

Introduction to applied bioinformatics

PETRA MATOUŠKOVÁ

2025/2026

7/10

Repetition from last time

- Open unknown sequence from Moodle
- Identify vector contamination
- Purify the sequence
- Identify the unknown sequence: what is it and probable source organism

Repetition from last time

download sequence

open in chromas

run vecscreen

Use range extractor DNA

BLASTn purified sequence

Range Extractor DNA

Range Extractor DNA accepts a DNA sequence along with a set of positions or ranges as lowercase text. Use Range Extractor DNA to obtain subsequences using position in

Paste a raw sequence or one or more FASTA sequences into the

```
CTTATTGAAGAACTTCAGGTTGCTCTCATCAATCCAGGTGGAAGT
GTCAAATCTCATCCCTGCCGTGGTGTGAACAAAGTCATTACTAATTCA
GCAAGGGAACACGGCAAGCTGCACTCAGGAAGAAAGCTAAAGCTCAGTAG
AAGCTTCTACCTGAATCCAGCACACTGGCGGCCGTTACTAGTGGATCCG
AGCTCGGTACCAAGCTTGATGCATAGCTTGAGTATCTAACGGCTCAGCT
AAATAGCTTGGCGTAATCATGGTTCA
```

Enter the base positions or ranges, separated by commas, in the middle, and length of the sequence in the last field. The center base along with the center base along with the center base

77..1062

Submit Clear Reset

- Obtain bases from the
- Sequence segments starting at

*This page requires JavaScript

*You can [mirror this page](#) or [download the source](#)

Range Extractor DNA results

```
>results for 1179 residue
AGGTGATGGATCCGGAGAACCTGTAC
TGGGATATTGTGCTCTAGGGTTCGTT
TTTATCCTTATCTAATTTGTGCACCT
CAGTTGTCAACGGGGCGACGGATGGC
AAGGCTTCAACATATACCTCGTTTCT
ATCTCTGCCAAAAATATAATAAGGTT
GCTCTGTAGATGCTTACAAACCACT?
TTGTCAATAACGTCGGCATGAGTTA
```

BLAST » vector contamination » RID-ZHCNRHE1013

Formatting options Download

Job title: 22207006

RID ZHCNRHE1013 (Expires on 04-12 14:12 pm)
Query ID lcl|Query_408397
Description 22207006
Molecule type dna
Query Length 1179

Other reports: Search Summary Taxonomy reports Distance tree of results MSA viewer

Graphic Summary

Distribution of Vector Matches on the Query Sequence



Match to Vector: Strong Moderate Weak
Segment of suspect origin:

Segments matching vector:
Strong match: 10-76, 1063-1173

BLAST » blastn suite » results for RID-ZHCXKD62013

Home Recent Results Saved Strategies Help

Edit Search Save Search Search Summary

How to read this report? BLAST Help Videos Back to Traditional Results Page

Job Title Nucleotide Sequence

RID ZHCXKD62013 Search expires on 04-12 14:16 pm Download All

Program BLASTN Citation

Database core_nt See details

Query ID lcl|Query_5391011

Description None

Molecule type dna

Query Length 986

Other reports Distance tree of results MSA viewer

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments

Download Select columns Show 100

select all 3 sequences selected

GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
PREDICTED: Drosophila virilis hydroxysteroid 17-beta dehydrogenase 12 splicev (splicev), transcript variant ...	Drosophila virilis	75.0	75.0	5%	2e-08	92.45%	1827	XM_015169541.3
PREDICTED: Drosophila virilis hydroxysteroid 17-beta dehydrogenase 12 splicev (splicev), transcript variant ...	Drosophila virilis	75.0	75.0	5%	2e-08	92.45%	1737	XM_015169543.3
PREDICTED: Drosophila virilis hydroxysteroid 17-beta dehydrogenase 12 splicev (splicev), transcript variant ...	Drosophila virilis	75.0	75.0	5%	2e-08	92.45%	1831	XM_015169542.3

