Introduction to applied bioinformatics

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2023/2024

„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

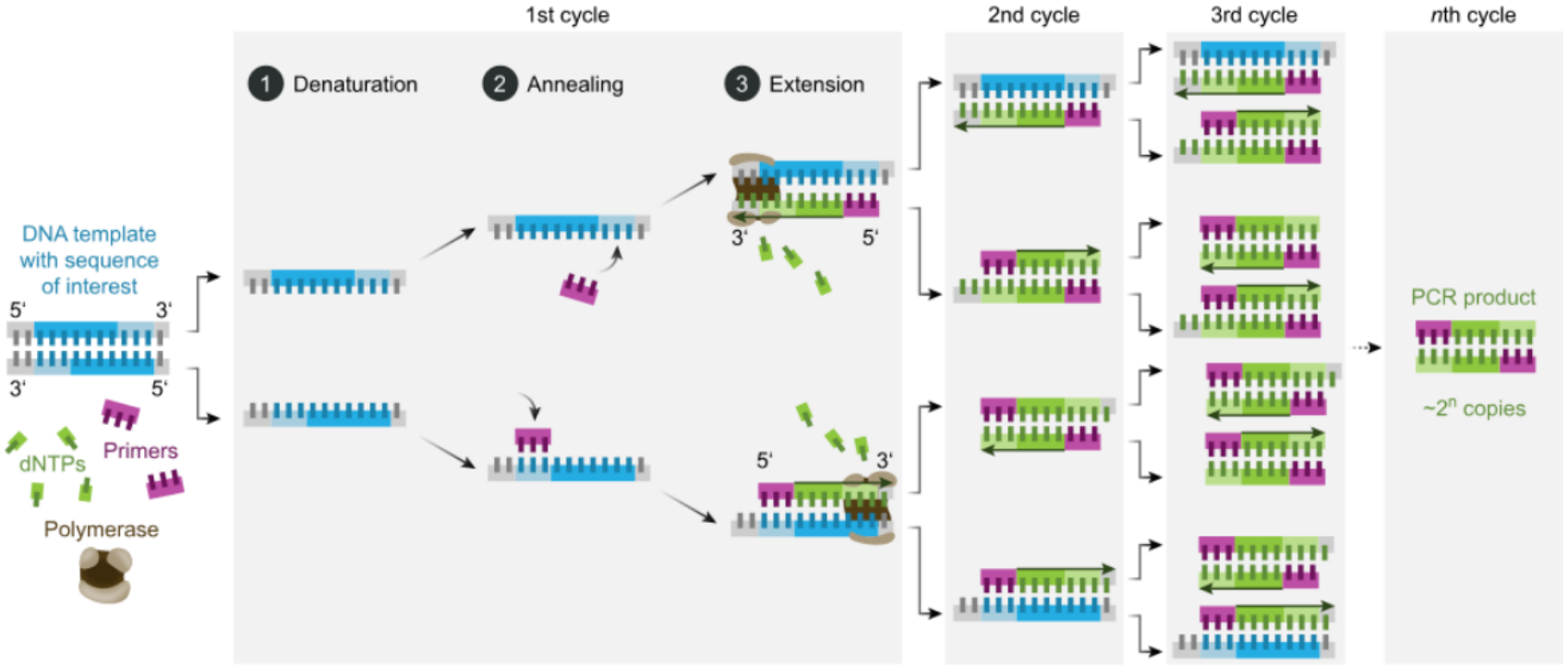
microRNA

Genomes….

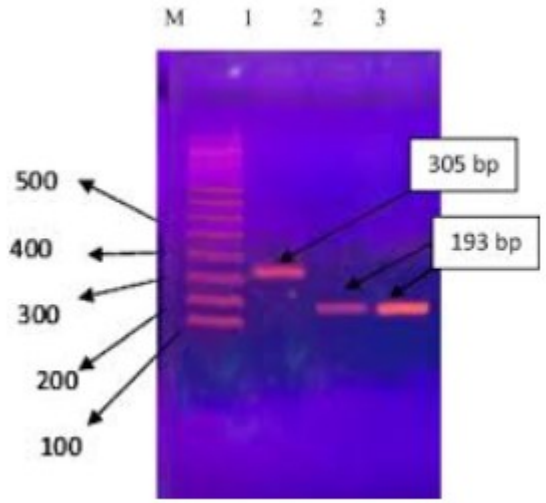
….

