Základy praktické Bioinformatiky

PETRA MATOUŠKOVÁ

2023/2024

Základy praktické bioinformatiky

Téma 7/10

Nukleotidová bioinformatika III

Cíle:

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

„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

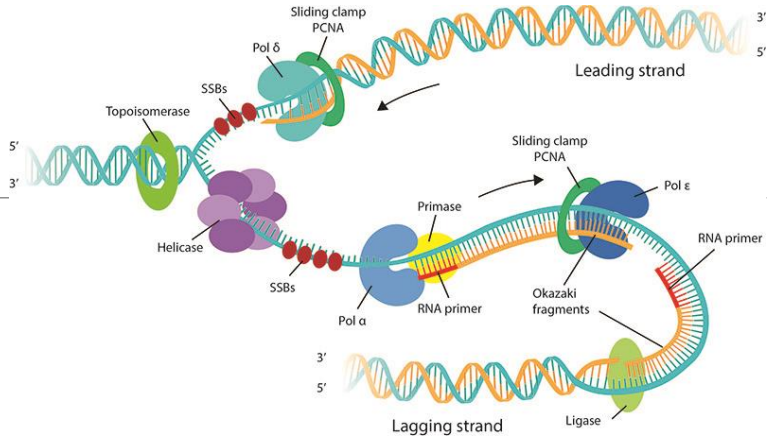
mikroRNA

Celé genomy

….

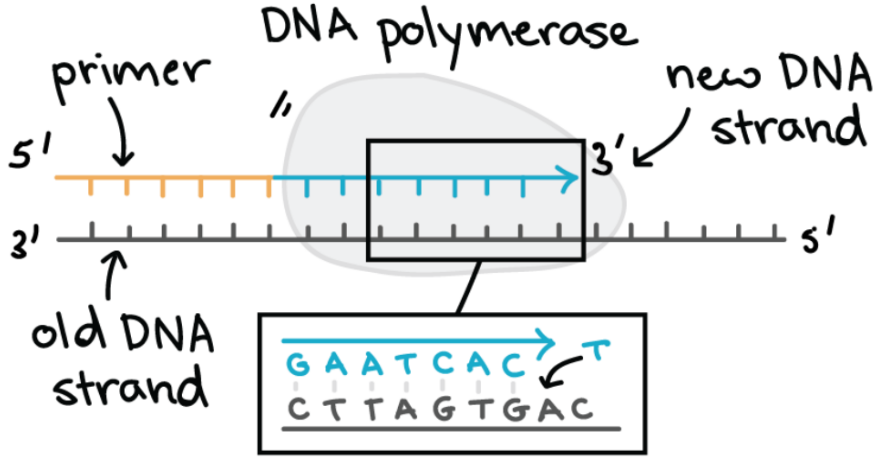
Primer = oligonukleotid

= krátký jednořetězcový úsek DNA



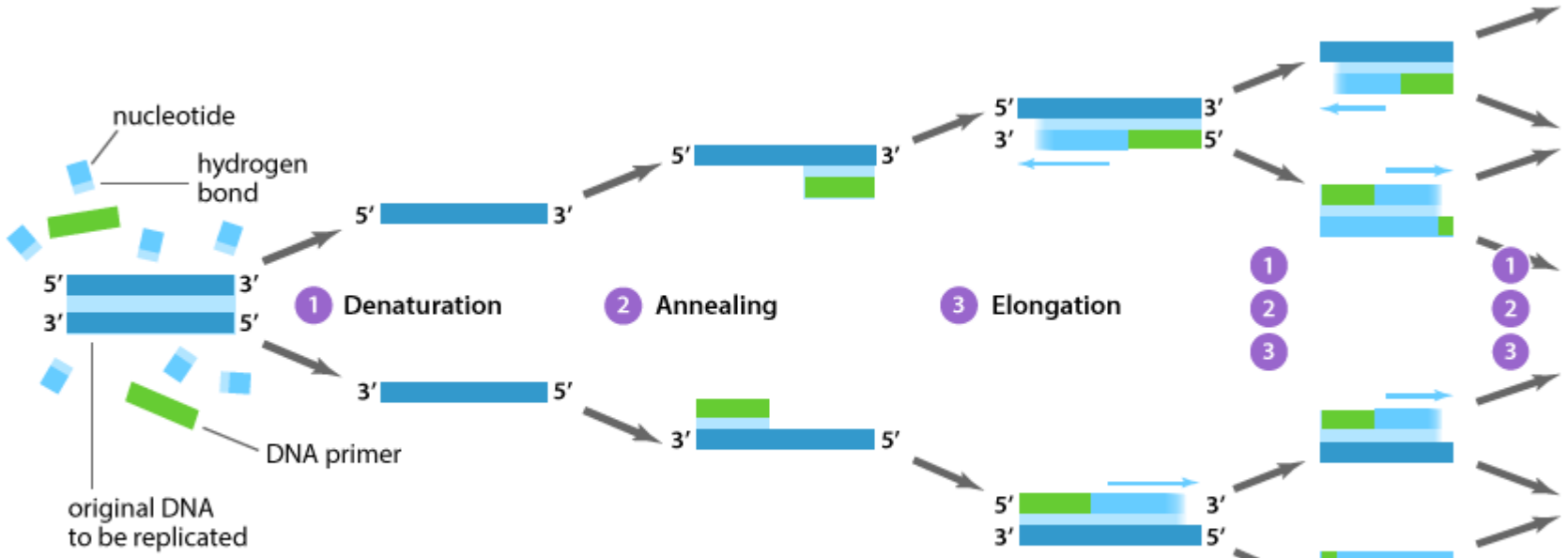
Použití:

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

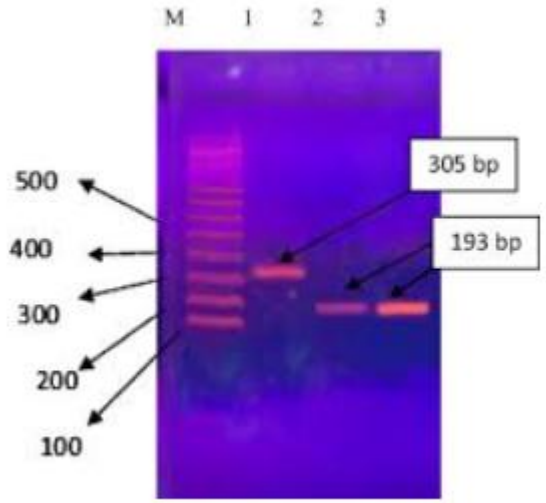


Polymerázová řetězová reakce

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

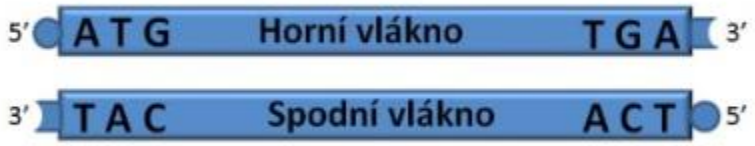


→ „End point“ detekce

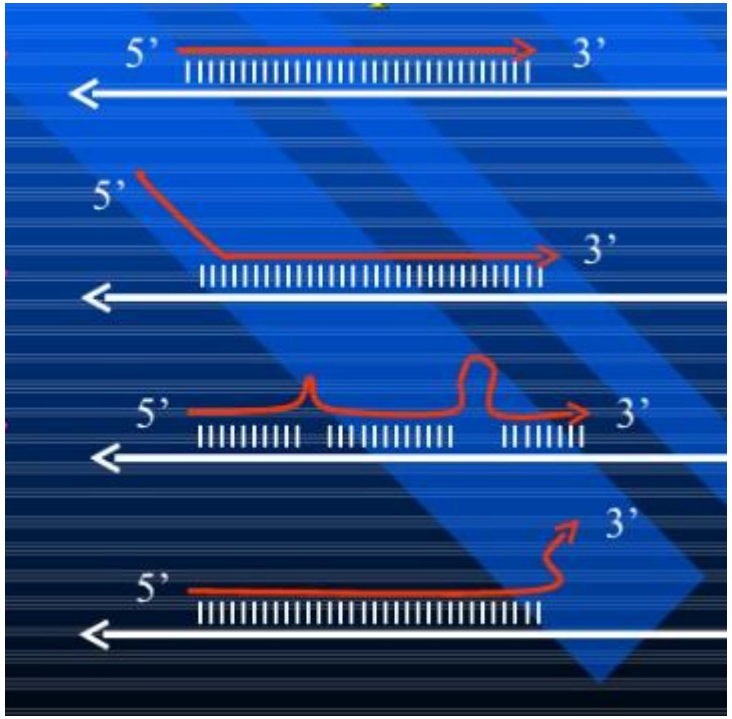


Polymerázová řetězová reakce

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



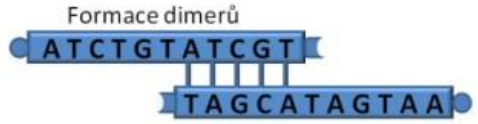
Syntéza: 5´→ 3´

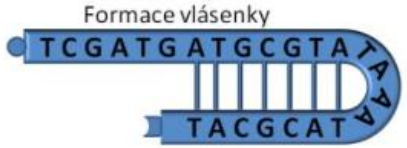


Polymerázová řetězová reakce

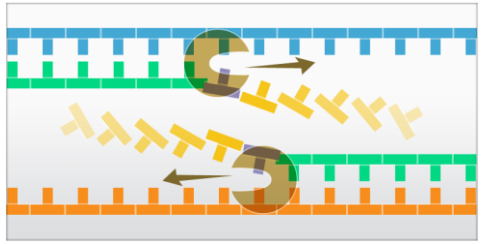
Charakteristiky „dobré“ dvojice primerů:

- 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)