„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

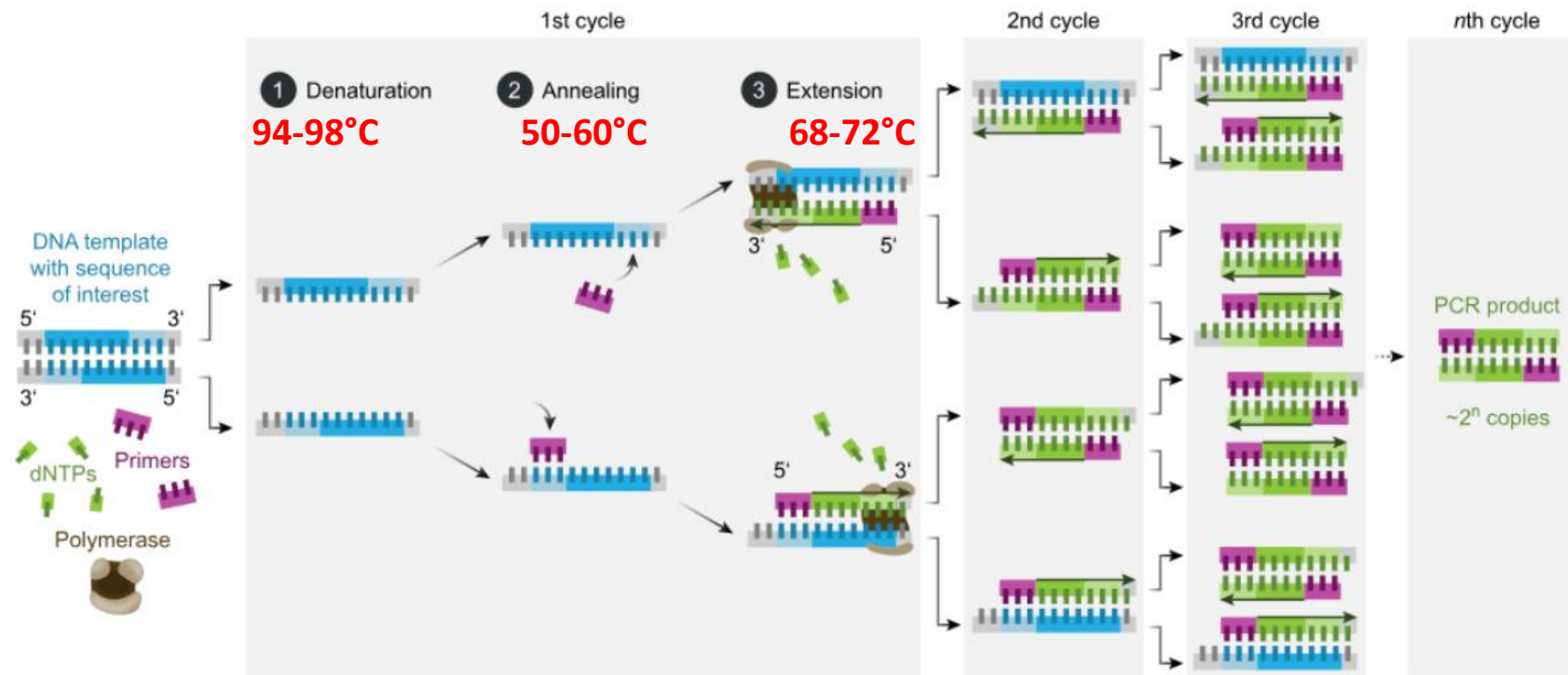
microRNA

Genomes....

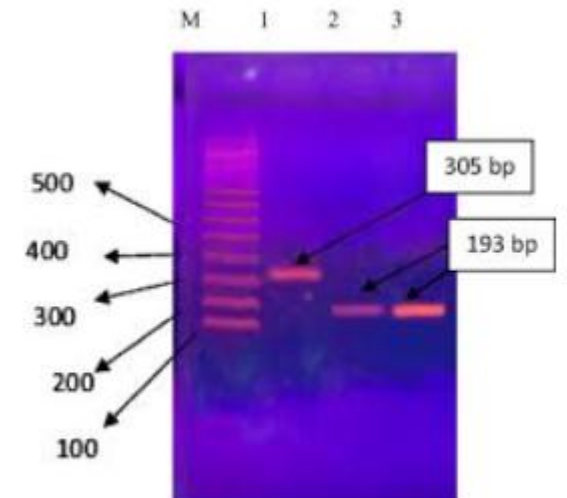
....

Polymerase chain reaction

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



→ „End point“ detection



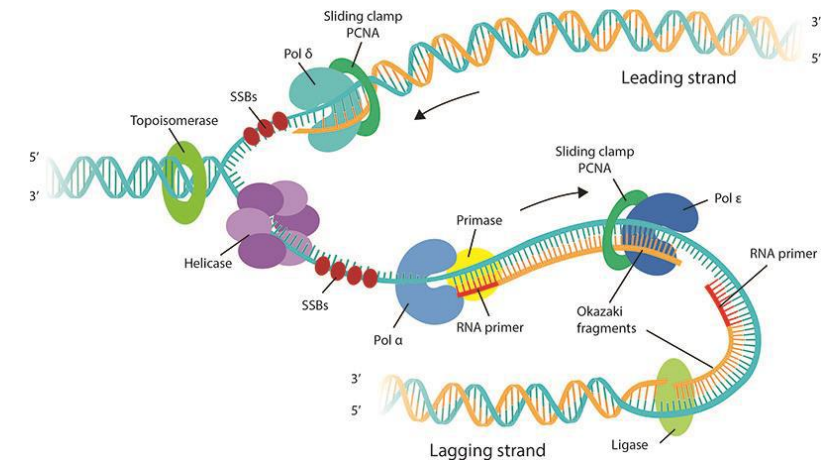
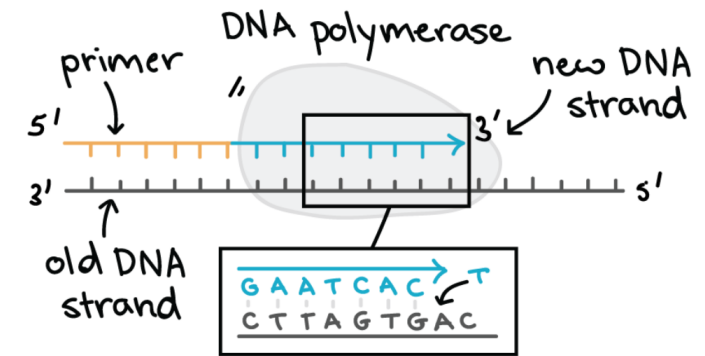
[Polymerase chain reaction \(PCR\) \(article\) | Khan Academy](#)