Polymerase chain reaction

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



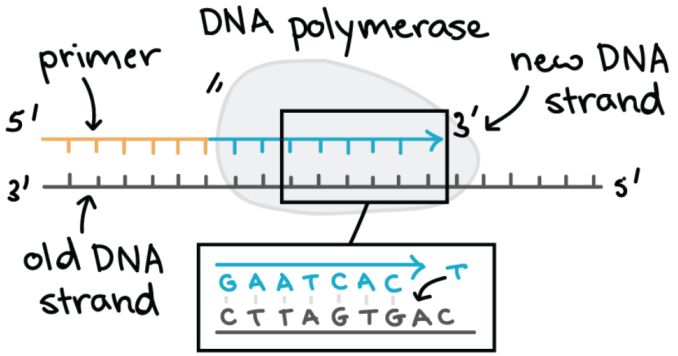
→ „End point“ detection



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

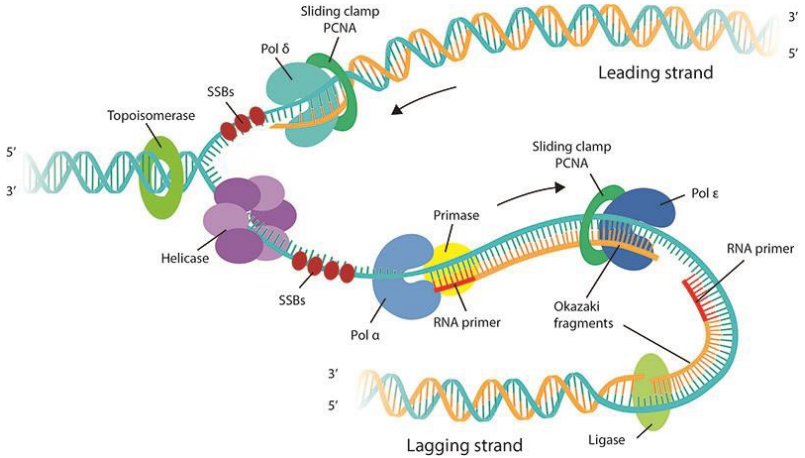
Primer = oligonucleotide

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



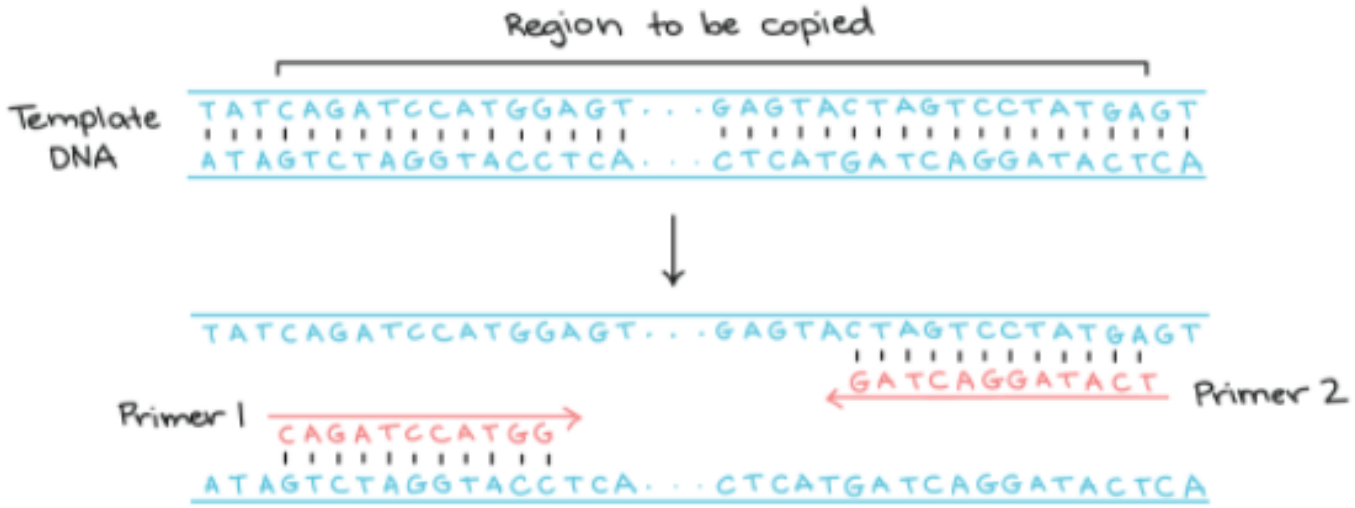
Usage:

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

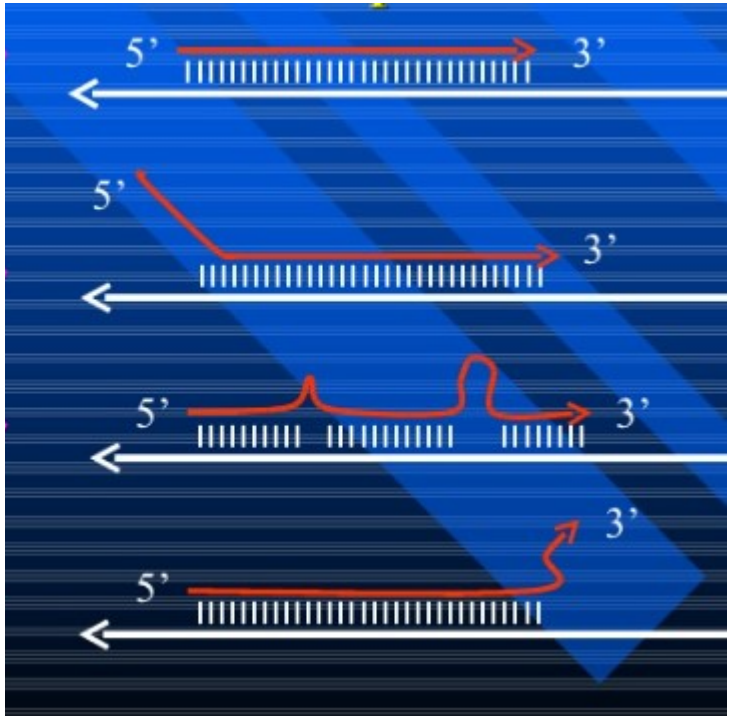


Polymerase chain reaction

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



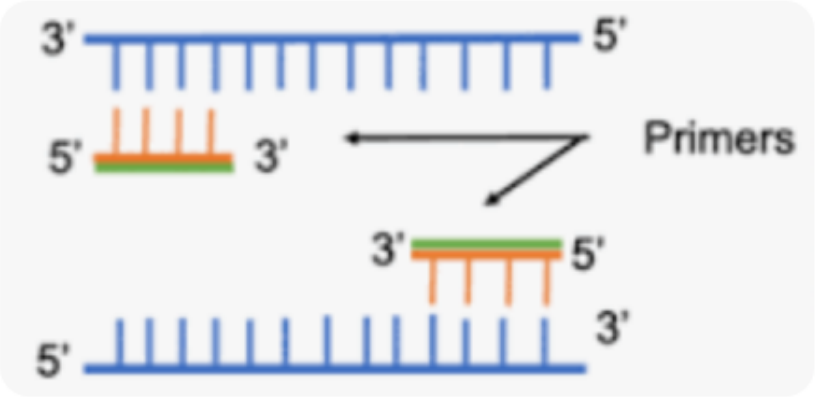
Synthesis: 5´→ 3´

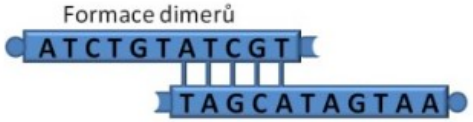


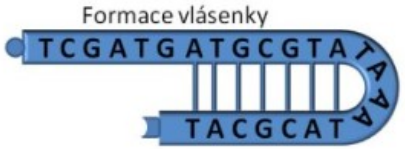
Polymerase chain reaction

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

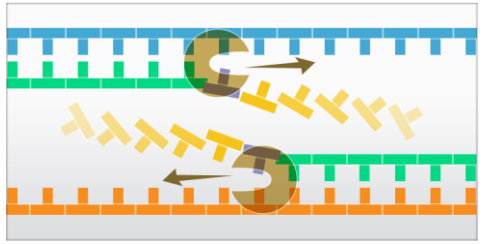
- Length of 18-24 bases.
- 40-60% G/C content.
- Start and end with 1-2 G/C pairs.
- Melting temperature (Tm) of 50-60°C.
- **Primer** pairs should have a Tm within 5°C of each other.
- **Primer** pairs should not have complementary regions.





