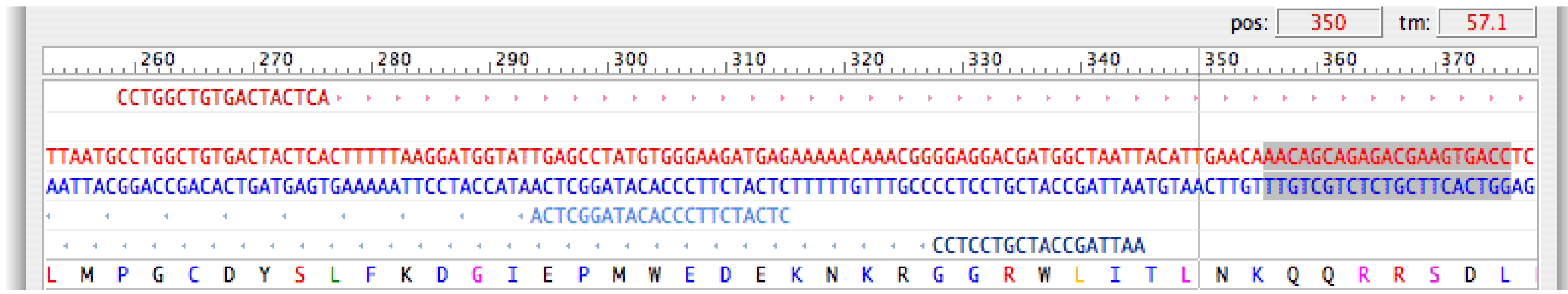


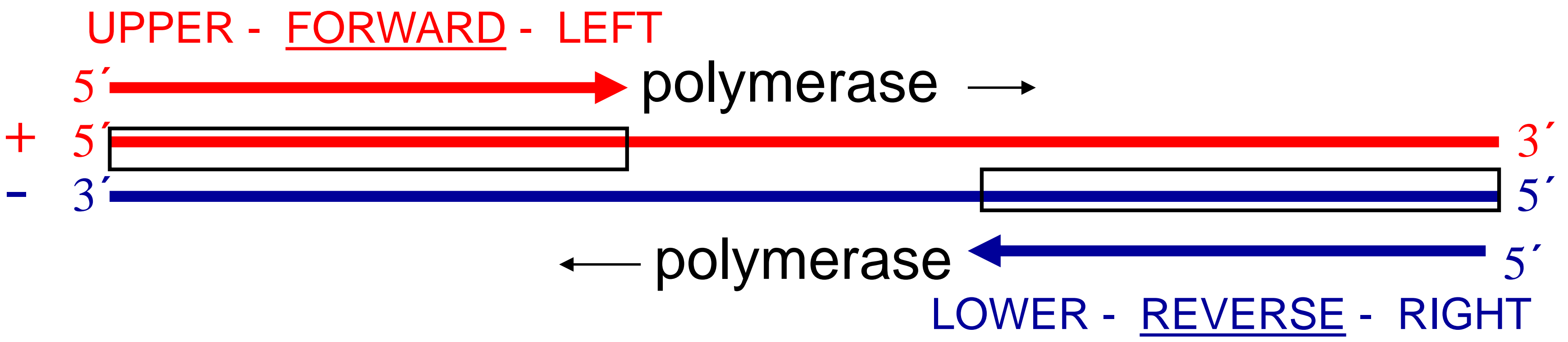
M U N I

Terminology

forward primer… part of the + string

reverse primer… part of the - string





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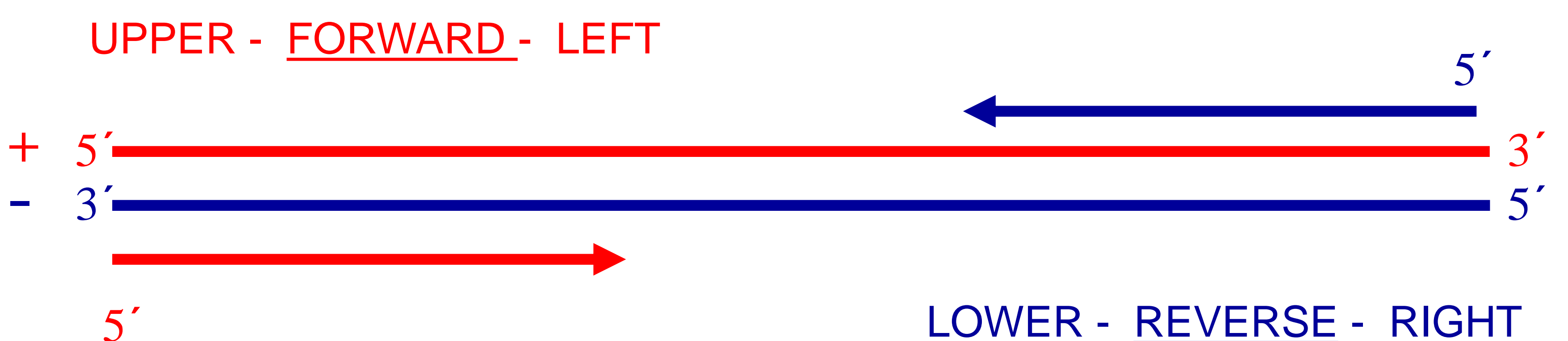
Annealing of PCR primers