Primer = oligonucleotide

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

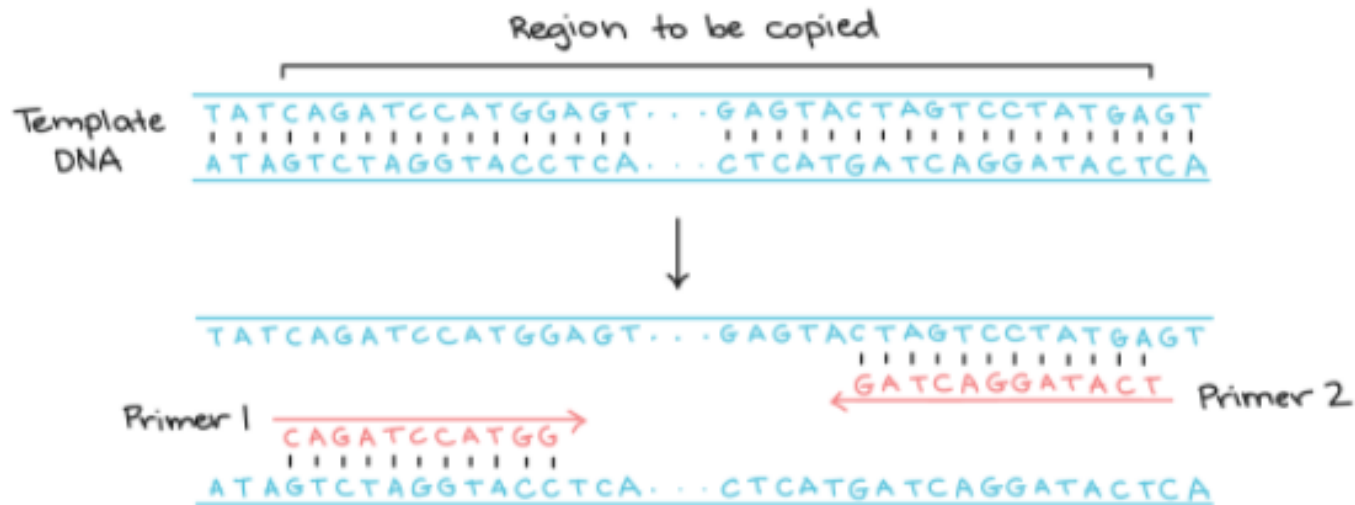
Usage:

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

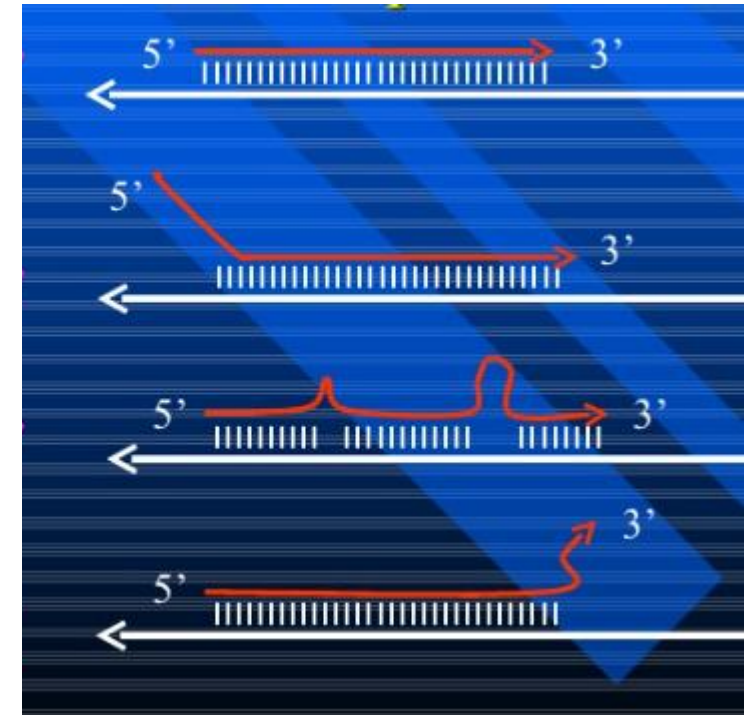


Polymerase chain reaction

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



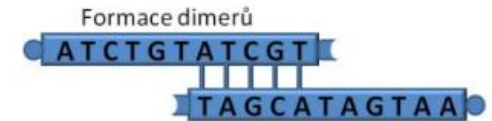
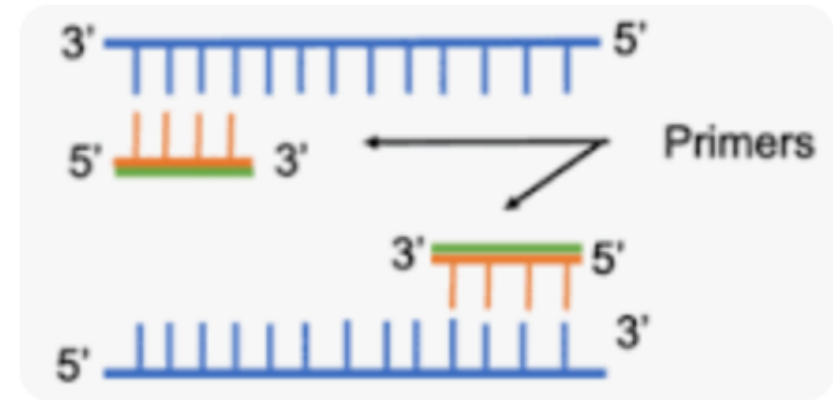
Synthesis: 5' → 3'