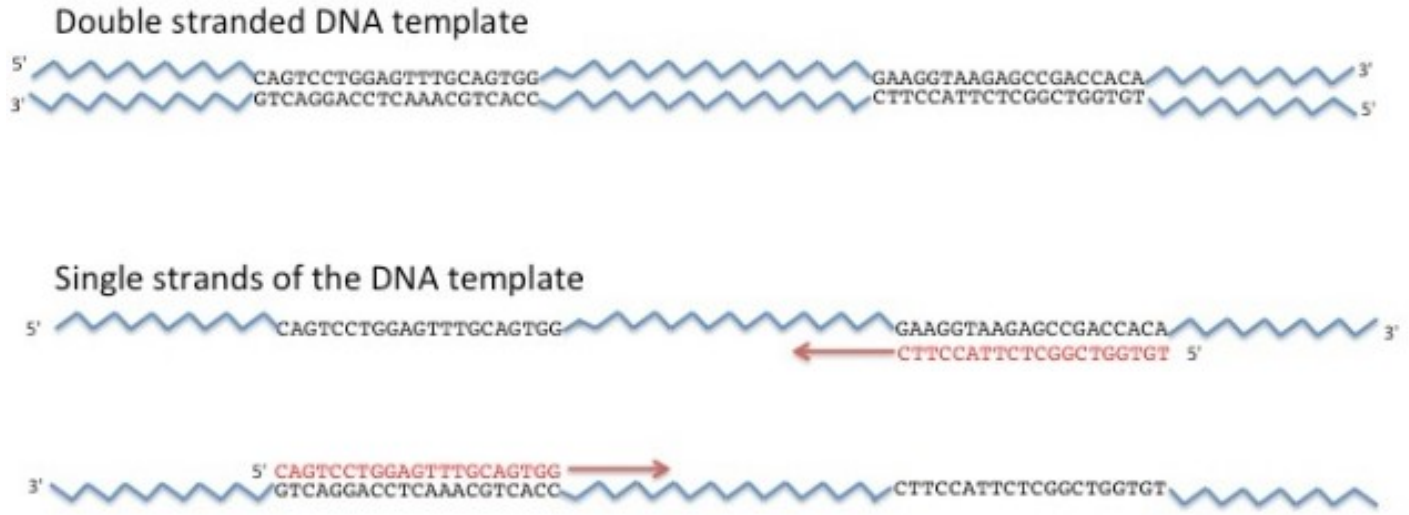


Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design



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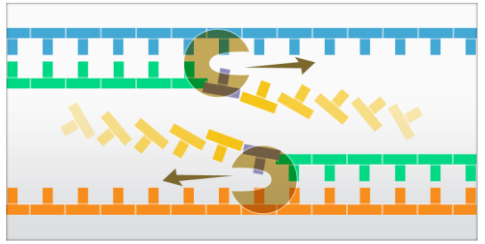


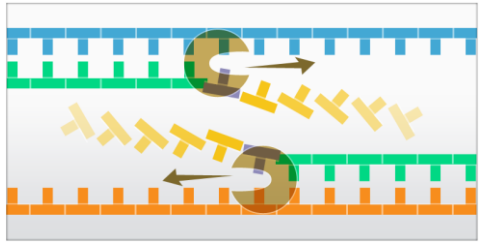
1) Amplification of desired gene / DNA fragment

→ manual design:

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

5´-ATGCCCTTTCnnnnnnnnnnnnnnnTAAATCCCGC-3´

Polymerase chain reaction



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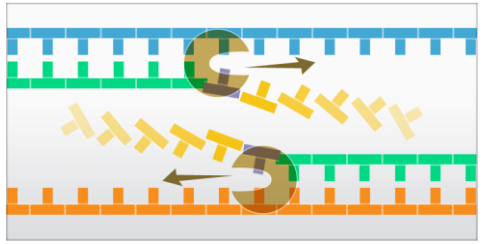
3´-TACGGGAAAGnnnnnnnnnnnnnnATTTAGGGCG-5´

Polymerase chain reaction



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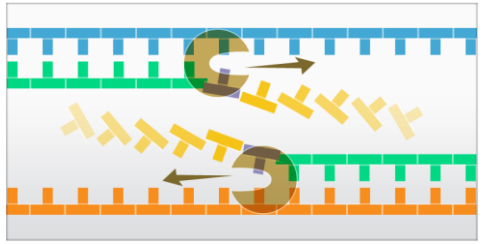
ATTTAGGGCG-5

5´-**ATGCCCTTTC-**

3´-TACGGGAAAGnnnnnnnnnnnnnnATTTAGGGCG-5´

Polymerase chain reaction



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5´-ATGCCCTTTCnnnnnnnnnnnnnnTAAATCCCGC-3´

ATTTAGGGCG-5 R_primer: **GCGGGATTTA**

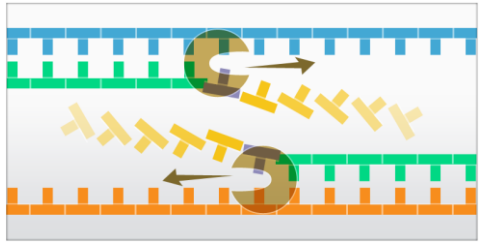
F_primer: **ATGCCCTTTC**

5´-**ATGCCCTTTC-**

3´-TACGGGAAAGnnnnnnnnnnnnnnATTTAGGGCG-5´

when you have the final primers they must be written in the correct direction 5´-3´ !

