

# Základy praktické Bioinformatiky

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PETRA MATOUŠKOVÁ

2023/2024

7/10

## Nukleotidová bioinformatika III

### Cíle:

Student bude schopen navrhnout primery pro namnožení požadovaného úseku genu, udělat restriční analýzu a zkontrolovat jak by vypadal výsledek štěpení.

# „Bioinformatika nukleových kyselin II“

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Vyhledávání NK sekvencí

Analýza vlastností sekvencí-složení, reverse complement, **identifikace restrikčních míst (Palindromy)**

Práce s kódující DNA=práce s proteiny / překlad DNA sekvence-otvírání čtecího rámce

**Návrh primerů pro PCR**, rt-PCR, bodovou mutagenezi

Předpověď sekundárních struktur

Porovnávání sekvencí, identifikace neznámé sekvence

(Vyhledání SNPs)

„čtení“ sekvenačních dat a spojování fragmentů

Vyhledávání hladin expresí jednotlivých genů

mikroRNA

Celé genomy

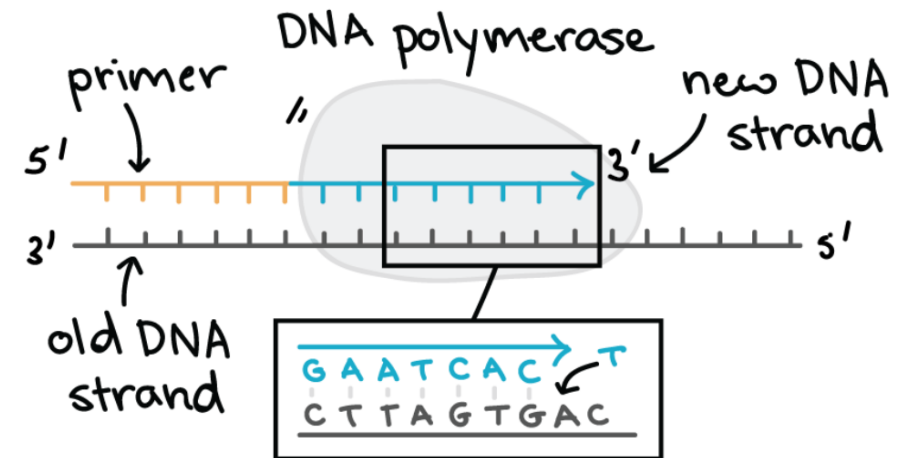
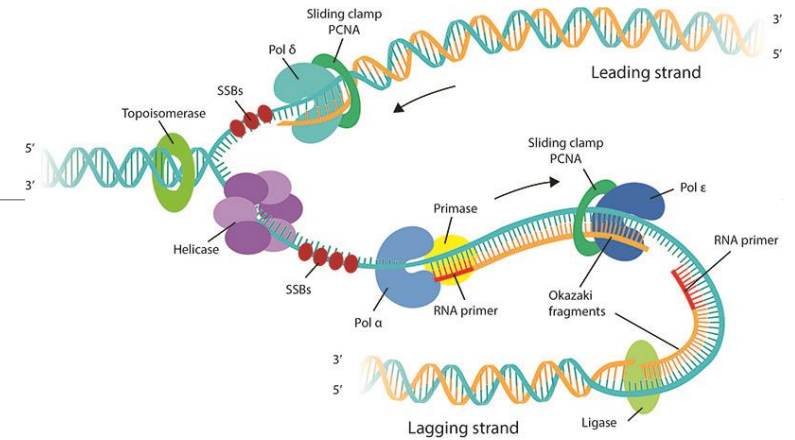
....

# Primer = oligonukleotid

= krátký jednořetězcový úsek DNA

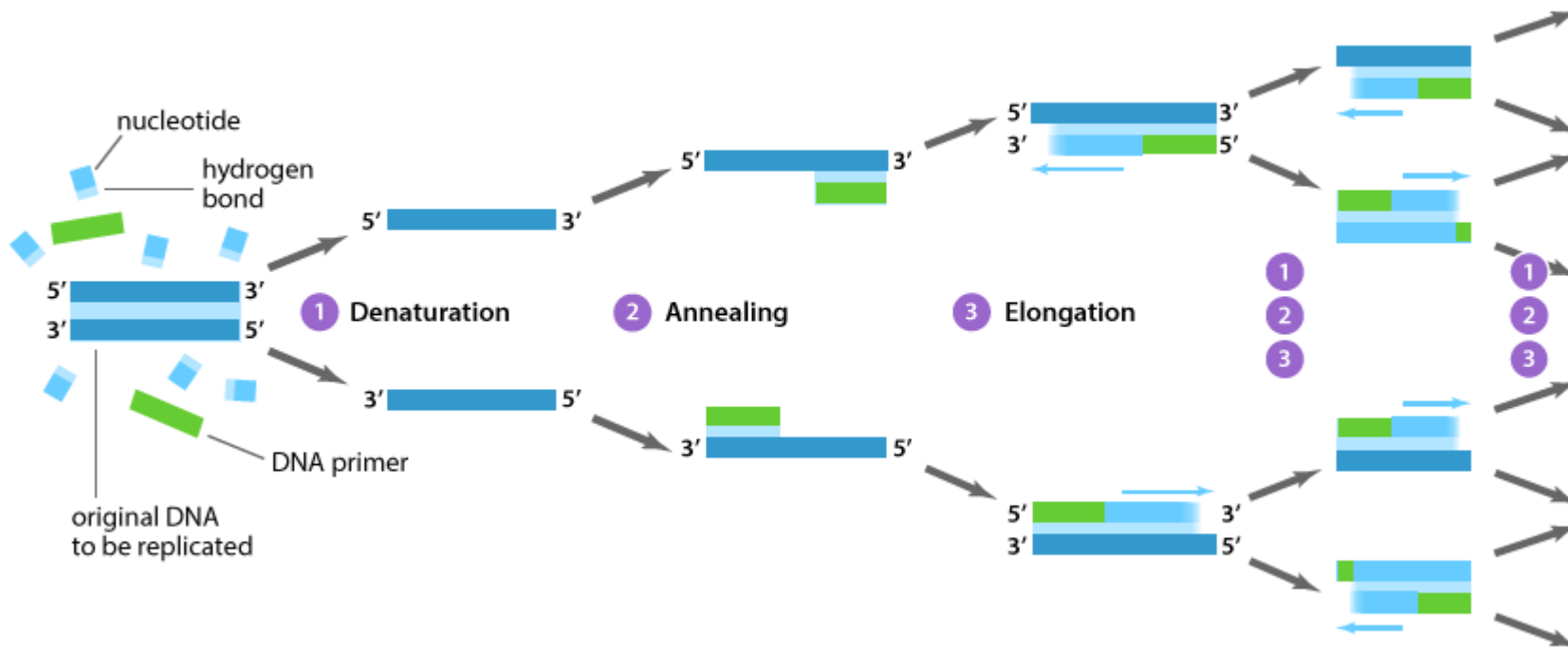
Použití:

- PCR (namnožení genu/úseku, detekce genu, mutace, RT-PCR...)
- Reverzní transkripce (oligo(dT), GSP, hexamers)
- sekvenování

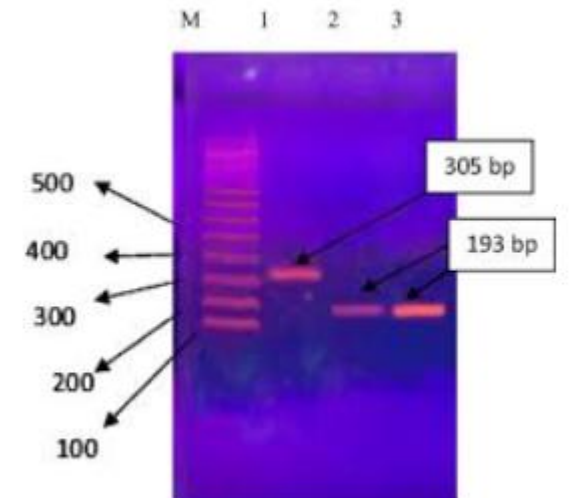


# Polymerázová řetězová reakce

Polymerase chain reaction (PCR) → namnožení (amplifikace) úseku DNA vymezeného primery

