

Introduction to applied bioinformatics

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7/10

„Nucleotide bioinformatics III“

Retrieving nucleotide sequences from databases (Genbank/NCBI)

Feature analysis: statistics, reverse complement, restriction analysis

Translation, identifying open reading frame

PCR primer design, rt-PCR

Secondary structure prediction

Sequence comparison, unknown sequence identification

Single Nucleotide Polymorphisms

DNA sequencing

Gene expression

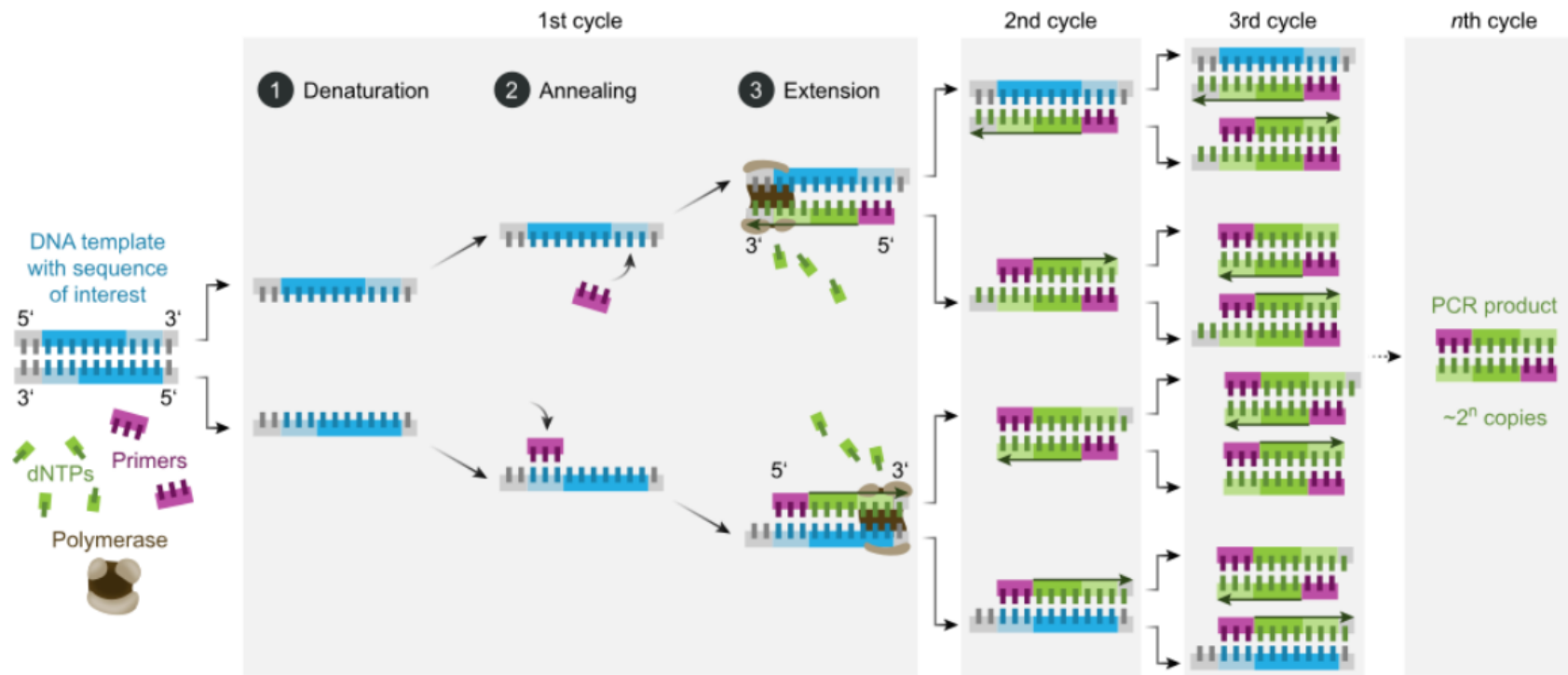
microRNA

Genomes....

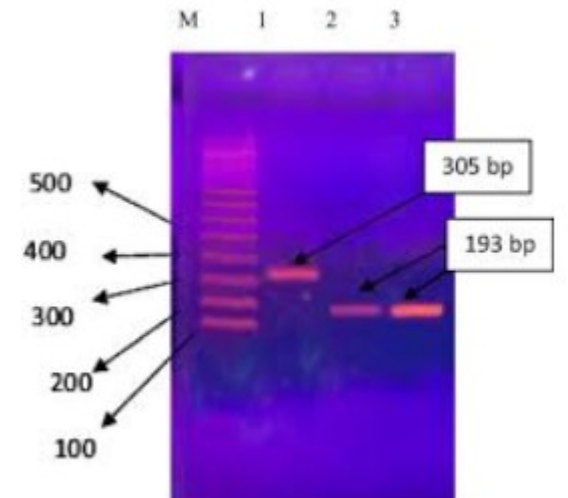
....

Polymerase chain reaction

Polymerase chain reaction (PCR) → amplification of required gene, between two primers