5´ CTT CTG CTC AAT CTT TCT AC 3´ **FORWARD**

read from left to right

+ 5´

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

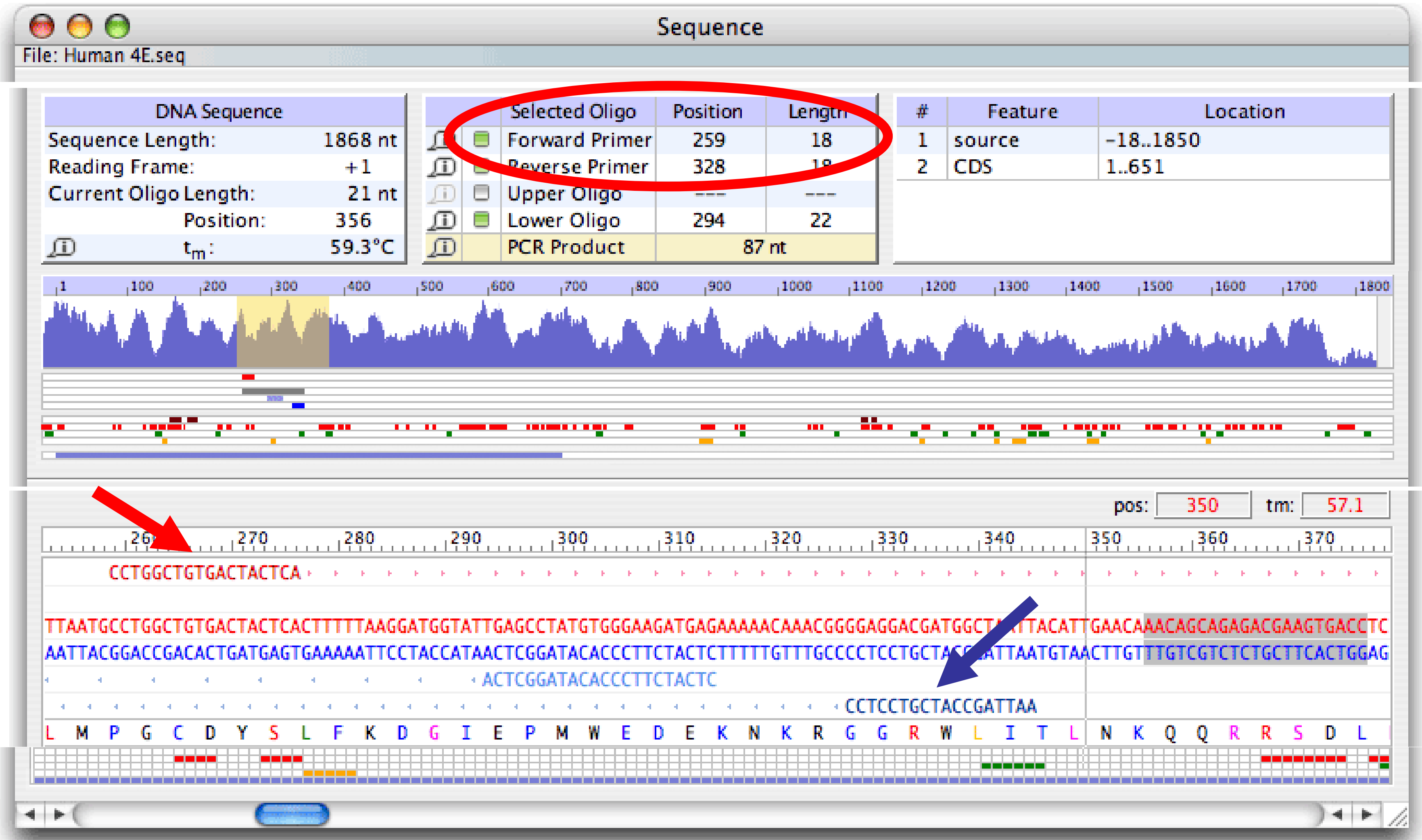
3´

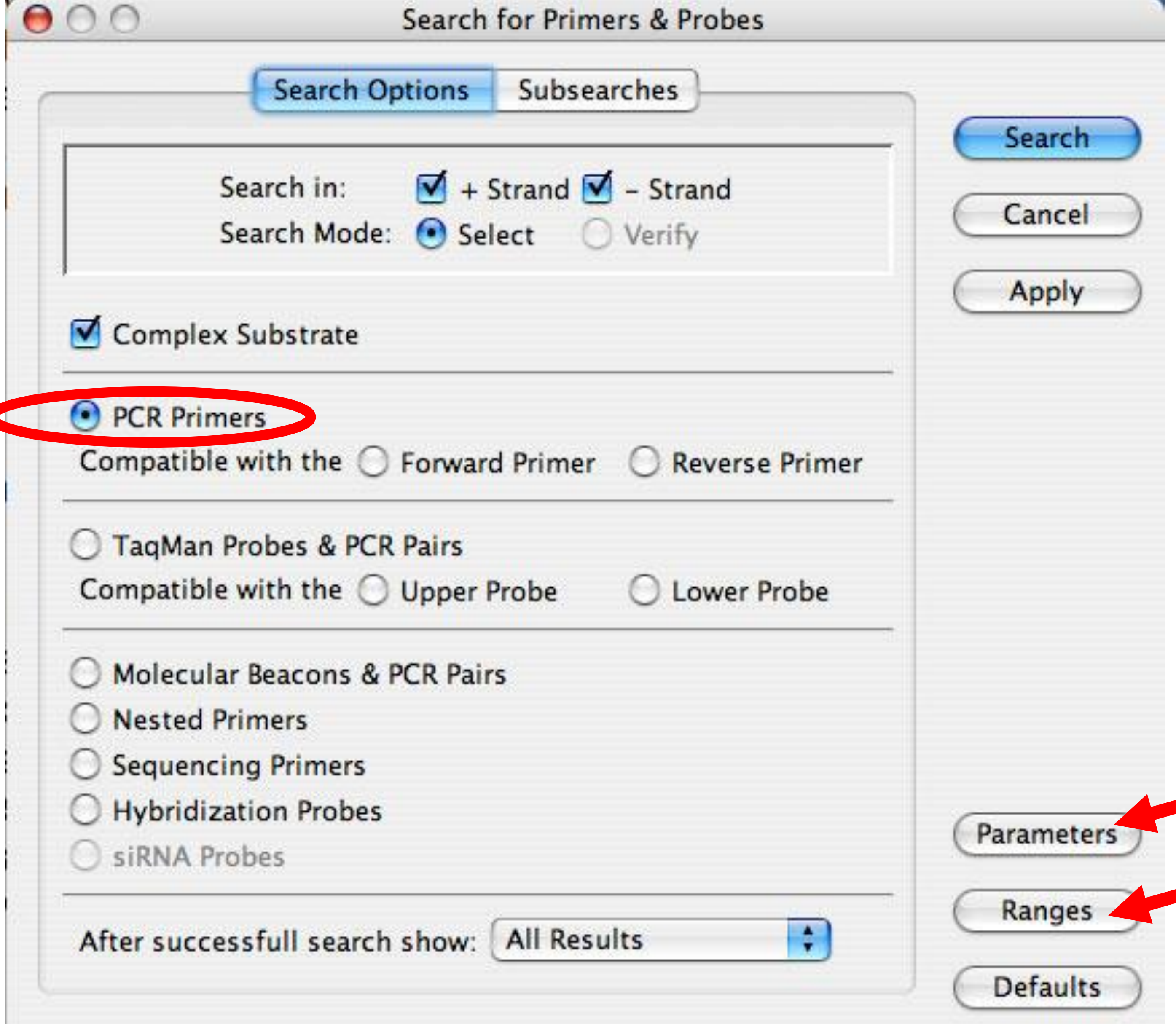
5´ TGA AGA ATA TCA GCT AGT TT 3´ **REVERSE**

read from right to left

complementary

M U N I




Search for Primers & Probes
Search Options
