Základy praktické Bioinformatiky

PETRA MATOUŠKOVÁ

2025/2026

Základy praktické bioinformatiky

Téma 7/10

Nukleotidová bioinformatika III

Cíle:

Student bude schopen navrhnout primery pro namnožení požadovaného úseku genu a navrhnout vhodné enyzmy pro restrikční analýzu.

„Bioinformatika nukleových kyselin II“

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

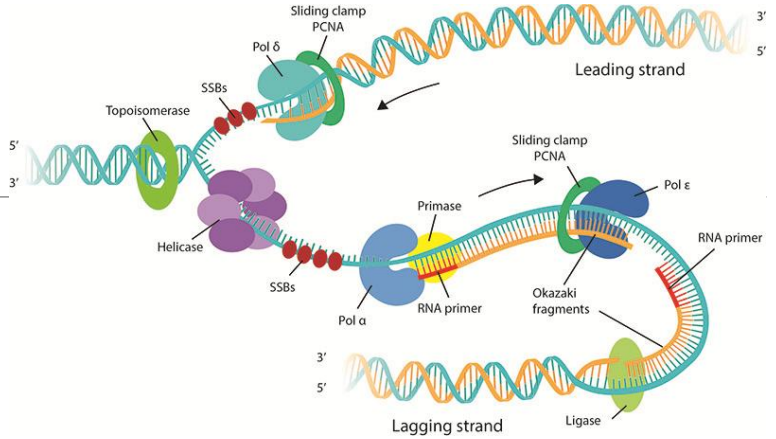
mikroRNA

Celé genomy

….

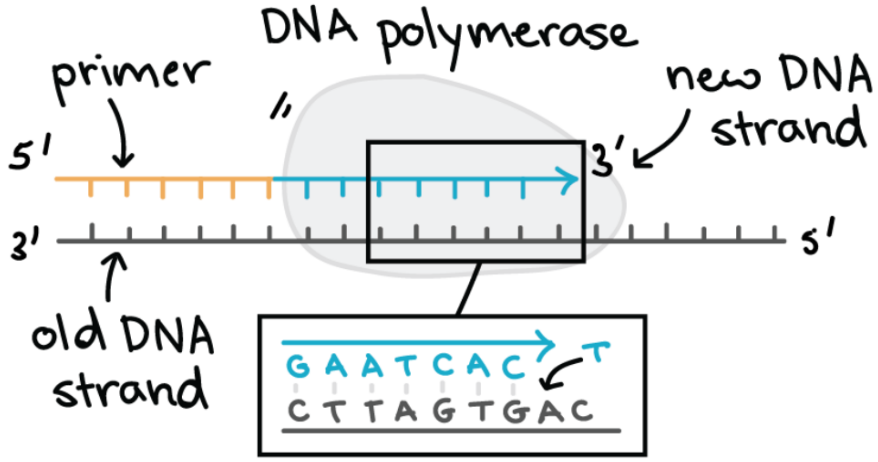
Primer = oligonukleotid

= krátký jednořetězcový úsek DNA



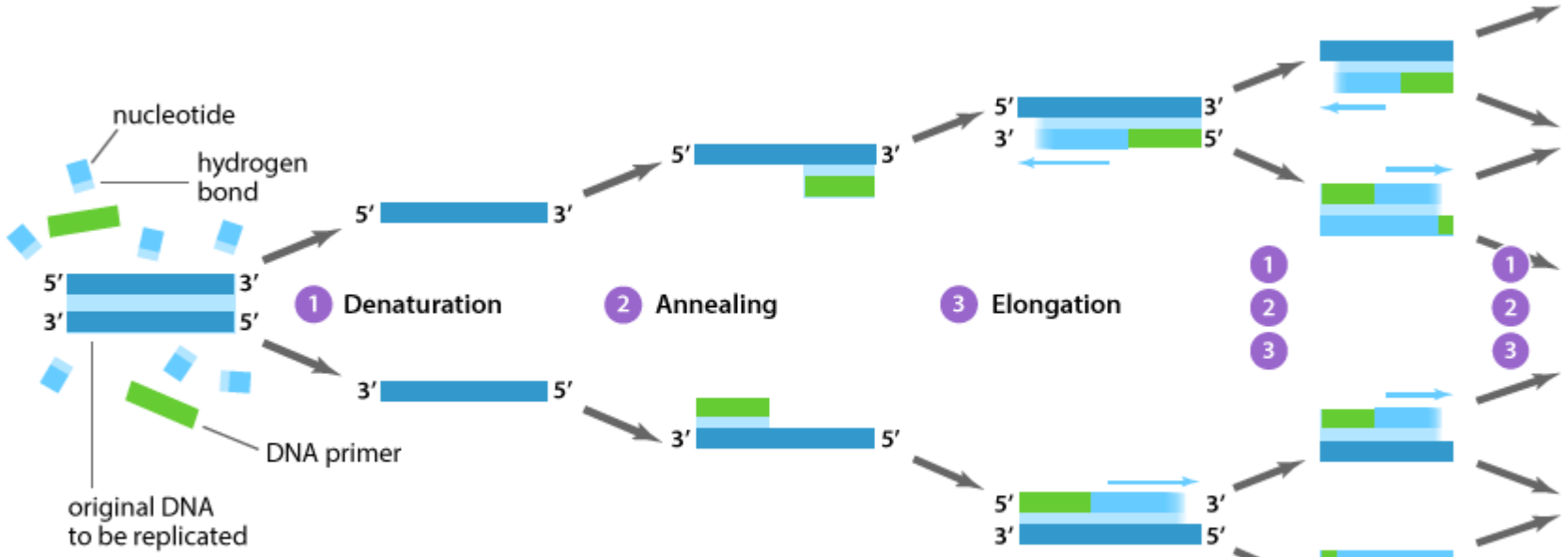
Použití:

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

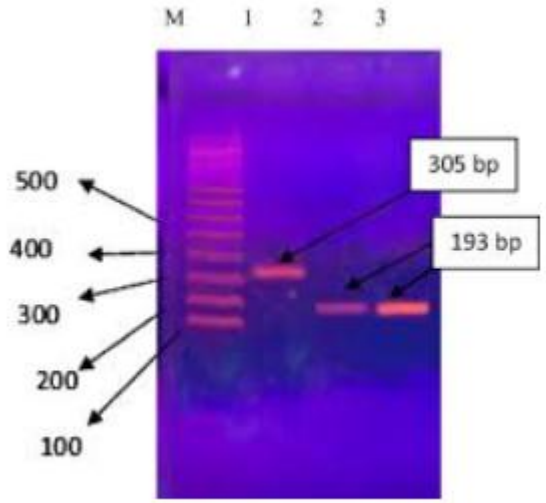


Polymerázová řetězová reakce

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

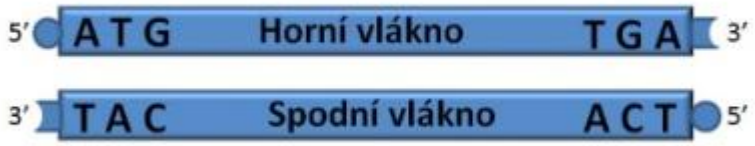


→ „End point“ detekce

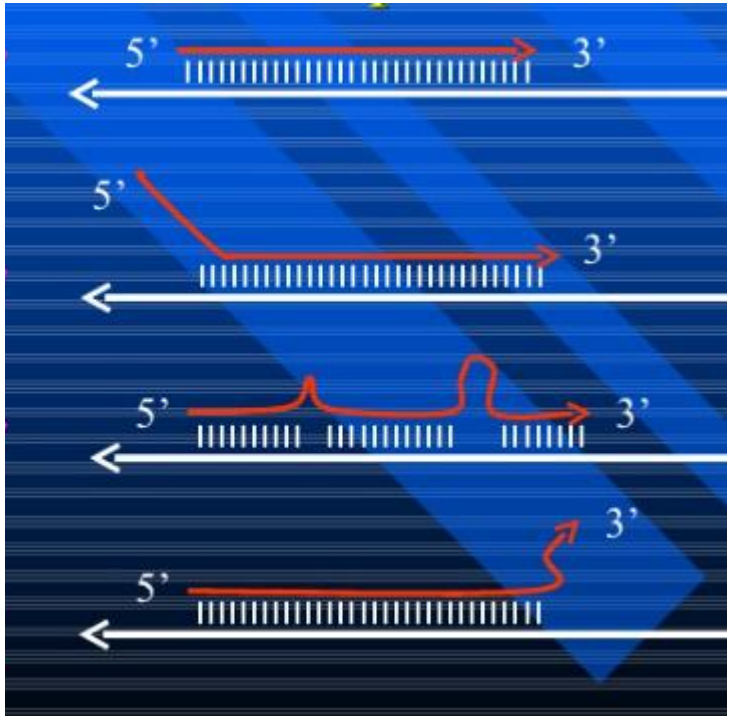


Polymerázová řetězová reakce

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



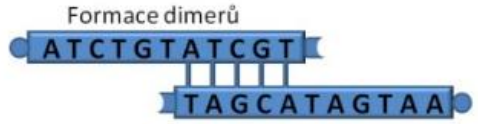
Syntéza: 5´→ 3´

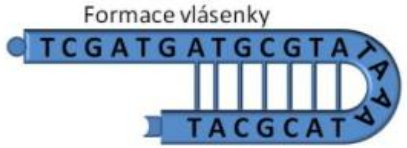


Polymerázová řetězová reakce

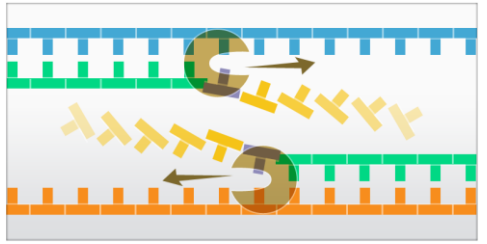
Charakteristiky „dobré“ dvojice primerů:

- **Tm rozdíl (<1-3°C)**
- Podobná délka (17-28nt)
- Podobné složení bazí (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)



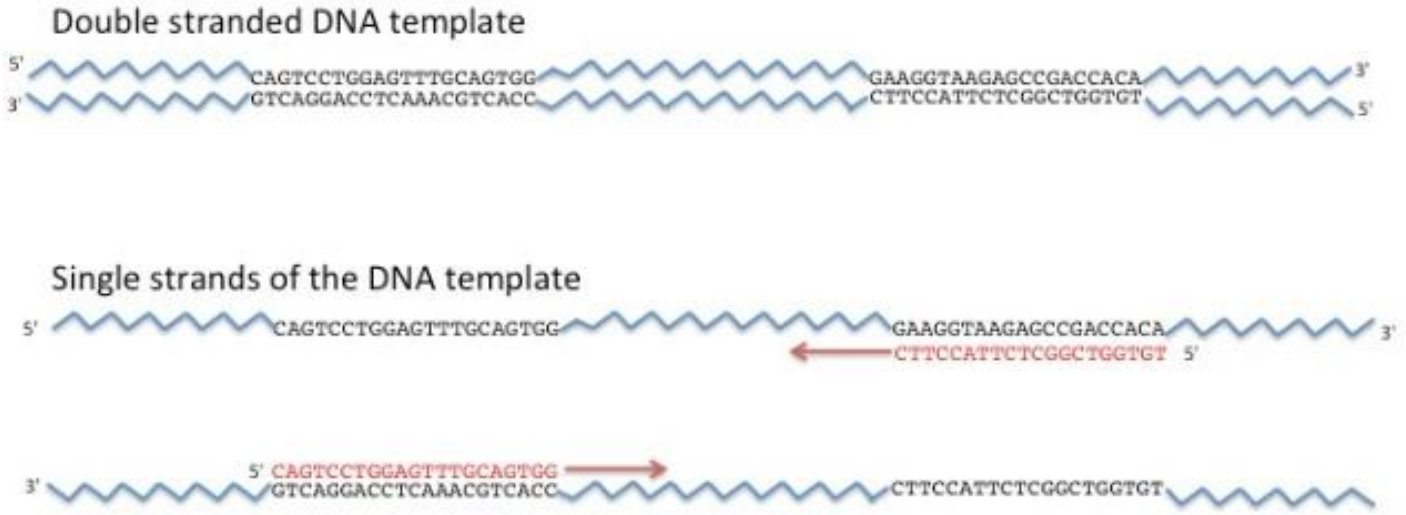


Polymerázová řetězová reakce

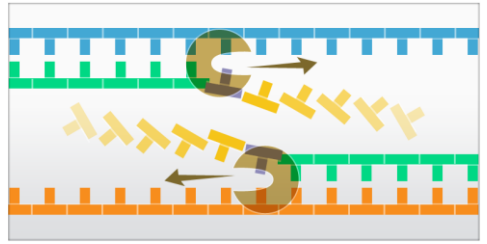


1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh



Polymerázová řetězová reakce



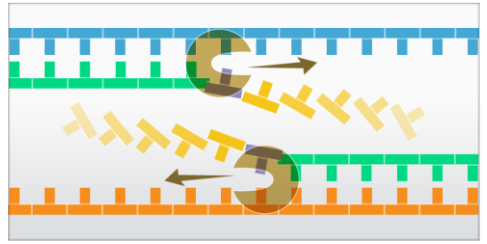
1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh

- **navrhněte (krátké) primery pro sekvenci:**

5´-ATGCCCTTTCnnnnnnnnnnnnnnnTAAATCCCGC-3´

Polymerázová řetězová reakce



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

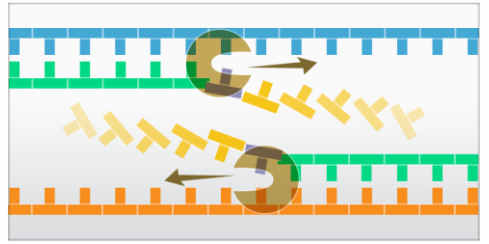
→ manuální návrh

- **navrhněte (krátké) primery pro sekvenci:**

5´-ATGCCCTTTCnnnnnnnnnnnnnnTAAATCCCGC-3´

3´-TACGGGAAAGnnnnnnnnnnnnnnATTTAGGGCG-5´

Polymerázová řetězová reakce



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

→ manuální návrh

➢ **navrhněte (krátké) primery pro sekvenci:**

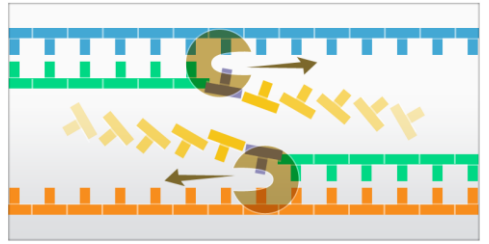
5´-ATGCCCTTTCnnnnnnnnnnnnnnTAAATCCCGC-3´