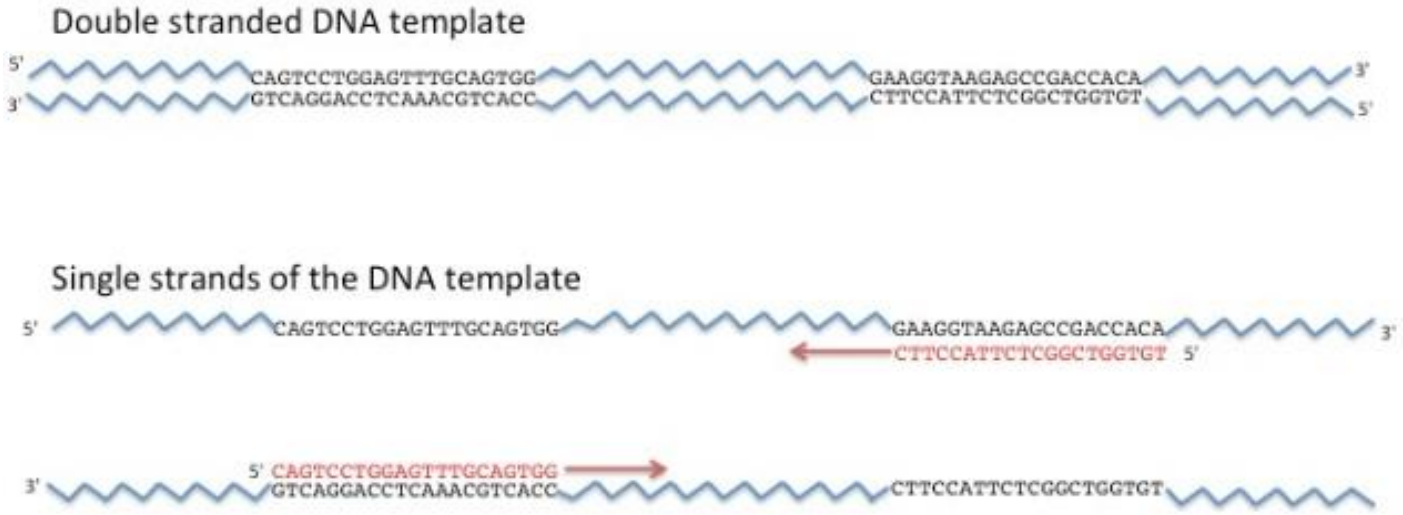


Polymerázová řetězová reakce

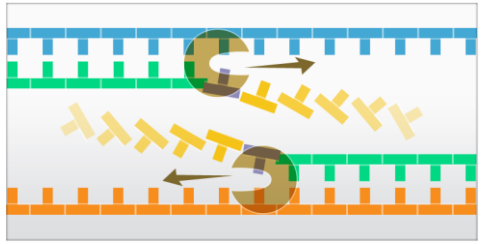


1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh

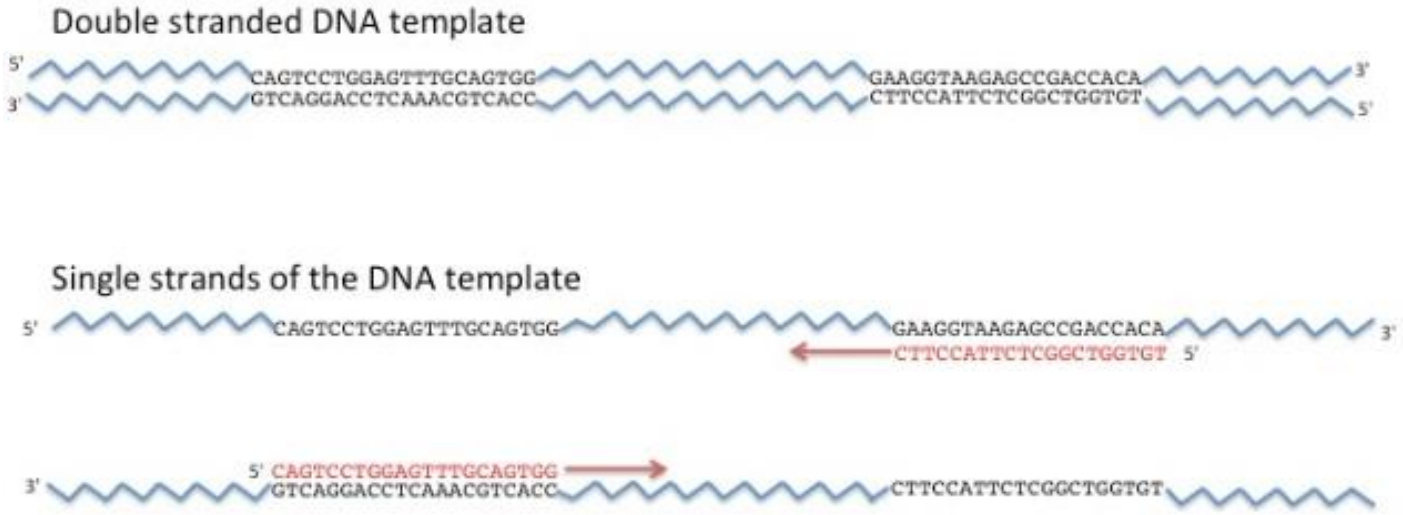


Polymerázová řetězová reakce

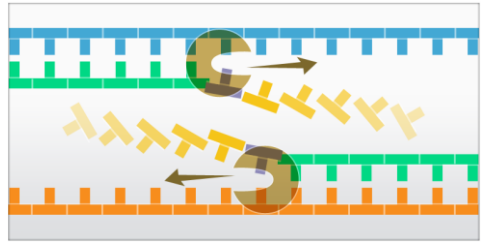


1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh



Polymerázová řetězová reakce



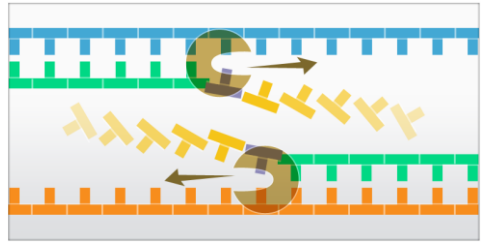
1) Amplifikace (namnožení) požadovaného úseku DNA (genu, fragmentu):

→ manuální návrh

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

5´-ATGCCCTTTCnnnnnnnnnnnnnnnTAAATCCCGC-3´

Polymerázová řetězová reakce



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

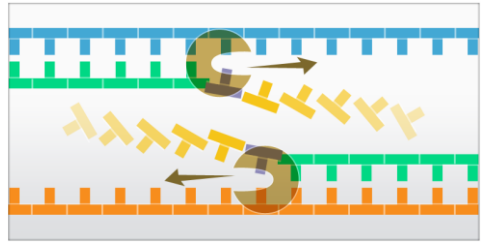
→ manuální návrh

- **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:**

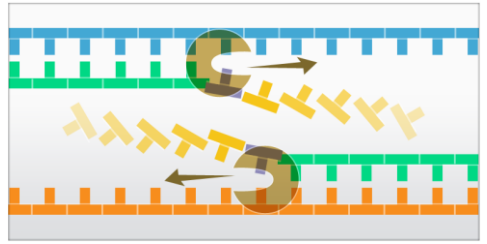
5´-ATGCCCTTTCnnnnnnnnnnnnnnTAAATCCCGC-3´

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

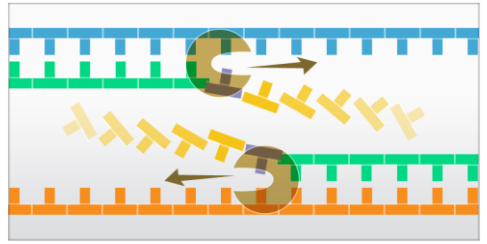
F_primer: **ATGCCCTTTC**

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**

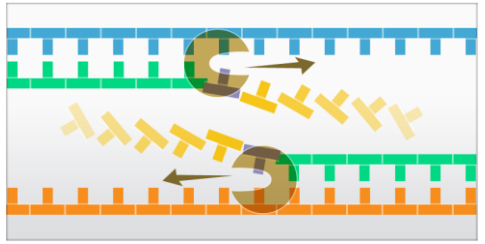
5´-ATGCCCTTTCnnnnnnnnnnnnnnTAAATCCCGC-3´