→ „End point“ detection



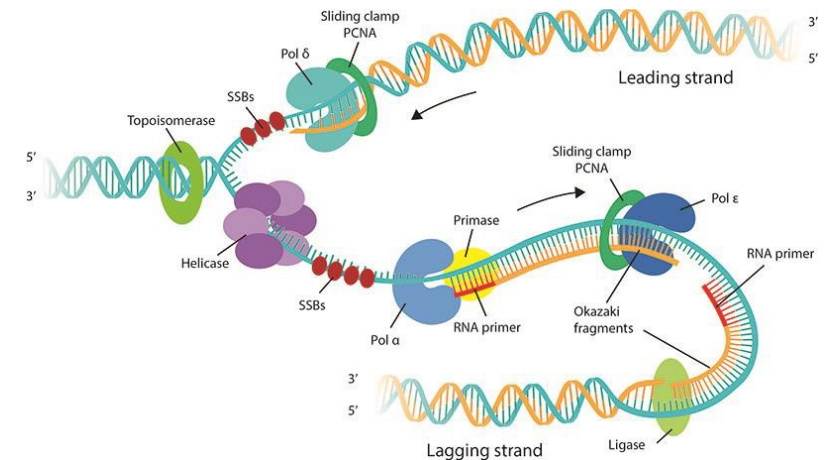
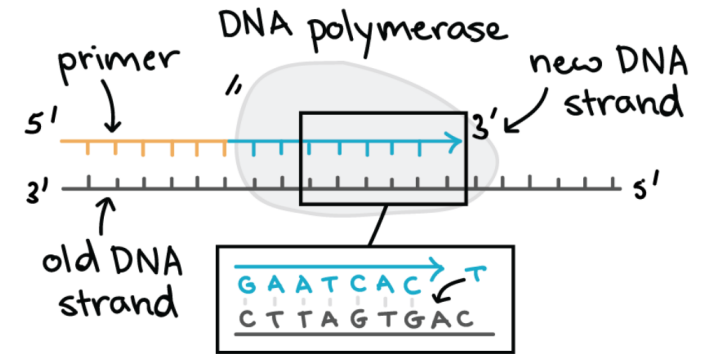
[Polymerase chain reaction \(PCR\) \(article\) | Khan Academy](#)

Primer = oligonucleotide

-short (15-50nt) single-strand DNA

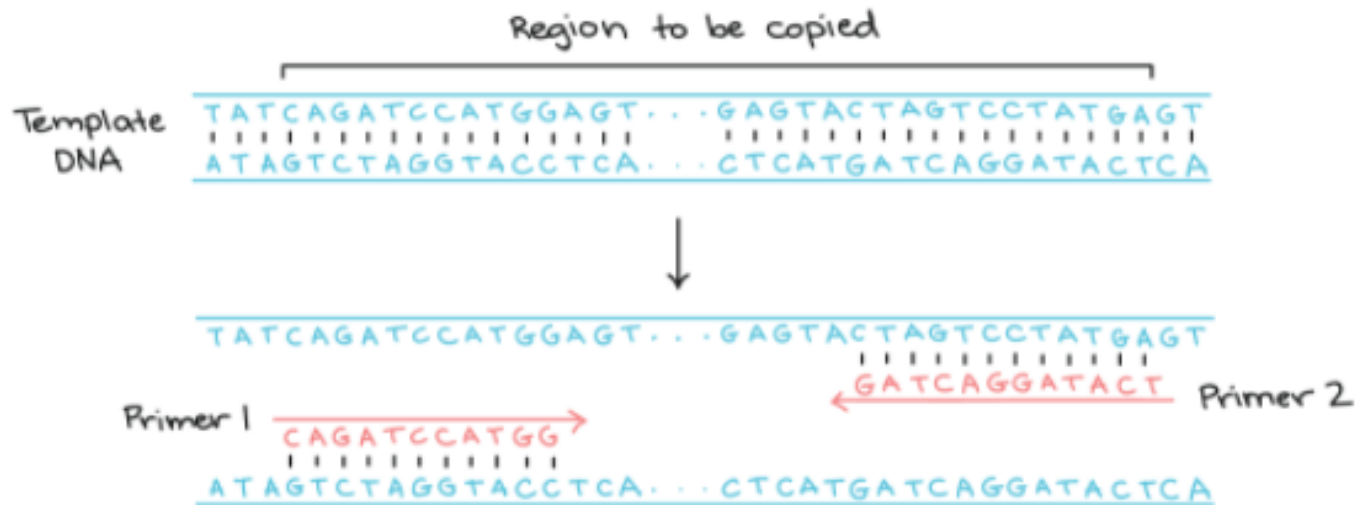
Usage:

- PCR (fragment amplification, gene detection, expression quantification, mutagenesis...)
- reverse transcriptiod (oligo(dT), hexamers..)
- sequencing

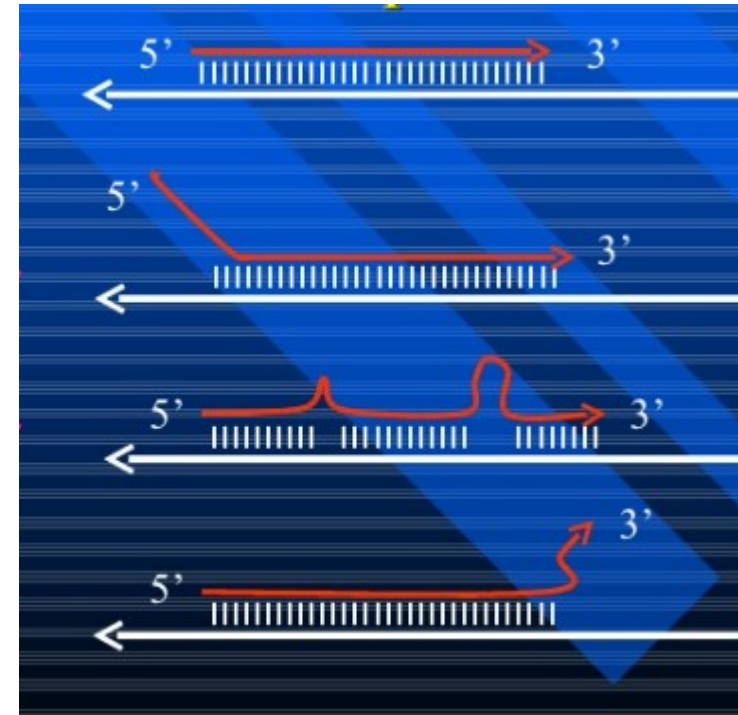


Polymerase chain reaction

Polymerase chain reaction (PCR) — amplification of required gene, between two primers



