Introduction to applied bioinformatics

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2024/2025

7/10

Repetition from last time

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

Repetition from last time

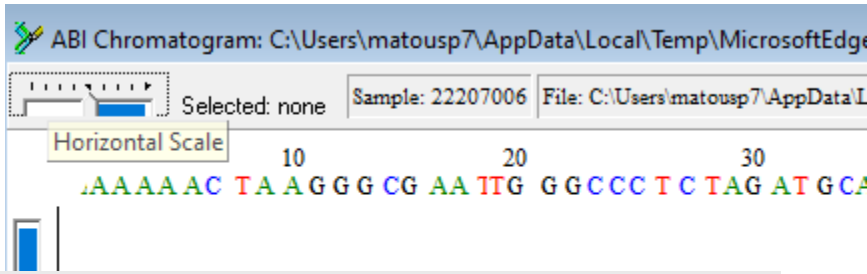
download sequence

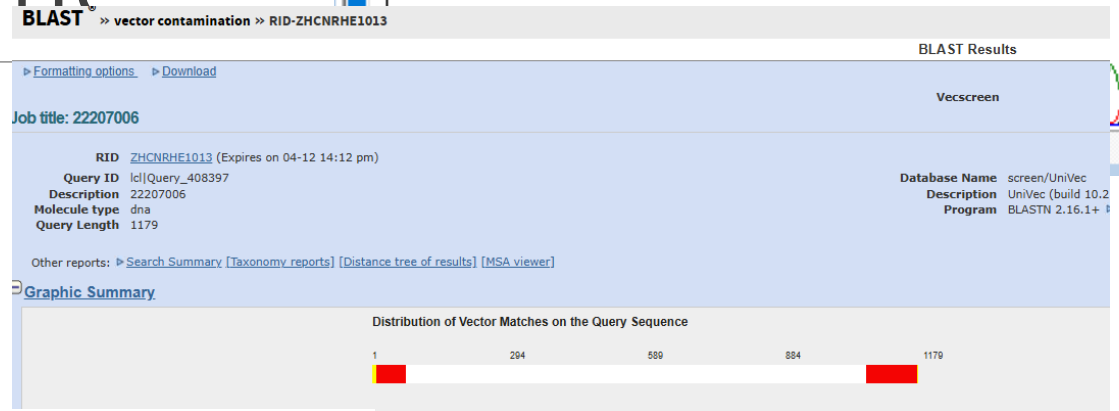