Subsearches
Search
Cancel
Apply
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
Molecular Beacons & PCR Pairs
Nested Primers
Sequencing Primers
Hybridization Probes
siRNA Probes
After successfull search show:
All Results
Parameters
Ranges
Defaults

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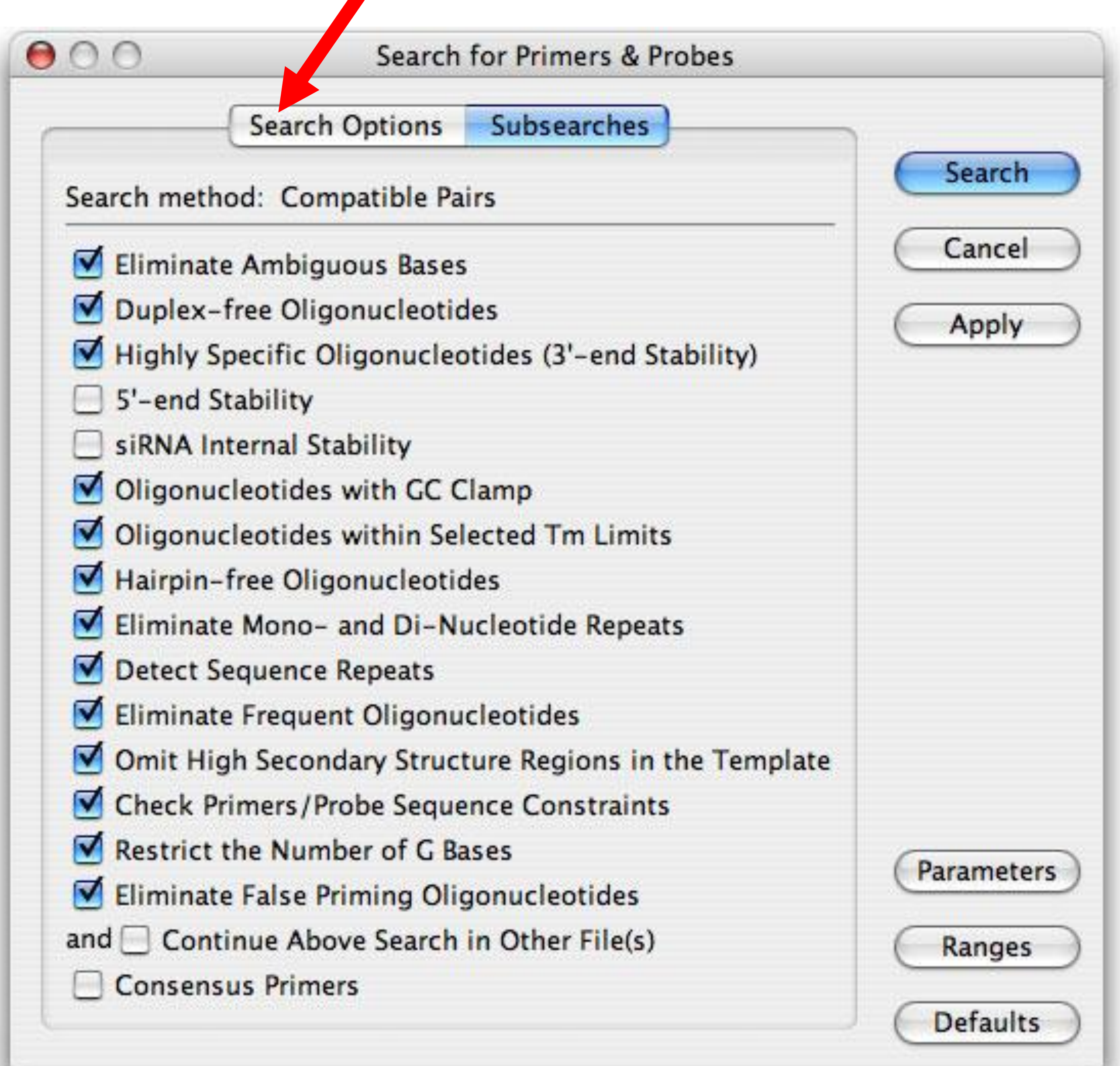