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

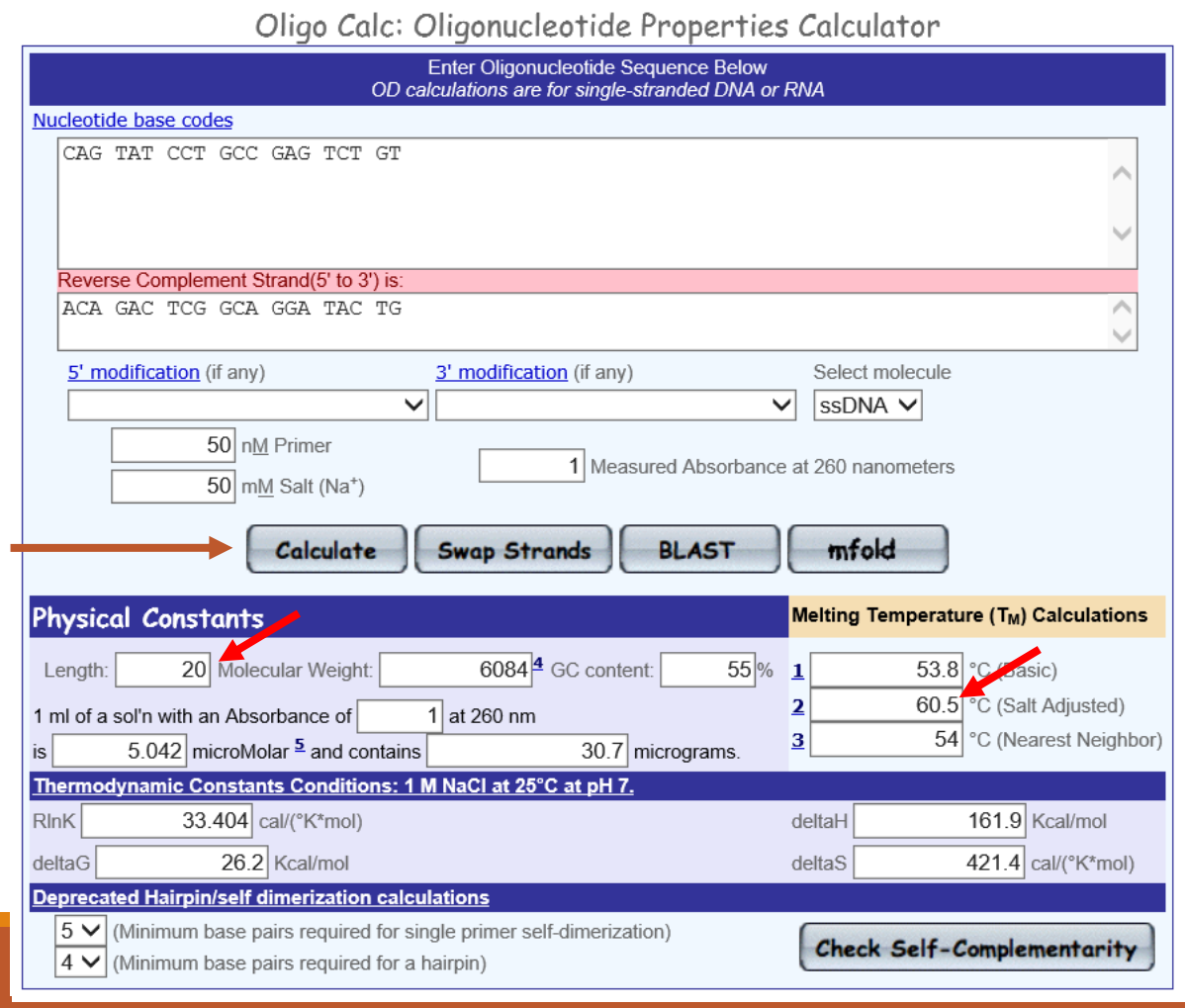
→ **manuální návrh: 2 primery**

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

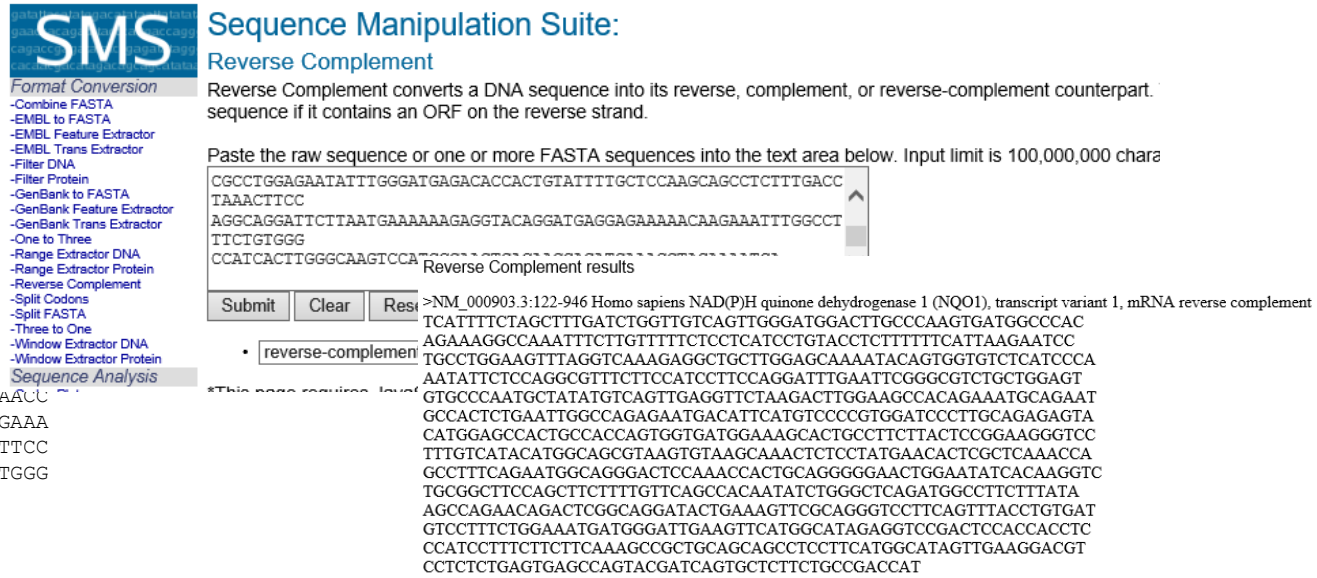
→ **manuální návrh: 2 primery**

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

ATGGTCGGCAGAAGACACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATG
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