open in chromas

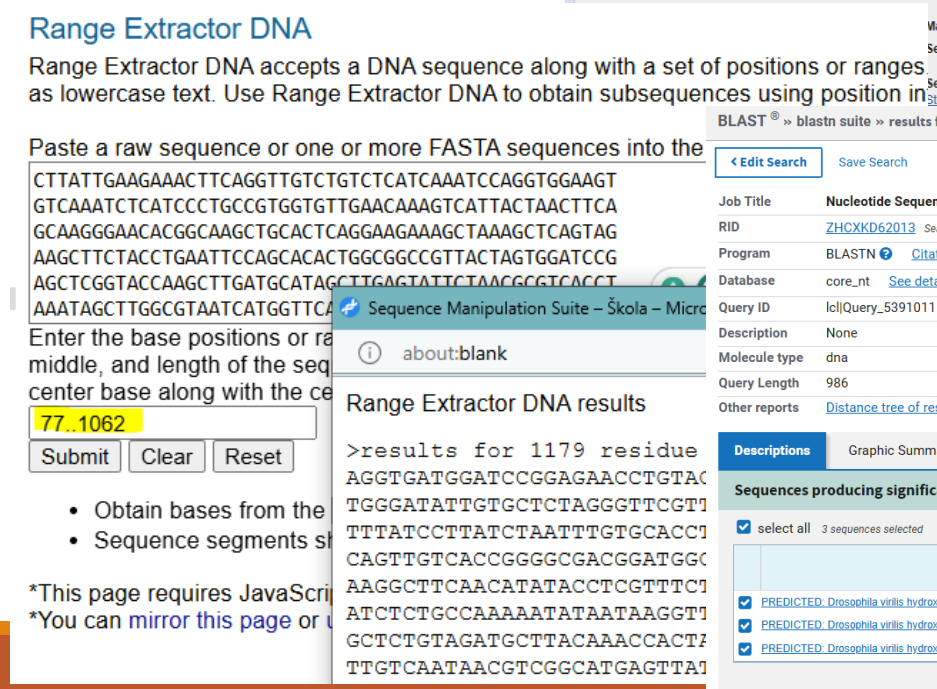
run vecscreen

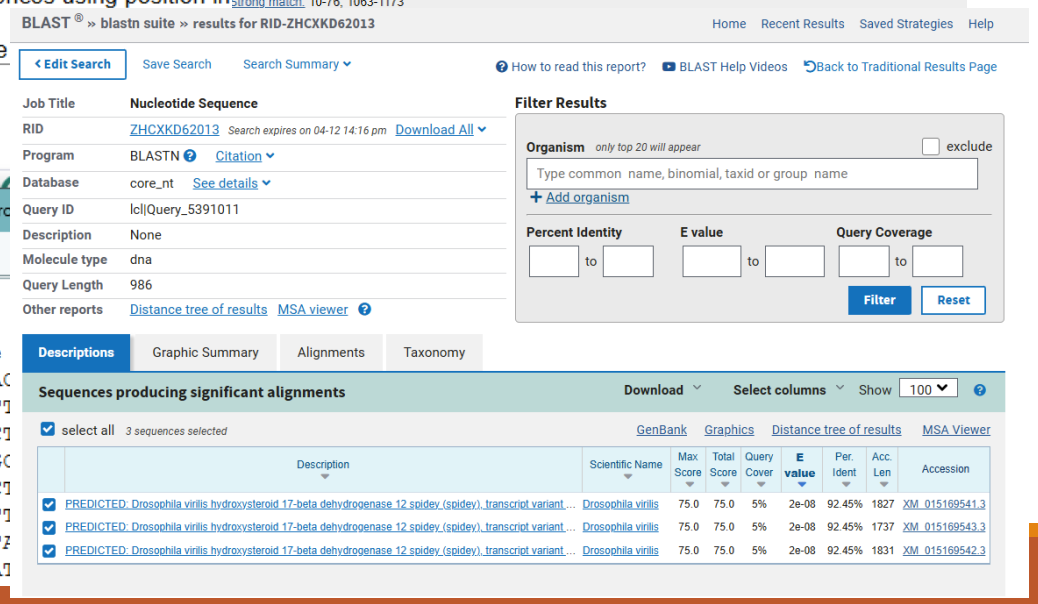
Use range extractor DNA

BLASTn purified sequence









„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

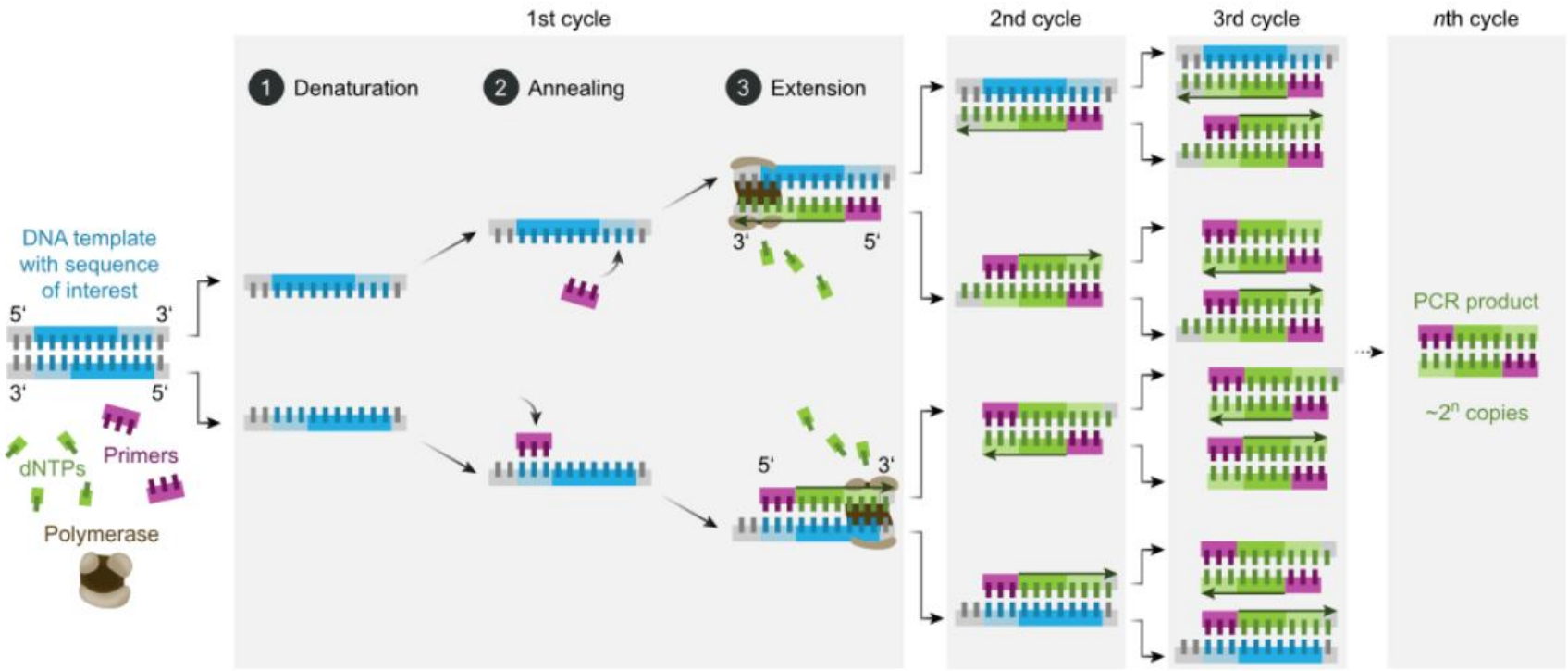
microRNA

Genomes….

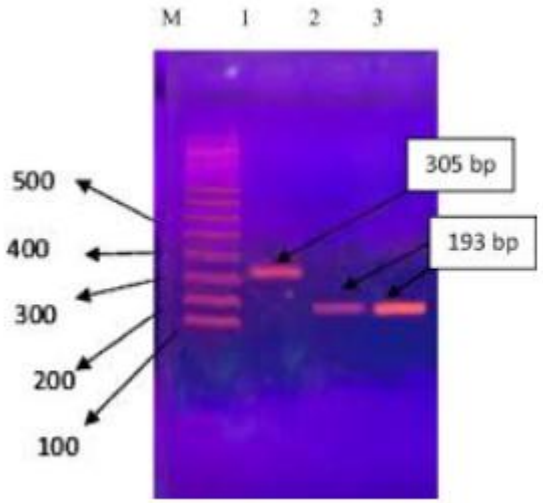
….