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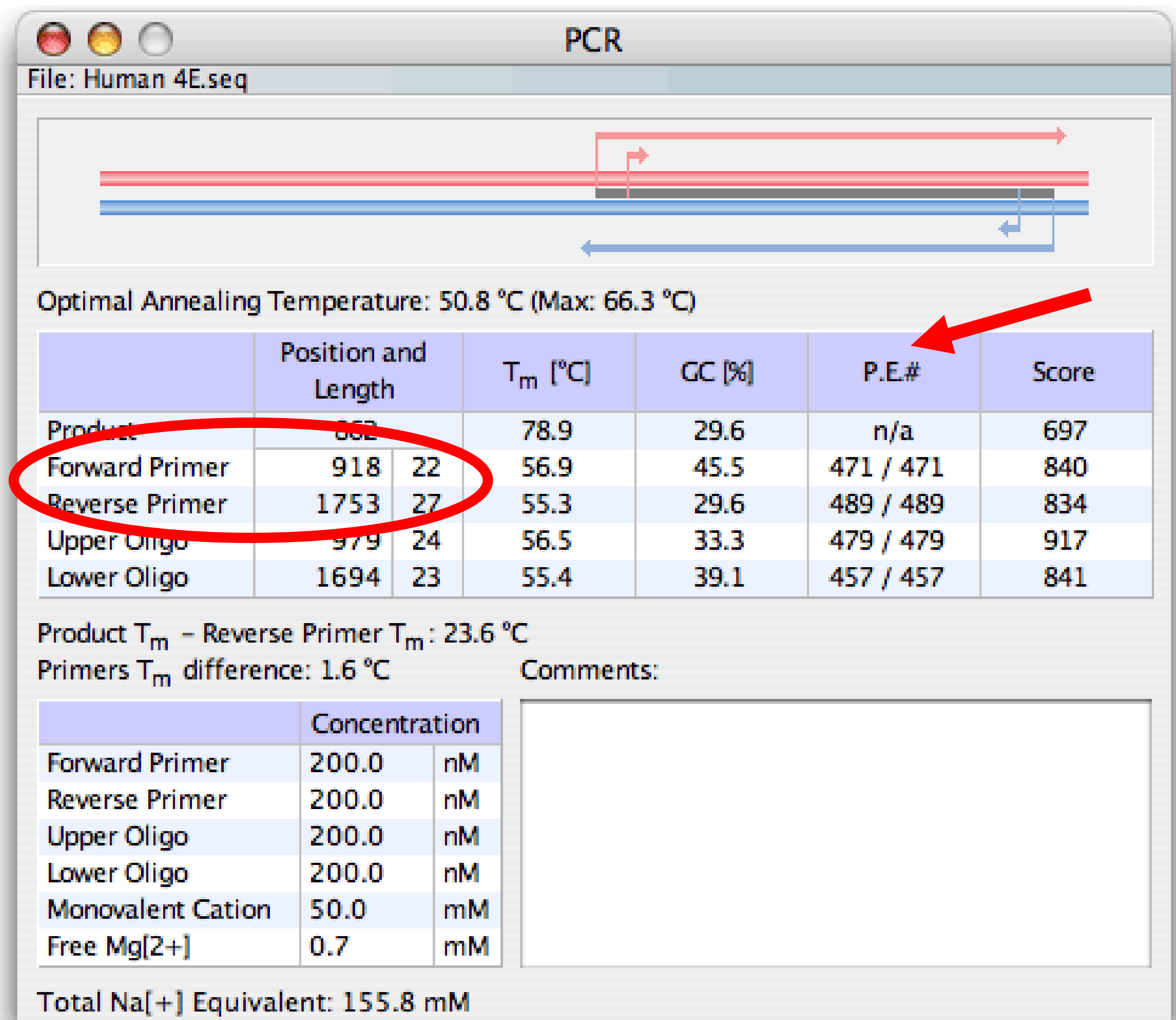