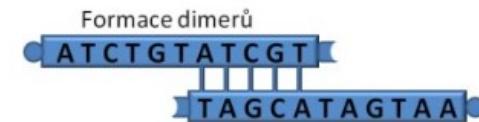
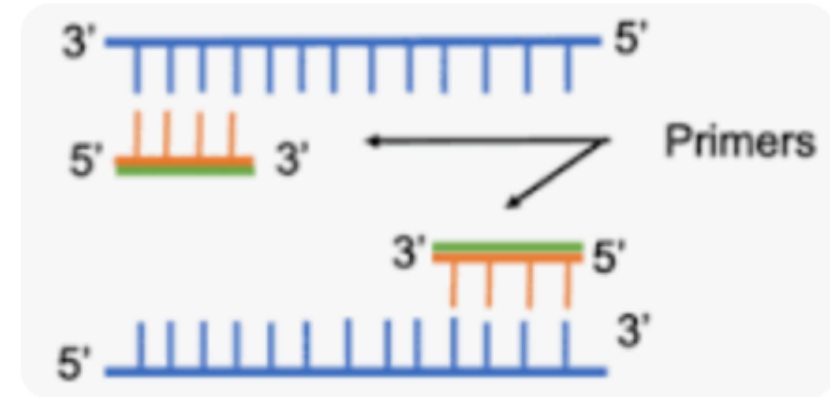
Synthesis: 5' → 3'



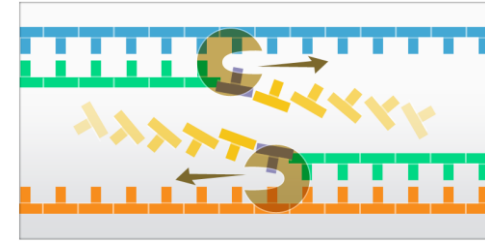
Polymerase chain reaction

Taking into consideration the information above, primers should generally have the following properties:

- Length of 18-24 bases.
- 40-60% G/C content.
- Start and end with 1-2 G/C pairs.
- Melting temperature (T_m) of 50-60°C.
- **Primer** pairs should have a T_m within 5°C of each other.
- **Primer** pairs should not have complementary regions.



Polymerase chain reaction



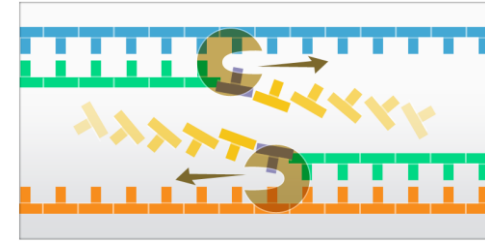
1) Amplification of desired gene / DNA fragment

→ manual design:

➤ design (short) primers for amplification of the following sequence:

5'-ATGCCCTTTCnnnnnnnnnnnnnnnnTAAATCCCGC-3'

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

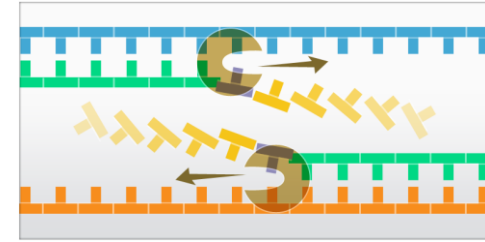
→ manual design:

➤ design (short) primers for amplification of the following sequence:

5'-ATGCCCTTTCnnnnnnnnnnnnnnnnTAAATCCCGC-3'

3'-TACGGGAAAGnnnnnnnnnnnnnnnnATTAGGGCG-5'

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design:

➤ design (short) primers for amplification of the following sequence:

5'-ATGCCCTTTCnnnnnnnnnnnnnnnnTAAATCCCGC-3'

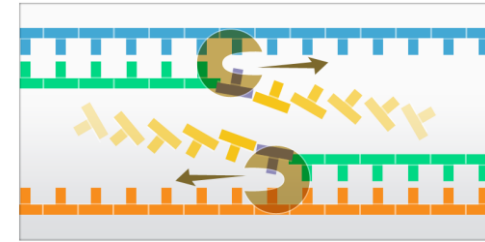
ATTAGGGCG-5'



5'-ATGCCCTTTC-

3'-TACGGGAAAGnnnnnnnnnnnnnnnnATTAGGGCG-5'

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design:

➤ design (short) primers for amplification of the following sequence:

5'-ATGCCCTTTCnnnnnnnnnnnnnnnnTAAATCCCGC-3'

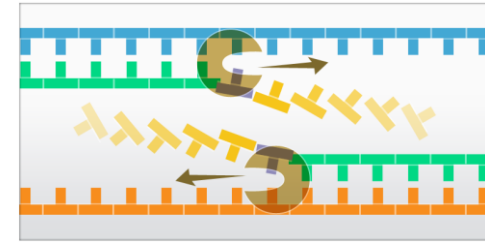
reverse complement:

5'-GCGGGATTAnnnnnnnnnnnnnnnGAAAGGGCAT-3'
(3'-TACGGGAAAGnnnnnnnnnnnnnnnnATTAGGGCG-5')

R_primer: GCGGGATTTA

reverse

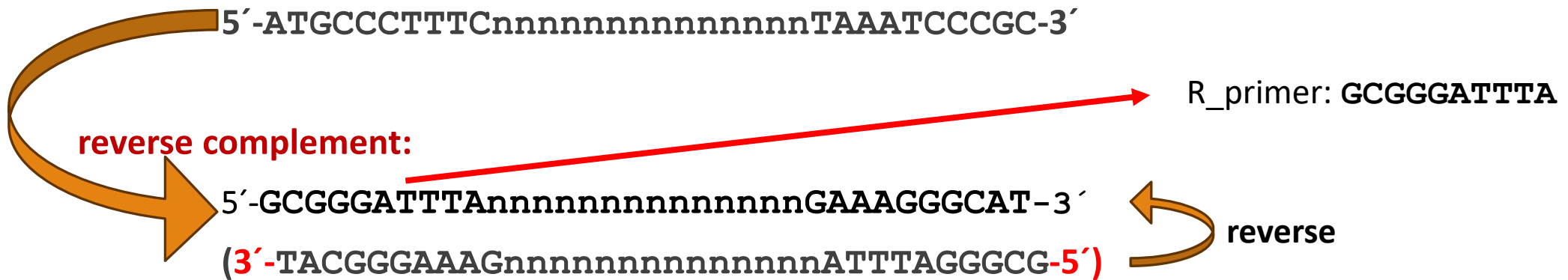
Polymerase chain reaction



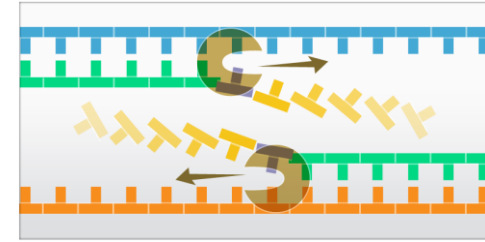
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Polymerase chain reaction