Polymerase chain reaction

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



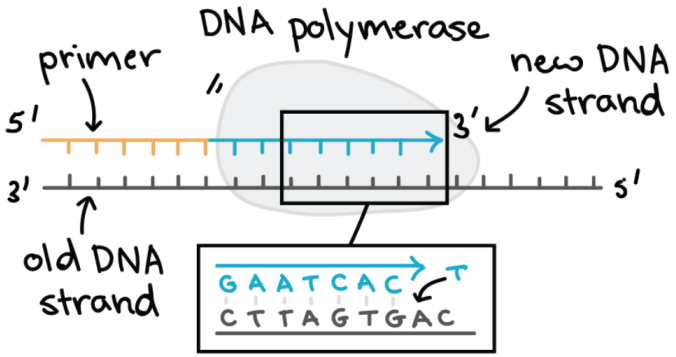
→ „End point“ detection



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

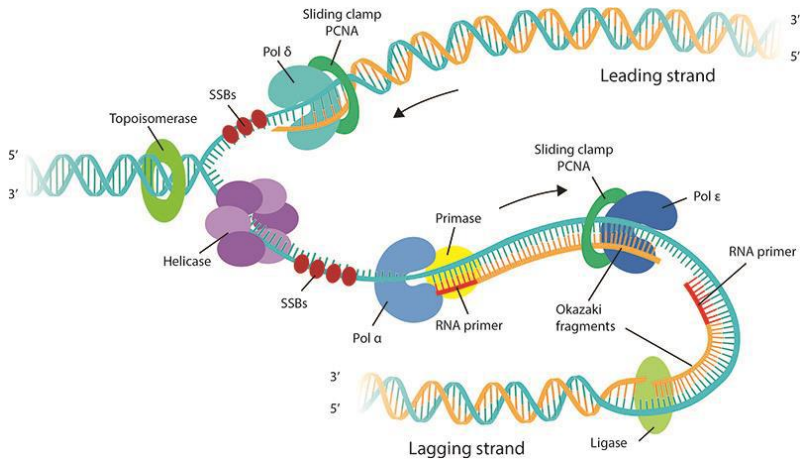
Primer = oligonucleotide

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



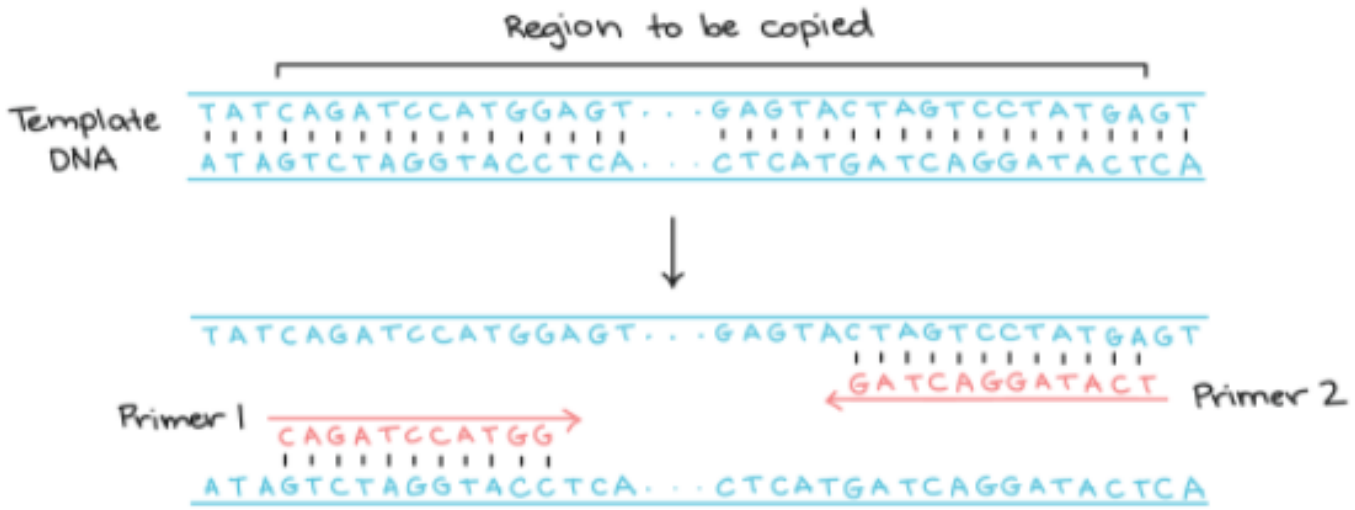
Usage:

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

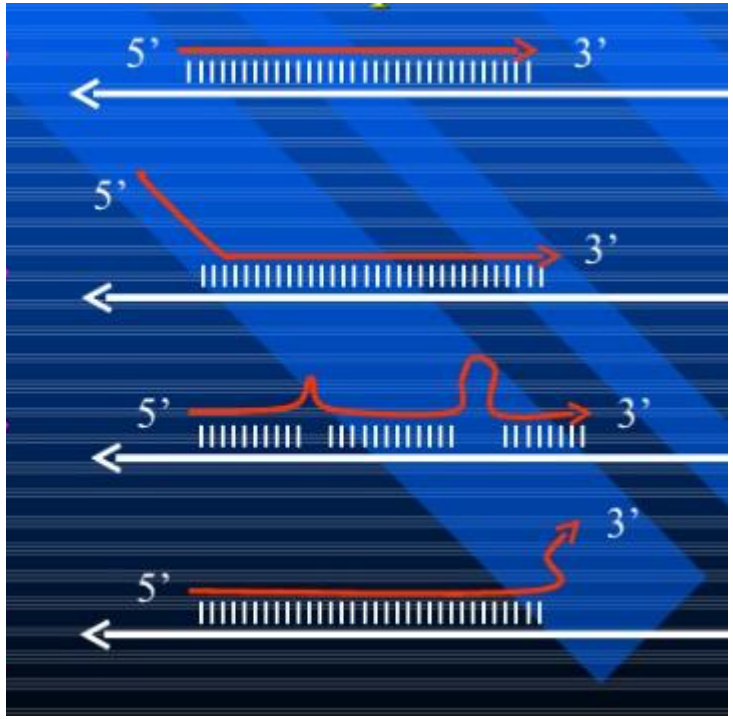


Polymerase chain reaction

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



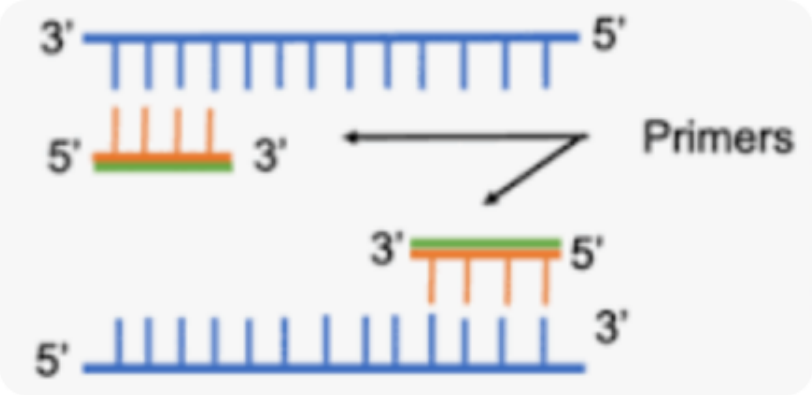
Synthesis: 5´→ 3´

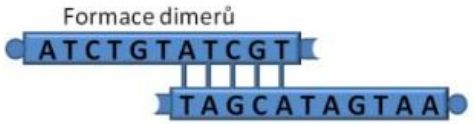


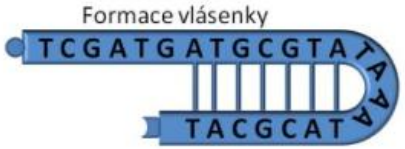
Polymerase chain reaction

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

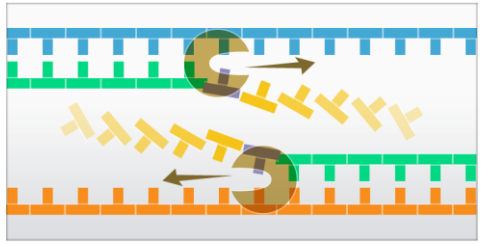
- Length of 18-24 bases.
- 40-60% G/C content.
- Start and end with 1-2 G/C pairs.
- Melting temperature (Tm) of 50-60°C.
- **Primer** pairs should have a Tm within 5°C of each other.
- **Primer** pairs should not have complementary regions.





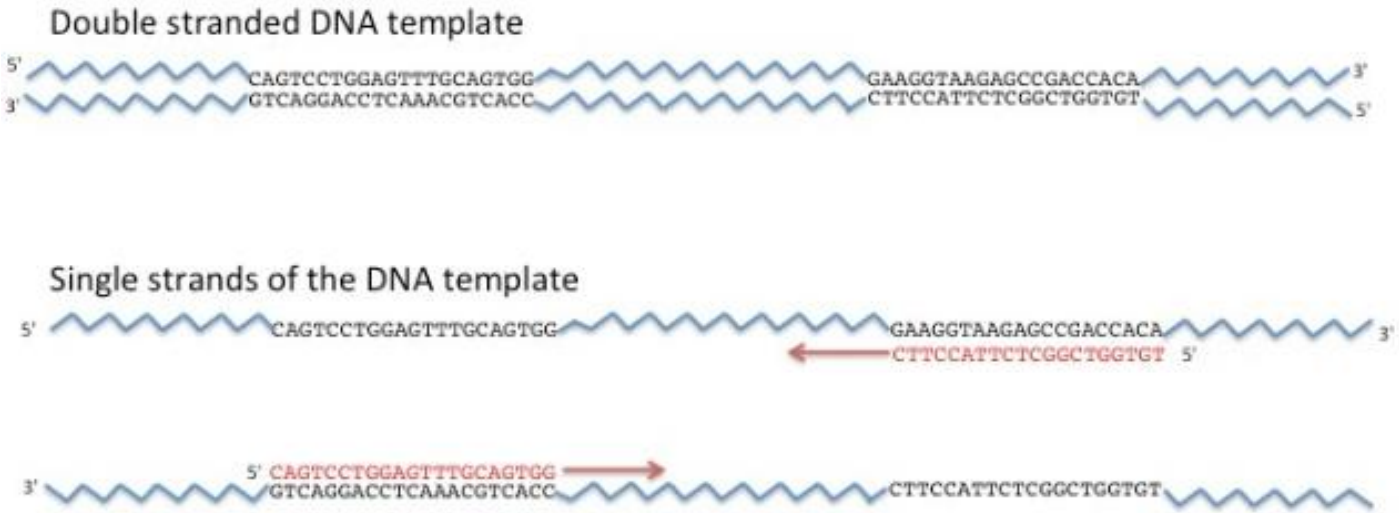


Polymerase chain reaction

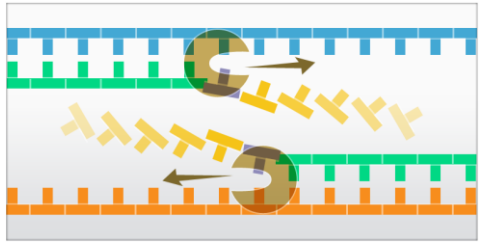


1) Amplification of desired gene / DNA fragment

→ manual design



Polymerase chain reaction



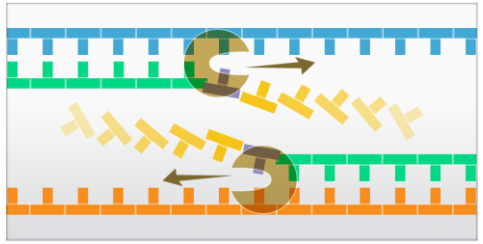
1) Amplification of desired gene / DNA fragment