Polymerase chain reaction



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5´-ATGCCCTTTCnnnnnnnnnnnnnnnTAAATCCCGC-3´

R_primer: **GCGGGATTTA**

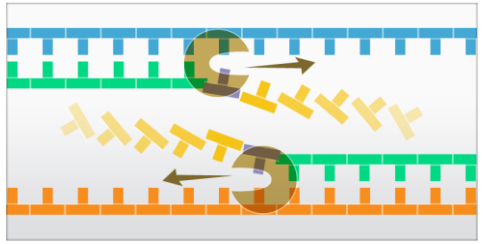
reverse complement:

5´-**GCGGGATTTAnnnnnnnnnnnnnnnGAAAGGGCAT**-3´

(**3´-TACGGGAAAGnnnnnnnnnnnnnnnATTTAGGGCG-5´**)

reverse

Polymerase chain reaction



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R_primer: **GCGGGATTTA**

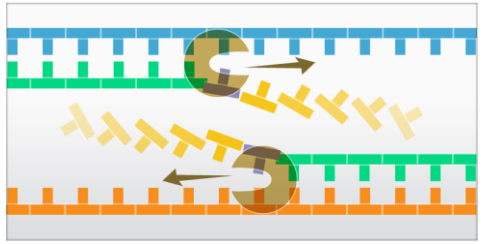
reverse complement:

5´-**GCGGGATTTA**nnnnnnnnnnnnnnn**GAAAGGGCAT**-3´

(**3´-**TACGGGAAAGnnnnnnnnnnnnnnn**ATTTAGGGCG-5´**)

reverse

Polymerase chain reaction



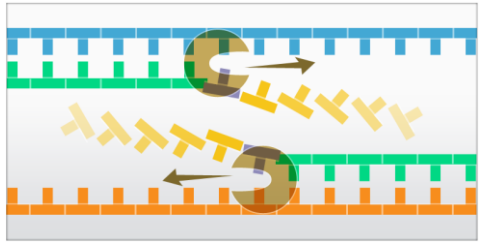
1) Amplification of desired gene / DNA fragment

→ 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)**

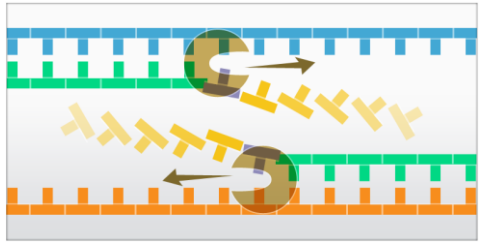
– R (reverse)

Aim: amplification of the specific product

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

ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
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)

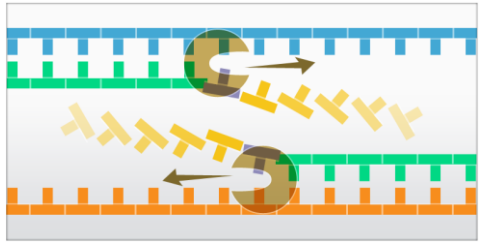
ATGGTCGGCAGAAGAGCACT

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
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

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

– R (reverse)

→ re-write sequence from the beginning cca 20nt

ATGGTCGGCAGAAGAGCACT

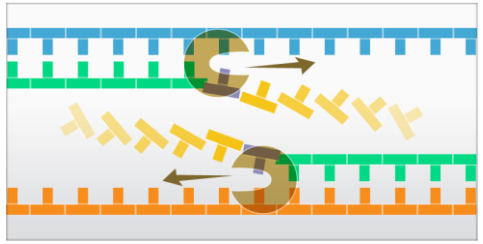
→check the primer (Tm) - OligoCalc

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
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

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

– R (reverse)

→ re-write sequence from the beginning cca 20nt

ATGGTCGGCAGAAGAGCACT (Tm= 60.5°C)

→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
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
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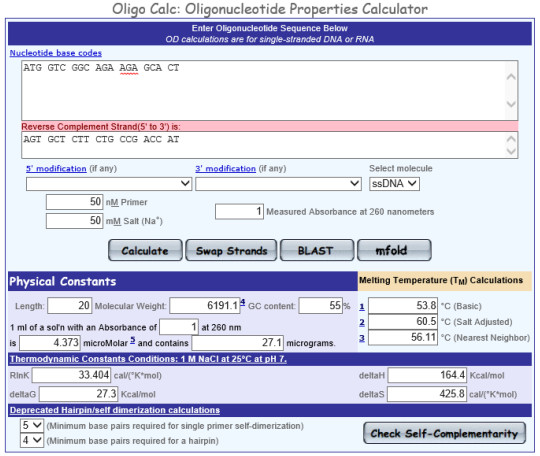
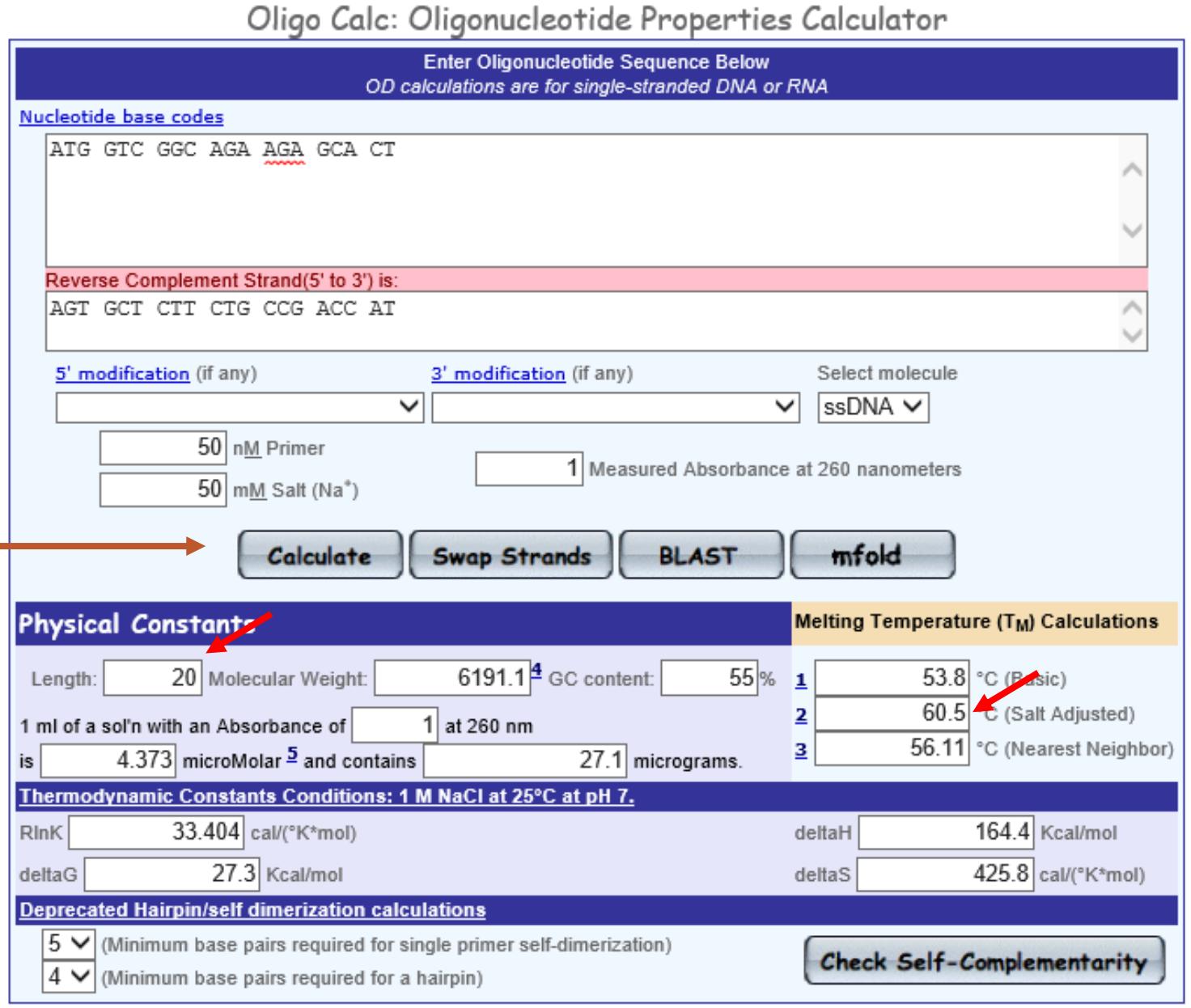
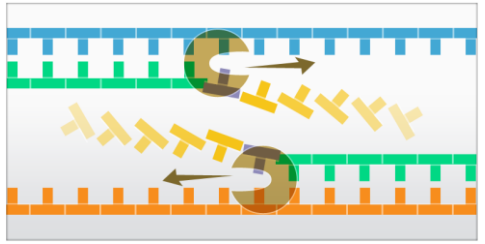