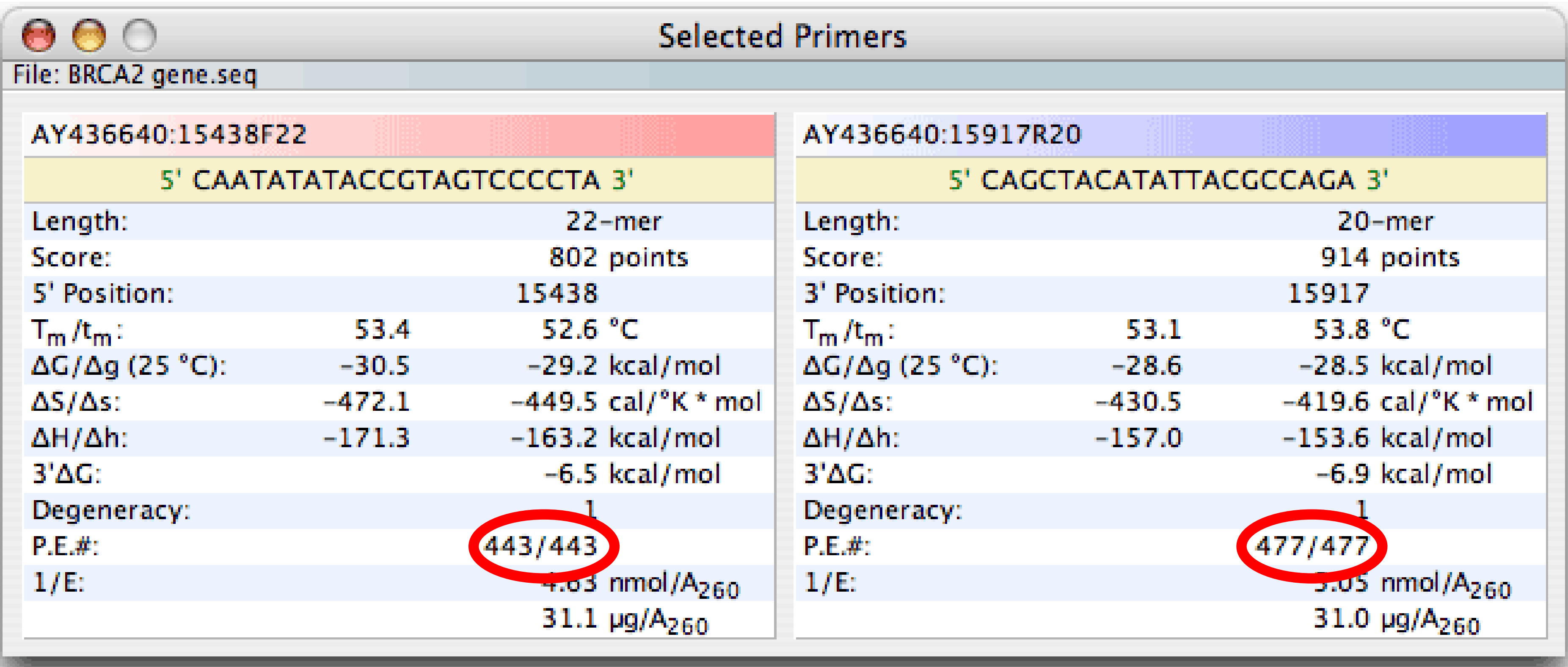

Priming **E**fficiency PE
Score

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Secondary structures

Current Oligo Duplexes

File: BRCA2 gene.seq

Current Oligo 21-mer [5042]

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

```
5' GAATTAGATAAATTCAAATTA 3'
           ||| || || |||
      3' ATTAAACTTAAATAGATTAAG 5'
```

DIMER intermolecular

[Current– Oligo] – The most stable 3'-dimer: # of hydrogen bonds = 10; ΔG = –7.3 kcal/mol; T_m = 2.9°C

```
5' TAATTTGAATTTATCTAATTC 3'
         |||||     |||||
    3' CTTAATCTATTTAAGTTTAAT 5'
```

The most stable dimer overall: # of hydrogen bonds = 10; ΔG = –7.4 kcal/mol; T_m = 2.2°C

```
    5' GAATTAGATAAATTCAAATTA 3'
       |||||     |||||
3' ATTAAACTTAAATAGATTAAG 5'
```

Hairpin: loop = 5 nt; ΔG = –3.0 kcal/mol; T_m = 54.6 °C

```
    5'GAATTAG-┐
       |||||   A
3'ATTAAACTTAAAT-┘
```

HAIRPIN intramolecular

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Current Oligo Hairpin Stems

File: BRCA2 gene.seq

Current Oligo 21-mer [5042]

1. # of paired bases = 5; loop = 5 nt; ΔG = −3.0 kcal/mol; T_m = 54.6 °C

```
5042 GAATT 5046        5'GAATTAG┐
     |||||               |||||   A
5056 CTTAA 5052    3'ATTAAACTTAAAT┘
```

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

```
5043 AATTAGA 5049  5'GAATTAGATA┐
     |||| ||          |||| ||   A
5061 TTAAACT 5055  3'ATTAAACTTA┘
```

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

```
5052 AATT 5055  5'GAATTAGATAAATTC┐
     ||||                  ||||  |
5061 TTAA 5058          3'ATTAAA┘
```