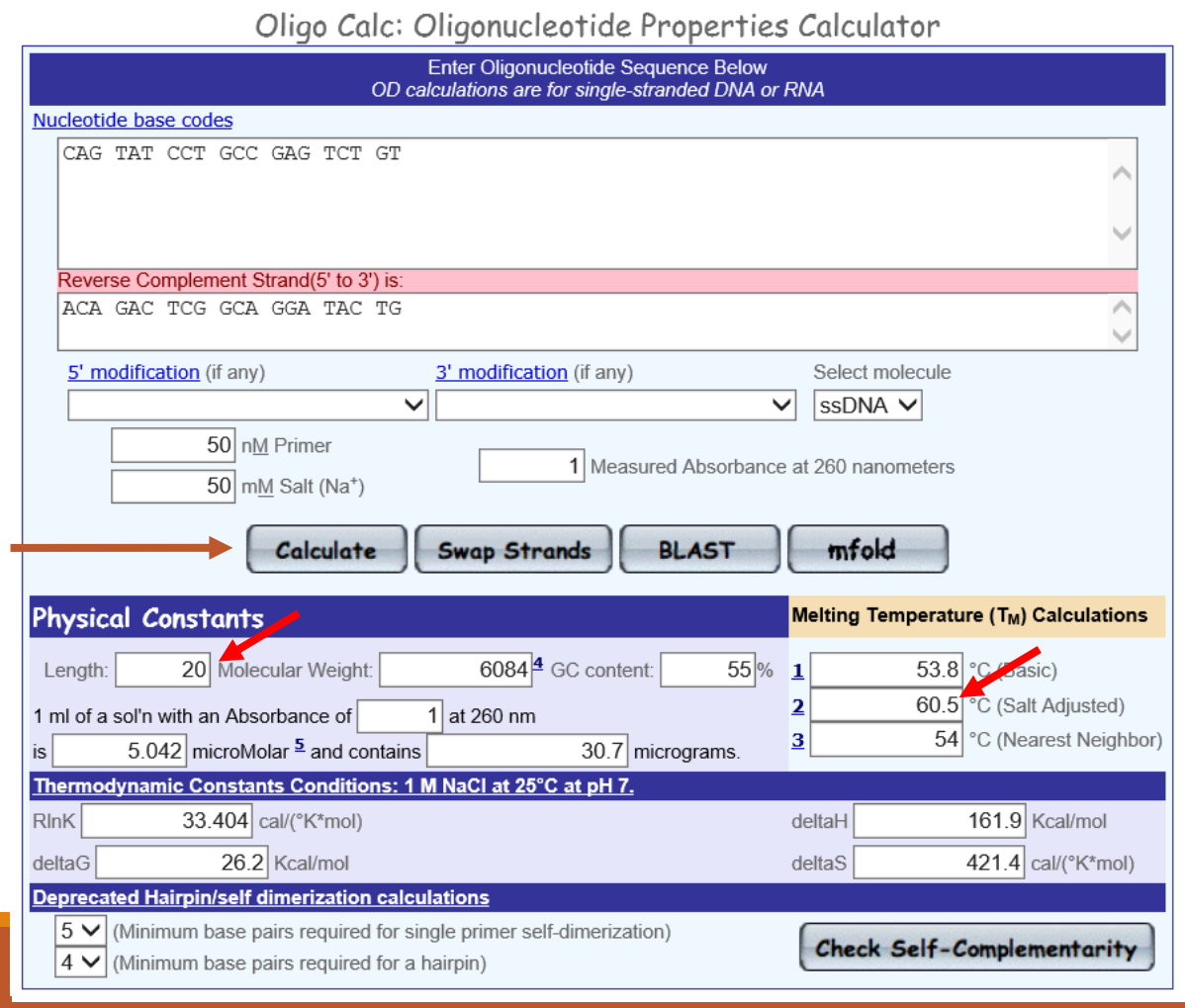
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCCAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
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

2) zkontrolujte pozice pomoci 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

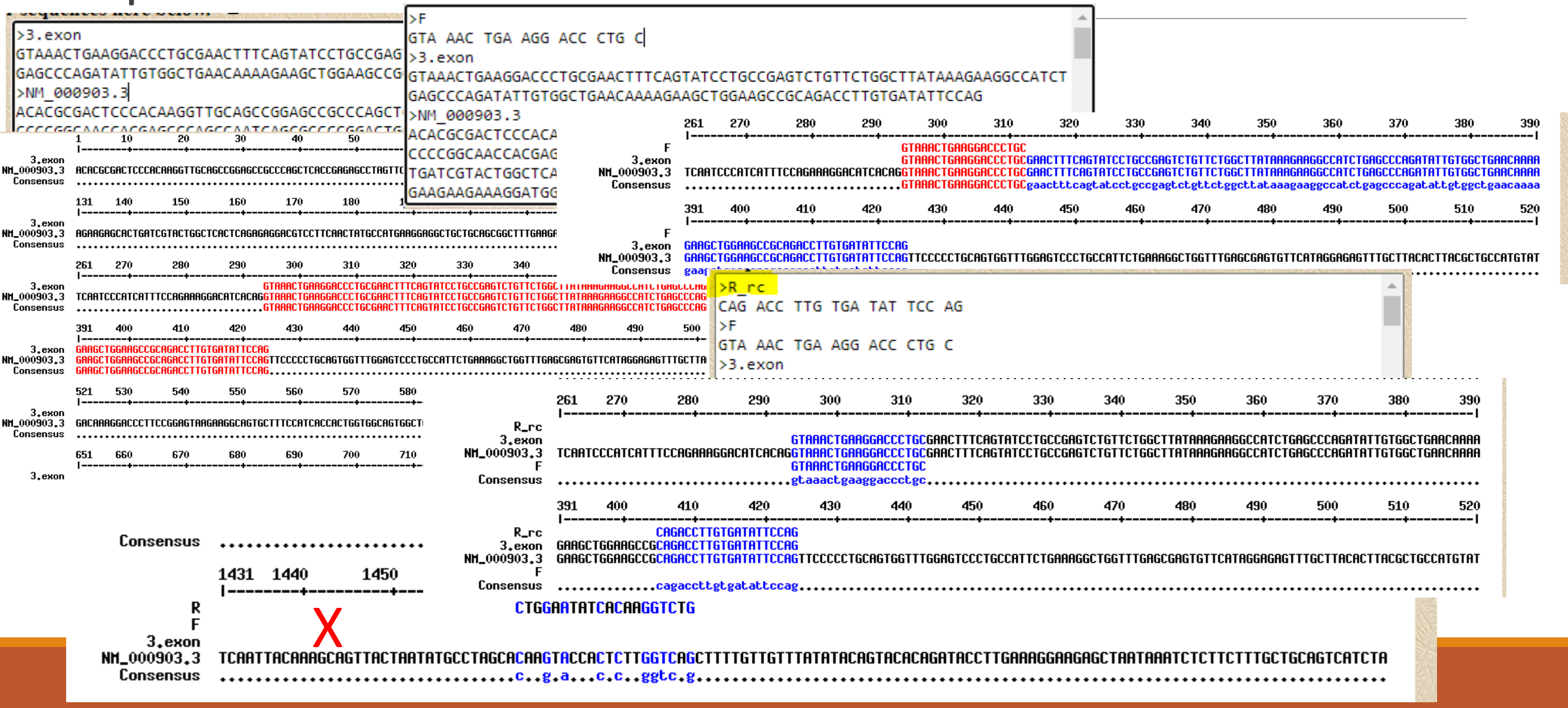
Výsledek:

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 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
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 (DU7)

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

- délka:18-24nt
- Tm: 55-60°C

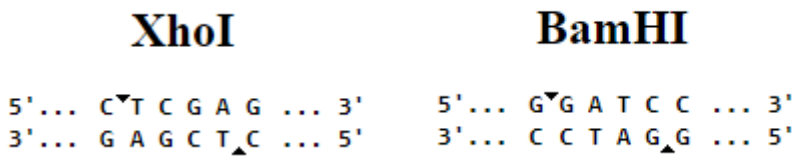
+ zkontrolujte pozice pomoci multalin:

(porovnejte) celou mRNA, CDS a oba primery

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

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

Sequence Manipulation Suite:

Restriction Summary

Restriction Summary accepts a DNA sequence and returns the number and ly 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
ctaaattgtaagcgttaatattttgttaaaattcgcgttaaatttttgttaaatcagctca
ttttttaaccaataggccgaaatcggcaaaatcccttataaatcaaaagaatagaccgaga
tagggttgagtgttgttccagtttggaacaagagtccactattaaagaacgtggactccaa
cgtcaaagggcgaaaaaccgtctatcagggcgatggcccactacgtgaaccatcaccctaa
tcaagtttttttggggtcgaggtgccgtaaagcactaaatcggaaccctaaagggagccccc