OligoCalc:


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
Melting Temperature (TM) Calculations
Length: 20 Molecular Weight: 6191.1 GC content: 55 %
1 ml of a sol'n 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 56.11 °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

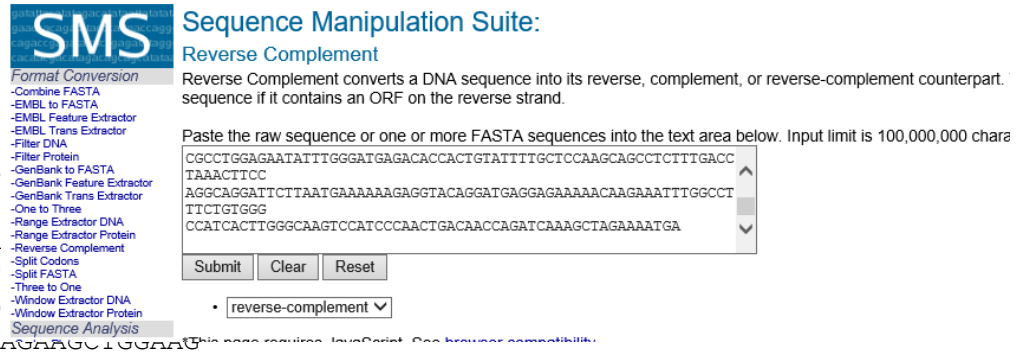


1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) `ATGGTCGGCAGAAGAGCACT (Tm= 60.5°C)`

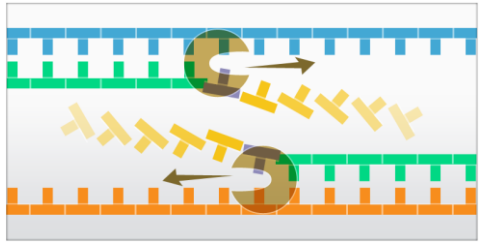
– **R (reverse) → reverse complement sequence (SMS)**

Aim: amplification of the specific product




```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydro
transcript variant 1, mRNA
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACT
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTA
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTT
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAA
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGA
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCA
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTG
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Polymerase chain reaction



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

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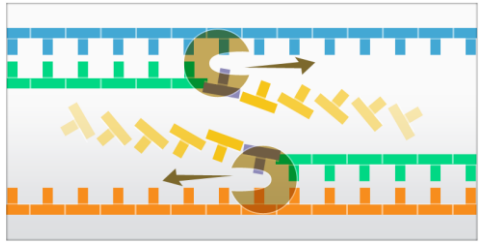
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCATACATGGCAGCGTAAGTGTAAGCAAACTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) `ATGGTCGGCAGAAGAGCACT (Tm= 60.5°C)`

– **R (reverse) → reverse complement**

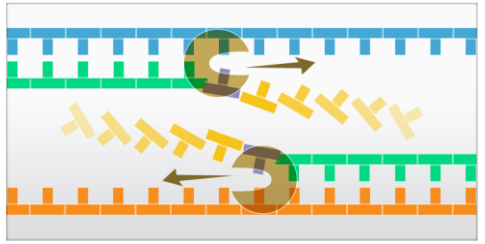
→ re-write sequence from the beginning cca 20nt

`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
AGAAAGGCCAAATTTCTTGTTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCATACATGGCAGCGTAAGTGTAAGCAAACTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT
```

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) ATGGTCGGCAGAAGAGCACT (Tm= 60.5°C)