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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) tctaagtggtcagtg-tgc (5108)5'
```

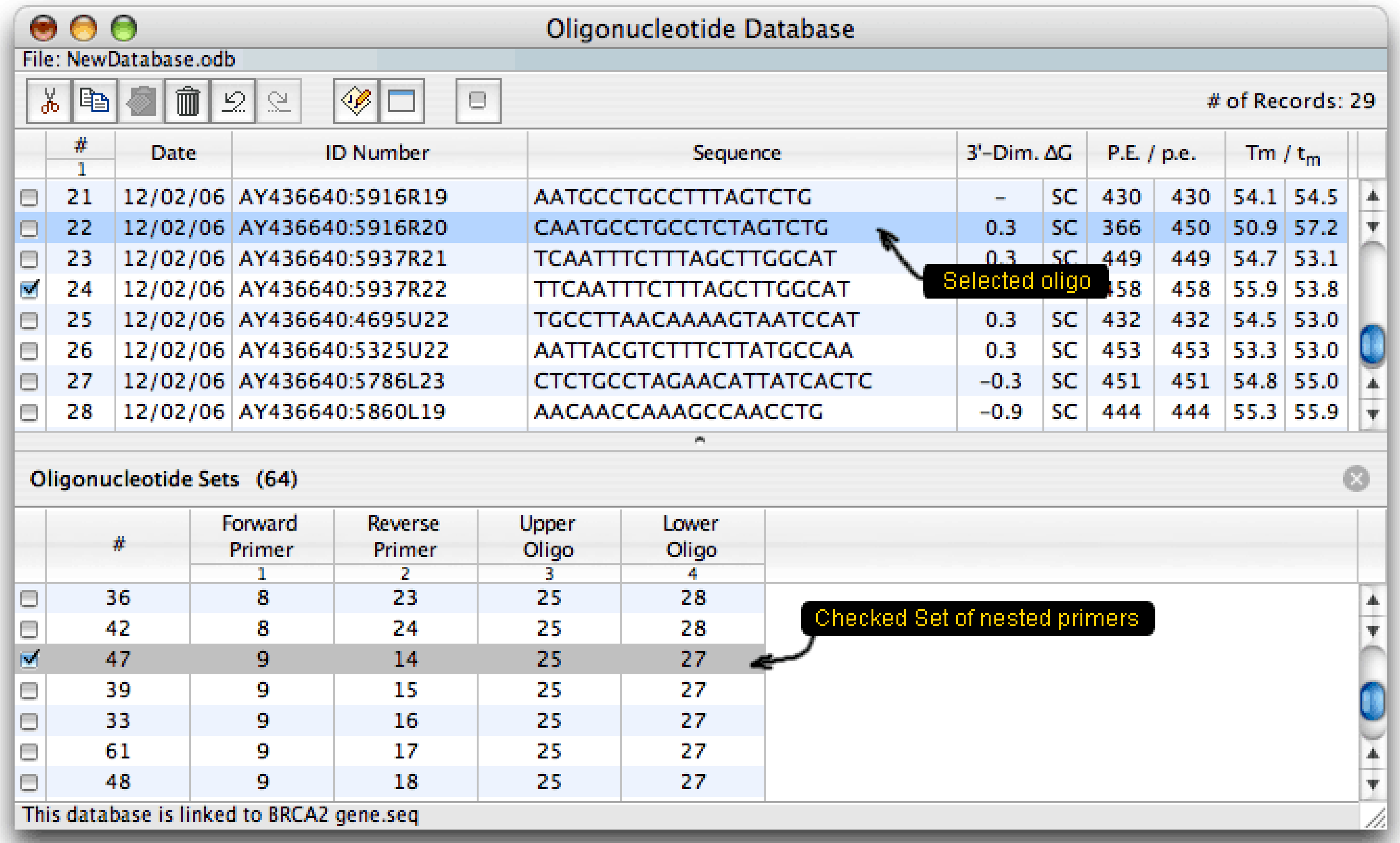
Forward Primer Composition

File: BRCA2 gene.seq

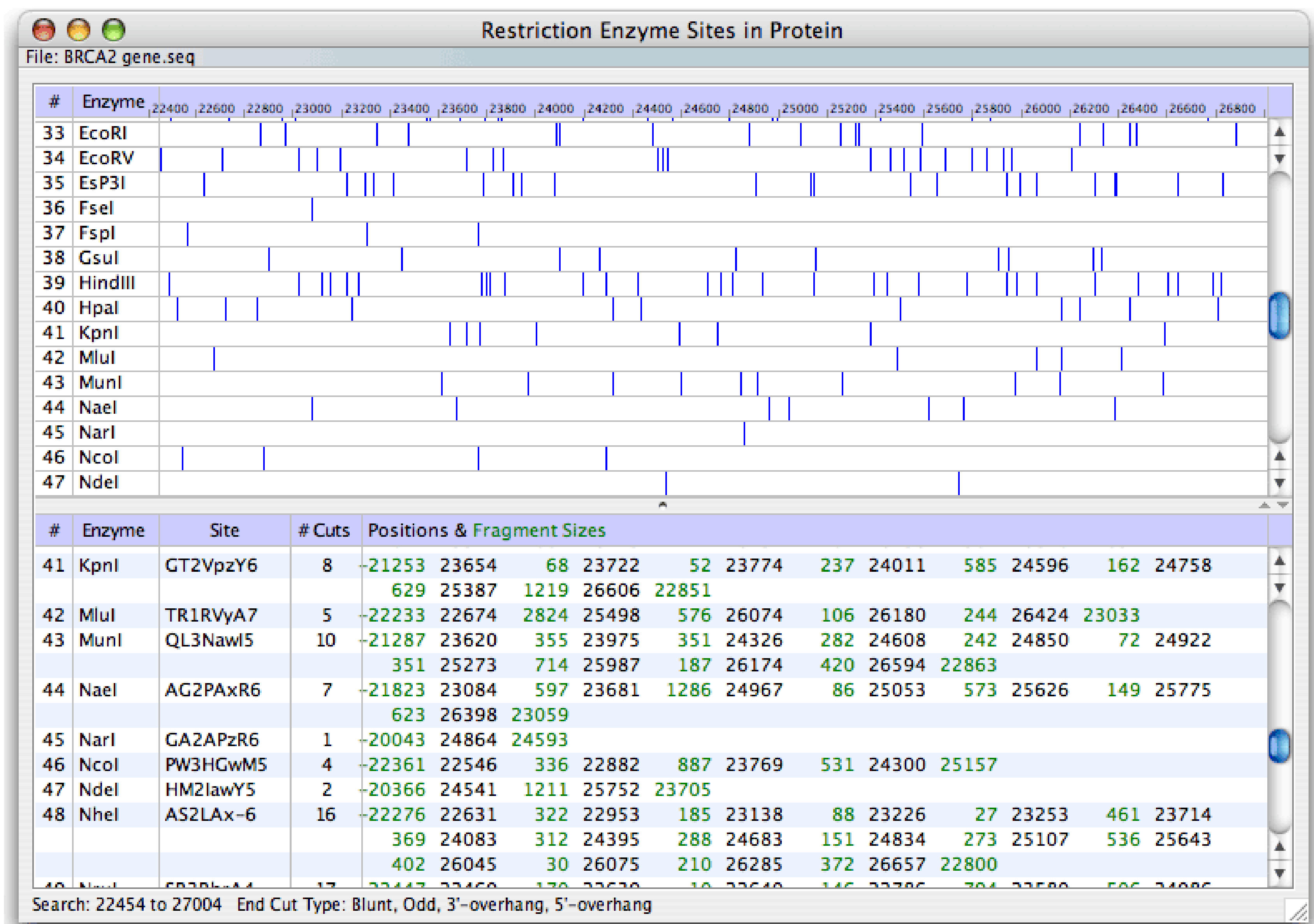
Forward Primer AY436640:6275F19

T_d	64.2°	[nearest neighbor method]
T_m	56.5°	[nearest neighbor method]
T_m	70.8°	[%GC method]
T_m	56°	[2(A+T)° + 4(G+C)° method]
T_m (RNA)[1M Na]	81°	[%GC method]
T_m (DNA:RNA)[1M Na]	74.7°	[%GC method]
A_{260}/A_{280}	1.59	[single strand]
Molecular Weight	5.8K	[one strand]
Molecular Weight	11.7K	[two strands]
µg/OD	47.4	[dsDNA]

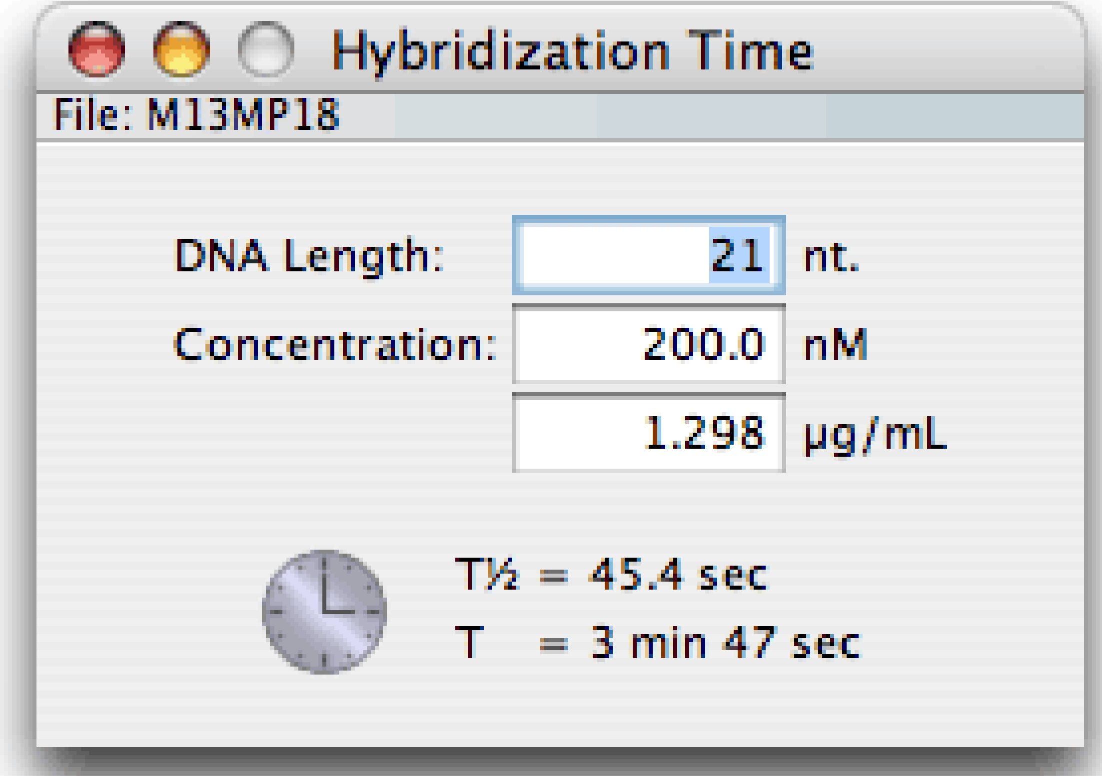
Base	Number & %	
A	2	[10.5%]
C	5	[26.3%]
G	4	[21.1%]
T	8	[42.1%]
A + T	10	[52.6%]
G + C	9	[47.4%]


Oligonucleotide Database
File: NewDatabase.odb
# of Records: 29
| Date | ID Number | Sequence | 3'-Dim. ΔG | P.E. / p.e. | Tm / tm
21 12/02/06 AY436640:5916R19 AATGCCTGCCTTTAGTCTG – SC 430 430 54.1 54.5
22 12/02/06 AY436640:5916R20 CAATGCCTGCCTCTAGTCTG 0.3 SC 366 450 50.9 57.2
23 12/02/06 AY436640:5937R21 TCAATTTCTTTAGCTTGGCAT 0.3 SC 449 449 54.7 53.1
24 12/02/06 AY436640:5937R22 TTCAATTTCTTTAGCTTGGCAT 458 458 55.9 53.8
25 12/02/06 AY436640:4695U22 TGCCTTAACAAAAGTAATCCAT 0.3 SC 432 432 54.5 53.0
26 12/02/06 AY436640:5325U22 AATTACGTCTTTCTTATGCCAA 0.3 SC 453 453 53.3 53.0
27 12/02/06 AY436640:5786L23 CTCTGCCTAGAACATTATCACTC -0.3 SC 451 451 54.8 55.0
28 12/02/06 AY436640:5860L19 AACAACCAAAGCCAACCTG -0.9 SC 444 444 55.3 55.9
Selected oligo
Oligonucleotide Sets (64)
| Forward Primer 1 | Reverse Primer 2 | Upper Oligo 3 | Lower Oligo 4
36 8 23 25 28
42 8 24 25 28
47 9 14 25 27
39 9 15 25 27
33 9 16 25 27
61 9 17 25 27
48 9 18 25 27
Checked Set of nested primers
This database is linked to BRCA2 gene.seq

MUNI