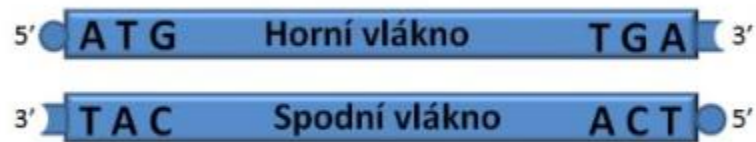


→ „End point“ detekce



# Polymerázová řetězová reakce

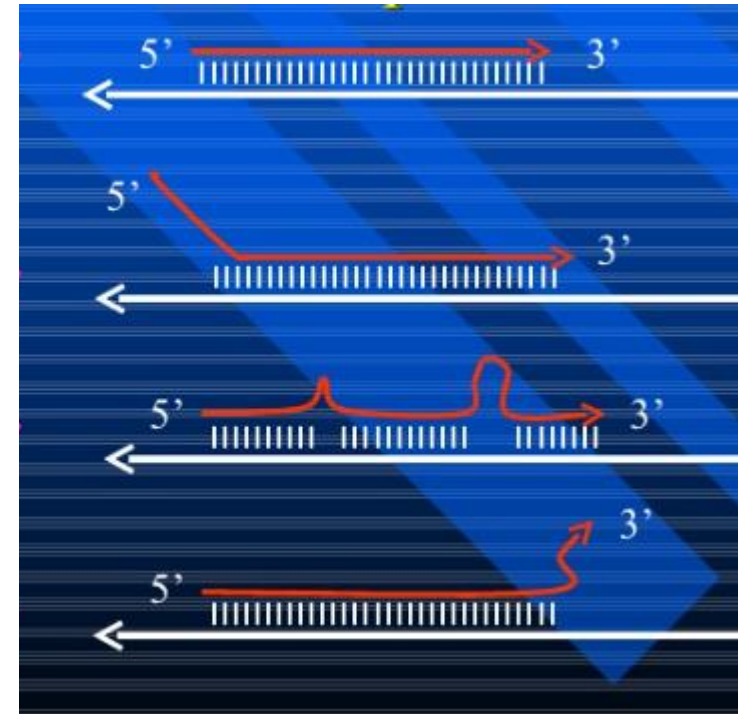
Polymerase chain reaction (PCR) → namnožení (amplifikace) úseku DNA vymezeného primery



Nasedání primerů a směr syntézy



Syntéza: 5' → 3'



# Polymerázová řetězová reakce

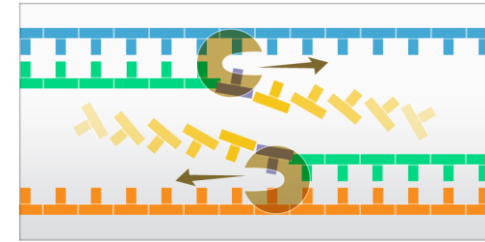
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## Charakteristiky „dobré“ dvojice primerů:

- T<sub>m</sub> rozdíl (<1-3°C)
- Podobná délka (17-28nt)
- Podobné složení bází (GC≅50-60%)
- Nepřítomnost delších úseků ze stejných bází (>4)
- Nepřítomnost sekundárních struktur (vnitřní vlásenky)
- Nepřítomnost tvorby dimerů
- (Málo GC nukleotidů na 3' konci) ALE jeden G/C na úplném konci ano
  - (Specifičnost)

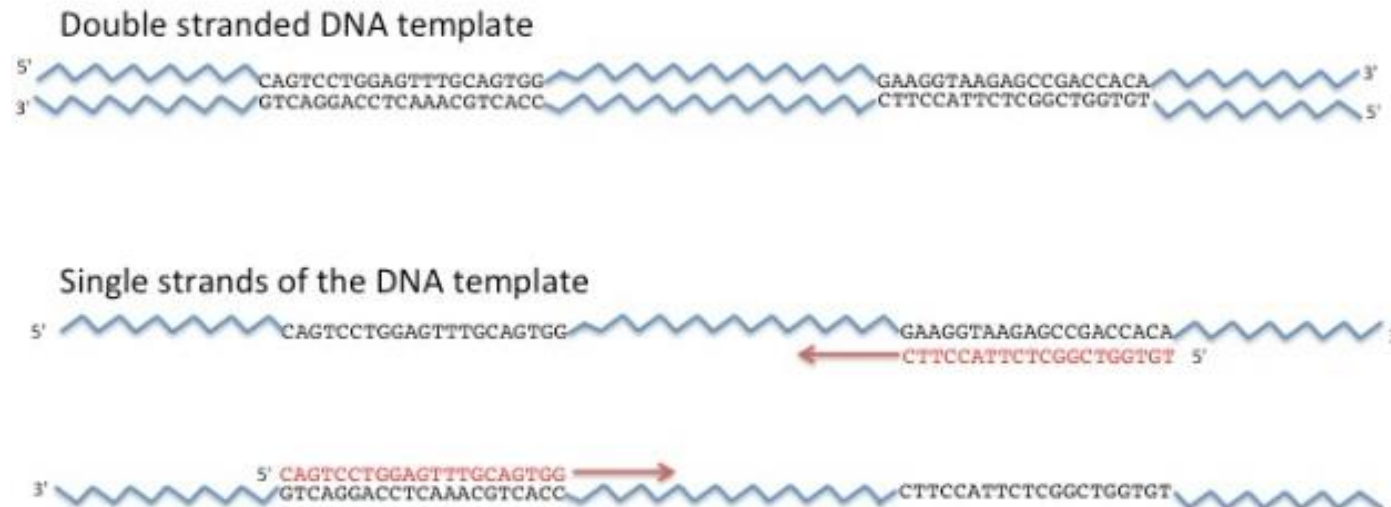


# Polymerázová řetězová reakce



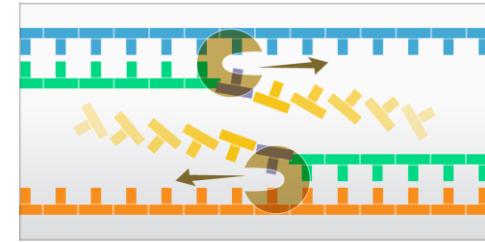
1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh



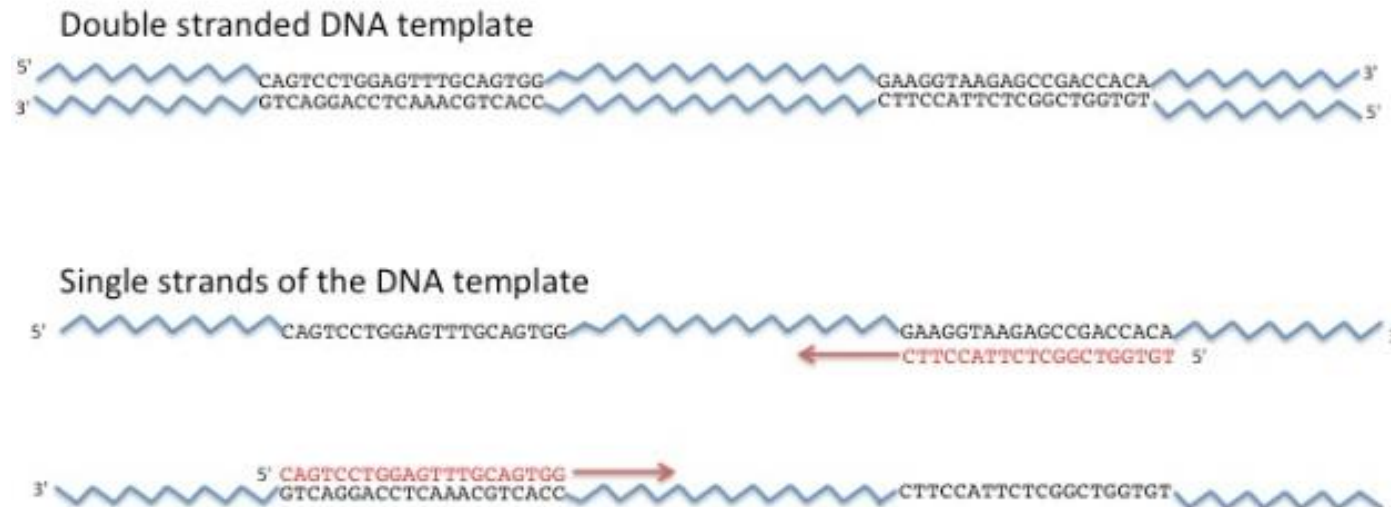


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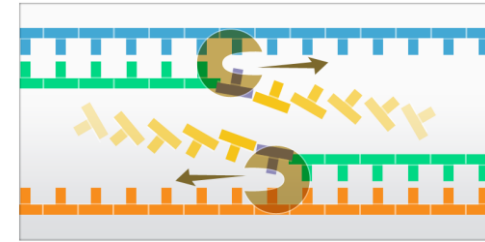


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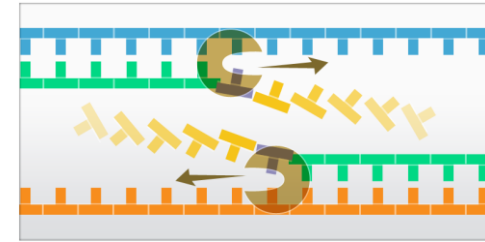
1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh

➤ navrhňte (krátké) primery pro sekvenci:

**5'-ATGCCCTTTCnnnnnnnnnnnnnnnnTAAATCCCGC-3'**

# Polymerázová řetězová reakce



1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

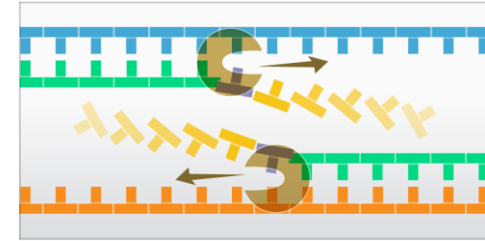
→ manuální návrh

➤ navrhňte (krátké) primery pro sekvenci:

5'-ATGCCCTTTCnnnnnnnnnnnnnnnnnnTAAATCCCGC-3'

3'-TACGGGAAAGnnnnnnnnnnnnnnnnnnATTAGGGCG-5'

