

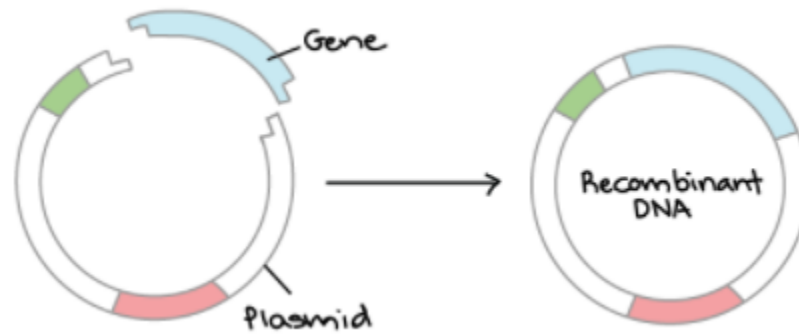
Introduction to applied bioinformatics

PETRA MATOUŠKOVÁ

2023/2024

8/10

Cloning

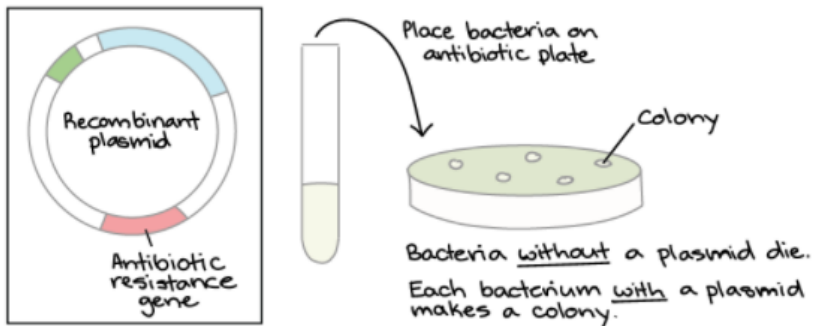
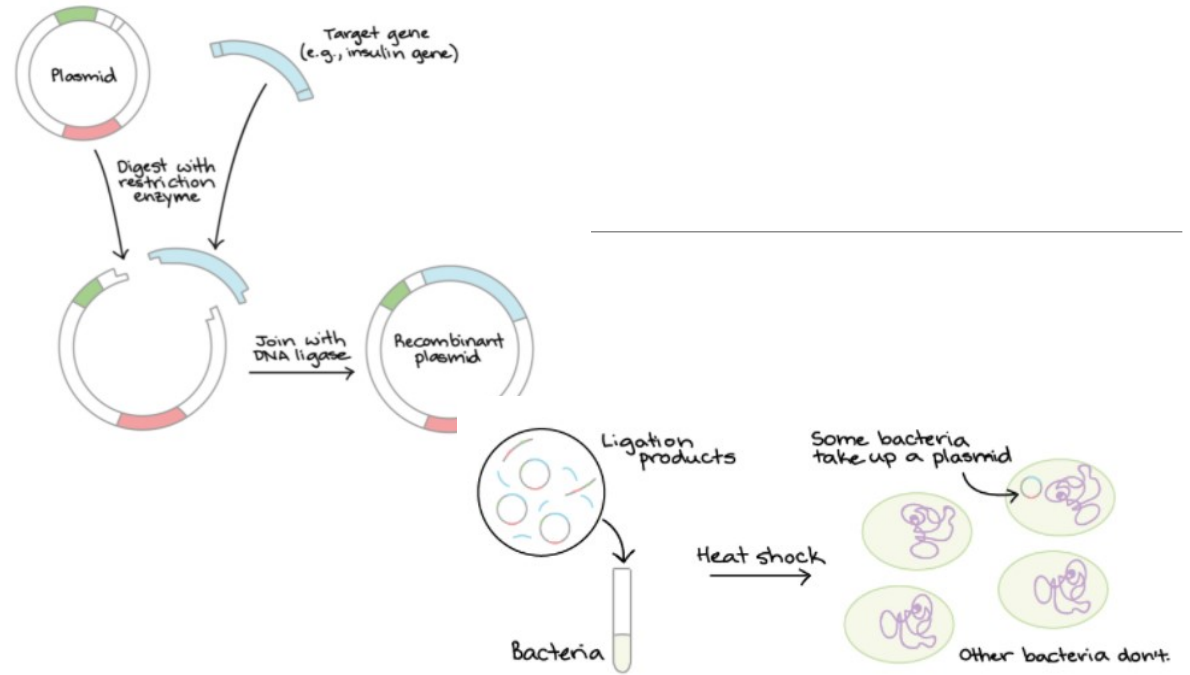