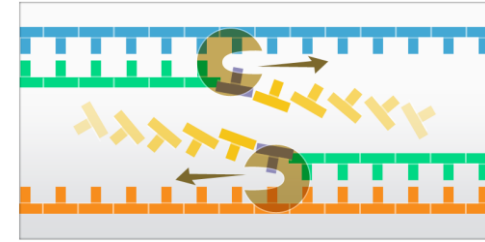
1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward)

– R (reverse)

Aim: amplification of the specific product

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – **F (forward)**

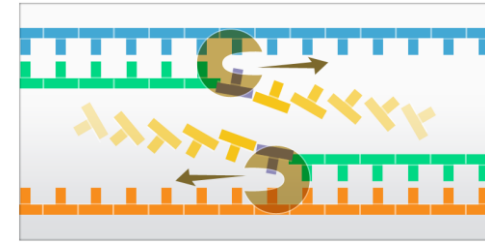
– R (reverse)

Aim: amplification of the specific product

>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

```
ATGGTCGGCAGAAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGTCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – **F (forward)** → re-write sequence from the beginning cca 20nt

– R (reverse)

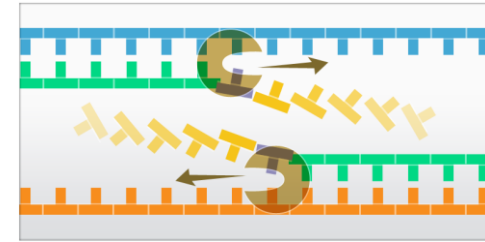
ATGGTCGGCAGAAGAGCACT

Aim: amplification of the specific product

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),  
transcript variant 1, mRNA
```

```
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG  
AGGCTGCTGCAGCGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACCT  
CAATCCCATCATTTCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC  
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG  
CCGCAGACCTTGTGATATTCAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT  
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT  
AAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG  
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC  
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA  
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGTCTCCAAGCAGCCTCTTTGACCTAAACTTCC  
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG  
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```


Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – **F (forward)**

→ re-write sequence from the beginning cca 20nt

– R (reverse)

ATGGTCGGCAGAAGAGCACT

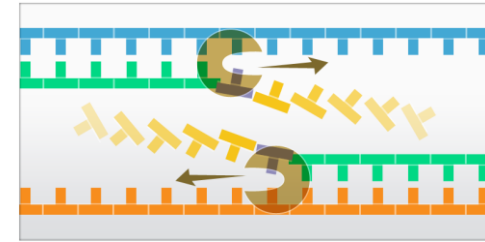
→ check the primer (Tm) - OligoCalc

Aim: amplification of the specific product

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),  
transcript variant 1, mRNA
```

```
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG  
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT  
CAATCCCATCATTTCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC  
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG  
CCGCAGACCTTGTGATATTCAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT  
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT  
AAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG  
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC  
TCAACTGACATATAGCATTTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA  
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGTCTCCAAGCAGCCTCTTTGACCTAAACTTCC  
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG  
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – **F (forward)**

→ re-write sequence from the beginning cca 20nt

– R (reverse)

ATGGTCGGCAGAAGAGCACT (T_m= 60.5 °C)

→ check the primer (T_m) - OligoCalc

Aim: amplification of the specific product

>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

```
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACCTT
CAATCCCATCATTTCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Physical Constants		Melting Temperature (T _m) Calculations	
Length:	20	Molecular Weight:	6191.1
		GC content:	55%
1 ml of a softn with an Absorbance of 1 at 260 nm is 4.373 micromolar and contains 27.1 micrograms.		1	53.8 °C (Basic)
		2	60.5 °C (Salt Adjusted)
		3	56.1 °C (Nearest Neighbor)
Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7			
Rink	33.404 kcal/(K·mol)	deltaH	164.4 Kcal/mol
deltaG	27.3 Kcal/mol	deltaS	425.8 cal/(K·mol)
Deprecated Hairpin/self dimerization calculations			
5	(Minimum base pairs required for single primer self-dimerization)		
4	(Minimum base pairs required for a hairpin)		

OligoCalc:

Oligo Calc: Oligonucleotide Properties Calculator

Enter Oligonucleotide Sequence Below
OD calculations are for single-stranded DNA or RNA

Nucleotide base codes

ATG GTC GGC AGA AGA GCA CT

Reverse Complement Strand(5' to 3') is:
AGT GCT CTT CTG CCG ACC AT

5' modification (if any) 3' modification (if any) Select molecule
[] [] ssDNA []

50 nM Primer
50 mM Salt (Na⁺) 1 Measured Absorbance at 260 nanometers

Calculate **Swap Strands** **BLAST** **mfold**

Physical Constants

Length: 20 Molecular Weight: 6191.1⁴ GC content: 55%
1 ml of a sol'n with an Absorbance of 1 at 260 nm
is 4.373 microMolar⁵ and contains 27.1 micrograms.

Melting Temperature (T_M) Calculations

1	53.8 °C (Basic)
2	60.5 °C (Salt Adjusted)
3	56.11 °C (Nearest Neighbor)

Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7.