# Polymerázová řetězová reakce



1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh

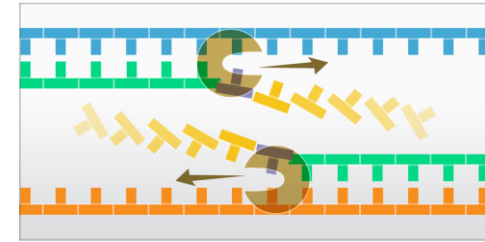
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5'-ATGCCCTTTCnnnnnnnnnnnnnnnnnnTAAATCCCGC-3'  
ATTAGGGCG-5'  
←

→  
5'-ATGCCCTTTC-

3'-TACGGGAAAGnnnnnnnnnnnnnnnnnnATTAGGGCG-5'

# Polymerázová řetězová reakce



1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh

➤ navrhňte (krátké) primery pro sekvenci:

R\_primer: **GCGGGATTTA**

**5'-ATGCCCTTTCnnnnnnnnnnnnnnnnTAAATCCCGC-3'**

F\_primer: **ATGCCCTTTC**

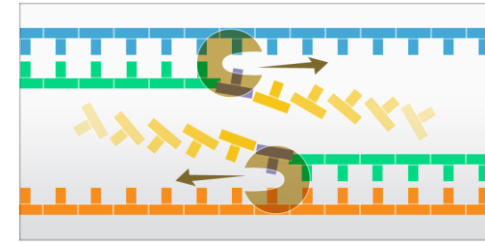
**ATTAGGGCG-5'**

5' - **ATGCCCTTTC** -

**pro zápis finálních F a R primerů směr psaní 5'-3' !**

**3'-TACGGGAAAGnnnnnnnnnnnnnnnnATTAGGGCG-5'**

# Polymerázová řetězová reakce



1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh

➤ navrhňte (krátké) primery pro sekvenci:

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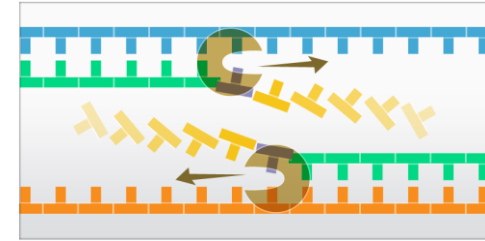
**5'-ATGCCCTTTCnnnnnnnnnnnnnnnnnnTAAATCCCGC-3'**

reverse complement:

**5'-GCGGGATTTAnnnnnnnnnnnnnnnnnGAAAGGGCAT-3'**

**(3'-TACGGGAAAGnnnnnnnnnnnnnnnnATTAGGGCG-5')**

# Polymerázová řetězová reakce



1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh

➤ navrhňte (krátké) primery pro sekvenci:

R\_primer: GCGGGATTTA

5'-ATGCCCTTTCnnnnnnnnnnnnnnnnTAAATCCCGC-3'

reverse complement:

5'-GCGGGATTTAnnnnnnnnnnnnnnnnnGAAAGGGCAT-3'

(3'-TACGGGAAAGnnnnnnnnnnnnnnnnATTAGGGCG-5')

# PCR-amplifikace požadovaného úseku

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→ manuální návrh: 2 primery

Forward

-horní primer

Reverse

-dolní primer (z „reverse complement“)

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1  
(NQO1), transcript variant 1, mRNA  
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG  
AGGCTGCTGCAGCGGCTTTGAAGAAGAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT  
CAATCCCATCATTCCAGAAAGGACATCACAGGTAAGTGAAGGACCTGCGAACTTTCAGTATCCTGCC  
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG  
CCGCAGACCTTGTGATATCCAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT  
TGAGCGAGTGTTCATAGGAGAGTTTGGCTTACACTTACGCTGCCATGTATGACAAAGGACCTTCCGGAGT  
AAGAAGGCAGTGTCTTCCATCACCACCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG  
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC  
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCCGAATTCAAATCCTGGAAGGATGGAAGAAA  
CGCCTGGAGAATATTTGGGATGAGACACCCTGTATTTTGTCCAAGCAGCCTCTTTGACCTAAACTTCC  
AGGCAGGATCTTAAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGG  
CCATCACTTGGGCAAGTCCATCCCACTGACAACCAGATCAAAGCTAGAAAATGA
```



# PCR-amplifikace požadovaného úseku

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→ manuální návrh: 2 primery

**Forward primer**

→ primer se „opíše“ – cca 20-22 (18-24) nt  
→ a zkontroluje

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1  
(NQO1), transcript variant 1, mRNA  
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG  
AGGCTGCTGCAGGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT  
CAATCCCATCATTTCCAGAAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC  
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG  
CCGCAGACCTTGTGATATCCAGTTCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT  
TGAGCGAGTGTTCATAGGAGAGTTTGGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTCCGGAGT  
AAGAAGGCAGTGTCTTCCATCACCACCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG  
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC  
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCCGAATTCAAATCCTGGAAGGATGGAAGAAA  
CGCTGGAGAATATTTGGGATGAGACACCCTGTATTTTGTCCAAGCAGCCTTTTGACCTAAACTTCC  
AGGCAGGATCTTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGG  
CCATCACTTGGGCAAGTCCATCCCACTGACAACCAGATCAAAGCTAGAAAATGA
```

# PCR-kontrola manuálně navržených primerů: OligoCalc

## Oligo Calc: Oligonucleotide Properties Calculator

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

**Nucleotide base codes**

CAG TAT CCT GCC GAG TCT GT

Reverse Complement Strand(5' to 3') is:  
ACA GAC TCG GCA GGA TAC TG

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

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

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

**Physical Constants** **Melting Temperature (T<sub>M</sub>) Calculations**

Length:  Molecular Weight:  GC content:  %

1 ml of a sol'n with an Absorbance of  at 260 nm  
is  microMolar and contains  micrograms.

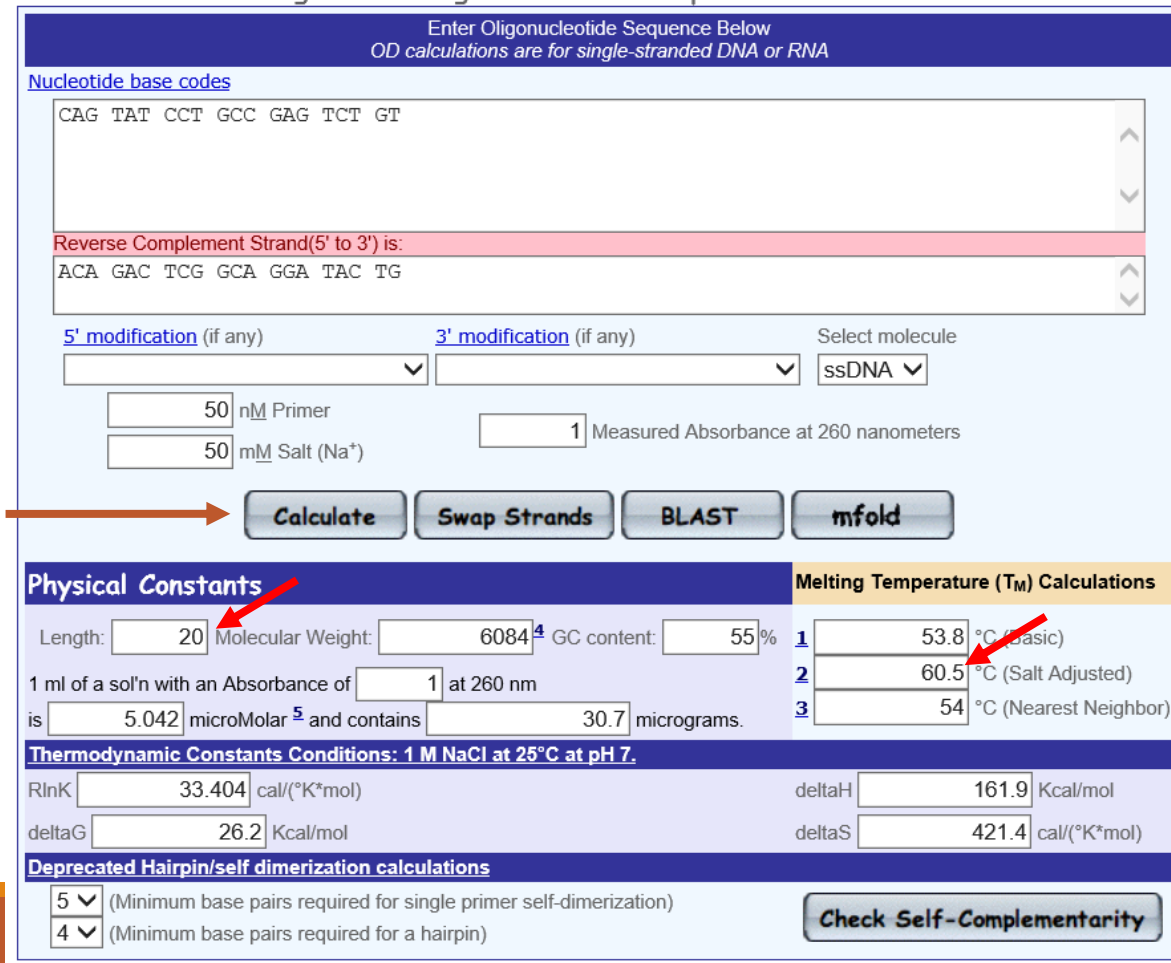
**Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7.**

RlnK  cal/(°K\*<sup>4</sup>mol) deltaH  Kcal/mol  
deltaG  Kcal/mol deltaS  cal/(°K\*<sup>3</sup>mol)