[* DNA cloning and recombinant DNA \(video\) | Khan Academy](#)

Cloning

Steps:

- 1) amplification, restriction and ligation
- 2) transformation
- 3) selection
- 4) ...



Cloning of desired products (gene)

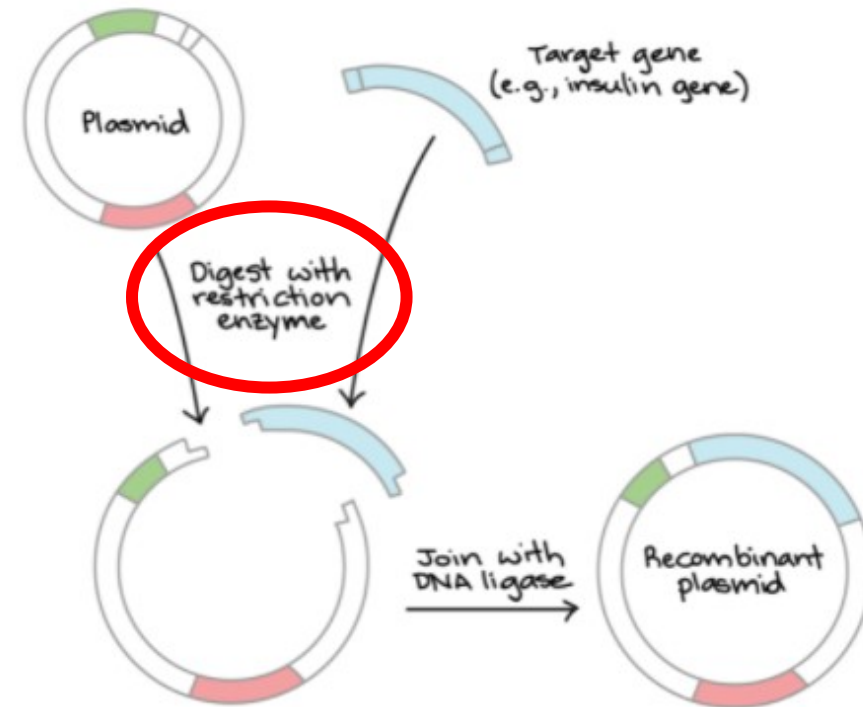
„manual design“ Forward primer

Reverse primer (sequence „reverse complement“)

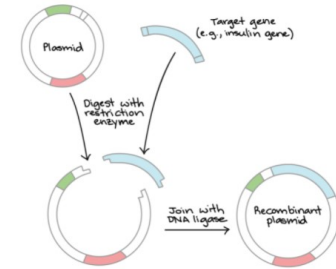
- Specific (cloning CDS, promotor sequence anal
→ plasmid of choice