Polymerase chain reaction

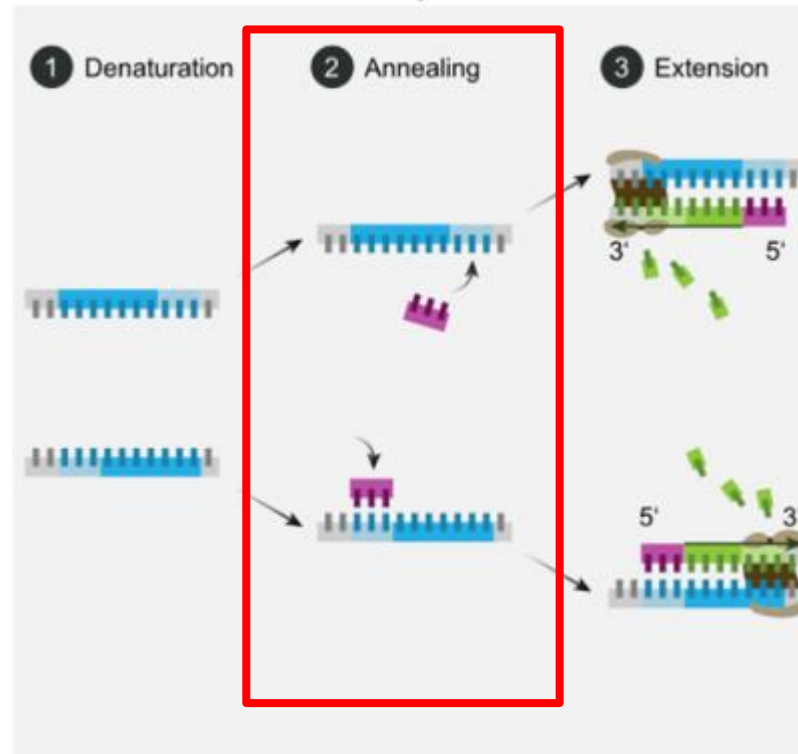
Taking into consideration the information above, primers should generally have the following properties:

- 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.

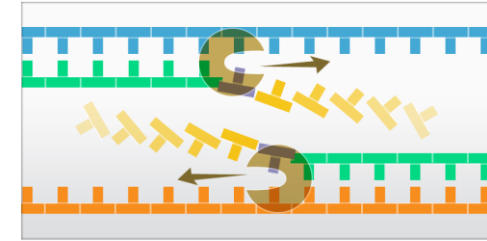


Why?

- **Primer** pairs should have a T_m within 5°C of each other.

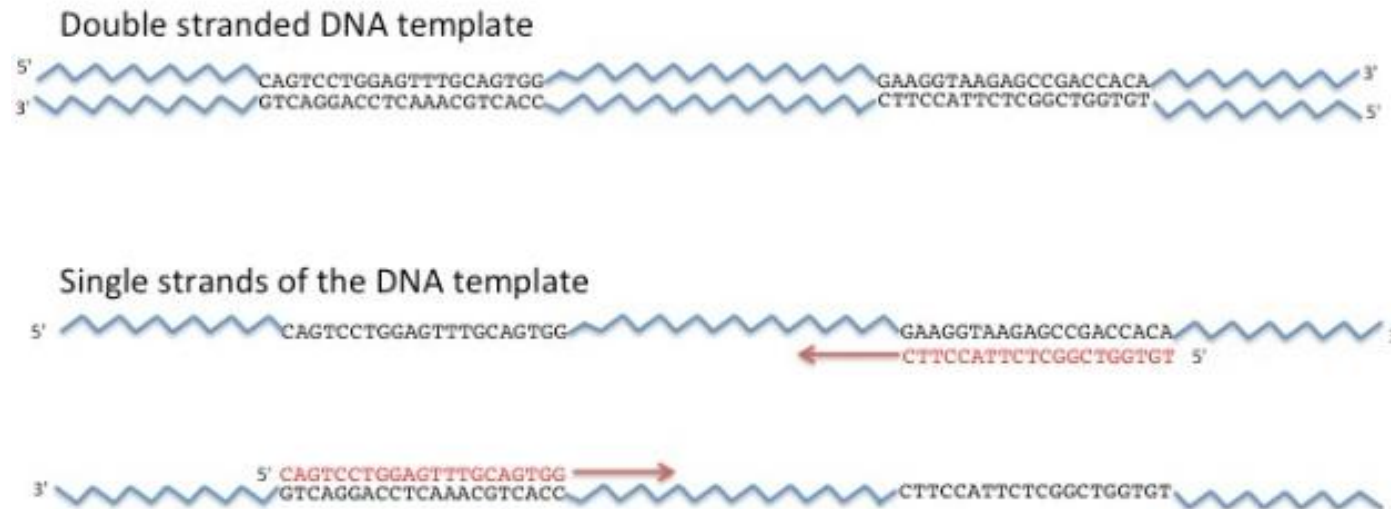


Polymerase chain reaction

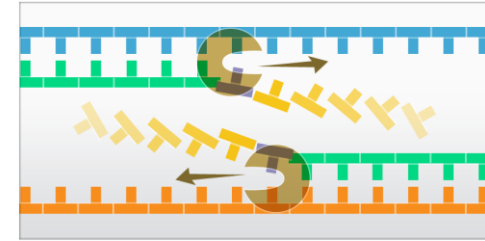


1) Amplification of desired gene / DNA fragment

→ manual design



Polymerase chain reaction



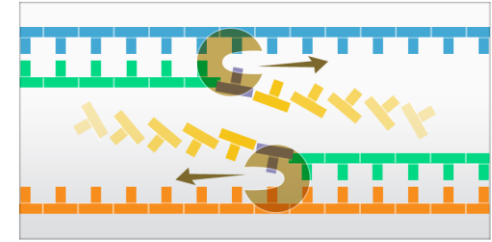
1) Amplification of desired gene / DNA fragment