**Deprecated Hairpin/self dimerization calculations**

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

**Check Self-Complementarity**



# PCR-amplifikace požadovaného úseku

→ manuální návrh: 2 primery

Forward primer

**Reverse primer** → celá sekvence „reverse complement“

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCAGAGAGGACGTCCTTCAACTATGCCATG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGA
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAGTGAAGGACCTGCGAAGCTTTCAGTATCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTTGGCTGAACAAAAGAAGCTG
CCGCAGACCTTGTGATATCCAGTTCACCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCT
TGAGCAGTGTTCATAGGAGAGTTTGGCTTACACTTACGCTGCCATGTATGACAAAGGACCTTCCG
AAGAAGGCAGTGTTCATCACCACCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCAC
ACATGAATGTCATTTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGA
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCCGAATTCAAATCCTGGAAGGATGGAAGAA
CGCCTGGAGAATATTTGGGATGAGACACCACCTGTATTTTGCCTCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATCTTAAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACAGATCAAGCTAGAAAAATGA
```

SMS  
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

## Sequence Manipulation Suite:

### Reverse Complement

Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart. Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart. Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart. Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart.

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

```
CGCCTGGAGAATATTTGGGATGAGACACCACCTGTATTTTGTCTCCAAGCAGCCTCTTTGACC
TAAACTTCC
AGGCAGGATCTTAAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCT
TTCTGTGGG
CCATCACTTGGGCAAGTCCAT
```

Reverse Complement results

Submit Clear Resi >NM\_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement

```
TCATTTTCTAGCTTTGATCTGGTTGTCAGTTGGGATGGACTTGCCCAAGTATGGCCAC
AGAAAGGCCAAATTTCTTTTCTCTCCTACCTGTACCTCTTTTTCATTAAGAATCC
TGCCCTGGAAGTTTAGTCAAAGAGGCTGCTTGGAGCAAATACAGTGGTGTCTCATCCCA
AATATTTCCAGGCGTTTCTTCCATCCTTCCAGGATTTGAATTCGGGCGTCTGTCTGGAGT
GTGCCCAATGCTATATGTCAGTTGAGGTTCTAAGACTTGAAGCCACAGAAATGCAGAAT
GCCACTTGAATTGGCCAGAGAATGACATTCATGCCCCGGATCCCTTGCAGAGAGTA
CATGGAGCCACTGCCACCAAGTGGTATGGAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTACATACATGGCAGCGTAAGTGAAGCAAACTCTCTATGAACACTCGCTCAAACA
GCCTTTCAGAATGGCAGGACTCCAAACCACTGCAGGGGAACTGGAATATCACAAGGTC
TGCCGCTTCCAGCTCTTTTGTTCAGCCACAATATCTGGGCTCAGATGGCCTCTTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTTCGACGGTCTCTCAGITTACCTGTGAT
GTCCTTCTGGAAATGATGGGATTGAAGTTCATGGCATAGAGGTCGACTCCACCACCTC
CCATCCTTCTTCTTCAAAGCCGCTGCAGCAGCCTCTTTCATGGCATAGTTGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTCTCTTCTGCCACCAT
```

# PCR-amplifikace požadovaného úseku

→ manuální návrh: 2 primery

Forward primer

**Reverse primer** → celá sekvence „reverse complement“

→ primer se „opíše“ – cca 20-22 (18-24) nt

→ a zkontroluje

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTCCAGAAAGGACATCACAGGTAAACTGAAGGACCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATCCAGTTCGCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCTTCCGGAGT
AAGAAGGCAGTGTTCATCACCACCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAAC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCCTGTATTTTGTCCAAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Reverse Complement results

```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA reverse complement
TCATTTCTAGCTTTGATCTGGTTGTCAGITGGGATGGACTTGCCCAAGTGATGGCCAC
AGAAAGGCCAAATTTCTGTTTTCTCCTCATCCTGTACCTCTTTTTTCATTAAGAATCC
TGCTGGAAGTTTAGGTCAAAGAGGCTGCTTGGAGCAAAATACAGTGGTGTCTCATCCCA
AATATTCAGAGCGTTTCTCCATCCTCCAGGATTTGAATTCGGGCGTCTGCTGGAGT
GTGCCAATGCTATATGTCAGITGAGGTTCTAAGACTTGGAAAGCCACAGAAATGCAGAA
GCCACTTGAATGGCCAGAGAATGACATTCATGCCCCGGGATCCCTTGACAGAGATA
CATGGAGCCACTGCCACAGTGGTATGGAAGCACTGCCTTCTTACTCCGGAAGGGTCC
TTTGTACATACATGGCAGCGTAAGTGTAAAGCAAATCTCCTATGAACACTCGCTCAAACCA
GCCTTTCAGAATGGCAGGGACTCCAACCCTGCAGGGGAACTGGAATATACAAGGTC
TGCGGCTTCCAGCTTCTTTGTTGAGCCCAATATCTGGGCTCAGATGGCCTTCTTATA
AGCCAGAACAGACTCGGCAGGATACTGAAAGTTCGAGGGTCTTACGTTTACCTGTGAT
GTCTTTCTGGAATGATGGGATGAAAGTTCATGGCATAGAGGTCAGCTCCACCACTC
CCATCCTTCTTCTTCAAAGCCGCTGCAGCAGCCTCCTTCATGGCATAGITGAAGGACGT
CCTCTCTGAGTGAGCCAGTACGATCAGTGCTCTTCTGCCGACCAT
```

# PCR-kontrola manuálně navržených primerů: OligoCalc

## Oligo Calc: Oligonucleotide Properties Calculator

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

Nucleotide base codes

CAG TAT CCT GCC GAG TCT GT

Reverse Complement Strand(5' to 3') is:  
ACA GAC TCG GCA GGA TAC TG

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

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

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

**Physical Constants** **Melting Temperature (T<sub>M</sub>) Calculations**

Length:  Molecular Weight:  GC content:  %  
1 ml of a sol'n with an Absorbance of  at 260 nm  
is  microMolar and contains  micrograms.

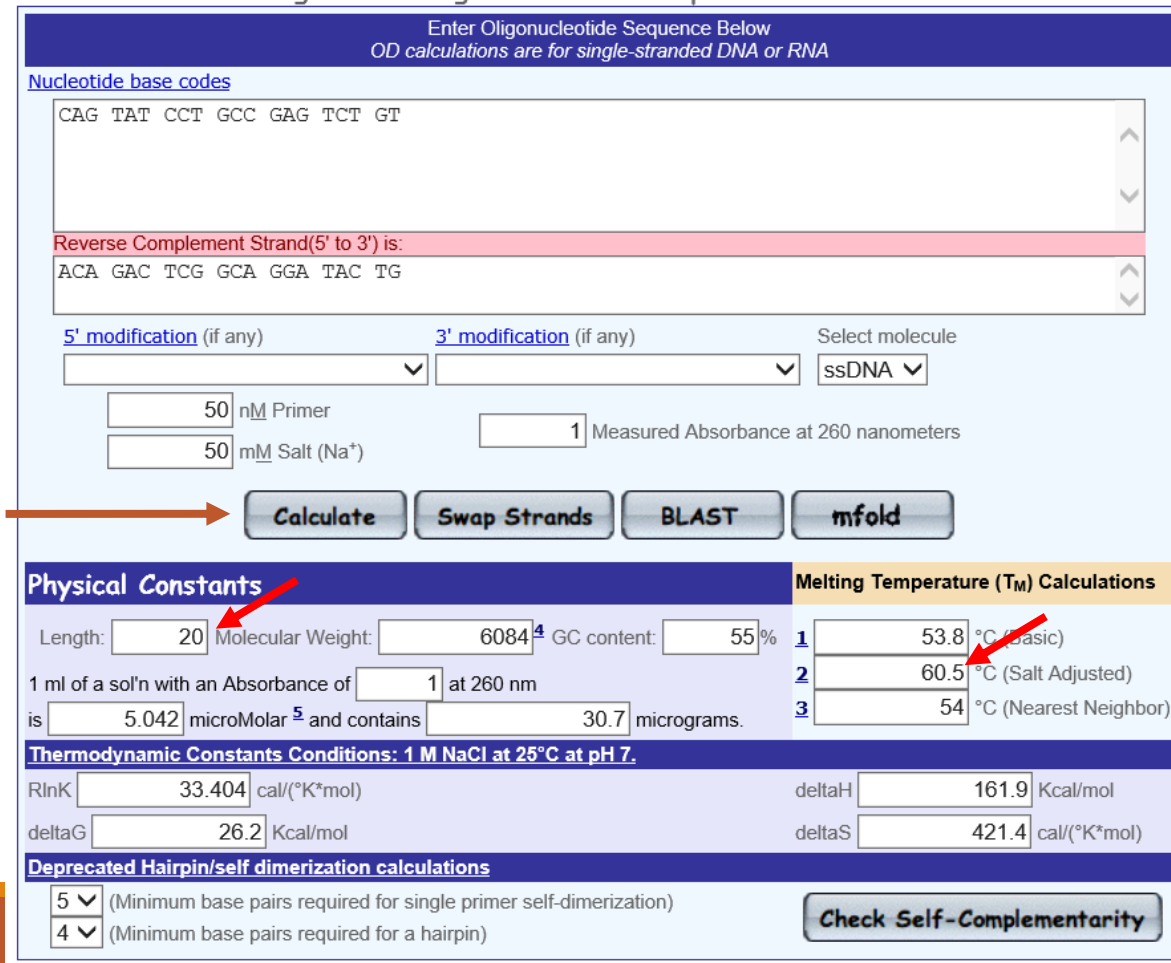
**Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7.**

RlnK  cal/(°K\*<sup>4</sup>mol) deltaH  Kcal/mol  
deltaG  Kcal/mol deltaS  cal/(°K\*<sup>3</sup>mol)

**Deprecated Hairpin/self dimerization calculations**

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

**Check Self-Complementarity**



# PCR-kontrola manuálně navržených primerů: OligoCalc

---

→ manuální návrh: 2 primery

Forward primer

Reverse primer

→ primery je nutné „**vyladit**“ aby seděly Tm (**prodloužením**, či **krácením** podle sekvence)