ATTTAGGGCG–5

5´- **ATGCCCTTTC–**

3´-TACGGGAAAGnnnnnnnnnnnnnnATTTAGGGCG-5´

Polymerázová řetězová reakce



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

→ manuální návrh

➢ **navrhněte (krátké) primery pro sekvenci:**

R_primer: **GCGGGATTTA**

5´-ATGCCCTTTCnnnnnnnnnnnnnnTAAATCCCGC-3´

ATTTAGGGCG–5

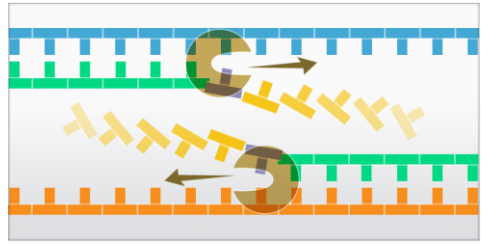
F_primer: **ATGCCCTTTC**

5´- **ATGCCCTTTC**–

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

3´-TACGGGAAAGnnnnnnnnnnnnnnATTTAGGGCG-5´

Polymerázová řetězová reakce



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

→ manuální návrh

➢ **navrhněte (krátké) primery pro sekvenci:**

R_primer: **GCGGGATTTA**

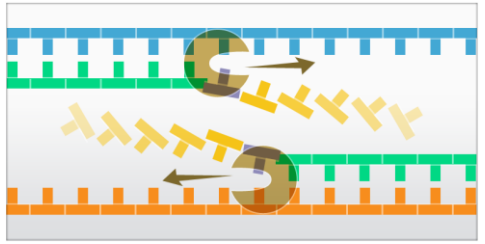
5´-ATGCCCTTTCnnnnnnnnnnnnnnTAAATCCCGC-3´

reverse complement:

5´-**GCGGGATTTAnnnnnnnnnnnnnnGAAAGGGCAT**-3´

(3´-**TACGGGAAAGnnnnnnnnnnnnnnATTTAGGGCG**-5´)

Polymerázová řetězová reakce



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

→ manuální návrh

➢ **navrhněte (krátké) primery pro sekvenci:**

R_primer: **GCGGGATTTA**

5´-ATGCCCTTTCnnnnnnnnnnnnnnTAAATCCCGC-3´

reverse complement:

5´-**GCGGGATTTAnnnnnnnnnnnnnnGAAAGGGCAT**-3´

(3´-**TACGGGAAAGnnnnnnnnnnnnnnATTTAGGGCG**-5´)

PCR-amplifikace požadovaného úseku

→ **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
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA

PCR-amplifikace požadovaného úseku

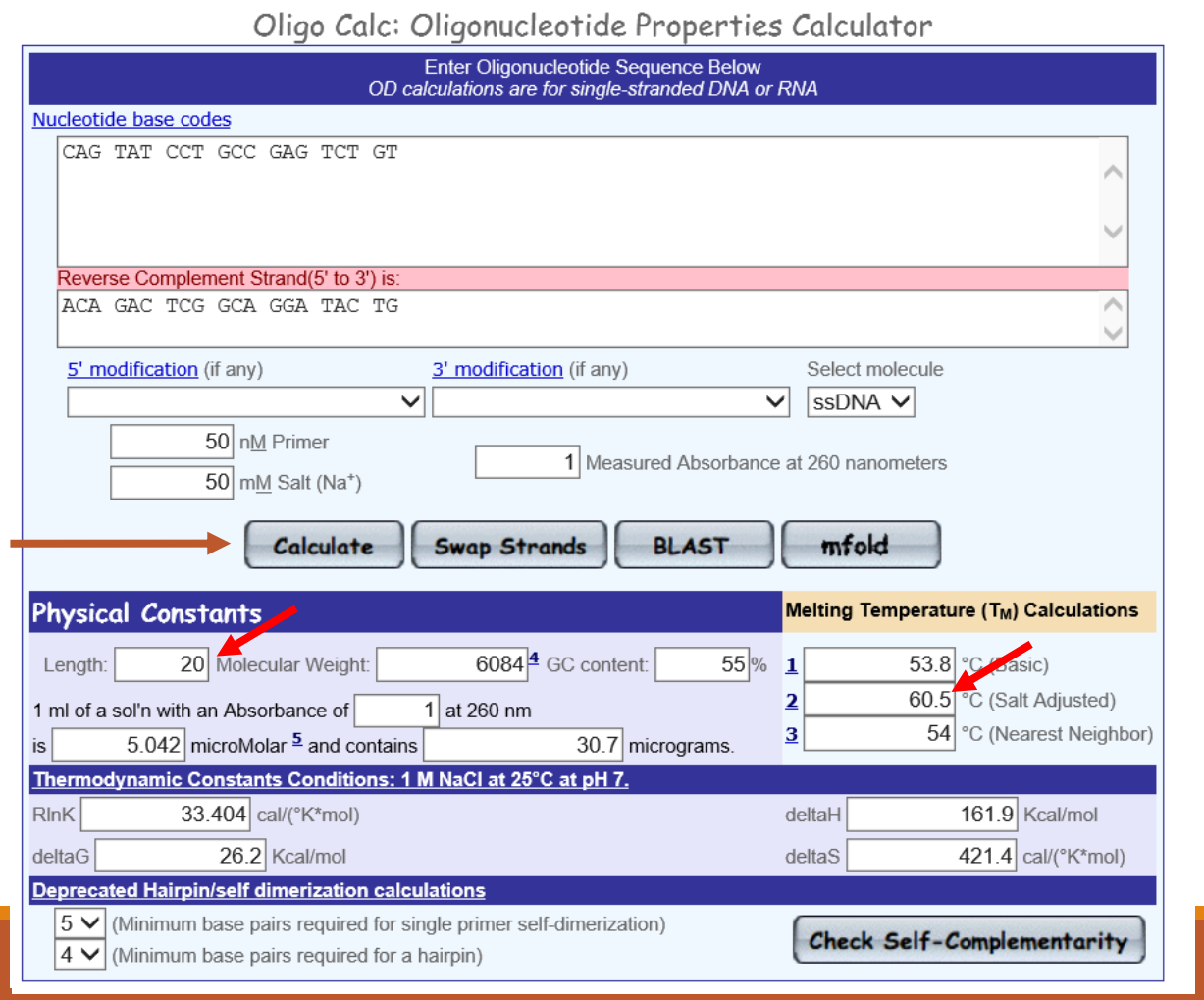
→ **manuální návrh: 2 primery**

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
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA

PCR-kontrola manuálně navržených primerů: OligoCalc



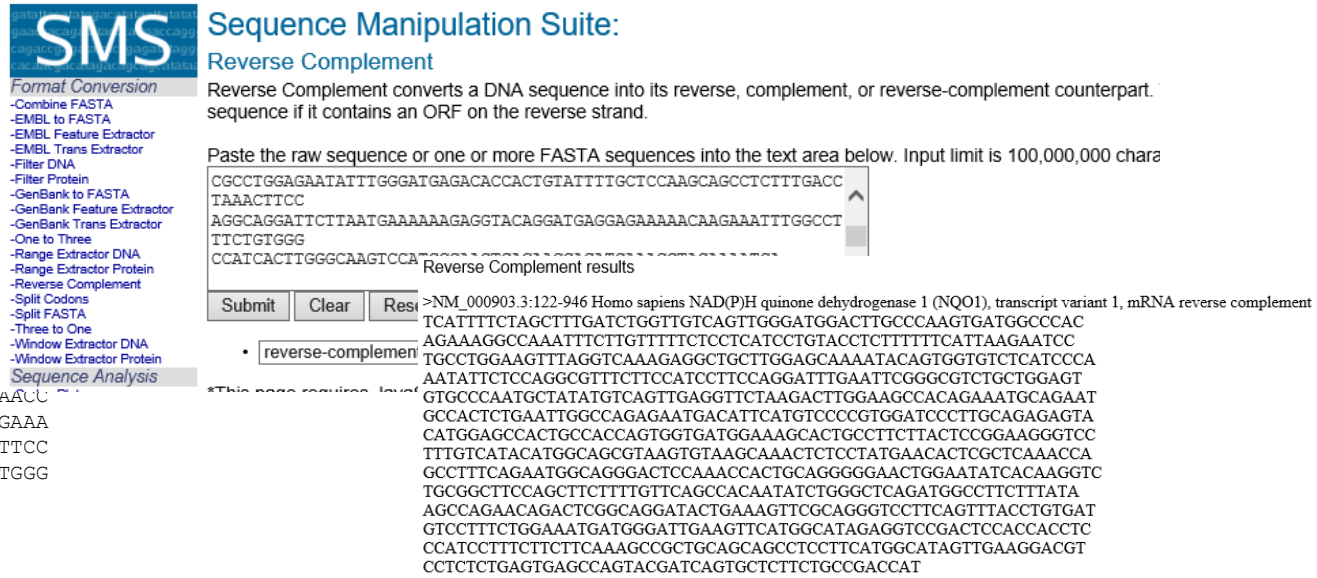
PCR-amplifikace požadovaného úseku

→ **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
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGA
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCG
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCAC
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```