→ manual design:

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

5´-ATGCCCTTTCnnnnnnnnnnnnnnnTAAATCCCGC-3´

Polymerase chain reaction



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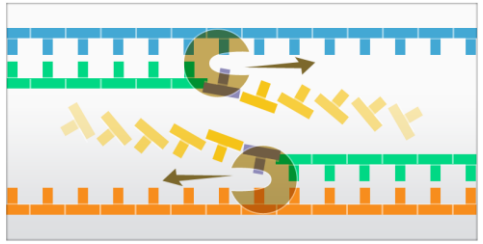
Polymerase chain reaction



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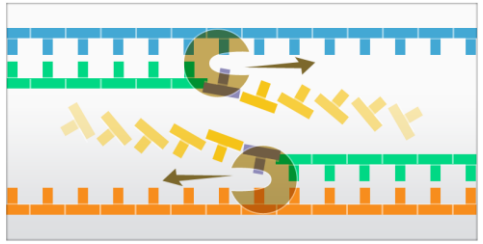
ATTTAGGGCG-5

5´-**ATGCCCTTTC-**

3´-TACGGGAAAGnnnnnnnnnnnnnnnATTTAGGGCG-5´

Polymerase chain reaction



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R_primer: **GCGGGATTTA**

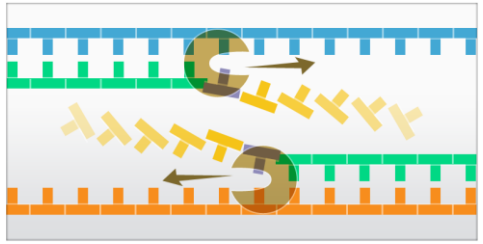
F_primer: **ATGCCCTTTC**

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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R_primer: **GCGGGATTTA**

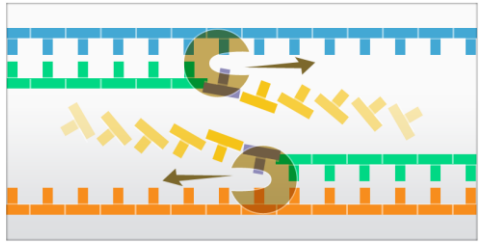
reverse complement:

5´-**GCGGGATTTAnnnnnnnnnnnnnnnGAAAGGGCAT**-3´

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

reverse

Polymerase chain reaction



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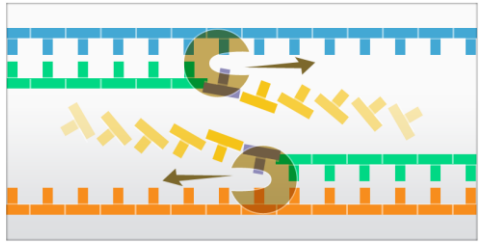
(**3´-TACGGGAAAGnnnnnnnnnnnnnnnATTTAGGGCG-5´**)

reverse

Polymerase chain reaction



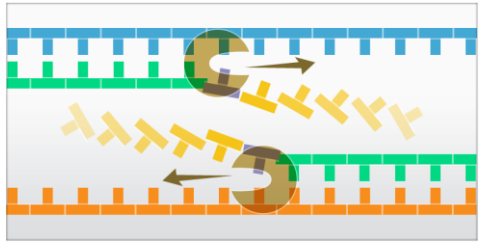
1) Amplification of desired gene / DNA fragment

→ manual design: 2 primers – F (forward)

– R (reverse)

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

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

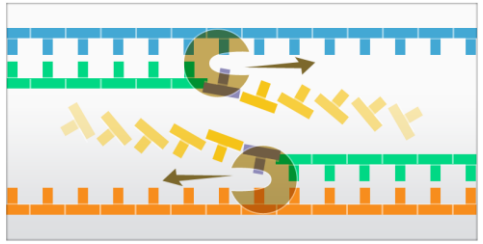
→ manual design: 2 primers – **F (forward)**

– 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
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**

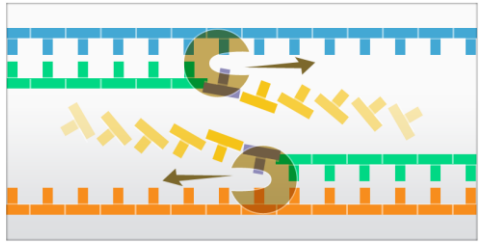
– R (reverse)

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

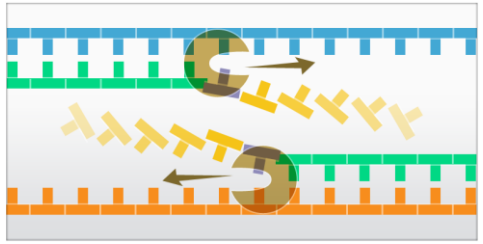
ATGGTCGGCAGAAGAGCACT

→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)

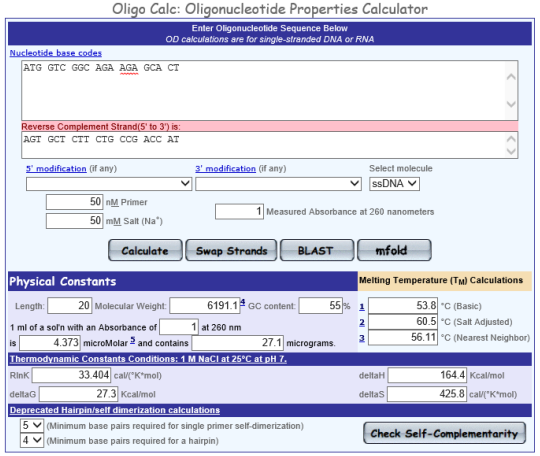
→ re-write sequence from the beginning cca 20nt

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
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```



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: 6191.1 [4] GC content: 55 %

1 ml of a sol'n with an Absorbance of 1 at 260 nm
is 4.373 microMolar [5] 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.