#	Enzyme	Site	# Cuts	Positions & Fragment Sizes
41	KpnI	GT2VpzY6	8	-21253 23654 68 23722 52 23774 237 24011 585 24596 162 24758 629 25387 1219 26606 22851
42	MluI	TR1RVyA7	5	-22233 22674 2824 25498 576 26074 106 26180 244 26424 23033
43	MunI	QL3NawI5	10	-21287 23620 355 23975 351 24326 282 24608 242 24850 72 24922 351 25273 714 25987 187 26174 420 26594 22863
44	NaeI	AG2PAxR6	7	-21823 23084 597 23681 1286 24967 86 25053 573 25626 149 25775 623 26398 23059
45	NarI	GA2APzR6	1	-20043 24864 24593
46	NcoI	PW3HGwM5	4	-22361 22546 336 22882 887 23769 531 24300 25157
47	NdeI	HM2lawY5	2	-20366 24541 1211 25752 23705
48	NheI	AS2LAx-6	16	-22276 22631 322 22953 185 23138 88 23226 27 23253 461 23714 369 24083 312 24395 288 24683 151 24834 273 25107 536 25643 402 26045 30 26075 210 26285 372 26657 22800


Hybridization Time
File: M13MP18
DNA Length:
21
nt.
Concentration:
200.0
nM
1.298
µg/mL
T½ = 45.4 sec
T = 3 min 47 sec

Concentrations

File: BRCA2 gene.seq

(•) Constant Concentration ( ) Constant Volume

(•) Current +Oligo:	5.08 nmol/OD, 32.5 μg/OD
( ) Current −Oligo:	4.67 nmol/OD, 30.9 μg/OD
( ) Entire Sequence (ds):	0.001 nmol/OD, 48.1 μg/OD
( ) Forward Primer:	5.98 nmol/OD, 35.0 μg/OD
( ) Reverse Primer:	5.31 nmol/OD, 34.0 μg/OD
( ) PCR Product (ds):	0.146 nmol/OD, 48.1 μg/OD
( ) Upper Oligo:	4.83 nmol/OD, 31.2 μg/OD
( ) 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

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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 TCTC**ATG**GAC 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 AC**TAA**AGAGA
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

MUNI

101 CCCAATCTCC GCTTCACTCT TCTC**ATG**GAC 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 TGAGAAACAC
551 ATCATTCAAG CTGGTGGTAT AGTTCCTCAA GTGGATATTA AC**TAA**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’