```

Please check the browser compatibility page before using this program.

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.

Fri Jun 17 16:17:06 2016
Valid XHTML 1.0; Valid CSS

Restriction Summary results

cuts once
cuts twice

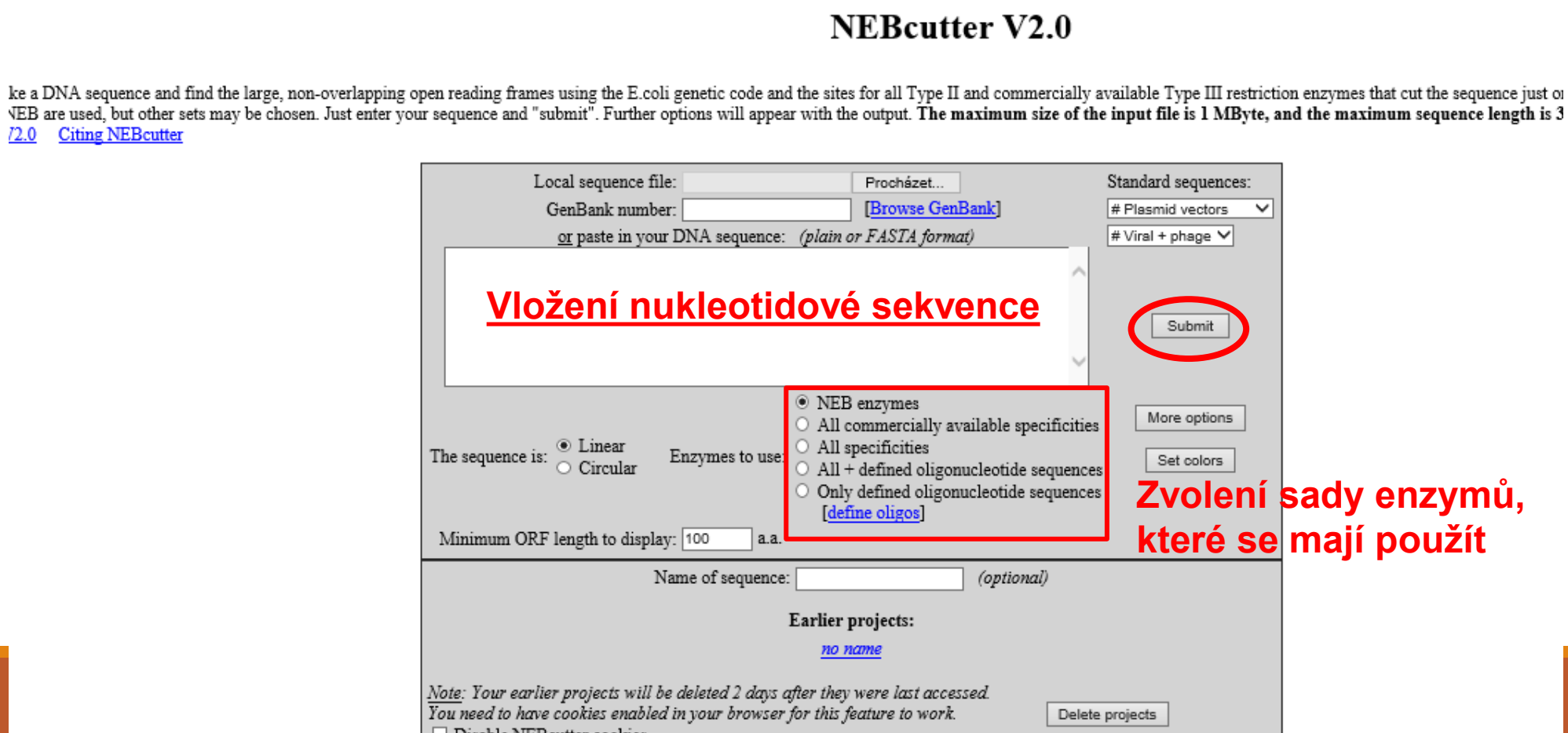
Results for linear 2961 residue sequence "sample sequence" starting "ctaaattgta"

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
AflII c|ttaag	none
AgeI a|ccggt	none
AhlI a|ctagt	684
Alw441 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
AscI gg|cgcgcc	none

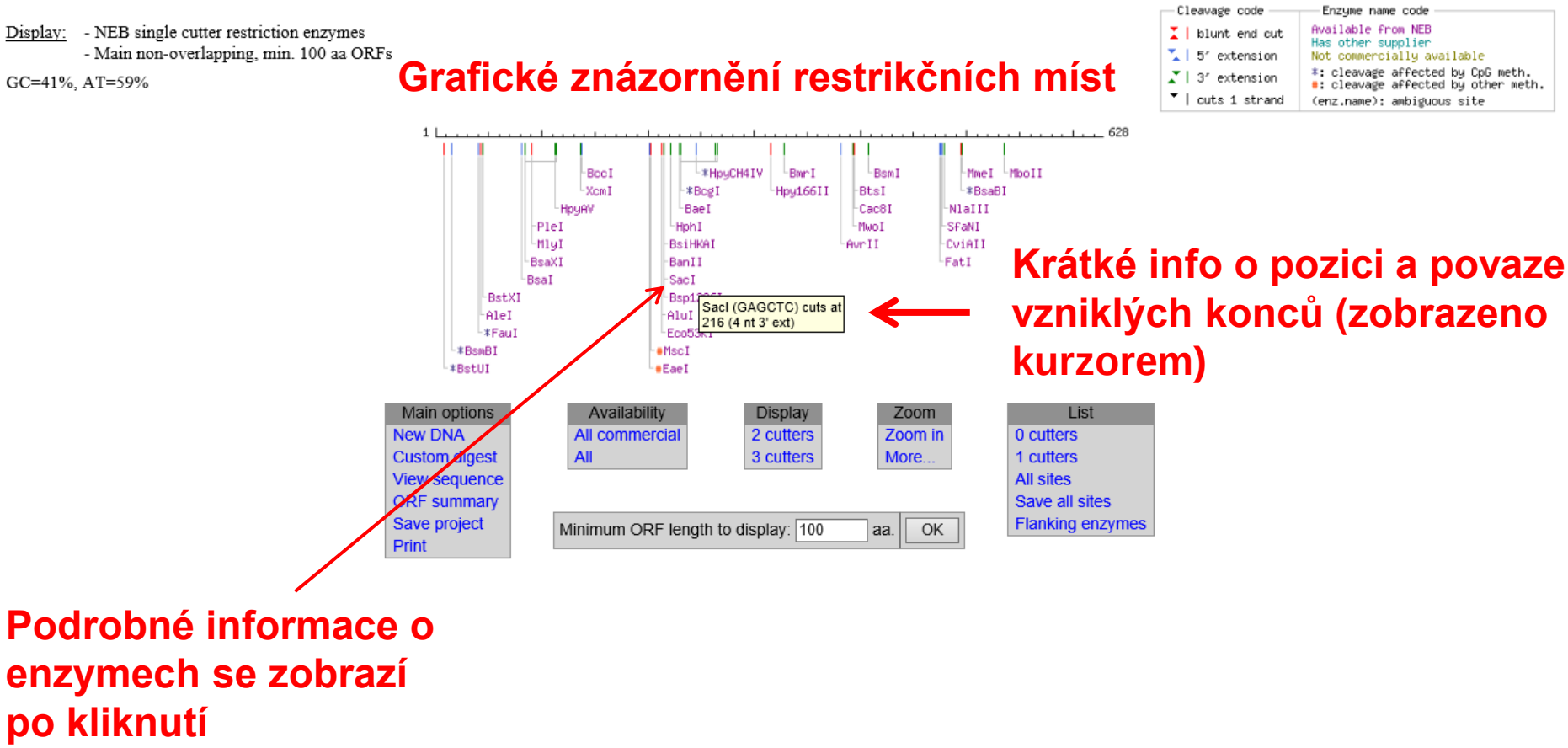
Restrikční analýza

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

NEBcutter



Restrikční analýza



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

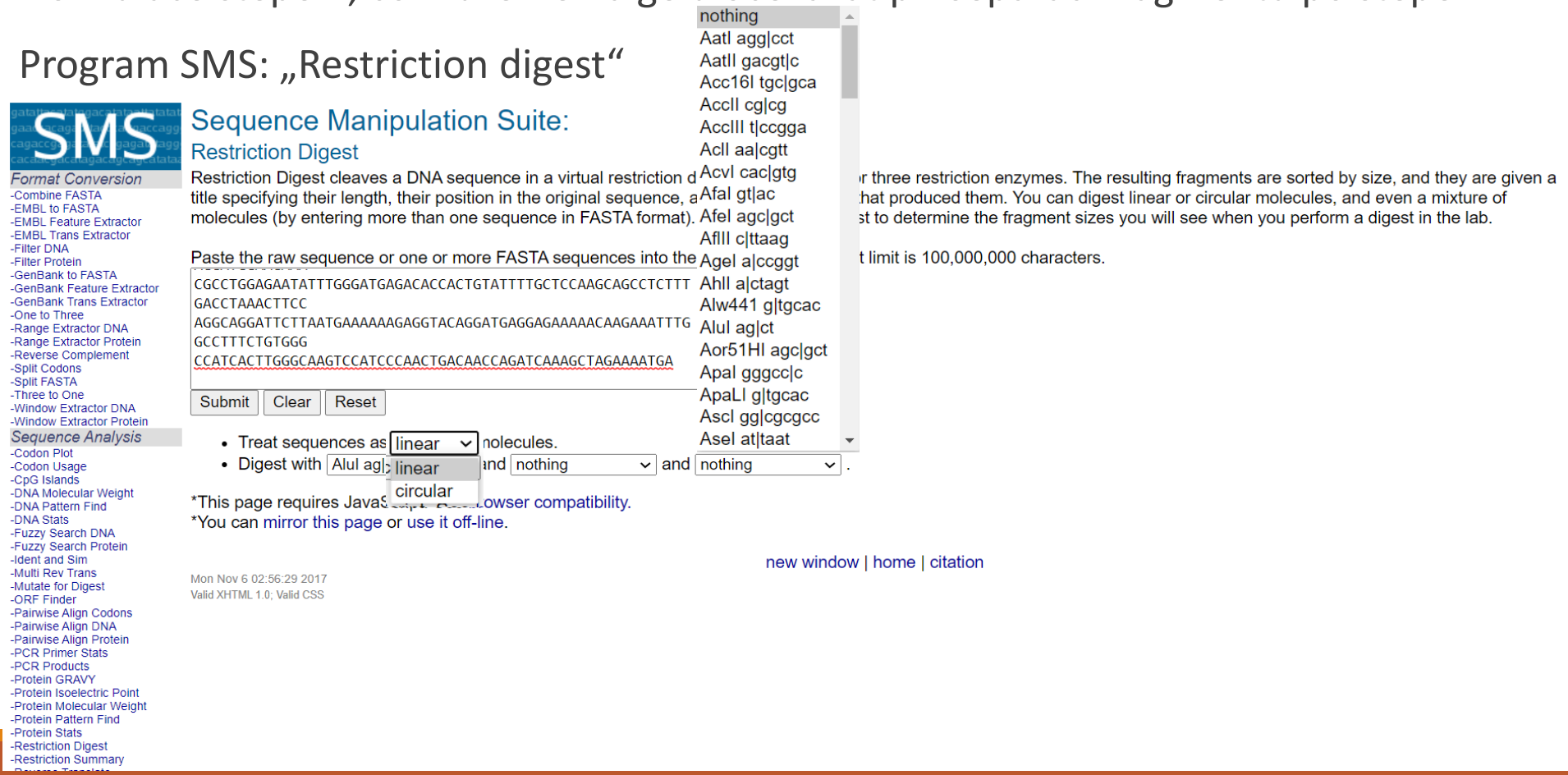
a které štěpí 1x, 2x…těmto RE je nutné se při klonování vyhnout

(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
```