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

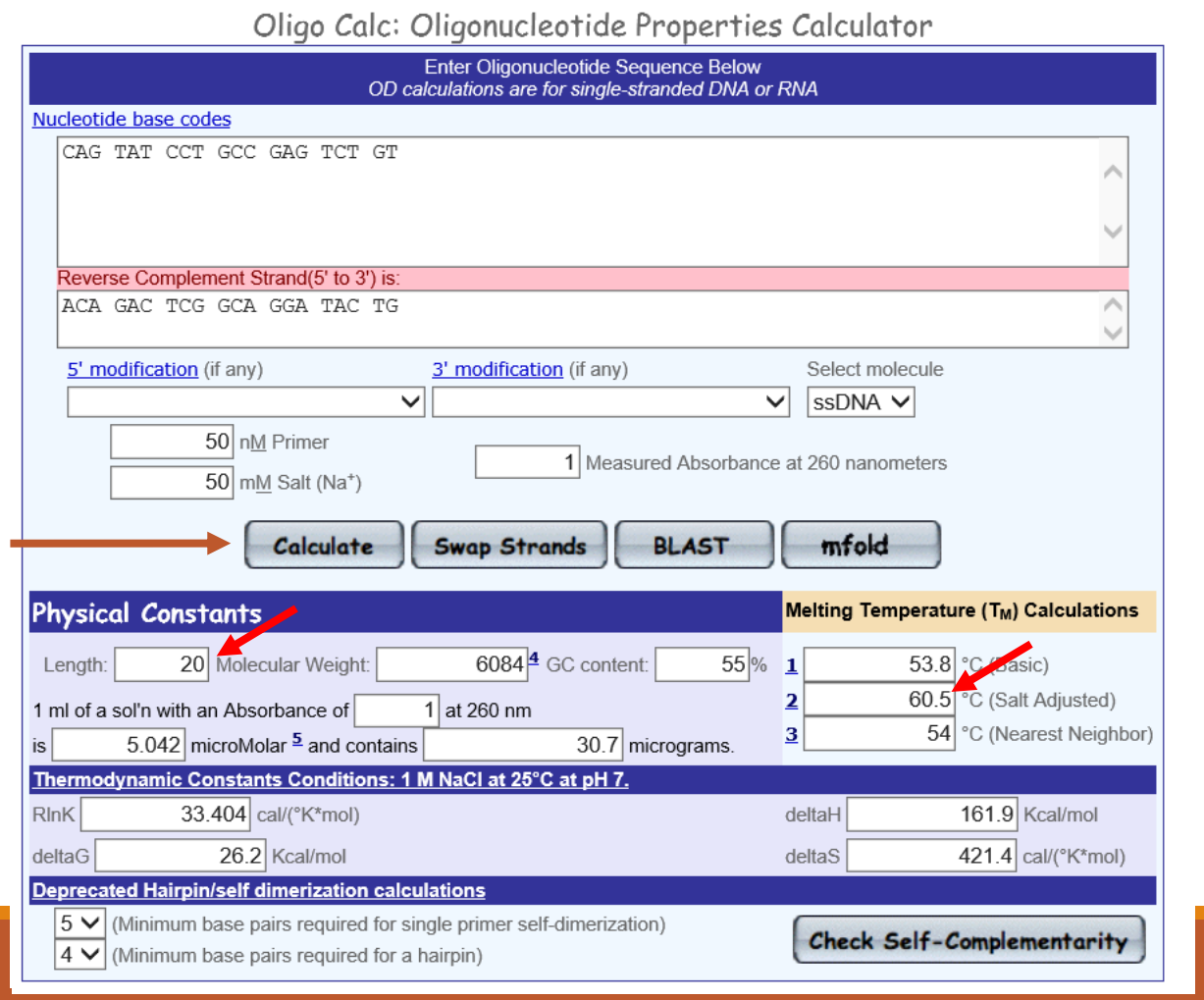
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA

Reverse Complement results

>NM_000903.3:122-946 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

PCR-kontrola manuálně navržených primerů: OligoCalc



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
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA

Vyzkoušejte si

1) Manuálně navrhnout primery pro amplifikaci:

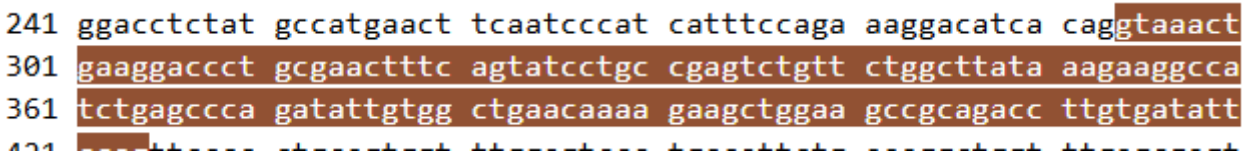
3. exonu NQO1 (NM_000903.3)

- délka:18-24nt
- Tm: 55-60°C

Vyzkoušejte si

1) Manuálně navrhnout primery pro amplifikaci:

3. exonu NQO1 (NM_000903.3)



- délka:18-24nt

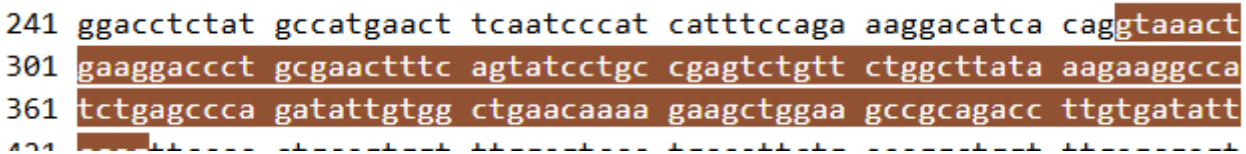
- Tm: 55-60°C

Výsledek:

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)

Ověření PCR produktů:

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

Sequence Manipulation Suite:

PCR Products

PCR Products accepts one or more DNA sequence templates and two primer sequences. The program searches for perfectly matching primer annealing sites that can generate a PCR product. Any resulting products are sorted by size, and they are given a title specifying their length, their position in the original sequence, and the primers that produced them. You can use linear or circular molecules as the template. Use PCR Products to determine the product sizes you can expect to see when you perform PCR in the lab.

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

```
GCTCTCGACAGTATCCACAATAGCTGACGGCTGGGTGTTTCAGTTTGAAAATATTTTGTTGCC
TTCATCT
TCACTGCAATTTTGTGTAAATTTCTCAAAGATCTGAATTAAATAAATAAAATTCATTTCTACA
GACCCAC
A
```

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

F GTA AAC TGA AGG ACC C

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

R CTG GAA TAT CAC AAG G

Submit Clear Reset

- Treat sequences as linear molecules.

*This page requires JavaScript. See browser compatibility.
*You can mirror this page or use it off-line.