```
>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1  
(NQO1), transcript variant 1, mRNA  
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG  
AGGCTGCTGCAGCGGCTTTGAAGAAGAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT  
CAATCCCATCATTCCAGAAAGGACATCACAGGTAAGTGAAGGACCTGCGAACTTTCAGTATCCTGCC  
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG  
CCGCAGACCTTGTGATATCCAGTTCGCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT  
TGACGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCTTCCGGAGT  
AAGAAGGCAGTGTTCATCACCACCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG  
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAAC  
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCCGAATTCAAATCCTGGAAGGATGGAAGAAA  
CGCCTGGAGAATATTTGGGATGAGACACCCTGTATTTTGTCCAAGCAGCCTCTTTGACCTAAACTTCC  
AGGCAGGATCTTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGG  
CCATCACTTGGGCAAGTCCATCCCACTGACAACCAGATCAAAGCTAGAAAATGA
```

# Vyzkoušejte si

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1) Manuálně navrhnout primery pro amplifikaci:

**3. exonu NQO1 (NM\_000903.3)**

- délka:18-24nt

- Tm: 55-60°C

2) zkontrolujte pozice pomocí multalin:

**(porovnejte) celou mRNA, 3. exon a oba primery**

# Vyzkoušejte si

---

1) Manuálně navrhnout primery pro amplifikaci:

## 3. exonu NQO1 (NM\_000903.3)

- délka:18-24nt

- Tm: 55-60°C

**Výsledek:**

R:CTG GAA TAT CAC AAG GTC TGC (59,5°C)  
F:GTA AAC TGA AGG ACC CTG CG (60,5°C)

2) zkontrolujte pozice pomocí multalin:

**(porovnejte) celou mRNA, 3. exon a oba primery**



# porovnání - multalin

```

>3.exon
GTA AACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAG
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCG
>NM_000903.3
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCT
CCCCGGCAACCACGAG

```

3.exon  
NH\_000903.3  
Consensus

131 140 150 160 170 180

```

AGARAGGACCTGATCGTACTGGCTCACTCAGAGAGGAGCTCTTCACTATGCCATGARAGGAGGCTGCGAGCGCTTTGAGG

```

3.exon  
NH\_000903.3  
Consensus

261 270 280 290 300 310 320 330 340

```

TCATCCCATCATTTCCAGAAAGGACATCACAG
GTA AACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCGAGACCTTGTGATATTCAG

```

3.exon  
NH\_000903.3  
Consensus

391 400 410 420 430 440 450 460 470 480 490 500 510 520

```

GAGCTGGARAGCCGAGACCTTGTGATATCCAG
GAGCTGGARAGCCGAGACCTTGTGATATCCAGTTCCCCCTGCAGTGGTTGGAGTCCCTGCCATTCTGARAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTTGTCTTACACTTACGCTGCCATGTAT

```

3.exon  
NH\_000903.3  
Consensus

521 530 540 550 560 570 580

```

GACARAGGACCCCTCCGGAGTAGARAGGAGGCTGCTTCCATCACCCTGGTGGCAGTGGCT

```

3.exon  
NH\_000903.3  
Consensus

651 660 670 680 690 700 710

3.exon

Consensus

1431 1440 1450

R  
F

3.exon  
NH\_000903.3  
Consensus

```

>F
GTA AAC TGA AGG ACC CTG C
>3.exon
GTA AACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCT
GAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCGAGACCTTGTGATATTCAG
>NM_000903.3
ACACGCGACTCCCACA
CCCCGGCAACCACGAG
TGATCGTACTGGCTCA
GAAGAAGAAAGGATGG

```

261 270 280 290 300 310 320 330 340 350 360 370 380 390

F

3.exon  
NH\_000903.3  
Consensus

391 400 410 420 430 440 450 460 470 480 490 500 510 520

F

3.exon  
NH\_000903.3  
Consensus

>R rc  
CAG ACC TTG TGA TAT TCC AG

>F  
GTA AAC TGA AGG ACC CTG C

>3.exon

261 270 280 290 300 310 320 330 340 350 360 370 380 390

R\_rc

3.exon  
NH\_000903.3  
F

Consensus

391 400 410 420 430 440 450 460 470 480 490 500 510 520

R\_rc

3.exon  
NH\_000903.3  
F

Consensus

CTGGATATCACAGGCTCTG

c.g.a..c.c.ggtc.g

# porovnání - multalin

```

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

Pro porovnání nutné R primer vložit jako „reverse complement“

	261	270	280	290	300	310	320	330	340	350	360	370	380	390	
R_rc	-----														
3.exon	-----														
NM_000903.3	TCAATCCCATCATTTCAGAAAGGACATCACAG	GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTGTGGCTGACAAAA													
F	GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTGTGGCTGACAAAA														
Consensus	.....gt.aaact.gaaggaccct.gc.....														
	391	400	410	420	430	440	450	460	470	480	490	500	510	520	
R_rc	-----														
3.exon	-----														
NM_000903.3	GAACTGGAGCCGCAGACCTTGTGATATCCAG	CAGACCTTGTGATATCCAG													
F	GAACTGGAGCCGCAGACCTTGTGATATCCAGTTCACCCCTGCAGTGGTTTGAGTCCCTGCCATTCTGAAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTACGCTGCCATGTAT														
Consensus	.....cagaccttgtgatattccag.....														

Consensus	-----														
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560	
R	-----														
F	-----														
3.exon	-----														
NM_000903.3	TCAATTACAAGCAGTTACTAATATGCCTAGCAC	CTGGATATCACAGGCTCTG										CAGTACCACTCTTGGTCAGCTTTTGTGTTTATATACAGTACACAGATACCTTGAAGGAGAGCTAATAATCTCTTCTTTGCTGCAGTCATCTA			
Consensus	.....c.g.a.c.c.ggctc.g.....														

# Vyzkoušejte si (DU7)

---

Manuálně navrhnout primery pro vaši **CDS** (kódující sekvenci).

- délka:18-24nt
- T<sub>m</sub>: 55-60°C

+ zkontrolujte pozice pomocí multalin:

**(porovnejte) celou mRNA, CDS a oba primery**

# Restrikční analýza

---

= hledání cílových míst restrikčních endonukláz

Např: XhoI CTCGAG; BamHI GGATCC (palindromy)

**XhoI**

5'... C<sup>▼</sup>T C G A G ... 3'  
3'... G A G C T<sup>▲</sup>C ... 5'

**BamHI**

5'... G<sup>▼</sup>G A T C C ... 3'  
3'... C C T A G<sup>▲</sup>G ... 5'

Důvody:

- pro kontrolu produktu po PCR
- analýza mutací
- před klonováním (!)

# Restrikční analýza

SMS

## Sequence Manipulation Suite:

### Restriction Summary

Restriction Summary accepts a DNA sequence and returns the number and determine whether or not an enzyme cuts a particular segment of DNA.

Paste the raw sequence or one or more FASTA sequences into the text area

```
>sample sequence
ctaaattgtaagcgttaatatTTTTGTAAAATTCGCGTTAAATTTGTAAATCAGCTCA
TTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTATAAATCAAAGAATAGACCGAGA
TAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAGAACGTGGACTCAA
CGTCAAAGGGCGAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTAA
TCAAGTTTTTGGGGTCGAGGTGCCGTAAGCACTAAATCGGAACCCCTAAGGGGAGCCCC
```

Please check the [browser compatibility page](#) before using this program.

- Treat sequences as  molecules.

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

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

Fri Jun 17 16:17:06 2016

Valid XHTML 1.0; Valid CSS

### Restriction Summary results

cuts once

cuts twice

Results for linear 2961 residue sequence "sample sequence" starting "ctaaattgta"

Site:	Positions:
AatI agg cct	none
AatII gacgt c	none
Acc16I tgc gca	480, 2269
AccII cg cg	36, 412, 432, 456, 622, 624, 664, 795, 1001, 1003, 1201, 1782, 2112, 2605, 2937
AccIII t ccgga	none
AclI aa cgtt	2273, 2646
AcvI cac gtg	none
AfaI gt ac	758, 2527
AfeI agc gct	none
AfIII c ttaag	none
AgeI a ccggt	none
AhII a ctagt	684
Alw44I g tgcac	1468, 2714
AluI ag ct	58, 315, 530, 656, 722, 764, 819, 914, 978, 1096, 1322, 1412, 1458, 1715, 2236, 2336, 2399
Aor51HI agc gct	none
ApaI gggcc c	754
ApalI g tgcac	1468, 2714
Ascl aal cacacc	none

# Restrikční analýza



<http://nc2.neb.com/NEBcutter2/>

## NEBcutter

### NEBcutter V2.0

Enter a DNA sequence and find the large, non-overlapping open reading frames using the E.coli genetic code and the sites for all Type II and commercially available Type III restriction enzymes that cut the sequence just or NEB are used, but other sets may be chosen. Just enter your sequence and "submit". Further options will appear with the output. **The maximum size of the input file is 1 MByte, and the maximum sequence length is 3 /2.0 [Citing NEBcutter](#)**

Local sequence file:  Procházet...