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

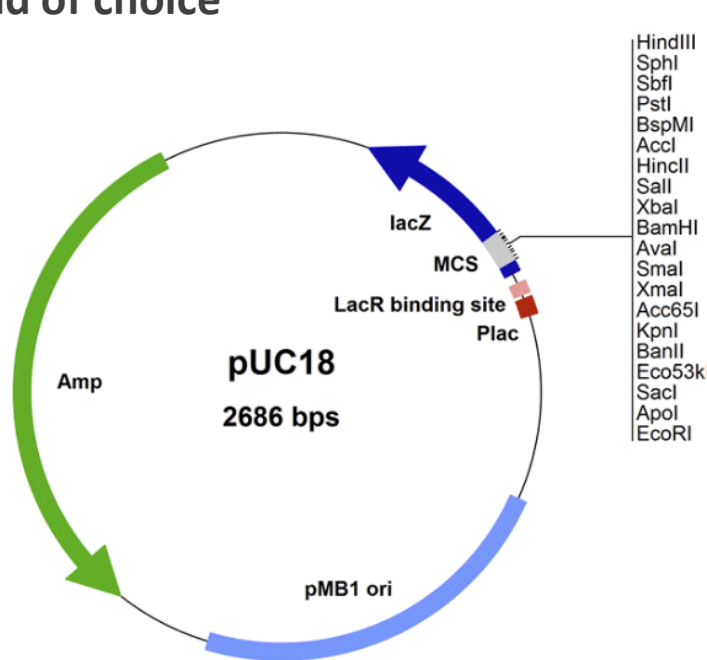
```
ATGGTCGGCAGAAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACCTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAAACTGAAGGACCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATCCAGTTCACCCCTGCAGTGGTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCCTTCCGGAGT
AAGAAGGCAGTGTCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCAATCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCCTGTATTTTGTCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATTCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCACTGACAACCAGATCAAGCTAGAAAATGA
```



Cloning



- Specific (cloning CDS, promoter sequence analysis, 3' UTR...)
- plasmid of choice



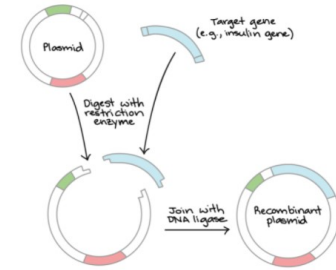
MCS (multiple cloning site)
Recognition sites for restriction endonucleases

M13/pUC sequencing primer (-20), 17-mer 399 HindIII PaeI SdaI BvuI PstI HincII Sall XbaI BamHI Cfr9I Eco58I SmaI KpnI Acc65I Ecl136II Eco24I SacI EcoRI XapI 455

5' G TAA AAC GAC GGC CAG TGC CAA GCT TGC ATG CCT GCA GGT CGA CTC TAG AGG ATC CCC GGG TAC CGA GCT CGA ATT CGT
3' C ATT TTG CTG CCG GTC ACG GTT CGA ACG TAC GGA CGT CCA GCT GAG ATC TCC TAG GGG CCC ATG GCT CGA GCT TAA GCA
LacZ ← Val val Ala Leu Ala Ser Ala His Arg Cys Thr Ser Glu Leu Pro Asp Gly Pro Val Ser Ser Ser Asn Thr
AAT CAT GGT CAT AGC TGT TTC CTG 3'
TTA GTA CCA GTA TCG ACA AAG GAC 5'
Ile Met Thr Met

M13/pUC reverse sequencing primer (26), 17-mer

Cloning



- Specific (cloning CDS, promoter sequence analysis, 3' UTR...)

→ plasmid of choice

→ primers with RE target sites overhangs

→ 1) restriction analysis of a sequence



>NM_000903.2:192-1016 Homo sapiens NAD(P)H quinone dehydrogenase 1

(NQO1), transcript variant 1, mRNA

```
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTT
CAATCCCATCATTTCCAGAAAGGACATCACAGGTAACCTGAAGGACCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGCAGACCTTGTGATATTCAGTTCCCCCTGCAGTGGTTGGAGTCCCTGCCATTCGAAAGGCTGGTT
TGAGCGAGTGTTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAGGACCTTCCGGAGT
AAGAAGGCAGTGCCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGGATCCACGGGG
ACATGAATGTCATTCTCTGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAA
CGCCTGGAGAATATTTGGGATGAGACACCCTGTATTTTGCTCCAAGCAGCCTCTTTGACCTAAACTTCC
AGGCAGGATCTTAAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCACTGACAACCCAGATCAAAGCTAGAAAATGA
```