Vyzkoušejte si

1) Manuálně navrhnout primery pro amplifikaci:

3. exonu NQO1 (NM_000903.3)

- délka:18-24nt
- Tm: 55-60°C

Výsledek:

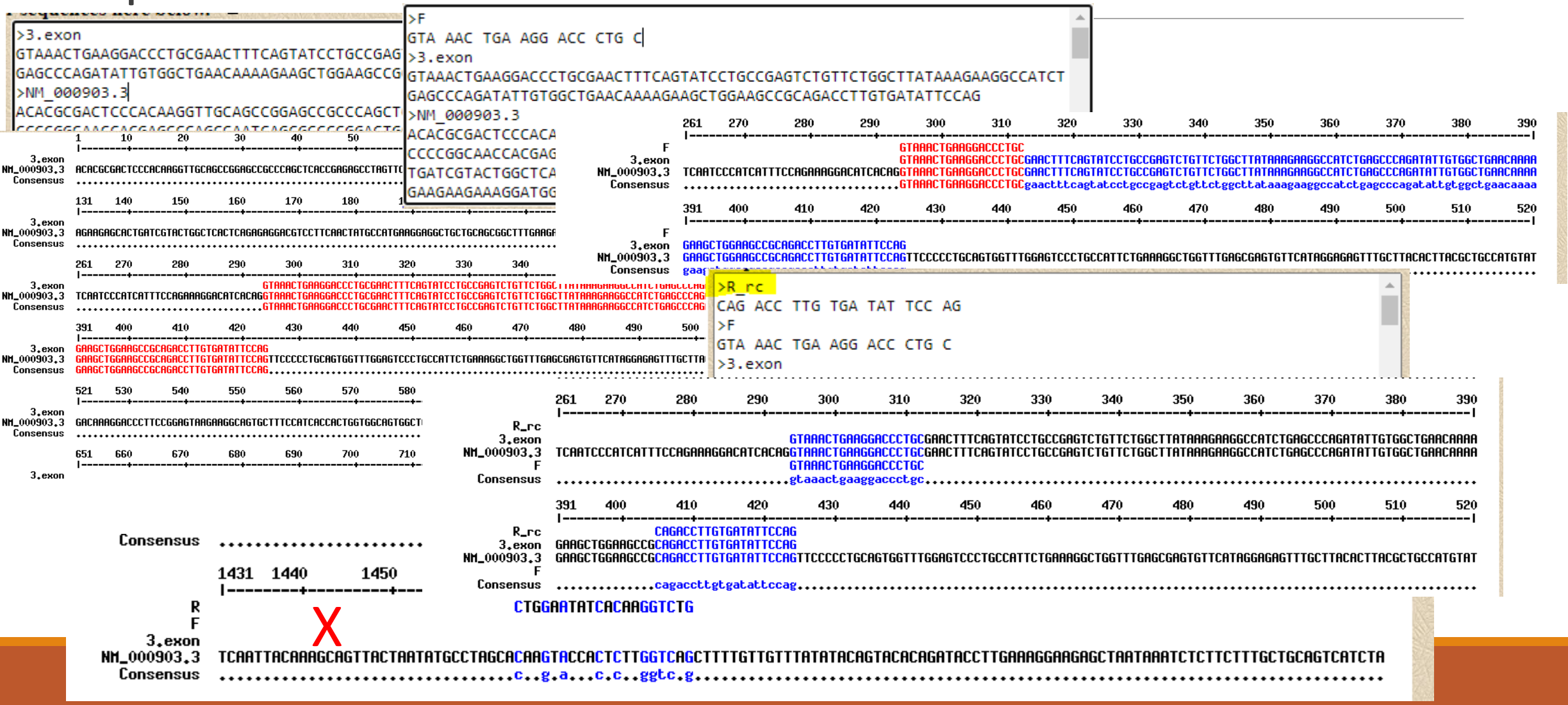
F: GTA AAC TGA AGG ACC CTG CG 60°C

R: CTG GAA TAT CAC AAG GTC TGC 60°C

2) zkontrolujte pozice pomoci multalin:

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

porovnání - multalin



porovnání - multalin

```
>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
```