– **R (reverse) → reverse complement**

→ re-write sequence from the beginning cca 20nt

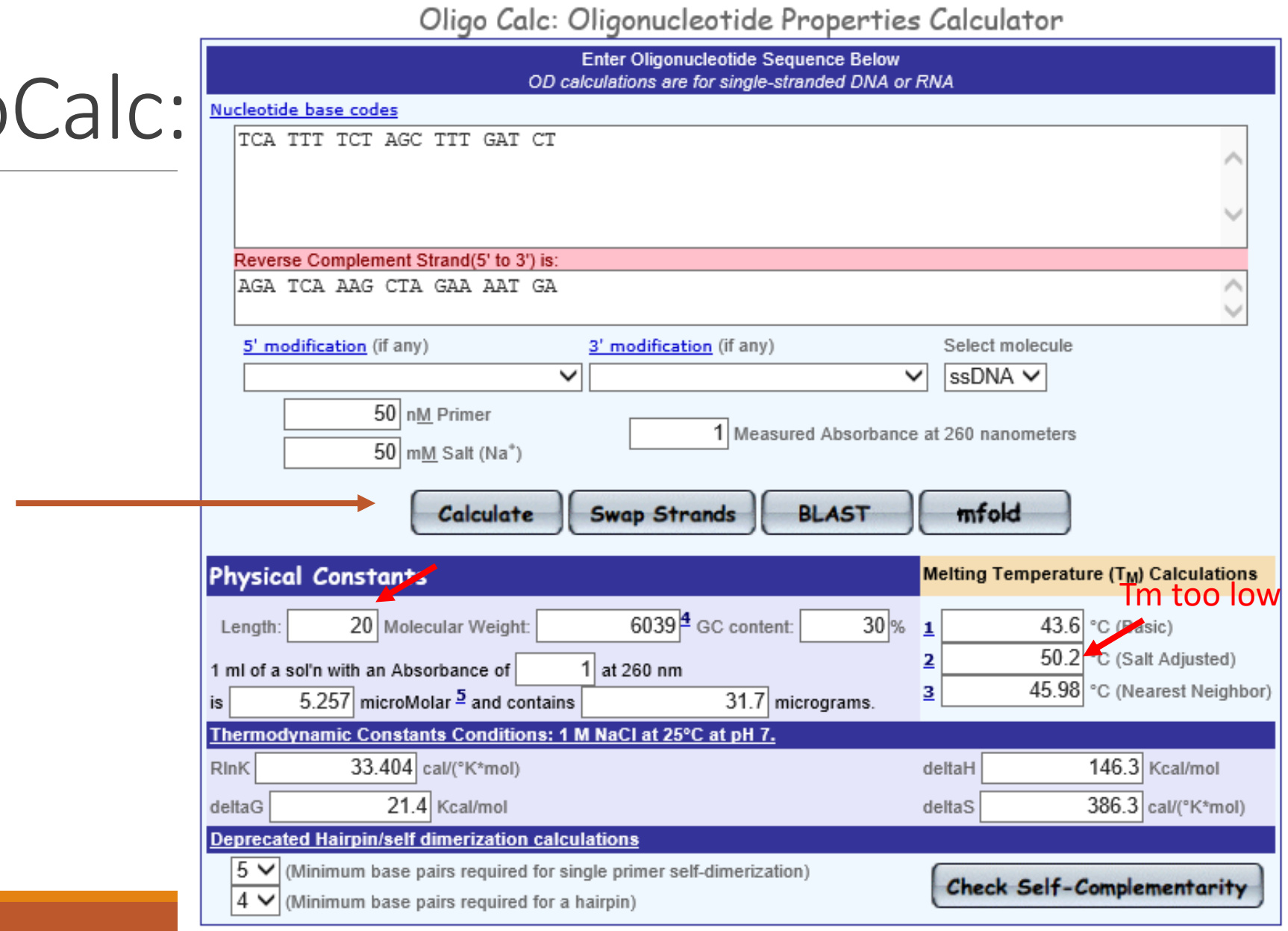
TCATTTTCTAGCTTTGATCT

Aim: amplification of the specific product

→check the primer (Tm) - OligoCalc

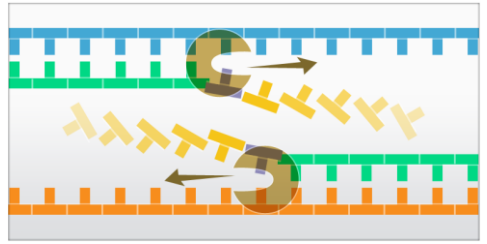
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCATACATGGCAGCGTAAGTGTAAGCAAACTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT

OligoCalc:



Tm too low →longer primer

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) ATGGTCGGCAGAAGAGCACT (Tm= 60.5°C)

– **R (reverse) → reverse complement**

→ re-write sequence from the beginning cca 20nt

TCATTTTCTAGCTTTGATCT

Aim: amplification of the specific product

→check the primer (Tm) - OligoCalc

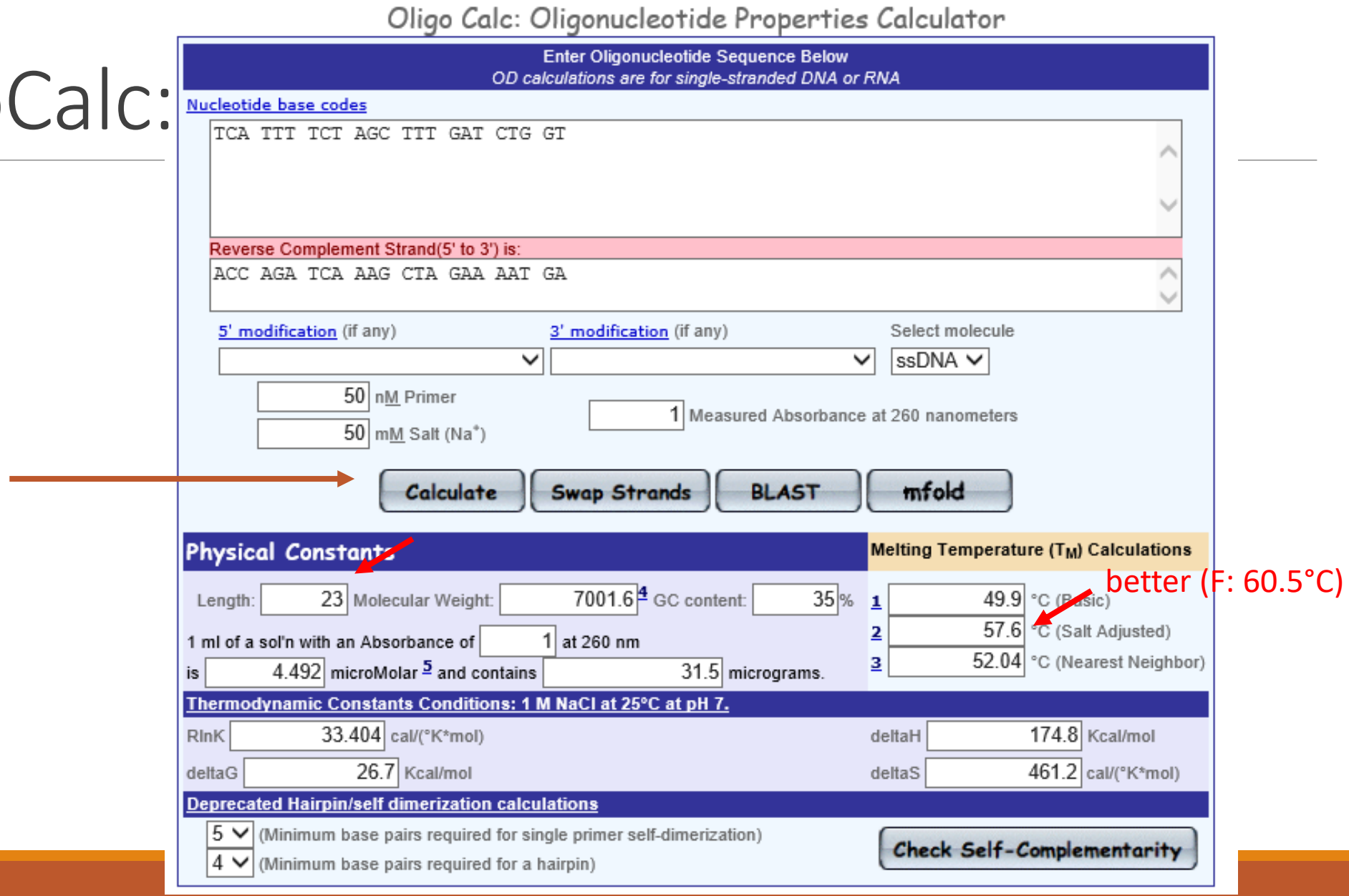
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement

TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCATACATGGCAGCGTAAGTGTAAGCAAACTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT

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

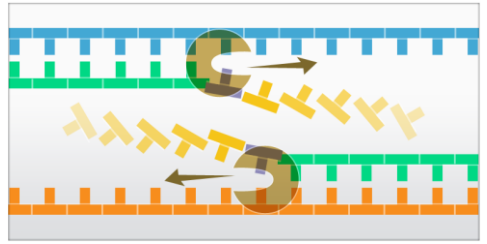
TCATTTTCTAGCTTTGATCTGGT

OligoCalc:



better (F: 60.5°C)

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward) ATGGTCGGCAGAAGAGCACT (Tm= 60.5°C)

– **R (reverse) → reverse complement**

→ re-write sequence from the beginning cca 20nt

TCATTTTCTAGCTTTGATCT

Aim: amplification of the specific product

→check the primer (Tm) - OligoCalc

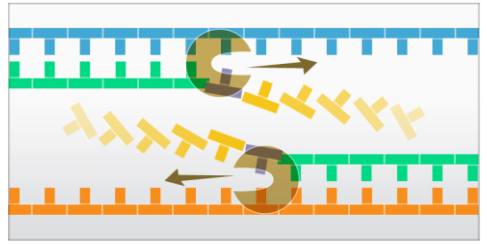
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement

TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCATACATGGCAGCGTAAGTGTAAGCAAACTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT

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

TCATTTTCTAGCTTTGATCTGGT

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – **F (forward)** ATGGTCGGCAGAAGAGCACT (Tm= 60.5°C)

– **R (reverse)** TCATTTTCTAGCTTTGATCTGGT (Tm= 57.6°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
AGAAAGGCCAAATTTCTTGTTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCATACATGGCAGCGTAAGTGTAAGCAAACTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT

Practical part

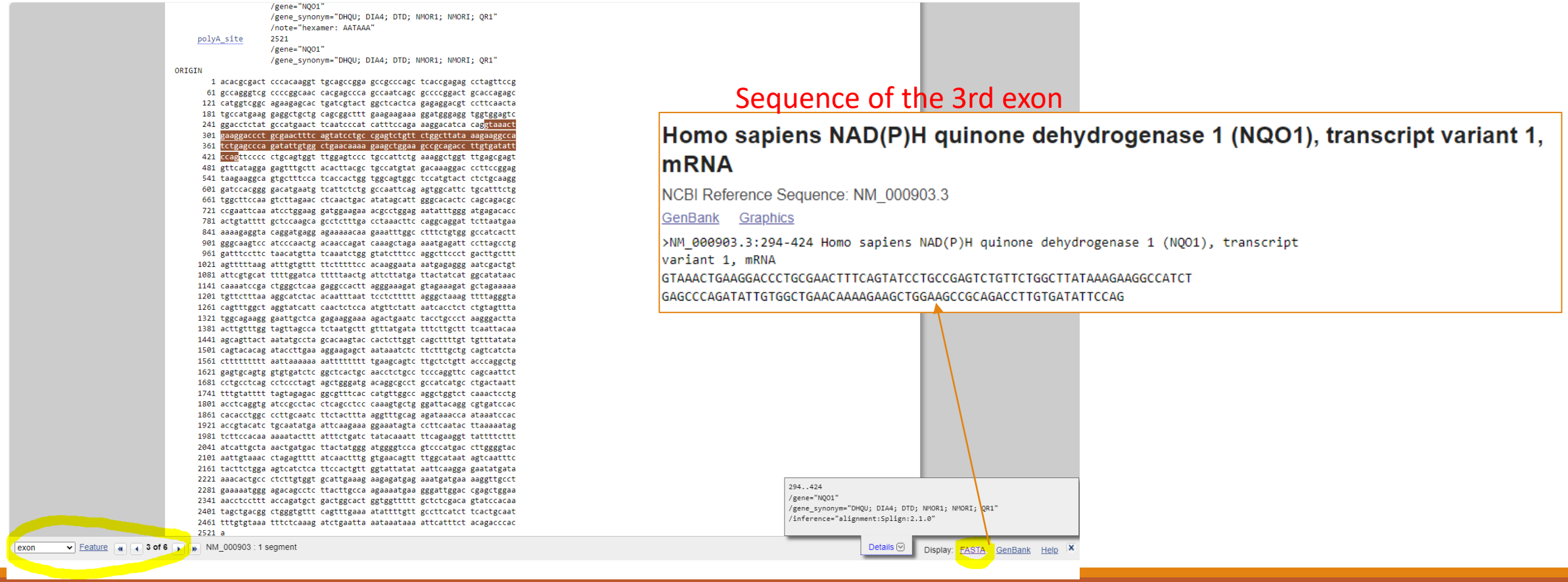
1) Design primers manually for amplification of the 3rd exon of NQO1 (NM_00903.3)