MUNI

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’

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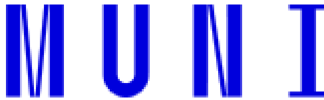
Additional resources for interested

- **Computational and Structural Biotechnology Journal 17, 2019, 1056-1065. In-silico Design of DNA Oligonucleotides: Challenges and Approaches.** *includes a list of oligo design tools* https://www.sciencedirect.com/science/article/pii/S2001037019300844
- www.protocol-online.org/prot/Research_Tools/Online_Tools/Oligo_Design/index.html
- **OLIGO 7 Power Point Presentation** http://oligo.net/tutorials.html
- **OLIGO Primer analysis software, Version 7** http://oligo.net/tutorials.html
- https://langdalelab.files.wordpress.com/2015/07/degenerate-primer-design.pdf
- http://www.genomecompiler.com/tips-for-efficient-primer-design/ (2015)
- Nature Methods 2014, 11 (5): 499. Large-scale de novo DNA synthesis: technologies and applications
- *Expert Opin Ther Pat.* 2014, 24(7):801-19. Oligonucleotide delivery: a patent review (2010 - 2013).
- Laboratory Methods in Enzymology 529: DNA Explanatory Chapter: PCR Primer Design (2013)
- PCR Primer Design; IMBB Workshop, N. Ndegwa (2013)
- PCR Primer Design; A. Yuryev (2010)
- *AAPS Journal* 2009, 11(1): 195 - 203. Targeted Delivery Systems for Oligonucleotide Therapeutics
- **iCODEHOP** (COnsensus-DEgenerate Hybrid Oligonucleotide Primer)PCR primer design (2009) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2703993/
- Bioinformatic tools and guideline for PCR primer design. Kamel A. Abd-Elsalam (2003)
- Artificial DNA: Methods and Applications; Khudyakov, Y.E., Fields, W.A., Ed. (2003)
- PCR Primer: A Laboratory Manual (2003)



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

Marcel Proust