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                                                    GTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAA
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.....................................................................................
```

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

Vyzkoušejte si

1) Manuálně navrhnout primery pro amplifikaci úseku:

Sekvence v Moodlu: **nácvik manuálního návrhu primerů-nefunkční NCBI**

- délka:18-24nt
- Tm: 55-60°C

Vyzkoušejte si

1) Manuálně navrhnout primery pro amplifikaci úseku:

Sekvence v Moodlu: **nácvik manuálního návrhu primerů-nefunkční NCBI**

- délka:18-24nt
- Tm: 55-60°C

Výsledek: F: GGT TTC CAA TGC GGC CGT
R: GAT TGG CTG AGT GCT TTA TTG

Kontrola PCR produktu

(po manuálním návrhu primerů)

- agarosová elektroforéza – kontrola velikosti

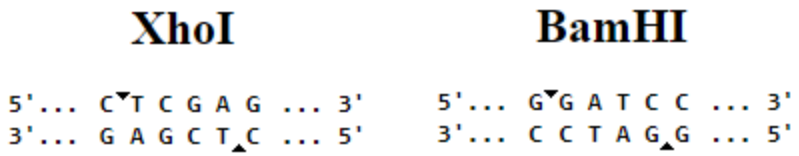
- sekvenace – kontrola vlastní sekvence

+ restrikční analýza („náznak“ sekvence a agarozová elektroforéza)

Restrikční analýza

= hledání cílových míst restrikčních endonukláz

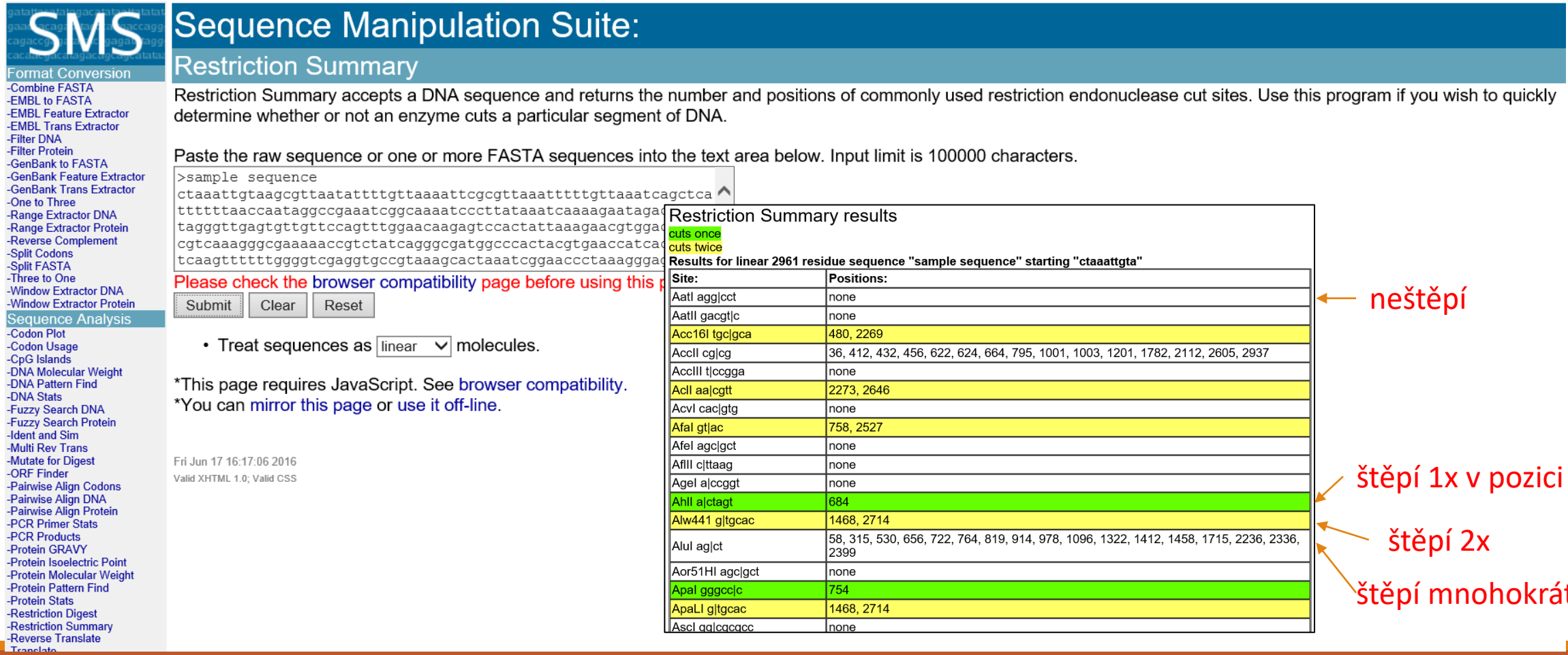
Např: XhoI CTCGAG; BamHI GGATCC (palindromy)



Důvody:

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

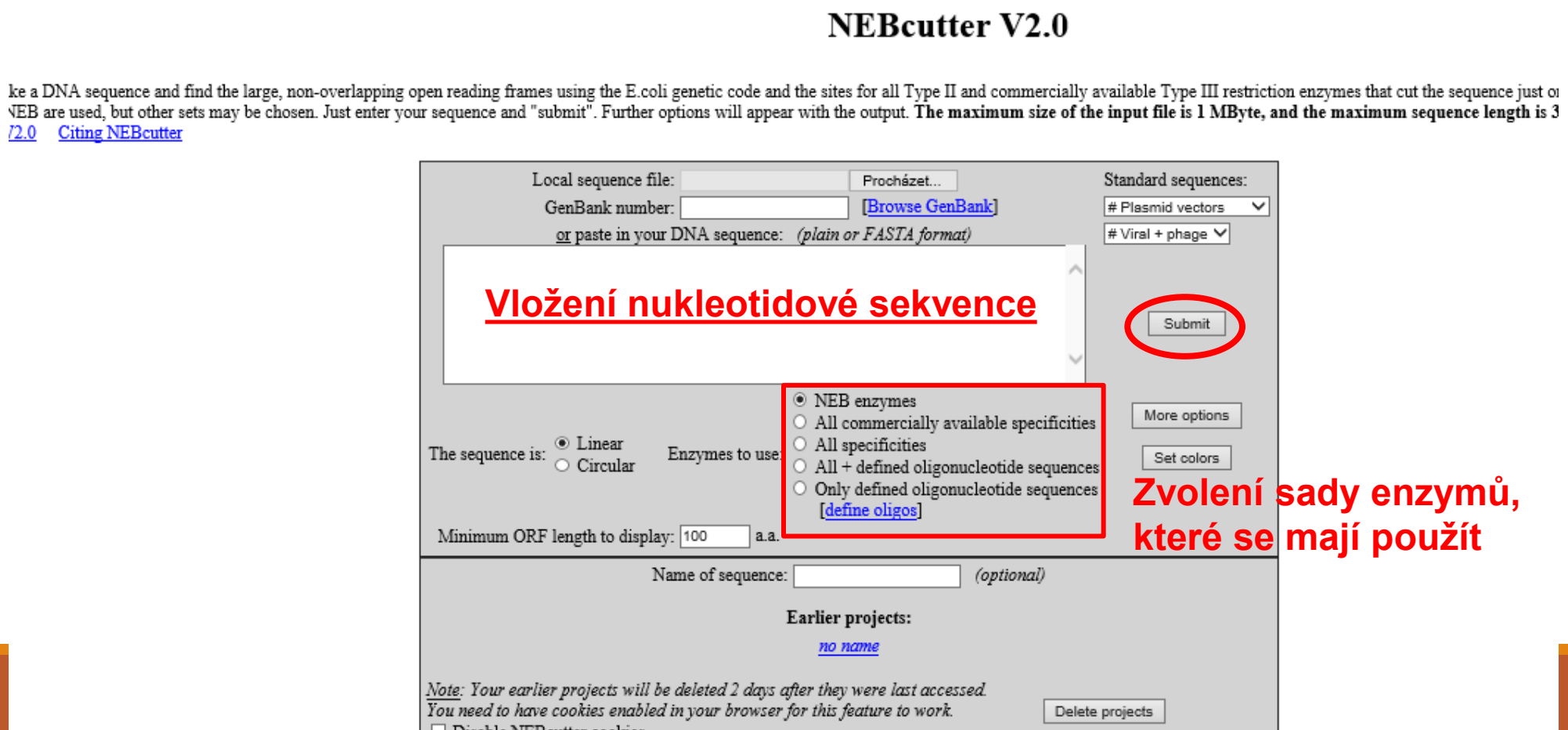
Restrikční analýza



Restrikční analýza

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

NEBcutter



Restrikční analýza

Display: - NEB single cutter restriction enzymes
- Main non-overlapping, min. 100 aa ORFs

GC=41%, AT=59%

Grafické znázornění restrikčních míst

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

Podrobné informace o enzymech se zobrazí po kliknutí

Restrikční analýza

[Back to main display]

SacI

```
5'... G A G C T˅C ... 3'
3'... C˄T C G A G ... 5'
```

Grafické znázornění štěpícího místa

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.

REBASE enzyme page | Methylation Sensitivity

Buffer name: NEBuffer 1.1

Main: 10 mM Bis-Tris-Propane-HCl
pH: 7.0
Mg: 10 mM MgCl2
BSA: 100

Reaction temperature: 37 °C

Overlapping methylation:

NONE

Neoschizomers:

Eco53kI	5'... G A G˅C T C ... 3' 3'... C T C˄G A G ... 5'

Enzymy rozpoznávající sekvenci jako SacI →

Isoschizomers:

NONE

Sites in sequence: 1

End produced at 212:

3' overhang: AGCT

Enzymy produkující kompatibilní konce →

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
BsiHKAI	GWGCWC	all sites	25	100	100	100
Bsp1286I	GDGCHC	all sites	25	25	25	100
SacI	GAGCTC	all sites	100	50	10	100

Vyzkoušejte si....

….udělejte restrikční analýzu „vašeho“ genu (celé CDS)

→ zjistěte, které neštěpí

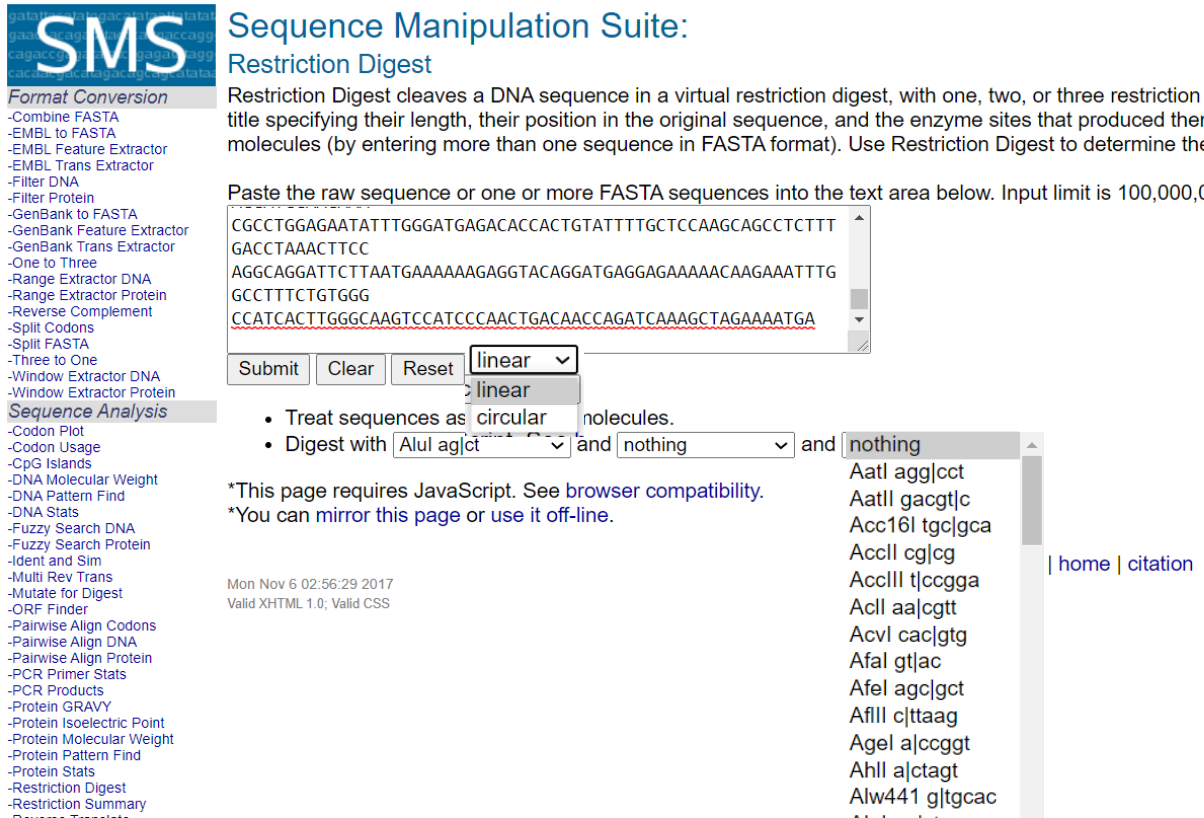
a které štěpí 1x, 2x…těmto RE je nutné se při klonování vyhnout

(DU: : XhoI, HindIII, SacI)

RA:kontrola PCR fragmentů

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

Program SMS: „**Restriction digest**“



RA:kontrola PCR fragmentů

NQO1:CDS-restiction summary