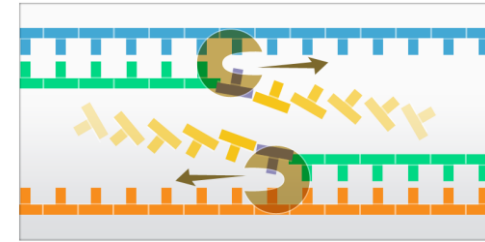
RlnK	33.404 cal/(°K*mol)	deltaH	164.4 Kcal/mol
deltaG	27.3 Kcal/mol	deltaS	425.8 cal/(°K*mol)

Deprecated Hairpin/self dimerization calculations

5 (Minimum base pairs required for single primer self-dimerization)
4 (Minimum base pairs required for a hairpin)

Check Self-Complementarity

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) ATGGTCGGCAGAAGAGCACT ($T_m = 60.5^\circ\text{C}$)

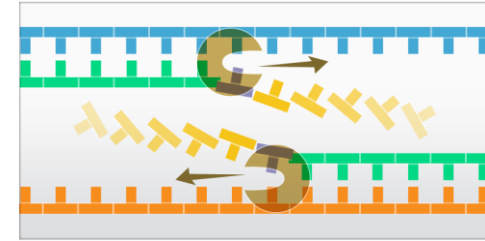
– R (reverse) → reverse complement sequence (SMS)

Aim: amplification of the specific product

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydro
transcript variant 1, mRNA
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACT
AGGCTGCTGCAGCGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTA
CAATCCCATCATTTCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTT
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAA
CCGCAGACCTTGTGATATTCAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

A screenshot of the SMS (Sequence Manipulation Suite) website. The page title is "SMS Sequence Manipulation Suite: Reverse Complement". Below the title, there is a description: "Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart, sequence if it contains an ORF on the reverse strand." There is a text input area with the following sequence: "CGCCTGGAGAAATTTGGGATGAGACACCCTGTATTTTGCTCCAAGCAGCCTCTTTGACC TAAACTTCC AGGCAGGATTTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCT TTCTGTGGG CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA". Below the input area are "Submit", "Clear", and "Reset" buttons. A dropdown menu is set to "reverse-complement".

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) ATGGTCGGCAGAAGAGCACT ($T_m = 60.5^\circ\text{C}$)

– R (reverse) → reverse complement (SMS)

Aim: amplification of the specific product

>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

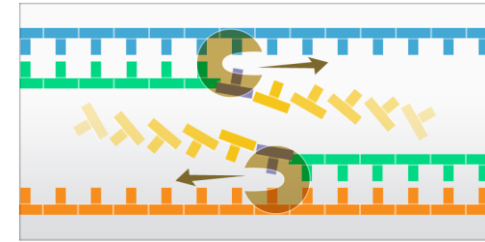
ATGGTCGGCAGA

AGGCTGCTGCAG
CAATCCCATCAT
GAGTCTGTTCTG
CCGCAGACCTTG
TGAGCGAGTGTT
AAGAAGGCAGTG
ACATGAATGTCA
TCAACTGACATA
CGCCTGGAGAAT
AGGCAGGATTCT
CCATCACTTGGG

>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTFTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGTATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCTATACATGGCAGCGTAAGTGTAAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACCTGGAATATCACAAGGTC
TGCCGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCCTTCAGTTTACCTGTGAT
GTCCTTTTCTGGAAATGATCGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGTCTTCTGCCCACCAT

reverse complement

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) ATGGTCGGCAGAAGAGCACT ($T_m = 60.5^\circ\text{C}$)

– R (reverse) → reverse complement

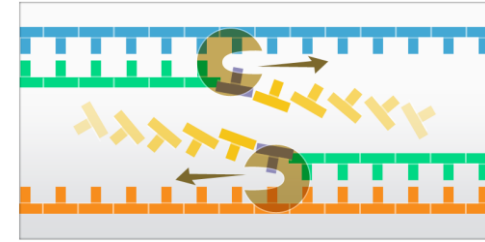
→ re-write sequence from the beginning cca 20nt

TCA TTTTCTAGCTTTGATCT

Aim: amplification of the specific product

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGTATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTGCATACATGGCAGCGTAAGTGTAAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACCTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCCTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT
```

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) ATGGTCGGCAGAAGAGCACT ($T_m = 60.5^\circ\text{C}$)

– R (reverse) → reverse complement

→ re-write sequence from the beginning cca 20nt

TCATTTTCTAGCTTTGATCT

→ check the primer (T_m) - OligoCalc

Aim: amplification of the specific product

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTFTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGTATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTTCATACATGGCAGCGTAAGTGTAAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACCTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCCTCAGTTTACCTGTGAT
GTCCTTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT
```

OligoCalc:

Oligo Calc: Oligonucleotide Properties Calculator

Enter Oligonucleotide Sequence Below
OD calculations are for single-stranded DNA or RNA

Nucleotide base codes

TCA TTT TCT AGC TTT GAT CT

Reverse Complement Strand(5' to 3') is:
AGA TCA AAG CTA GAA AAT GA

5' modification (if any) 3' modification (if any) Select molecule
[] [] ssDNA []

[50] nM Primer [1] Measured Absorbance at 260 nanometers
[50] mM Salt (Na⁺)

Calculate **Swap Strands** **BLAST** **mfold**

Physical Constants

Length: [20] Molecular Weight: [6039⁴] GC content: [30] %
1 ml of a sol'n with an Absorbance of [1] at 260 nm
is [5.257] microMolar⁵ and contains [31.7] micrograms.

Melting Temperature (T_M) Calculations

1	[43.6] °C (Basic)
2	[50.2] °C (Salt Adjusted)
3	[45.98] °C (Nearest Neighbor)

Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7.