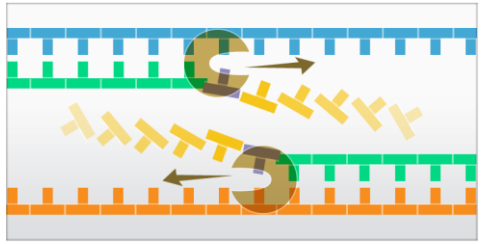
RlnK 33.404 cal/(°K*mol) | deltaH 164.4 Kcal/mol
deltaG 27.3 Kcal/mol | deltaS 425.8 cal/(°K*mol)

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

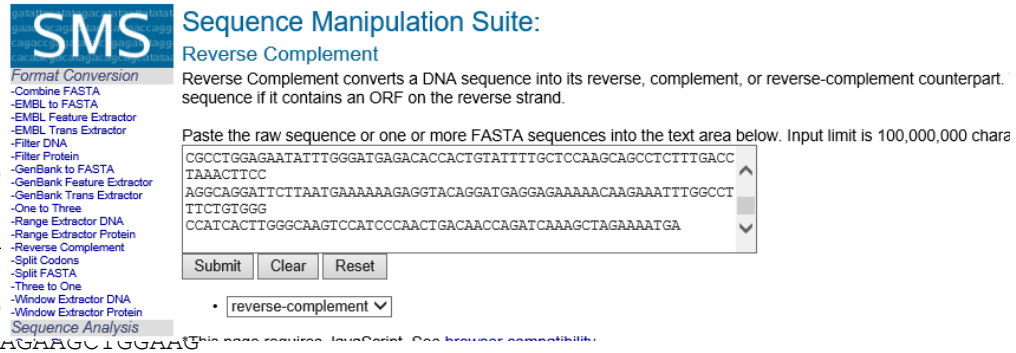
Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

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

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



Aim: amplification of the specific product

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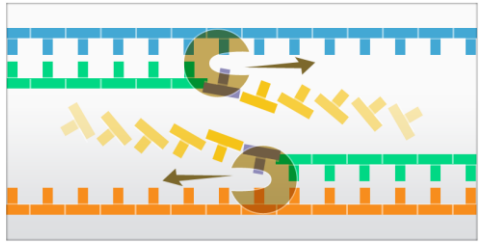
transcript variant 1, mRNA
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACT
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTA
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTT
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Polymerase chain reaction



1) Amplification of desired gene / DNA fragment

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

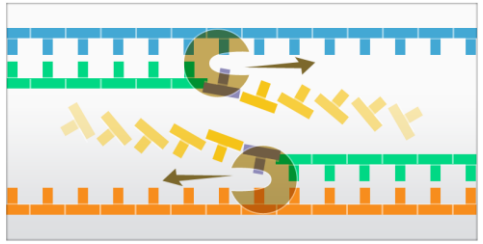
– **R (reverse) → reverse complement (SMS)**

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  reverse complement
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTGATGGCCCAC
AGAAAGGCCAAATTTCTTGTTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGGAAGCCACAGAAATGCAGAAT
GCCACTCTGAATTGGCCAGAGAATGACATTCATGTCCCCGTGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAGTGGTGATGGAAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTCATACATGGCAGCGTAAGTGTAAGCAAACTCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAAACCACTGCAGGGGGAACTGGAATATCACAAGGTC
TGCGGCTTCCAGCTTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGCAGGGTCCTTCAGTTTACCTGTGAT
GTCCTTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCCGACTCCACCACCTC
CCATCCTTTCTTCTTCAAAGCCGGTGCAGCAGCCTCCTTCATGGCATAGTTGAAGGACGT
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**

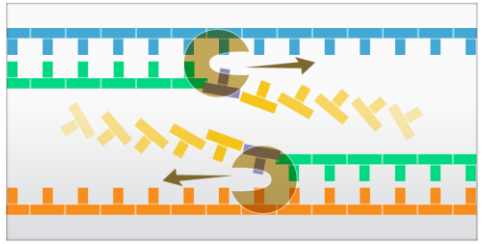
→ re-write sequence from the beginning cca 20nt

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

Aim: amplification of the specific product

TCATTTTCTAGCTTTGATCT

→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:

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

Calculate | Swap Strands | BLAST | mfold

Physical Constants

Length: 20 Molecular Weight: 6039[4] GC content: 30 %

1 ml of a sol'n with an Absorbance of 1 at 260 nm
is 5.257 microMolar[5] 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)

Tm too low →longer primer

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

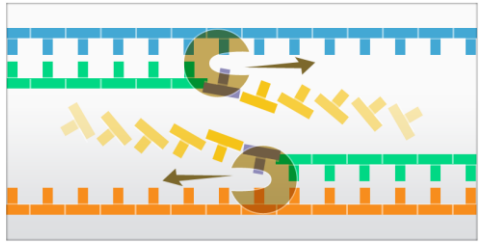
RlnK 33.404 cal/(°K*mol) | deltaH 146.3 Kcal/mol
deltaG 21.4 Kcal/mol | deltaS 386.3 cal/(°K*mol)