```
>NM_000903.3:122-946 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
```

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

NQO1:CDS-restiction 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

```
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTT
GACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTG
GCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

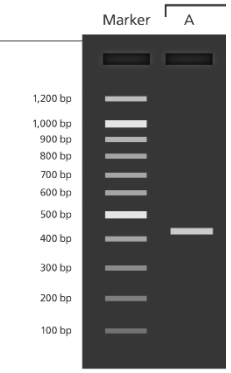
Submit Clear Reset

- Treat sequences as linear molecules.
- Digest with HpaII c|cgg and nothing and nothing .

```
>414 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACTTCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTG
AAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTC
CAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTTTGAGCGAGTG
TTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTC

>411 bp linear fragment from linear parent NM_000903.3:122-946
CGGAGTAAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTG
CAAGGGATCCACGGGGACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCAT
TTCTGTGGCTTCCAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCA
GACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAACGCCTGGAGAATATTTGGGATGAG
ACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCCAGGCAGGATTCTTA
ATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGGCCAT
CACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

A: 414+ 411nt

NQO1:CDS (825nt)



EcoRI

```
>602 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACTTCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTG
AAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTC
CAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTTTGAGCGAGTG
TTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGG
ATCCACGGGGACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGT
GGCTTCCAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCC
CG

>223 bp linear fragment from linear parent NM_000903.3:122-946
AATTCAAATCCTGGAAGGATGGAAGAAACGCCTGGAGAATATTTGGGATGAGACACCACT
GTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCCAGGCAGGATTCTTAATGAAAAA
AGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGGCCATCACTTGGG
CAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

B: 602+ 223nt

NQO1:CDS (825nt)

RA:kontrola PCR fragmentů

- Treat sequences as linear molecules.
- Digest with EcoRI g|aattc and HpaII c|cgg and nothing .

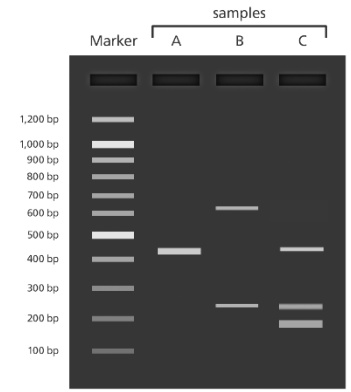
NQO1:CDS-restiction digest

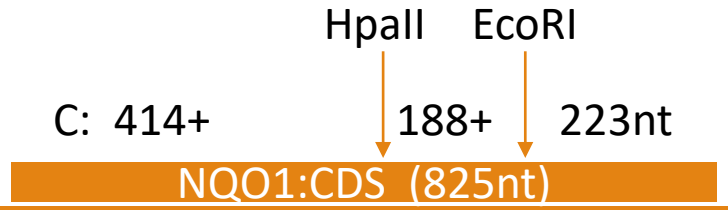
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

```
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

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

```
>414 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACTTCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTG
AAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTC
CAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTTTGAGCGAGTG
TTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTC

>223 bp linear fragment from linear parent NM_000903.3:122-946
AATTCAAATCCTGGAAGGATGGAAGAAACGCCTGGAGAATATTTGGGATGAGACACCACT
GTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCCAGGCAGGATTCTTAATGAAAAA
AGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGGCCATCACTTGGG
CAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA

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





RA:kontrola PCR fragmentů

NQO1:CDS-restiction digest

```
>NM_000903.3:122-946 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
```

HpaII c|cgg	415

EcoRI g|aattc	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

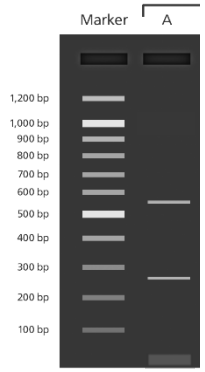
Paste the raw sequence or one or more FASTA sequences into the text area below. Input

```
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTT
GACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTG
GCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Submit Clear Reset

- Treat sequences as linear molecules.
- Digest with AluI ag|ct and nothing and nothing .

```
>541 bp linear fragment from linear parent NM_000903.3:122-946
CTGGAAGCCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCC
ATTCTGAAAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCC
ATGTATGACAAAGGACCCTTCCGGAGTAAGAAGGCAGTGCTTTCCATCACCACTGGTGGC
AGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGGACATGAATGTCATTCTCTGGCCA
ATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACCTCAACTGACATAT
AGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAACGC
CTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTA
AACTTCCAGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAA
TTTGGCCTTTCTGTGGGCCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAA
G

>273 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACTTCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTG
AAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAAG

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



A: 273+ 541+ 11nt

NQO1:CDS (825nt)

RA:kontrola PCR fragmentů

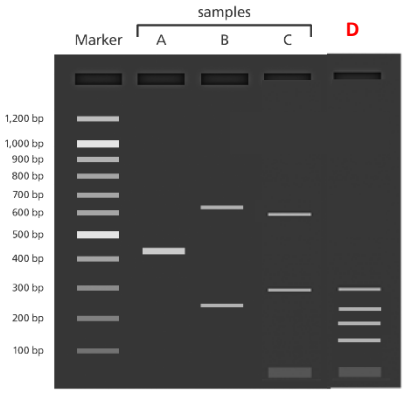
NQO1:CDS-restiction digest

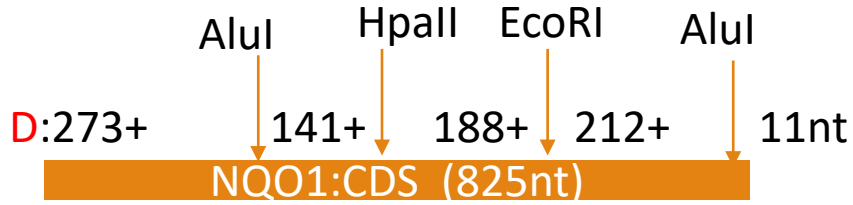
```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),
transcript variant 1, mRNA
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

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

- Treat sequences as linear molecules.
- Digest with EcoRI g|aattc and HpaII c|cgg and AluI ag|ct .