→ manual design:

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

5'-ATGCCCTTTCnnnnnnnnnnnnnnnnTAAATCCCGC-3'

Polymerase chain reaction



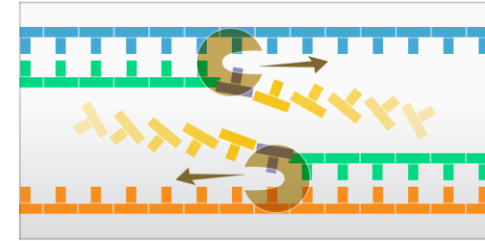
1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward)

– R (reverse)

Aim: amplification of the specific product: **the whole CDS**

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

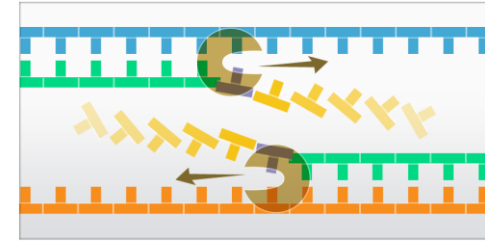
→ manual design: 2 primers – **F (forward)**

– 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  
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC  
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG  
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT  
TGAGCGAGTGTTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT  
AAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG  
ACATGAATGTCATTCTCTGGCCAATTCCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC  
TCAACTGACATATAGCATTTGGGCACACTCCAGCAGACGCCCCGAATTCAAATCCTGGAAGGATGGAAGAAA  
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC  
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)

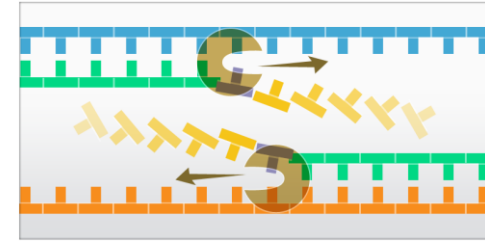
ATGGTCGGCAGAAGAGCACT

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  
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT  
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC  
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG  
CCGCAGACCTTGTGATATTCCAGTTCCTCCCTGTCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT  
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT  
AAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG  
ACATGAATGTCATTCTCTGGCCAATTACAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC  
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)

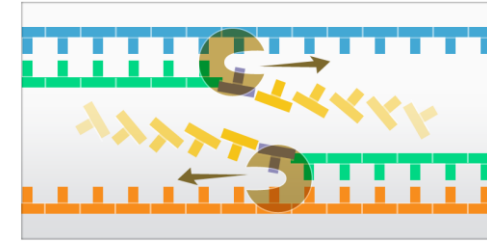
ATGGTCGGCAGAAGAGCACT

→ 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  
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC  
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG  
CCGCAGACCTTGTGATATTCCAGTTCCTCCCTGTCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT  
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 ($T_m = 60.5^\circ\text{C}$)

→ 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
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACCT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTACAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGTCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA

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 Strands (5' to 3') in:
AGT GCT CTT CTG CCG ACC AT

5' modification (if any) 3' modification (if any) Select molecule
50 mM Primer 1 Measured Absorbance at 260 nanometers
50 mM Salt (Na⁺)

Calculate Swap Strands BLAST mfold

Physical Constants

Length: 20 Molecular Weight: 6191.1 GC content: 55%
1 ml of a stock with an Absorbance of 1 at 260 nm is 4.373 microMolar and contains 27.1 micrograms

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

Check Self-Complementarity

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

50 nM Primer 1 Measured Absorbance at 260 nanometers

50 mM Salt (Na⁺)

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)

deltaG 27.3 Kcal/mol

deltaH 164.4 Kcal/mol

deltaS 425.8 cal/(°K*mol)

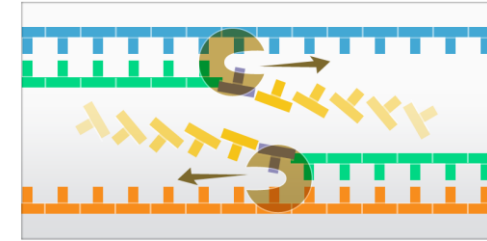
Deprecated Hairpin/self dimerization calculations

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  
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTA  
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAAGCTTT  
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAA  
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT  
TGAGCGAGTGTTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT  
AAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG  
ACATGAATGTCATTCTCTGGCCAATTCCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC  
TCAACTGACATATAGCATTTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA  
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC  
AGGCAGGATTCTTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG  
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

SMS

Sequence Manipulation Suite:

Reverse Complement

Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart. sequence if it contains an ORF on the reverse strand.