RlnK [33.404] cal/(°K²*mol) deltaH [146.3] Kcal/mol
deltaG [21.4] Kcal/mol deltaS [386.3] cal/(°K²*mol)

Deprecated Hairpin/self dimerization calculations

[5] (Minimum base pairs required for single primer self-dimerization)
[4] (Minimum base pairs required for a hairpin)

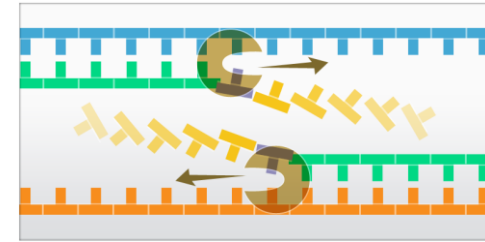
Check Self-Complementarity



T_m too low → longer primer



Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) ATGGTCGGCAGAAGAGCACT ($T_m = 60.5^\circ\text{C}$)

– R (reverse) → reverse complement

→ re-write sequence from the beginning cca 20nt

TCATTTTCTAGCTTTGATCT

→ check the primer (T_m) - OligoCalc

Aim: amplification of the specific product

>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement

```
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGTGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTTCATACATGGCAGCGTAAGTGTAAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAAGTGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTGTTCTAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGAGGGTCCCTCAGTTTACCTGTGAT
GTCCTTTCTGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT
```

→ prolong the primer and check again (T_m) - OligoCalc

TCATTTTCTAGCTTTGATCTGGT

OligoCalc:

Oligo Calc: Oligonucleotide Properties Calculator

Enter Oligonucleotide Sequence Below
OD calculations are for single-stranded DNA or RNA

Nucleotide base codes

TCA TTT TCT AGC TTT GAT CTG GT

Reverse Complement Strand(5' to 3') is:
ACC AGA TCA AAG CTA GAA AAT GA

5' modification (if any) 3' modification (if any) Select molecule
[] [] ssDNA []

50 nM Primer [] Measured Absorbance at 260 nanometers
50 mM Salt (Na⁺) []

Calculate **Swap Strands** **BLAST** **mfold**

Physical Constants **Melting Temperature (T_M) Calculations**

Length: [23] Molecular Weight: [7001.6⁴] GC content: [35%]

1 ml of a sol'n with an Absorbance of [1] at 260 nm
is [4.492] microMolar⁵ and contains [31.5] micrograms.

1	[49.9] °C (Basic)
2	[57.6] °C (Salt Adjusted)
3	[52.04] °C (Nearest Neighbor)

Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7.

RlnK [33.404] cal/(°K*mol) deltaH [174.8] Kcal/mol
deltaG [26.7] Kcal/mol deltaS [461.2] cal/(°K*mol)

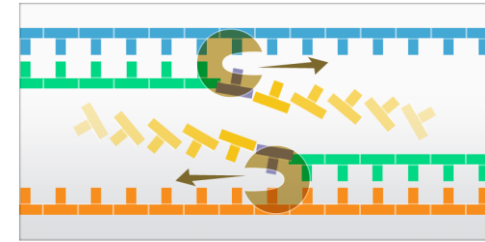
Deprecated Hairpin/self dimerization calculations

[5] (Minimum base pairs required for single primer self-dimerization)
[4] (Minimum base pairs required for a hairpin)

Check Self-Complementarity

better (F: 60.5°C)

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) ATGGTCGGCAGAAGAGCACT ($T_m = 60.5^\circ\text{C}$)

– R (reverse) → reverse complement

→ re-write sequence from the beginning cca 20nt

TCATTTTCTAGCTTTGATCT

→ check the primer (T_m) - OligoCalc

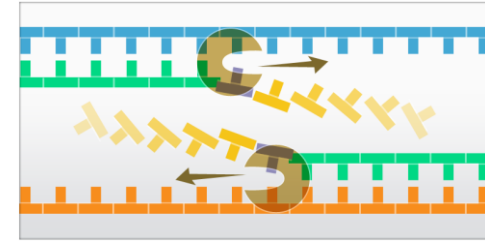
Aim: amplification of the specific product

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGTGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTTCATACATGGCAGCGTAAGTGTAAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAAGTGAATATCACAAGGTC
TGC GGCTTCCAGCTTCTTTGTTCTAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGAGGGTCCCTCAGTTTACCTGTGAT
GTCCTTCTGGAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT
```

→ prolong the primer and check again (T_m) - OligoCalc

TCATTTTCTAGCTTTGATCTGGT

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – **F (forward)** ATGGTCGGCAGAAGAGCACT ($T_m = 60.5^\circ\text{C}$)

– **R (reverse)** TCATTTTCTAGCTTTGATCTGGT ($T_m = 57.6^\circ\text{C}$)

Aim: amplification of the specific product

>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

ATGGTCGGCAGA
AGGCTGCTGCAG
CAATCCCATCAT
GAGTCTGTTCTG
CCGCAGACCTTG
TGAGCGAGTGTT
AAGAAGGCAGTG
ACATGAATGTCA
TCAACTGACATA
CGCCTGGAGAAT
AGGCAGGATTCT
CCATCACTTGGG

>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA **reverse complement**
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTFTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGTGAGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTTCATACATGGCAGCGTAAGTGTAAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCCTGCAGGGGGAACCTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGAGGGTCCCTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT

Practical part

1) Design primers manually for amplification of the 3rd exon of NQO1 (NM_00903.3)

- length: 18-24nt

- Tm: 55-60°C

2) check the primers position by Multalin: mRNA, 3rd exon, F primer, R primer

-getting 3rd exon

NCBI → NM_... → „highlight sequence features“ → select 3rd exon → „FASTA“ in the right bottom

polyA site
ORIGIN