```
>273 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACTTCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTG
AAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAAG

>212 bp linear fragment from linear parent NM_000903.3:122-946
AATTCAAATCCTGGAAGGATGGAAGAAACGCCTGGAGAATATTTGGGATGAGACACCACT
GTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCCAGGCAGGATTCTTAATGAAAAA
AGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGGCCATCACTTGGG
CAAGTCCATCCCAACTGACAACCAGATCAAAG

>188 bp linear fragment from linear parent NM_000903.3:122-946
CGGAGTAAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTG
CAAGGGATCCACGGGGACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCAT
TTCTGTGGCTTCCAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCA
GACGCCCG

>141 bp linear fragment from linear parent NM_000903.3:122-946
CTGGAAGCCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCC
ATTCTGAAAGGCTGGTTTGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCC
ATGTATGACAAAGGACCCTTC

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





Vyzkoušejte si:

- Kolik fragmentů vznikne, štěpíte-li 3. exon referenčního genu lidské GPX7 enzymem MseI?
- Kolik fragmentů vznikne, štěpíte-li 3. exon referenčního genu lidské GPX7 enzymem MscI?

Vyzkoušejte si:

- Kolik fragmentů vznikne, štěpíte-li 3. exon referenčního genu lidské GPX7 enzymem MseI?
- Kolik fragmentů vznikne, štěpíte-li 3. exon referenčního genu lidské GPX7 enzymem MscI?

Homo sapiens glutathione peroxidase 7 (GPX7), mRNA

NCBI Reference Sequence: NM_015696.5

GenBank Graphics

```
>NM_015696.5:440-1229 Homo sapiens glutathione peroxidase 7 (GPX7), mRNA
AGACTTCTGGGAAGGAGCCCACCTGGAACTTCTGGAAGTACCTAGTAGCCCCAGATGGAAAGGTGGTAGG
GGCTTGGGACCCAACTGTGTCAGTGGAGGAGGTCAGACCCCAGATCACAGCGCTCGTGAGGAAGCTCATC
CTACTGAAGCGAGAAGACTTATAACCACCGCGTCTCCTCCTCCACCACCTCATCCCGCCCACCTGTGTGG
GGCTGACCAATGCAAACTCAAATGGTGCTTCAAAGGGAGAGACCCACTGACTCTCCTTCCTTTACTCTTA
TGCCATTGGTCCCATCATTCTTGTGGGGGAAAAATTCTAGTATTTTGATTATTTGAATCTTACAGCAACA
AATAGGAACTCCTGGCCAATGAGAGCTCTTGACCAGTGAATCACCAGCCGATACGAACGTCTTGCCAACA
AAAATGTGTGGCAAATAGAAGTATATCAAGCAATAATCTCCCACCCAAGGCTTCTGTAAACTGGGACCAA
TGATTACCTCATAGGGCTGTTGTGAGGATTAGGATGAAATACCTGTGAAAGTGCCTAGGCAGTGCCAGCC
AAATAGGAGGCATTCAATGAACATTTTTTGCATATAAACCAAAAAATAACTTGTTATCAATAAAAACTTG
CATCCAACATGAATTTCCAGCCGATGATAATCCAGGCCAAAGGTTTAGTTGTTGTTATTTCCTCTGTATT
ATTTTCTTCATTACAAAAGAAATGCAAGTTCATTGTAACAATCCAAACAATACCTCACGATATAAAATAA
AAATGAAAGTATCCTCCTCA
```

MscI tgg|cca	366
MseI t|taa	none

Restriction Digest

Restriction Digest cleaves a DNA sequence in a virtual restriction digest, with one, two, or three restriction e
that produced them. You can digest linear or circular molecules, and even a mixture of molecules (by enterir

Paste the raw sequence or one or more FASTA sequences into the text area below. Input limit is 100,000,0(

```
AAAAACTTG
CATCCAACATGAATTTCCAGCCGATGATAATCCAGGCCAAAGGTTTAGTTGTTGTTATTTC
CTCTGTATT
ATTTTCTTCATTACAAAAGAAATGCAAGTTCATTGTAACAATCCAAACAATACCTCACGAT
ATAAAATAA
AAATGAAAGTATCCTCCTCA
```

Submit Clear Reset

- Treat sequences as linear molecules.
- Digest with MscI tgg|cca and MseI t|taa and nothing .

*This page requir
*You can mirror th

Sun 1 Oct 12:00:07 2023
Valid XHTML 1.0; Valid CSS

Sequence Manipulation Suite – Škola – Microsoft Edge

about:blank

Restriction Digest results

```
>425 bp linear fragment from linear parent NM_015696.5:440-1229 H
CCAATGAGAGCTCTTGACCAGTGAATCACCAGCCGATACGAACGTCTTGCCAACAAAAAT
GTGTGGCAAATAGAAGTATATCAAGCAATAATCTCCCACCCAAGGCTTCTGTAAACTGGG
ACCAATGATTACCTCATAGGGCTGTTGTGAGGATTAGGATGAAATACCTGTGAAAGTGCC
TAGGCAGTGCCAGCCAAATAGGAGGCATTCAATGAACATTTTTTGCATATAAACCAAAAA
ATAACTTGTTATCAATAAAAACTTGCATCCAACATGAATTTCCAGCCGATGATAATCCAG
GCCAAAGGTTTAGTTGTTGTTATTTCCTCTGTATTATTTTCTTCATTACAAAAGAAATGC
AAGTTCATTGTAACAATCCAAACAATACCTCACGATATAAAATAAAAATGAAAGTATCCT
CCTCA

>365 bp linear fragment from linear parent NM_015696.5:440-1229 H
AGACTTCTGGGAAGGAGCCCACCTGGAACTTCTGGAAGTACCTAGTAGCCCCAGATGGAA
AGGTGGTAGGGGCTTGGGACCCAACTGTGTCAGTGGAGGAGGTCAGACCCCAGATCACAG
CGCTCGTGAGGAAGCTCATCCTACTGAAGCGAGAAGACTTATAACCACCGCGTCTCCTCC
TCCACCACCTCATCCCGCCCACCTGTGTGGGGCTGACCAATGCAAACTCAAATGGTGCTT
CAAAGGGAGAGACCCACTGACTCTCCTTCCTTTACTCTTATGCCATTGGTCCCATCATTC
TTGTGGGGGAAAAATTCTAGTATTTTGATTATTTGAATCTTACAGCAACAAATAGGAACT
CCTGG
```

Vyzkoušejte si:

Pracujte s vaší CDS nebo ukázkovou sekvencí „**Nácvik štěpení**“

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

Vyzkoušejte si (DU7)

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

- délka:18-24nt
- Tm: 55-60°C

+ zkontrolujte pozice pomoci multalin:

(porovnejte) celou mRNA, CDS a oba primery

DÚ7 – Návrh primerů a štěpení RE

1) Navrhněte manuálně primery, tak aby se amplifikovala vaše CDS

- Navrhněte F a R primer tak aby Tm nebyla větší než 60°C
- zkontrolujte pozice pomoci multalin:
  (porovnejte) celou mRNA, CDS a oba primery

2) Štěpení

- Zjistěte zda a kolikrát štěpí **CDS** sekvenci vašeho genu enzymy: XhoI, HindIII, SacI
- Jaké fragmenty z vaší **CDS** vzniknou po štěpení všemi těmito enzymy?
- Vyberte RE, kterou byste štěpili vaši CDS jednou a výsledek na gelu by vhodně potvrdil vznik předpokládaného produktu