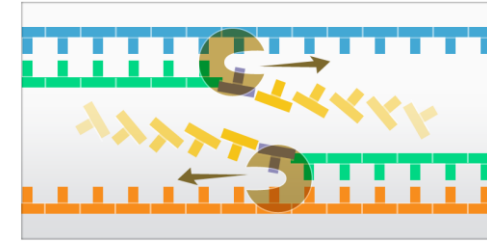
Paste the raw sequence or one or more FASTA sequences into the text area below. Input limit is 100,000,000 characters.

```
CGCCTGGAGAATATTTGGGATGAGACCACTGTATTTGCTCCAAGCAGCCTCTTGACC  
TAAACTTCC  
AGGCAGGATTCTTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCT  
TTCTGTGGG  
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Submit Clear Reset

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

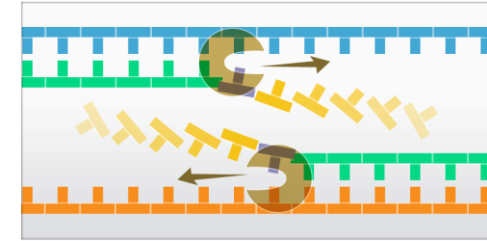
ATGGTCGGCAGA

AGGCTGCTGCAG
CAATCCCATCAT
GAGTCTGTTCTG
CCGCAGACCTTG
TGAGCGAGTGT
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
AGAAAGGCCAAATTTCTTGTFTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGTATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCATACATGGCAGCGTAAGTGTAAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTTCAAGATGGCAGGGACTCCAAACCACTGCAGGGGGAAGTGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCCTTCAGTTTACCTGTGAT
GTCCTTTTCTGGAAATGATCGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCCTTCTTGCCGACCAT

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

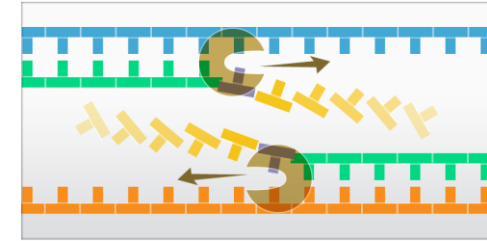
→ re-write sequence from the beginning cca 20nt

TCATTTTCTAGCTTTGATCT

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
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCTACATGGCAGCGTAAGTGTAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAAGTGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCTAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGAGGGTCCCTTCAGTTTACCTGTGAT
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

Aim: amplification of the specific product

→ check the primer (T_m) - OligoCalc

```
>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
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCATACATGGCAGCGTAAGTGTAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAAGTGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCTAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGAGGGTCCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
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
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/(°K*mol) deltaH 146.3 Kcal/mol
deltaG 21.4 Kcal/mol deltaS 386.3 cal/(°K*mol)

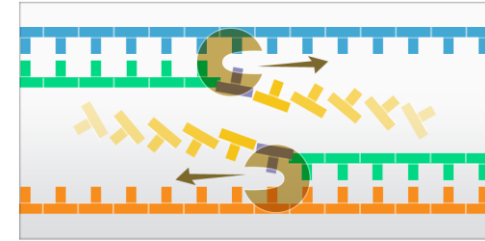
Deprecated Hairpin/self dimerization calculations

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

Check Self-Complementarity

Tm 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

Aim: amplification of the specific product

→ check the primer (T_m) - OligoCalc

>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
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCTACATGGCAGCGTAAGTGTAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAAGTGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
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
50 mM Salt (Na⁺) 1 Measured Absorbance at 260 nanometers

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

Physical Constants

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.

Melting Temperature (T_M) Calculations

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)

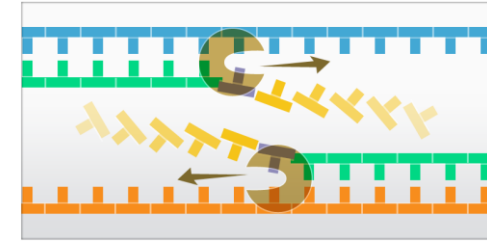
Deprecated Hairpin/self dimerization calculations

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

Aim: amplification of the specific product

→ check the primer (T_m) - OligoCalc

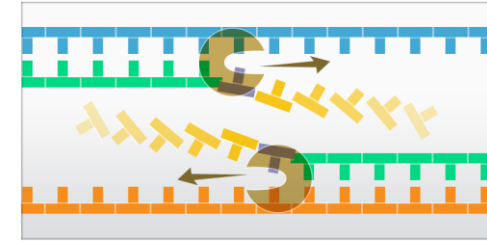
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement

```
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCTACATGGCAGCGTAAGTGTAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA
GCCTTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAAGTGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
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

TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC

AGAAAGGCCAAATTTCTTGTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC

TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA

AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT

GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT

GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA

CATGGAGCCACTGCCACCAGTGGTGTGAGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC

TTTGTCTATACATGGCAGCGTAAGTGTAAAGCAAACCTCTCCTATGAACACTCGCTCAAACCA

GCCTTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACTGGAATATCACAAGGTC

TGCGGCTTCCAGCTTCTTTTGTTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA

AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCCTTCAGTTTACCTGTGAT

GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC

CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT

CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT

reverse complement

Practical part

1) Design the primer to amplify sequence:

5' -TTTGGAAACnnnnnnnnnnnnnnnnnnnnCCCGCCCTTT-3'

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

- length: 18-24nt

- Tm: 55-60°C

Check PCR product

Format Conversion

- Combine FASTA
- EMBL to FASTA
- EMBL Feature Extractor
- EMBL Trans Extractor
- Filter DNA
- Filter Protein
- GenBank to FASTA
- GenBank Feature Extractor
- GenBank Trans Extractor
- One to Three
- Range Extractor DNA
- Range Extractor Protein
- Reverse Complement
- Split Codons
- Split FASTA
- Three to One
- Window Extractor DNA
- Window Extractor Protein

Sequence Analysis

- Codon Plot
- Codon Usage
- CpG Islands
- DNA Molecular Weight
- DNA Pattern Find
- DNA Stats
- Fuzzy Search DNA
- Fuzzy Search Protein
- Ident and Sim
- Multi Rev Trans
- Mutate for Digest
- ORF Finder
- Pairwise Align Codons
- Pairwise Align DNA
- Pairwise Align Protein
- PCR Primer Stats
- PCR Products

- Protein GRAVY
- Protein Isoelectric Point
- Protein Molecular Weight
- Protein Pattern Find
- Protein Stats
- Restriction Digest
- Restriction Summary
- Reverse Translate
- Translate

Sequence Figures

- Color Align Conservation
- Color Align Properties
- Group DNA
- Group Protein
- Primer Map
- Restriction Map
- Translation Map

Random Sequences

- Mutate DNA

Sequence Manipulation Suite:

PCR Products

PCR Products accepts one or more DNA sequence templates and two primer sequences. The program searches for perfectly matching primer annealing sites that can generate a PCR product, specifying their length, their position in the original sequence, and the primers that produced them. You can use linear or circular molecules as the template. Use PCR Products to determine the

Paste the raw sequence or one or more FASTA sequences into the text area below. Input limit is 200,000,000 characters.