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

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

Aim: amplification of the specific product

TCATTTTCTAGCTTTGATCT

→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:

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: 7001.6 [4] GC content: 35 %

1 ml of a sol'n with an Absorbance of 1 at 260 nm
is 4.492 microMolar [5] 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)

better (F: 60.5°C)

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

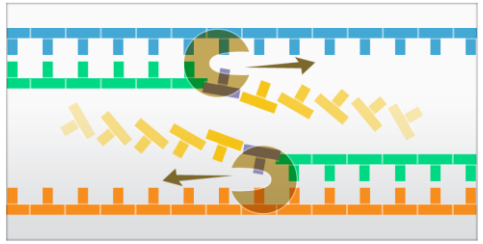
RlnK 33.404 cal/(°K*mol)
deltaG 26.7 Kcal/mol
deltaH 174.8 Kcal/mol
deltaS 461.2 cal/(°K*mol)

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

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

Aim: amplification of the specific product

TCATTTTCTAGCTTTGATCT

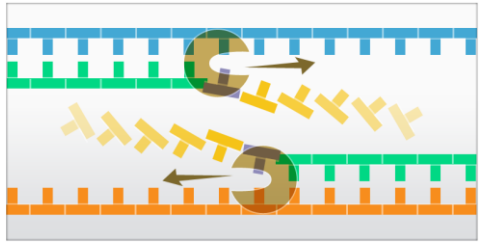
→check the primer (Tm) - OligoCalc

```
>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 the primer to amplify sequence:

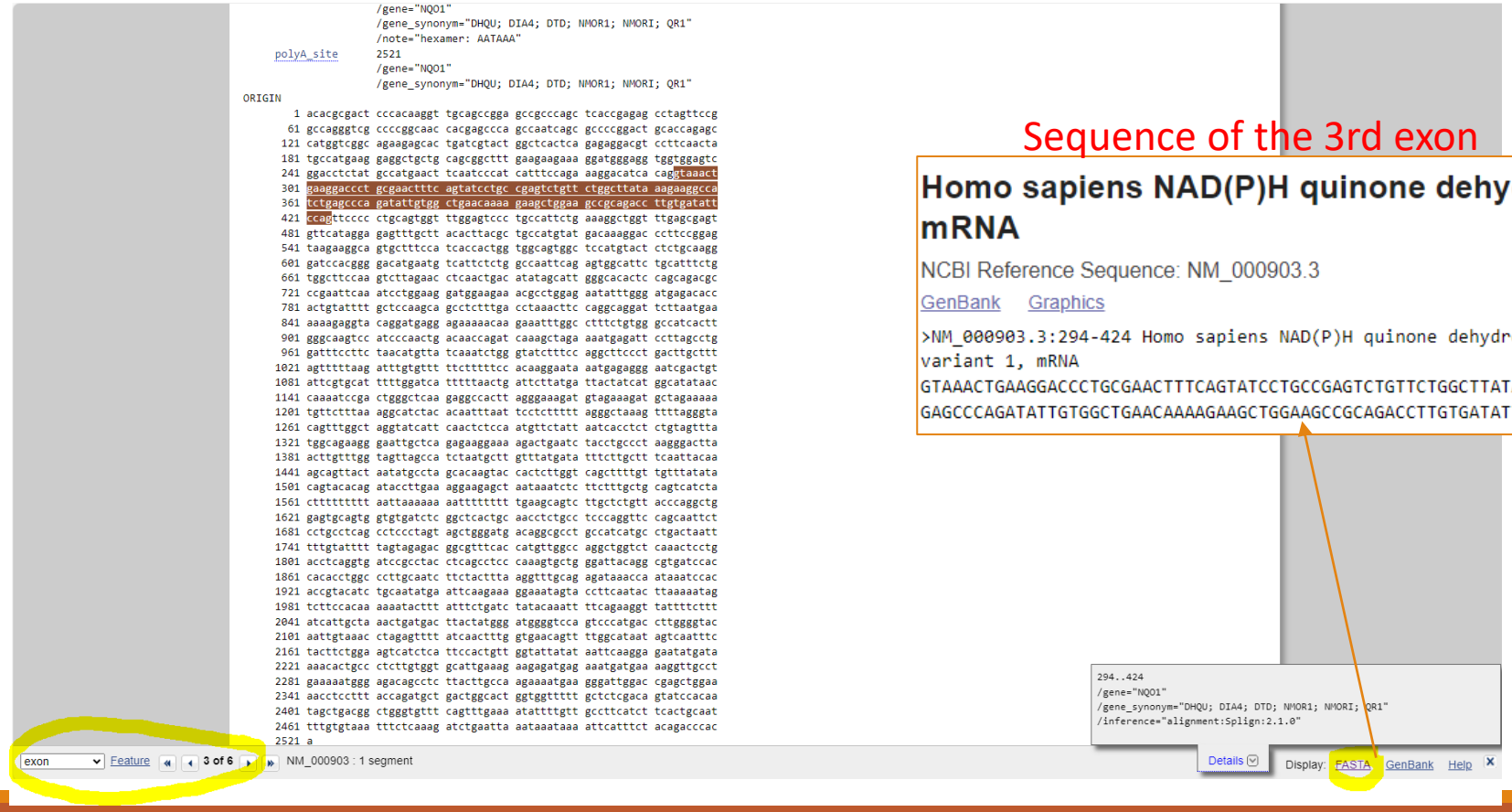
5´-TTTGGAAACCnnnnnnnnnnnnnnnCCCGCCCTTT-3´

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

- length: 18-24nt
- Tm: 55-60°C

-getting 3rd exon

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



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
GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTCCAG
```