- 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



Comparison by multalin

1) mRNA and 3rd exon:

```
>3.exon
GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG
>NM_000903.3
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCCAGCTCACCGAGAGCCTAGTTCCGGCCAGGGTCG
CCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
TGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTT
GAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTTCAATCCCATCATTTCCAGA
AAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATA
AAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAA
```

```
                1       10        20        30        40        50        60        70        80        90       100       110       120       130
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
3.exon
NM_000903.3     ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCCAGCTCACCGAGAGCCTAGTTCCGGCCAGGGTCGCCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGC
Consensus       ....................................................................................................................................

                131     140       150       160       170       180       190       200       210       220       230       240       250       260
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
3.exon
NM_000903.3     AGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACT
Consensus       ....................................................................................................................................

                261     270       280       290       300       310       320       330       340       350       360       370       380       390
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
3.exon                                                  GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAA
NM_000903.3     TCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAA
Consensus       ..............................GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAA

                391     400       410       420       430       440       450       460       470       480       490       500       510       520
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
3.exon          GAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG
NM_000903.3     GAAGCTGGAAGCCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTAT
Consensus       GAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG...................................................................................................

                521     530       540       550       560       570       580       590       600       610       620       630       640       650
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
3.exon
NM_000903.3     GACAAAGGACCCTTCCGGAGTAAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGGACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTC
Consensus       ....................................................................................................................................

                651     660       670       680       690       700       710       720       730       740       750       760       770       780
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
3.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
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCCAGCTCACCGAGAGCCTAGTTCCGGCCAGGGTCG
CCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
TGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTT
GAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTTCAATCCCATCATTTCCAGA
```

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

                391       400       410       420       430       440       450       460       470       480       490       500       510       520
                |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
F
3.exon        GAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG
NM_000903.3   GAAGCTGGAAGCCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTAT
Consensus     gaagctggaagccgcagaccttgtgatattccag.....................................................................................................
```

Comparison by multalin

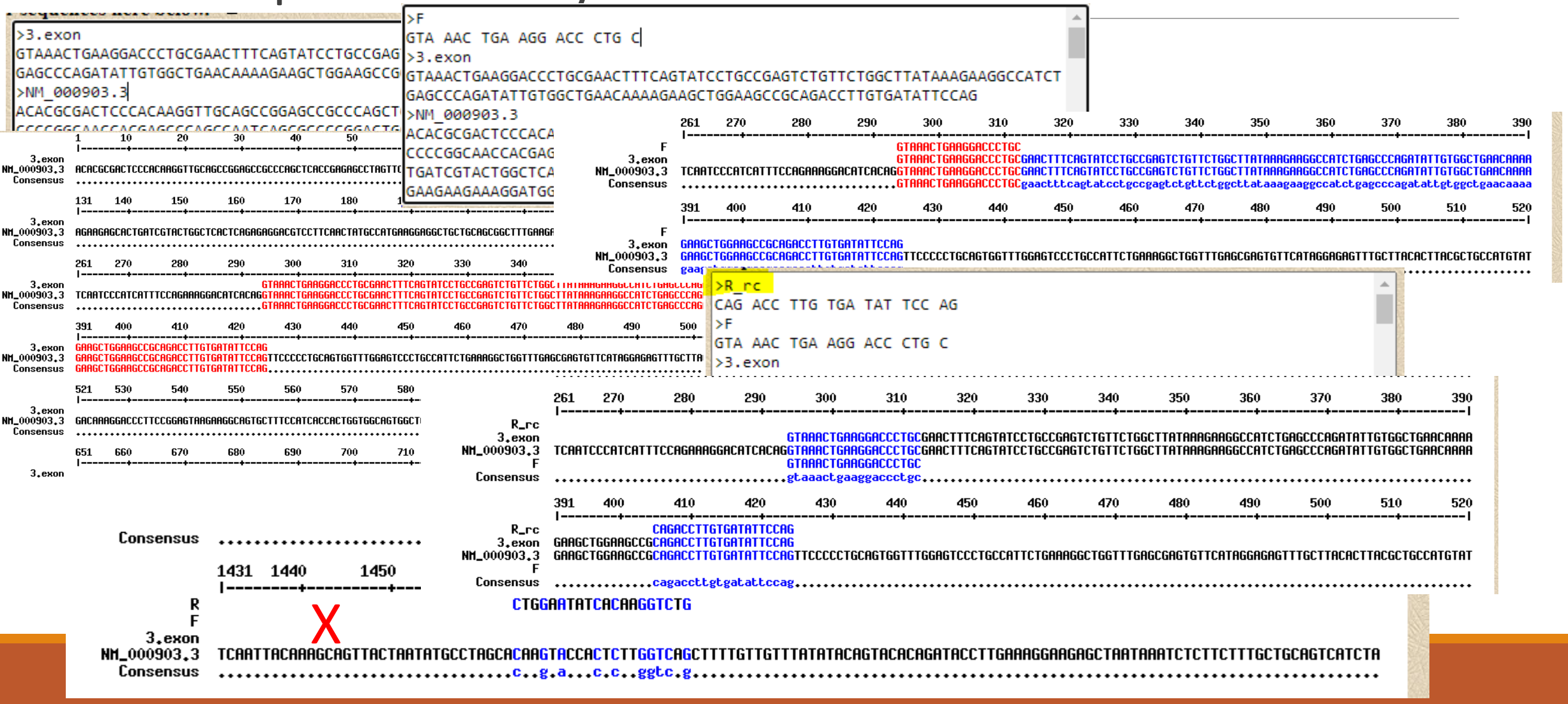
2) mRNA + 3rd exon + F primer +R primer (reverse complement)

```
>R_rc
CAG ACC TTG TGA TAT TCC AG
>F
GTA AAC TGA AGG ACC CTG C
>3.exon
GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG
>NM_000903.3
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCCAGCTCACCGAGAGCCTAGTTCCGGCCAGGGTCG
CCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
```

```
                261       270       280       290       300       310       320       330       340       350       360       370       380       390
                |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
R_rc
3.exon                                                    GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAA
NM_000903.3     TCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAA
F                                                         GTAAACTGAAGGACCCTGC
Consensus       ..................................gtaaactgaaggaccctgc..........................................................................

                391       400       410       420       430       440       450       460       470       480       490       500       510       520
                |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
R_rc                              CAGACCTTGTGATATTCCAG
3.exon          GAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG
NM_000903.3     GAAGCTGGAAGCCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTAT
F
Consensus       ..............cagaccttgtgatattccag..........................................................................
```

Comparison by multalin



Practical part

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

Homework 7

Work with „your“ nucleotide sequence.

---

1) Design manually primers for amplification of your CDS (primers should not have Tm>60°C)
2) Compare the designed primers with your mRNA sequence using Multalin

(don´t forget to reverse complement the R primer)

HW7: solution of NQO1

1) Design manually primers for amplification of your CDS (primers should not have Tm>60°C)

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

R: TCA TTT TCT AGC TTT GAT CTG GTT 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)