```
>sample sequence A
ctaaattgtaagcgttaatatattttgttaaaattcgcgttaattttgttaa
tttttaaccaataggccgaaatcgcaaaatccctataaatcaaaagaatagaccgaga
taggggttgagtgtgttcagtttggaacaagagtcactattaaagaacgtggactccaa
cgtcaaaagggcgaaaaaccgtctatcagggcgatggccactacgtgaaccatcacctaa
tcaagtttttggggtcgagggtccgtaaagcactaaatcggaaccctaaagggagcccc
```

Template (Sequence of the 3rd exon or full mRNA)

Enter the name of the first primer, followed by its sequence in the 5' to 3' direction. Degenerate sites can be represented using IUPAC DNA characters.

F aatacgactcactatag

Primer sequences

Enter the name of the second primer, followed by its sequence in the 5' to 3' direction. Degenerate sites can be represented using IUPAC DNA characters.

R attaacctcactaaag

Submit Clear Reset

• Treat sequences as molecules.

*This page requires JavaScript. See [browser compatibility](#).

*You can [mirror this page](#) or [use it off-line](#).

Sun 1 Oct 12:00:07 2023

Valid XHTML 1.0; Valid CSS

Primer names

Sequence Manipulation Suite – Škola – Microsoft Edge

about:blank

PCR Products results

```
>164 bp product from linear template sample sequence A, base 627 to base 790 (F - R).
aatacgactcactatagggcggaattggagctccaccgcggtggcgggcgctctagaacta
gtggatcccccggtgcaggaattcgatatcaagcttatcgataccgtcgacctcgagg
gggggcccgggtaccagcttttgttccttttagtgagggttaat
```

Sequence Manipulation Suite – Škola – Microsoft Edge

about:blank

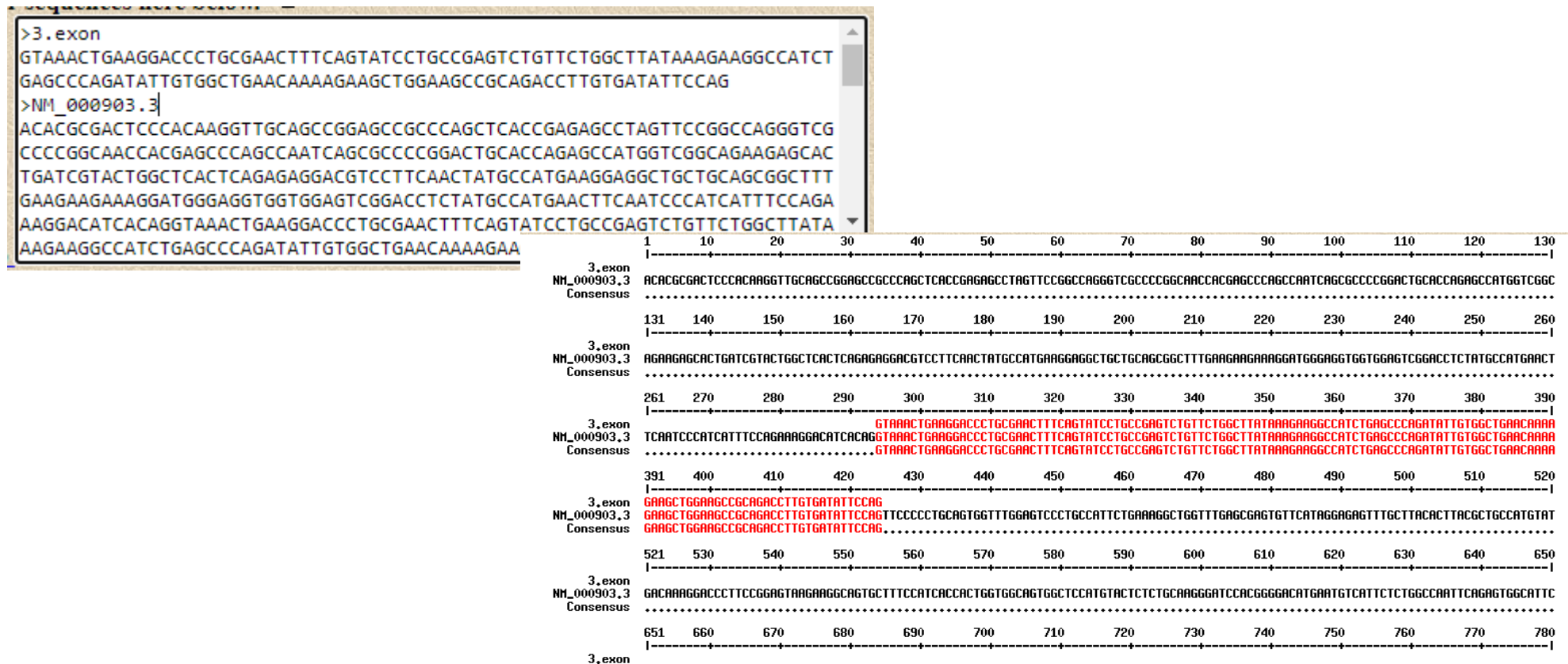
PCR Products results

No PCR products were obtained.

Wrong primers

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
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG
>NM_000903.3
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCTCACCAGAGCCTAGTTCCGGCCAGGGTCTG
CCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
TGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTT
GAAGAAGAAAGGATGGGAGGTGGTGGAGTCTATGCCATGAACTTCAATCCCATCATTCCACA
```