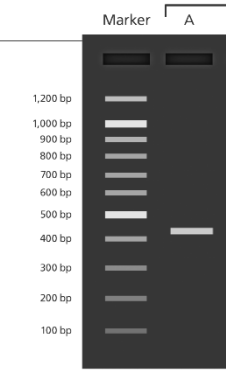
Submit Clear Reset

- 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 .

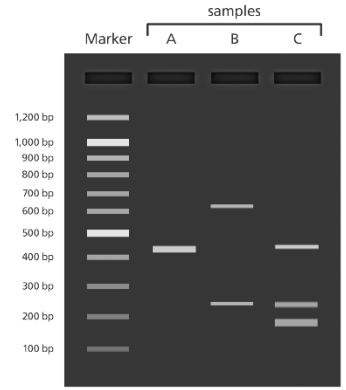
NQO1:CDS-restiction digest

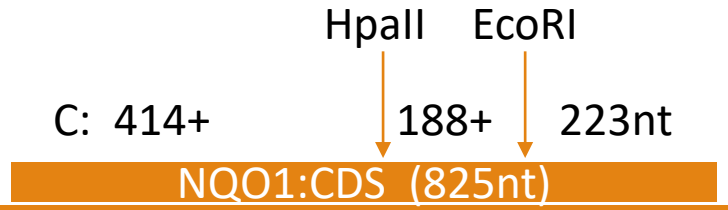
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

```
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

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

NQO1:CDS-restiction digest

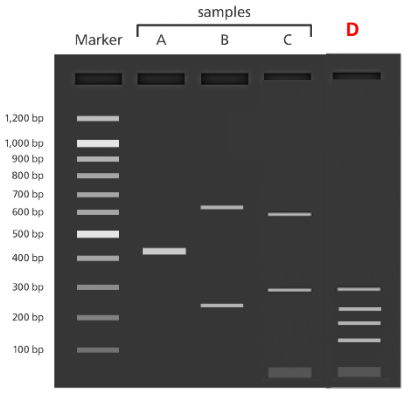
```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),
transcript variant 1, mRNA
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC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

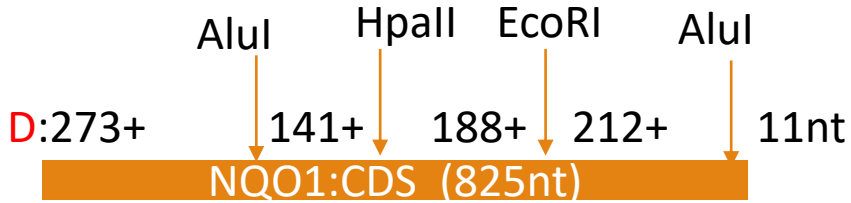
HpaII c|cgg	415

EcoRI g|aattc	603

AluI ag|ct	274, 815

```
>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:

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 restrikční 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 restrikční 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 Tm nebyla větší než 60°C
- zkontrolujte pozice pomoci 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|tcgag	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 (se
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACTTCAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTG
AAGGACCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGCAGACCTTGTGATATTC
CAGTTCCCCCTGCAGTGGTTTGGAGTCCCTGCCATTCTGAAAGGCTGGTTTGAGCGAGTG
TTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGCTTTCCATCACCACTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGG

>346 bp linear fragment from linear parent Untitled, base 480 to base 825 (
GATCCACGGGGACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTG
TGGCTTCCAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCAGACGC
CCGAATTCAAATCCTGGAAGGATGGAAGAAACGCCTGGAGAATATTTGGGATGAGACACC
ACTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCCAGGCAGGATTCTTAATGAA
AAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTTCTGTGGGCCATCACTT
GGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

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