GenBank number:  [Browse GenBank](#)

or paste in your DNA sequence: (plain or FASTA format)

**Vložení nukleotidové sekvence**

Standard sequences:  
# Plasmid vectors   
# Viral + phage

The sequence is:  Linear  Circular

Enzymes to use:

- NEB enzymes
- All commercially available specificities
- All specificities
- All + defined oligonucleotide sequences
- Only defined oligonucleotide sequences [\[define oligos\]](#)

Minimum ORF length to display:  a.a.

Name of sequence:  (optional)

Earlier projects:  
[no name](#)

*Note: Your earlier projects will be deleted 2 days after they were last accessed. You need to have cookies enabled in your browser for this feature to work.*

**Zvolení sady enzymů,  
které se mají použít**

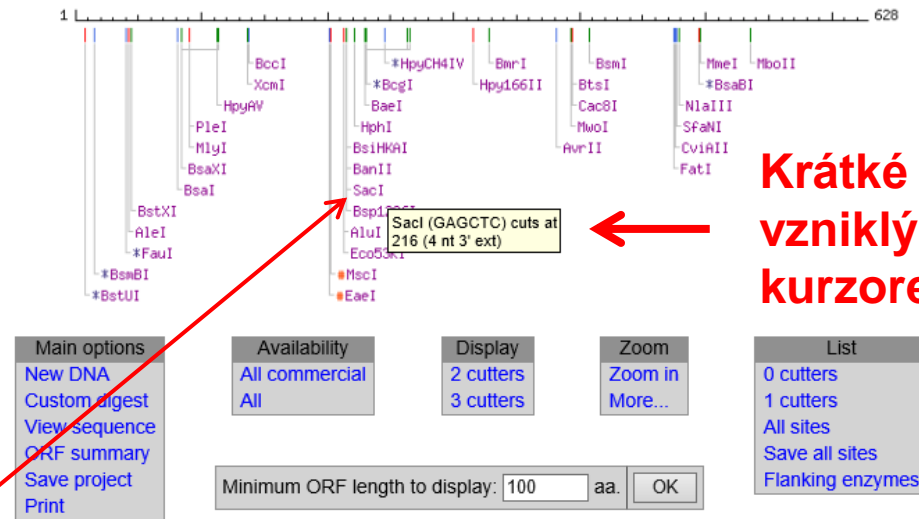
# Restrikční analýza



**Display:** - NEB single cutter restriction enzymes  
- Main non-overlapping, min. 100 aa ORFs  
GC=41%, AT=59%

## Grafické znázornění restrikčních míst

Cleavage code	Enzyme name code
⌞   blunt end cut	Available from NEB
⌞   5' extension	Has other supplier
⌞   3' extension	Not commercially available
⌞   cuts 1 strand	*: cleavage affected by CpG meth.
	o: cleavage affected by other meth.
	(enz.name): ambiguous site



**Krátké info o pozici a povaze vzniklých konců (zobrazeno kurzorem)**

**Podrobné informace o enzimech se zobrazí po kliknutí**

# Restrikční analýza



[Back to main display](#)

Available from NEB, Catalog # R0156

[View product page](#)

Time-Saver

SacI-HF (High-Fidelity version) NEB Catalog # R3156

[View product page](#)

Time-Saver Supplied with CutSmart buffer.

Buffer name: NEBuffer 1.1

Main: 10 mM Bis-Tris-Propane-HCl

pH: 7.0

Mg: 10 mM MgCl<sub>2</sub>

BSA: 100

Reaction temperature: 37 °C

Neoschizomers:

Eco53kI	5'... G A G C T C ... 3' 3'... C T C G A G ... 5'
---------	--

Sites in sequence: [1](#)

End produced at 212:

3' overhang: AGCT

SacI

5'... G A G C T C ... 3'  
3'... C T C G A G ... 5'

## Grafické znázornění štěpícího místa

[REBASE enzyme page](#)

[Methylation Sensitivity](#)

Overlapping methylation:

NONE

Isoschizomers:

NONE

Enzymes producing compatible ends:

Those cutting *unnamed sequence*:

Enzyme	Specificity	Compatible sites	% activity in			
			1.1	2.1	3.1	CS
BanII	GRGCYC	all sites	100	100	50	100
BsiHKA1	GWGCWC	all sites	25	100	100	100
Bsp1286I	GDGCHC	all sites	25	25	25	100
SacI	GAGCTC	all sites	100	50	10	100

Enzymy rozpoznávající sekvenci jako SacI



Enzymy produkující kompatibilní konce





# Vyzkoušejte si....

---

....udělejte restriční analýzu „vašeho“ genu (celé CDS)

→ zjistěte, které neštěpí

a které štěpí 1x, 2x...těmto RE je nutné se při klonování vyhnout

(DU: : XhoI, HindIII, SacI)

# RA:kontrola PCR fragmentů

= simulace štěpení, co můžeme na gelu očekávat při separaci fragmentů po štěpení

Program SMS: „Restriction digest“



## Sequence Manipulation Suite: Restriction Digest

Restriction Digest cleaves a DNA sequence in a virtual restriction digester, allowing you to specify the length, their position in the original sequence, and the number of molecules (by entering more than one sequence in FASTA format).

Paste the raw sequence or one or more FASTA sequences into the text box below.

```
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGTCCAAGCAGCCTCTTT  
GACCTAAACTTCC  
AGGCAGGATTTCTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTG  
GCCTTTCTGTGGG  
CCATCACTGGGCAAGTCCATCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

• Treat sequences as  molecules.  
• Digest with  and  and .

\*This page requires JavaScript. [Check your browser compatibility.](#)  
\*You can mirror this page or use it off-line.

Mon Nov 6 02:56:29 2017  
Valid XHTML 1.0; Valid CSS

- nothing
- AatI agg|cct
- AatII gacgt|c
- Acc16I tgc|gca
- AccII cg|cg
- AccIII t|ccgga
- AclI aa|cgtt
- AcvI cac|gtg
- AfaI gt|ac
- AfeI agc|gct
- AflII c|ttaag
- AgeI a|ccggt
- AhlI a|ctagt
- Alw441 g|tgcac
- AluI ag|ct
- Aor51HI agc|gct
- ApalI gggcc|c
- ApaLI g|tgcac
- AscI gg|cgcgcc
- AselI at|taat

For three restriction enzymes. The resulting fragments are sorted by size, and they are given a list of the restriction enzymes that produced them. You can digest linear or circular molecules, and even a mixture of molecules. The maximum character limit is 100,000,000 characters.

[new window](#) | [home](#) | [citation](#)

- 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

# RA:kontrola PCR fragmentů

## NQ01:CDS-restriction summary

```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),
transcript variant 1, mRNA
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTGGACCTCTATGCCATGAACCT
CAATCCCATCATTTCCAGAAAGGACATCACAGTAAACTGAAGGACCCCTGGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAAGCCATCTGAGCCAGATATTGTGGCTGAACAAAAGAAAGCTGGAAG
CCGACACCTTGTGATATCCAGTTCCTCCCTGCAGTGGTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTTCATAGGAGAGTTCCTTACACTTACGCTGCCATGTATGACAAAAGGACCCCTCCCGGAGT
AAGAGGCACTGCTTCCATCACCCTGGTGGCACTGGCTCCATGTAATCTCTGCAAGGATCCACGGG
ACATGAATGTCAATCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCGAATTCARATCCTGGAAGGATGGAAGAAA
CGCTGGAGAAATTTGGGATGAGACACCACTGATTTTGGCTCCAAAGCAGCCTCTTGCACCTAAACTCC
AGGCAGGATTCCTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCACTGACAACAGATCAAAGCTAGAAAAATGA
```

HpaII c cgg	415
EcoRI g aatc	603
AluI ag ct	274, 815

## NQ01:CDS-restriction digest

### Restriction Digest

Restriction Digest cleaves a DNA sequence in a virtual restriction digest, with one, two, or title specifying their length, their position in the original sequence, and the enzyme sites th molecules (by entering more than one sequence in FASTA format). Use Restriction Digest

Paste the raw sequence or one or more FASTA sequences into the text area below. Input

```
CGCTGGAGAATTTGGGATGAGACCACTGATTTTGTCCAAGCAGCTCTTT
GACCTAAACTCC
AGGCAGGATCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTG
GCCTTTCTGTGGG
CATCACTTGGGCAAGTCCATCCCACTGACAACAGATCAAAGCTAGAAAAATGA
```

Submit Clear Reset

- Treat sequences as  molecules.
- Digest with  and  and .