	261	270	280	290	300	310	320	330	340	350	360	370	380	390
F	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
3.exon														
NM_000903.3	GTAAACTGAAGGACCCTGC													
Consensus	GTAAACTGAAGGACCCTGC													
	TCATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGC													
	GTAAACTGAAGGACCCTGC													
	gaactttcagtcctgccgagtcgttctggcttataaagaaggccatctgagcccagatattgtggctgaacaaa													
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F	391	400	410	420	430	440	450	460	470	480	490	500	510	520
3.exon														
NM_000903.3	GAGCTGGAGCCGAGACCTTGTGATATTCCAG													
Consensus	GAGCTGGAGCCGAGACCTTGTGATATTCCAGTCCCCCTGCAGTGGTTGGAGTCCCTGCCATTCTGAAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTAT													
	gaagctggaagccgagaccttgcgatattccag.....													

Comparison by multalin

2) mRNA + 3rd exon + F primer +R primer

```
>R_rc
CAG ACC TTG TGA TAT TCC AG
>F
GTA AAC TGA AGG ACC CTG C
>3.exon
GTAAACTGAAGGACCCTGCGAACTTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT
GAGCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGACACCTTGTGATATTCCAG
>NM_000903.3
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCTCAGCTACCGAGAGCCTAGTTCCGGCCAGGGTCTG
CCCCGGCAACCACGAGCCAGCCAATCAGCGCCC
```

```
261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|
F          GTAAACTGAAGGACCCTGC
3.exon     GTAAACTGAAGGACCCTGC
NM_000903.3 TCAATCCCATCATTTCCGAAAGGACATCACAGGTAAACTGAAGGACCCTGC
Consensus  .....GTAAACTGAAGGACCCTGCgaactttcagtatcctgccgagctctgttctggcttataaagaaggccatctgagccagatattgtggctgaacaaaa

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|
F          GAGCTGGGAGCCGACACCTTGTGATATTCCAG
3.exon     GAGCTGGGAGCCGACACCTTGTGATATTCCAG
NM_000903.3 GAGCTGGGAGCCGACACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGAGTCCCTGCCATTCTGAAGGCTGTTTGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTAT
Consensus  gaagctggaagccgagaccttgtgatattccag.....
```

```
Consensus .....
1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|
R          CTGGATATCACAGGCTCTG
F          incorrect
3.exon     CTGGATATCACAGGCTCTG
NM_000903.3 TCAATTACAAAGCAGTTACTAATATGCCTAGCACAGTACCACTCTTGGTCAGCTTTTGTGTTTATATACAGTACACAGATACCTTGAAGGAGAGCTAATAATCTCTTCTTGTGCTGAGTCATCTA
Consensus  .....c..g.a...c..c..ggtc.g.....
```

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  
GTAAACTGAAGGACCCTGCGAACTTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT  
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG  
>NM_000903.3  
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCTCACCAGAGCCTAGTTCCGGCCAGGGTCG  
CCCCGGCAACCACGAGCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
```

	261	270	280	290	300	310	320	330	340	350	360	370	380	390
R_rc	-----													
3.exon														
NM_000903.3	TCAATCCCATCATTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCCTGCGAACTTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAA													
F	GTAAACTGAAGGACCCCTGCGAACTTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAA													
Consensus	gt.aactgaaggaccctgc.....													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
R_rc	-----													
3.exon														
NM_000903.3	GAGCTGGAGCCGCAGACCTTGTGATATTCCAGCAGACCTTGTGATATTCCAGTCCCCCTGCAGTGGTTTGAGTCCCTGCCATTCTGAAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTGCTTACCTTACGCTGCCATGTAT													
F	GAGCTGGAGCCGCAGACCTTGTGATATTCCAGTCCCCCTGCAGTGGTTTGAGTCCCTGCCATTCTGAAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTGCTTACCTTACGCTGCCATGTAT													
Consensus	cagaccttgtgatattccag.....													

Practical part HW:

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

- length: 18-24nt
- Tm: 55-60°C

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