```
1 acacgcgact cccacaaggt tgcagccgga gccgccccagc tcaccgagag cctagttccg
61 gccagggtcg ccccgcaac cagcagccca gccaatcagc gccccgact gcaccagagc
121 catggtcggc agaagagcac tgatcgtact ggctcactca gagaggagct ccttcaacta
181 tgccatgaag gaggtcgtc cagcgcctt gaagaagaaa ggatgggagb tggtagagtc
241 ggacctctat gccatgaact tcaatcccat catttcaga aaggacatca caaataaact
301 gaagaccct gcpaacttc aptatccctc cpagtctgtt ctggctata aapaaggcca
361 tctgagccca gatattgtcg ctgaacaaa gaactggaa gccgcagacc ttatgatatt
421 caagtcccc ctgcagtggt ttggagccc tgccattctg aaaggctggt ttgagcagt
481 gttcatagga gatttgcct acacttargc tgccatgtat gacaaaggac ccttccggag
541 taagaaggca gtgctttcca tcaaccactg ttgcagtgag tccatgtact ctctcaagg
601 gatccaggg gacatgaatg tcattctctg gccaatcag agtgctattc tgcatctctg
661 tggctccaaa gtcttagaac ctcaactgac atatagcatt gggcacactc cagcagagc
721 ccgaattcaa atcctgggaag gatggaagaa acgctggag aatattggg atgagacacc
781 actgtatttt gctccaagca gcctcttga cctaacttc caggcaggt tcttaatgaa
841 aaaaagagga caggatgag agaaaaacia gaaattggc ctctctggt gccatcactt
901 gggcaagtc atcccaactg acaaccagat caaagctaga aatagattt ccttagctgt
961 gatttccttc taacatgta tcaaatctgg gatctctccc aggtctccct gacttgcttt
1021 agtttttaag atttgtggtt tctctttccc acaaggaata aatgagagg aatcagctgt
1081 attcgtgcat tttggatca ttttaactg atcttatga ttactatcat ggcatataac
1141 caaaatcga ctgggtcaca gaggccactt agggaaagat gtagaagat gctgaaaaaa
1201 tgttctttaa aggcactcac acaatttaat tctctttttt agggctaag ttttaggta
1261 cagttggctt aggtatcatt caactctcca atgttctat aatcactctt ctgtagtta
1321 tggcagaagg gaattgctca gagaagaaa agactgaatc tacctccctt aaggactta
1381 actgtttgg tagttagcca tctaatgctt gtttatgata tttctgctt tcaattacaa
1441 agcagtact aatagctca gcacaagtac cactctggg cagctttgt tgtttatata
1501 cagtaacag atacctgaa aggaagagct aataaatctt tctttgctg cagctatca
1561 cttttttttt aattaaaaaa aattttttt tgaagcagtc ttgctctgtt acccagctg
1621 gaggcagtg gtgtgatctt ggctcactgc aacctctgcc tccaggttc cagcaattct
1681 cctgcctcag cctccctagt agctgggatg acaggcctc gccatcagc ctgactaatt
1741 tttgtattt tagtagagac ggctttcac catgtggccc aggtggtct caaactcctg
1801 acctcagtg atccgcctac ctcaacctc caaagtctg ggattacagg cgtgatccac
1861 cacactggc cttgcaactt tctacttta agtttgcag agataacca ataaatcac
1921 acgtacatc tgcaatatga attcaagaaa ggaatagta cctcaatac taaaaatag
1981 tcttccacaa aaaactcttt atttctgat tatacaaat ttcagaaggt tattttctt
2041 atcattgcta aactgatgac ttactatggg atgggttcca gtcccagac ctggggatc
2101 aattgtaac ctagatgttt atcaacttg gtgaacagtt ttggcataat agtcaattc
2161 tacttctgga agtcatctca ttccactgtt ggtattatat aattcaagga gaatatgata
2221 aaacactgc ccttctgtgt gcaatgaaag aagatgaaag aatgatgaa aaggttgctt
2281 gaaaaatggg agacagcttc ttacttcca agaaaatgaa gggattggac cgagctggaa
2341 aacctcttt accagatgct gactggcact ggtgtttttt cctctgaca gtatccacaa
2401 tagctagcg ctgggtgttt cagtttgaaa atatttggc gcctcactc tcaactgcaat
2461 tttgtgtaa tttctcaag atctgatta aataaaaaa attcattct acagaccac
2521 a
```

Sequence of the 3rd exon

Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

NCBI Reference Sequence: NM_000903.3

[GenBank](#) [Graphics](#)

>NM_000903.3:294-424 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

GTAAACTGAAGGACCTCGCAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGATATTTCCAG

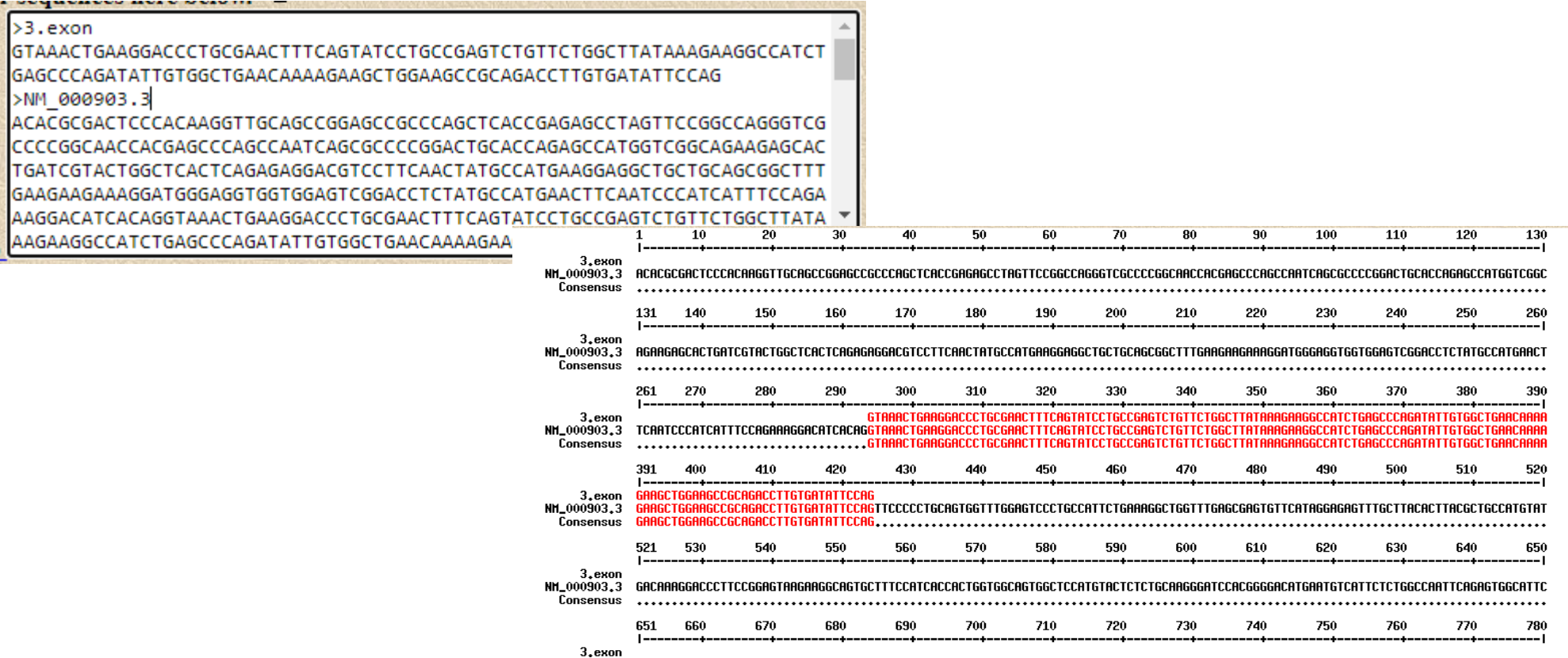
294..424
/gene="NQO1"
/gene_synonym="DHQU; DIA4; DTD; NMOR1; NMORI; QR1"
/inference="alignment:SpIign:2.1.0"

exon Feature 3 of 6 NM_000903 : 1 segment

Details Display FASTA GenBank Help

Comparison by multalin

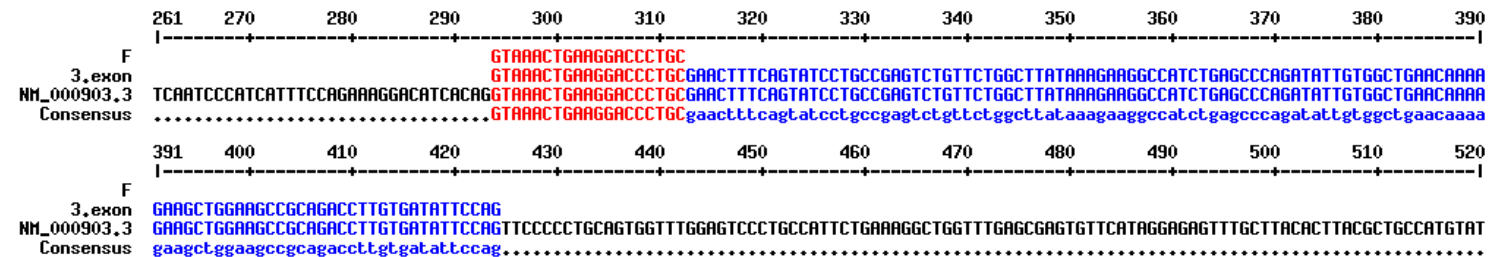
1) mRNA and 3rd exon:



Comparison by multalin

2) mRNA + 3rd exon + F primer:

```
>F
GTA AAC TGA AGG ACC CTG c|
>3.exon
GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATCCAG
>NM_000903.3
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCTCACCAGAGCCTAGTTCGGCCAGGGTCTG
CCCCGGCAACCACGAGCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
TGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTT
GAAGAAGAAAGGATGGGAGGTGGTGGAGTTCGACCTCTATGCCATGAACTTCAATCCCATCATTCCAA
```

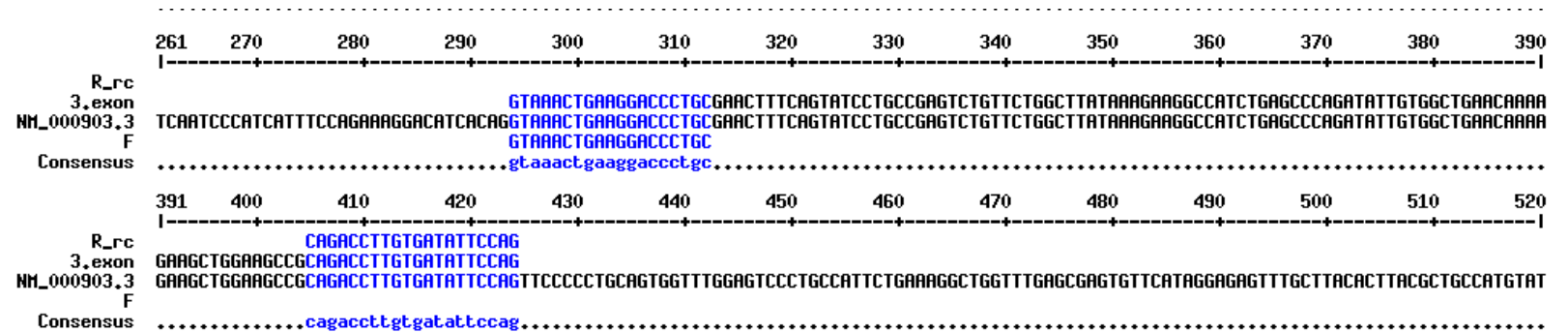


Comparison by multalin

2) mRNA + 3rd exon + F primer +R primer (reverse complement)

```

>R_rc
CAG ACC TTG TGA TAT TCC AG
>F
GTA AAC TGA AGG ACC CTG C
>3.exon
GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT
GAGCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG
>NM_000903.3
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCTCACCAGAGCCTAGTTCCGGCCAGGGTCCG
CCCCGGCAACCACGAGCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
  
```



Comparison by multalin



Practical part

1) Design primers manually for amplification of your **CDS**

- length: 18-24nt

- T_m: 55-60°C

2) check the primers position by Multalin: mRNA, CDS, F primer, R primer

Homework 7

Work with „your“ nucleotide sequence.

- 1) Design manually primers for amplification of your CDS (primers should not have $T_m > 60^\circ\text{C}$)
- 2) Compare the designed primers with your mRNA sequence using Multalin
(don't forget to reverse complement the R primer)

HW7: solution of NQ01

- 1) Design manually primers for amplification of your CDS (primers should not have $T_m > 60^\circ\text{C}$)

F: ATG GTC GGC AGA AGA GCA C 2 59.5 °C (Salt Adjusted)

R: TCA TTT TCT AGC TTT GAT CTG GTT 2 58.3 °C (Salt Adjusted)

- 2) Compare the designed primers with your mRNA sequence using Multalin (don't forget to reverse complement the R primer)