```
>414 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACCTCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAGTGC
AAGGACCCCTGCGAACTTTCAGTATCCTGCCAGTCTGTTCTGGCTTATAAAGAAAGGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAGCTGGAAGCCCGACAGCCTTGTGATATTC
CAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTTCTGAAAGGCTGGTTTGGAGGAGT
TTCATAGGAGAGTTTGGCTTACACTTACGCTGCCATGTATGACAAGGACCCCTTC
```

```
>411 bp linear fragment from linear parent NM_000903.3:122-946
CGGAGTAAGAAGGCAGTGTCTTCCATCACCCTGGTGGCAGTGGCTCCATGTAATCTCTG
CAAGGGATCCACGGGGACATGAATGTCAATCTCTGGCCAATTTCAGAGTGGCATTCTGCAT
TTCTGTGGCTTCCAAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCA
GACGCCGAATTCAAATCCTGGAAGGATGGAAGAAACGCTGGAGAAATTTGGGATGAG
ACACCCTGTATTTTGGCTCCAAAGCAGCCTCTTTCACCTAAACTTCCAGGCAGGATTTTA
ATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGGCCAT
CACTTGGGCAAGTCCATCCCACTGACAACCAATCAAAGCTAGAAAAATGA
```

A: 414+ 411nt

NQO1:CDS (825nt)



## EcoRI

```
>602 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACCTCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAGTGC
AAGGACCCCTGCGAACTTTCAGTATCCTGCCAGTCTGTTCTGGCTTATAAAGAAAGGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAGCTGGAAGCCCGACAGCCTTGTGATATTC
CAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTTCTGAAAGGCTGGTTTGGAGGAGT
TTCATAGGAGAGTTTGGCTTACACTTACGCTGCCATGTATGACAAGGACCCCTCCGGAGT
AAGAAGGCAGTGGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTCAAGGG
ATCCACGGGACATGAATGTCAATCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGT
GGCTTCCAAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCC
CG
```

```
>223 bp linear fragment from linear parent NM_000903.3:122-946
AATTCAAATCCTGGAAGGATGGAAGAAACGCTGGAGAAATTTGGGATGAGACACCCT
GTATTTTGGCTCCAAGCAGCCTTTTGACCTAACTCCAGGCAGGATTCCTAATGAAAA
AGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGGCCCTCACTTGGG
CAAGTCCATCCCACTGACAACAGATCAAAGCTAGAAAAATGA
```

B: 602+ 223nt

NQO1:CDS (825nt)

# RA:kontrola PCR fragmentů

- Treat sequences as  molecules.
- Digest with  and  and .

## NQ01:CDS-restiction digest

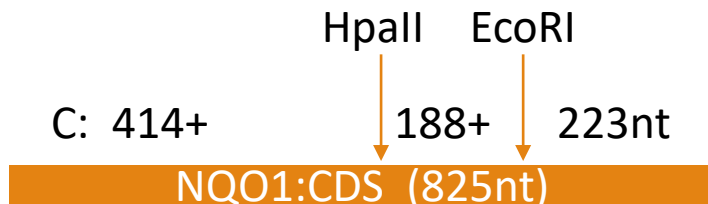
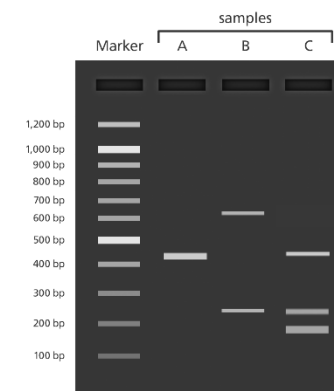
```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQ01),
transcript variant 1, mRNA
ATGGTCGGCAGAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACCTCCTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACCT
CAATCCCATCATTCCAGAAAGGACATCACAGTAAACTGAAGGACCCCTGCGAACTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGACACCTTGTGATATCCAGTCCCCCTGCAGTGGTTGGAGTCCCTGCCATTCTGAAAGGCTGGT
TGAGCGAGTTCATAGGAGAGTTGCTTACACTTACGCTGCCATGTATGACAAAAGGACCCCTCCCGGAGT
AAGAAGGCACTGCTTCCATCACCACCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGATCCACGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTCCAAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCGAATTCARATCCTGGAAGGATGGAAGAAA
CGCTGGAGATATTGGGATGAGACACCACTGTATTTGCTCCCAAGCAGCCTCTTGACCTAAACTCC
AGGCAGGATCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCACTGACAACCAAGATCAAAGCTAGAAAAATGA
```

HpaII c cgg	415	→
EcoRI g aatc	603	→
AluI ag ct	274, 815	

```
>414 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACCTCCTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACCTTCAATCCCATCATTTCAGAAAGGACATCACAGGTAACCTG
AAGGACCCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGACACCTTGTGATATTC
CAGTTCACCCCTGCAGTGGTGGAGTCCCTGCCATTCTGAAAGGCTGGTGGAGCGAGTG
TTCATAGGAGAGTTGCTTACACTTACGCTGCCATGTATGACAAAAGGACCCCTTC
```

```
>223 bp linear fragment from linear parent NM_000903.3:122-946
AATTCAAATCCTGGAAGGATGGAAGAAACGCCTGGAGAATATTTGGGATGAGACACCACT
GTATTTTGTCTCCAGCAGCCTCTTTGACCTAAACTTCCAGGCAGGATTCTTAATGAAAAA
AGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGGCCATCACCTTGGG
CAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAAATGA
```

```
>188 bp linear fragment from linear parent NM_000903.3:122-946
CGGAGTAAGAAGGCAGTGCTTCCATCACCACCTGGTGGCAGTGGCTCCATGTACTCTCTG
CAAGGGATCCACGGGGACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCAT
TTCTGTGGCTTCCAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCA
GACGCCCC
```



# RA:kontrola PCR fragmentů

## NQO1:CDS-restriction digest

```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),
transcript variant 1, mRNA
ATGGTCGGCAGAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACCT
CAATCCCATCATTTCCAGAAAGGACATCACAGTAAACTGAAGGACCCCTCGCAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTGTGGTGAACAAAAGAAAGCTGGAAG
CCGACACCTTGTGATATCCAGTCCCCCTGCAGTGGTTGGAGTCCCTGCCATTTGAAAGGCTGGT
TGAGCGAGTTCATAGGAGAGTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCCTCCCGAGT
AAGAAGGCACTGCTTCCATCACCACCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGATCCACGGG
ACATGAATGTCATTTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCGAAATTCARATCCTGGAAGGATGGAAGAAA
CGCTGGAGAAATTTGGGATGAGACACCACTGTATTTGCTCCCAAGACCCCTCTTGACCTAAACTCC
AGGCAGGATCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCACTGACCAACAGATCAAAGCTAGAAAAATGA
```

HpaII c cgg	415
EcoRI g aatc	603
AluI ag ct	274, 815

### Restriction Digest

Restriction Digest cleaves a DNA sequence in a virtual restriction digest, with one, two, or title specifying their length, their position in the original sequence, and the enzyme sites th molecules (by entering more than one sequence in FASTA format). Use Restriction Digest

Paste the raw sequence or one or more FASTA sequences into the text area below. Input

```
CGCTGGAGAATTTGGGATGAGACCACTGTATTTGTCTCAAGCAGCCTTT
GACCTAAACTTCC
AGGCAGGATTTCTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTG
GCCTTTCTGTTGG
CCATCACTTGGGCAAGTCCATCCCACTGACAACCCAGATCAAAGCTAGAAAAATGA
```

Submit Clear Reset

- Treat sequences as  molecules.
- Digest with  and  and .

```
>541 bp linear fragment from linear parent NM_000903.3:122-946
CTGGAAGCCGAGACCTTTGTGATATCCAGTTCGCCCTGCAGTGGTTTGGAGTCCCTGCC
ATTTCTGAAAGGCTGGTTTGTAGCGAGTGTTCATAGGAGAGTTTGTCTACACTTACGCTGCC
ATGTATGACAAAGGACCTTCCGGAGTAAGAAGGCAGTGTCTTCCATCACCCTGGTGGC
AGTGGCTCCATGTAATCTCTGCAAGGGATCCACGGGGACATGAATGTCATTTCTGGCCA
ATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACCTCAACTGACATAT
AGCATTGGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAACGC
CTGGAGAATATTTGGGATGAGACCACTGTATTTGCTCCCAAGCAGCCTCTTGTGACCTA
AACTTCCAGGCAGGATTTCTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAA
TTTGGCCTTTCTGTGGGCCATCACTTGGGCAAGTCCATCCCACTGACAACCCAGATCAAAG
G
```

```
>273 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGTCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCC
GACCTCTATGCCATGAACCTCAATCCCATCATTTCCAGAAAGGACATCACAGTAAACTG
AAGGACCCCTGCAGACTTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCAT
CTGAGCCCAGATATTTGGTCTGAACAAAAGAAG
```

```
>11 bp linear fragment from linear parent NM_000903.3:122-946
CTGAAATTA
```



# RA:kontrola PCR fragmentů

- Treat sequences as  molecules.
- Digest with  and  and .

## NQO1:CDS-restiction digest