Designing primers:

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

Oligonucleotide Properties Calculator

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

Nucleotide base codes
GTA AAC TGA AGG ACC CTG CG

Reverse Complement Strand(5' to 3') is:
CGC AGG GTC CTT CAG TTT AC

5' modification (if any) | 3' modification (if any) | select ss/ds and DNA or RNA
ssDNA

5 (Minimum base pairs required for single primer self-dimerization)
4 (Minimum base pairs required for a hairpin)
50 nM Primer
50 mM Salt (Na^+)

Calculate | Swap Strands | BLAST | Check Self-Complementarity

Physical Constants

Length: 20 bases
GC content: 55 %
Molecular Weight: 6151.1 4
1 ml of a sol'n with an Absorbance of 1 at 260 nm
is 4.47 microMolar 5 and contains 27.5 micrograms.

Melting Temperature (T_M) Calculations

1 54 °C (Basic)
2 60 °C (Salt Adjusted)
3 53 °C (Nearest Neighbor)

>3exon:reverse complement
CTGGAATATCACAAGGTCTGCGGCTTCCAGCTTCTT
TTGTTCAGCCACAATATCTGGGCT
CAGATGGCCTTCTTTATAAGCCAGAACAGACTCGGC
AGGATACTGAAAGTTCGCAGGGTC CTTCAGTTTAC

Oligonucleotide Properties Calculator

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

Nucleotide base codes
CTG GAA TAT CAC AAG GTC TGC

Reverse Complement Strand(5' to 3') is:
GCA GAC CTT GTG ATA TTC CAG

5' modification (if any) | 3' modification (if any) | select ss/ds and DNA or RNA
ssDNA

5 (Minimum base pairs required for single primer self-dimerization)
4 (Minimum base pairs required for a hairpin)
50 nM Primer
50 mM Salt (Na^+)

Calculate | Swap Strands | BLAST | Check Self-Complementarity

Physical Constants

Length: 21 bases
GC content: 48 %
Molecular Weight: 6430.2 4
1 ml of a sol'n with an Absorbance of 1 at 260 nm
is 4.376 microMolar 5 and contains 28.1 micrograms.

Melting Temperature (T_M) Calculations

1 52 °C (Basic)
2 60 °C (Salt Adjusted)
3 51 °C (Nearest Neighbor)

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

RlnK 33.404 cal/(°K*mol) | deltaH 163.9 Kcal/mol
deltaG 25.4 Kcal/mol | deltaS 430.5 cal/(°K*mol)

To use this calculator, you must be using Netscape 3.0 or later
or Internet Explorer version 3.0 or later, or another Javascript-capable browser
Self-Complementarity requires a 4.x browser. IE 5.0, Safari, and Mozilla supported.
This page was written in Javascript.
Extensively rewritten from 12/15/2000-12/19/2000 to isolate javascript Oligo object behaviors for teaching purposes.
This page may be freely distributed for any educational or non-commercial use.
Copyright Northwestern University, 1997-2006

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

```
>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
CCCCGGCAACCACGAGCCCAGCCAATCAGCGCCC
```

```
                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.........................................................................................................
```

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

X

incorrect

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.....................................................................
```

Practical part HW:

1) Design primers manually for amplification of

your CDS

- length: 18-24nt
- Tm: 55-60°C

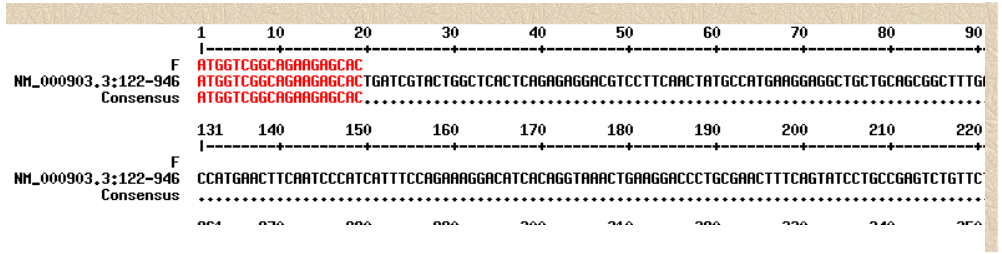
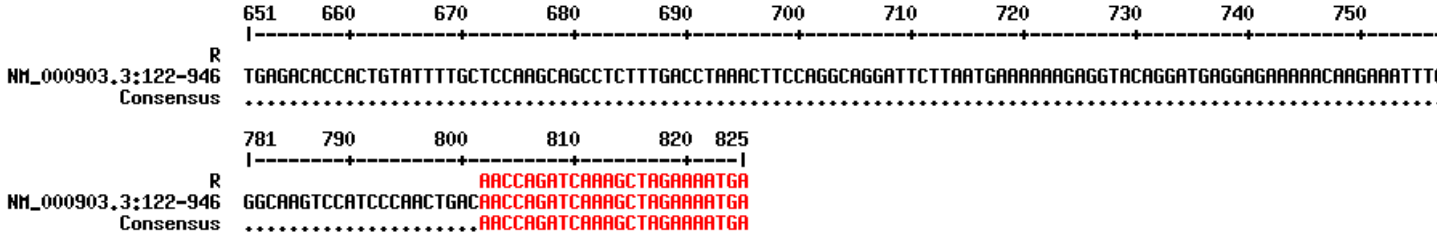
2) check the primers position by Multalin: mRNA, CDS, F primer, R primer

HW7: solution of 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 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)