```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),
transcript variant 1, mRNA
ATGGTCGGCAGAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAAC
CAATCCCATCATTTCCAGAAAGGACATCACAGTAAACTGAAGGACCCCTGCGAATTTAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTGGCTGAACAAAAGAAAGCTGGAAG
CCGACGACCTTGTGATATCCAGTTCCTCCCTGCAGTGGTTGGAGTCCCTGCCATTTGAAAGGCTGGT
TGAGCGAGTGTTCATAGGAGAGTTCCTTACACTTACGCTGCCATGTATGACAAAGGACCCCTCCCGAGT
AAGAGGCACTGCTTCCATCACCCTGCTGAGTGGCTCCATGTAATCTCTGCAAGGATCCACGSS
ACATGAATGTCATTTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCGAATTCARATCCTGGAAGGATGGAAGAAA
CGCTGGAGATATTGGGATGAGACACCACTGTATTTGCTCCAAAGACGCTCTTTGACCTAAACTCC
AGGCAGGATCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGG
CCATCACTTGGGCAAGTCCATCCCACTGACAAACAGATCAAGCTAGAAAAATGA
```

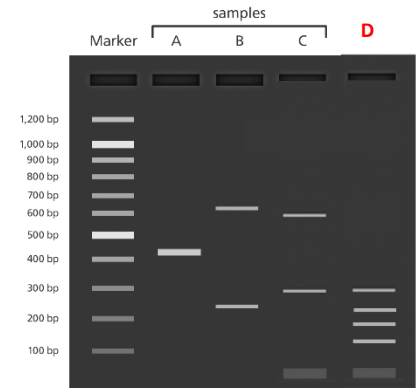
```
>273 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCC
GACCTCTATGCCATGAACCTCAATCCCATCATTTCCAGAAAGGACATCACAGTAAACTG
AAGGACCCCTGCGAATTTAGTATCCTGCCAGTCTGTTCTGGCTTATAAAGAAGGCCAT
CTGAGCCAGATATTGTGGCTGAACAAAAGAAAG
```

```
>212 bp linear fragment from linear parent NM_000903.3:122-946
AATTCAAATCCTGGAAGGATGGAAGAAACCGCTGGAGAAATATTTGGGATGAGACCCACT
GTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTCCAGGAGGATTCCTTAATGAAAAA
AGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGCCATCACTTGGG
CAAGTCCATCCCAACTGACAAACAGATCAAAAG
```

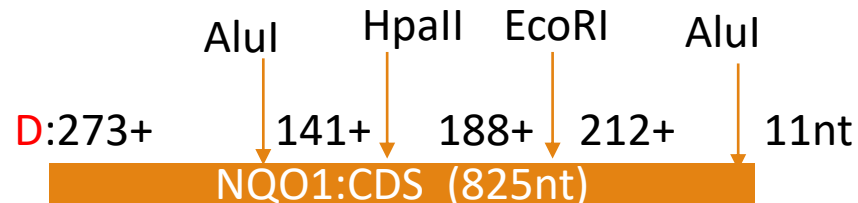
```
>188 bp linear fragment from linear parent NM_000903.3:122-946
CGGAGTAAGAAGGCACTGCTTTCCATCACCCTGGTGGCAGTGGCTCCATGTAATCTCTG
CAAGGGATCCACGCGGACATGAATGTATTCTCTGGCCAATTCAGAGTGGCATTCTGCAT
TTCTGTGGCTTCCAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCA
GACGCCCG
```

```
>141 bp linear fragment from linear parent NM_000903.3:122-946
CTGGAAGCCGACGACCTTGTGATATTCAGTTCCTCCCTGCAGTGGTTGGAGTCCCTGCC
ATTCTGAAAGGCTGGTTTGGAGGAGTGTTCATAGGAGAGTTTGGCTTACACTTACGCTGCC
ATGTATGACAAAGGACCTTC
```

```
>11 bp linear fragment from linear parent NM_000903.3:122-946
CTAGAAATGA
```



HpaII c cgg	415
EcoRI g aatc	603
AluI ag ct	274, 815



# Vyzkoušejte si:

---

Pomocí **Restriction summary** najděte RE, který štěpí 1x a další, který štěpí 2x, vyzkoušejte si kombinované štěpení v **Restriction Digest**

## DU7:

- 1) Zjistěte zda a kolikrát štěpí **CDS** sekvenci vašeho genu enzymy: XhoI, HindIII, SacI
- 2) Jaké fragmenty z vaší **CDS** vzniknou po štěpení **všemi** těmito enzymy?
- 3) Je nějaká restriční endonukleáza, která štěpí vaší CDS **právě jednou**? Jaké vzniknou fragmenty?

# DÚ7 – Návrh primerů

---

- 1) Zjistěte zda a kolikrát štěpí **CDS** sekvenci vašeho genu enzymy: XhoI, HindIII, SacI
- 2) Jaké fragmenty z vaší **CDS** vzniknou po štěpení **všemi** těmito enzymy?
- 3) Je nějaká restriční endonukleáza, která štěpí vaší CDS **právě jednou**? Jaké vzniknou fragmenty?
- 4) Navrhněte primery, tak aby se amplifikovala vaše CDS
  - Navrhněte F a R primer tak aby  $T_m$  nebyla větší než 60°C
  - zkontrolujte pozice pomocí multalin:  
(porovnejte) celou mRNA, CDS a oba primery



# DÚ7 – Návrh primerů-řešení

1-2) Celá sekvence NQO1 by byla štěpena pouze BamHI, který by byl nevhodný pro klonování

XhoI c tctgag	none
---------------	------

SacI gagct c	none
--------------	------

HindIII a agctt	none
-----------------	------

3) vzniknou dva fragmenty:

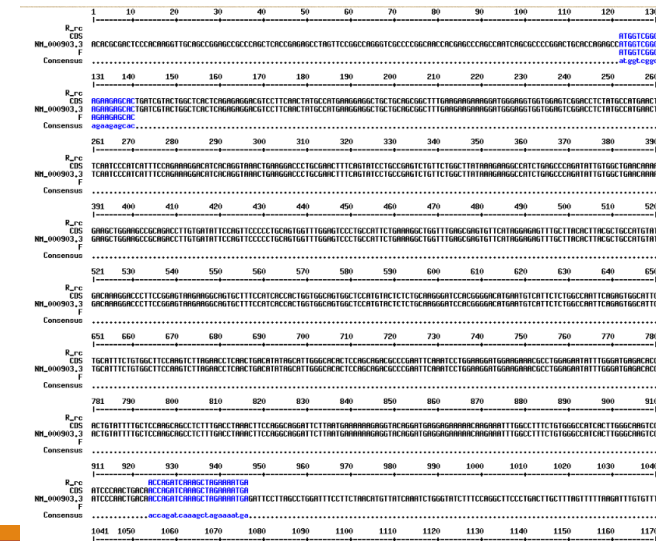
BamHI g gatcc	480
---------------	-----

Restriction Digest results

>479 bp linear fragment from linear parent Untitled, base 1 to base 479 (see...)  
 ATGGTCGGCAGAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTCAACTAT  
 GCCATGAAGGAGGCTGCTGCAGCGGCTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG  
 GACCTCTATGCCATGAACCTTCATCCCATCATTTCCAGAAGGACATCACAGTAAACTG  
 AAGGACCCCTGGCAACTTTCAGTATCCTGCCGAGTGTGTTTGGCTTATAAAGAGGCCAT  
 CTGAGCCAGATATTGGCTGAACAAAAGAGCTGGAAGCCGACAGCCTTGTGATATTC  
 CAGTCCCCCTGCAGTGGTTGGAGTCCCTGCCATTTCTGAAAGGCTGGTTTGGAGGAGTG  
 TTCATAGGAGAGTTTGGCTTACACTTACGCTGCCATGTATGACAAGGACCCCTCCGGAGT  
 AAGAAGGCAGTCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGG

>346 bp linear fragment from linear parent Untitled, base 480 to base 825 (1)  
 GATCCACGGGACATGAATGTCACTCTCTGCCCAATTGAGAGTGGCATTCTGCAATTTCTG  
 TGGCTTCCAAGTCTTGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCAGACGC  
 CCGAATTCAAATCCTGGAGGATGGAGAAACGCCTGGGAAATATTTGGGATGAGACACC  
 ACTGATTTTGTCCAAAGCAGCCTCTTTCACCTAACTTCCAGGCAGGATTTCTAATGAA  
 AAAAGAGGTACAGGATGAGGAGAAAACAAGAAATTTGGCCTTTCTGTGGCCATCACTT  
 GGGCAAGTCCATCCCAACTGACAACAGATCAAAGCTAGAAAATGA

4) F: ATG GTC GGC AGA AGA GCA C  
 R: TCA TTT TCT AGC TTT GAT CTG GT



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 C

Reverse Complement Strand(s) to 3' is:  
 CTG CTC TTC TGC CGA CCA T

5' modification (if any) 3' modification (if any) Select molecule  
 50 nM Primer ssDNA Measured Absorbance at 260 nanometers

Calculate Swap Strands BLAST mfold

Physical Constants Melting Temperature (T<sub>m</sub>) Calculations  
 Length: 19 Molecular Weight: 5886.9 GC content: 58% 1 53.2 °C (Basic) 2 59.5 °C (Salt Adjusted)

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(s) to 3' is:  
 ATG GTC GGC AGA AGA GCA C

5' modification (if any) 3' modification (if any) Select molecule  
 50 nM Primer ssDNA Measured Absorbance at 260 nanometers

Calculate Swap Strands BLAST mfold

Physical Constants Melting Temperature (T<sub>m</sub>) Calculations  
 Length: 23 Molecular Weight: 7261.4 GC content: 35% 1 49.5 °C (Basic) 2 52.8 °C (Salt Adjusted)