Cloning-Restriction analysis

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
ctaaattgtaagcgttaatatTTTTGTAAATTCGCGTTAAATTTGTAAATCAGTCA
TTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAGAATAGACCGAGA
TAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAAGAAGCTGGACTCAA
CGTCAAAGGGCGAAAACCGTCTATCAGGGCGATGGCCACTACGTGAACCATCACCTAA
TCAAGTTTTTGGGGTCGAGGTGCCGTAAGCACTAAATCGGAACCCCTAAAGGGAGCCCC
```

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

Submit Clear Reset

- 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 aalcacacc	none

Format Conversion

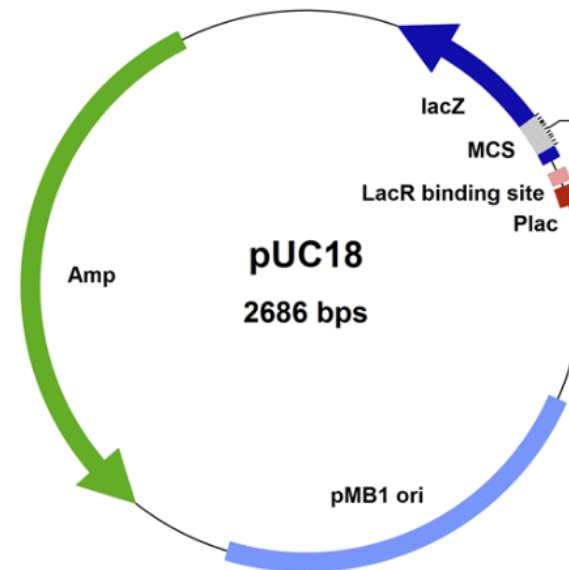
- Combine FASTA
- EMBL to FASTA
- EMBL Feature Extractor
- EMBL Trans Extractor
- Filter DNA
- Filter Protein
- GenBank to FASTA
- GenBank Feature Extractor
- GenBank Trans Extractor
- One to Three
- Range Extractor DNA
- Range Extractor Protein
- Reverse Complement
- Split Codons
- Split FASTA
- Three 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

Practical part

Analyze your sequence for presence of RE target
- check REs from multiple cloning site



HindIII
SphI
SbfI
PstI
BspMI
AccI
HincII
SalI
XbaI
BamHI
AvaI
SmaI
XmaI
XmaI
XmaI
Acc65I
KpnI
BanII
Eco53k
SacI
ApeI
EcoRI

HindIII
SphI
SbfI
PstI
BspMI
AccI
HincII
SalI
XbaI
BamHI
AvaI
SmaI
XmaI
Acc65I
KpnI
BanII
Eco53k
SacI
ApeI
EcoRI

Cloning-Fragment analysis

= restriction simulation (what can be expected on the agarose gel after separation)

Program SMS: „Restriction digest“



- 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

Sequence Manipulation Suite: Restriction Digest

Restriction Digest cleaves a DNA sequence in a virtual restriction digest. The user specifies the restriction enzyme, the length of the fragments, the 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
AGGCAGGATCTTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTG
GCCTTCTGTGGG
CCATCACTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Submit Clear Reset

- Treat sequences as molecules.
- Digest with and 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 agIcct
- AatII gacgtIc
- Acc16I tgcIgca
- AccII cgIcg
- AccIII tIccgga
- AcI aalcggt
- AcvI cacIgtg
- AfaI gtIac
- AfeI agcIgct
- AflII cIttaag
- AgeI aIccggt
- AhlI aIctagt
- Alw441 gIgtcac
- AluI agIct
- Aor51HI agcIgct
- ApalI gggccIc
- ApaLI gIgtcac
- AscI ggIcgcgcc
- Asel atItaat

For three restriction enzymes. The resulting fragments are sorted by size, and they are given a title that specifies their length, their position in the original sequence, and the number of molecules (by entering more than one sequence in FASTA format). 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](#)

Cloning-Fragment analysis

NQ01:CDS-restriction summary

```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),
transcript variant 1, mRNA
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACCT
CAATCCCATCATTTCAGAAAGGACATCACAGSTAACTGAAGGACCCCTGCGAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAAGCCATCTGAGCCAGATATTGTGGCTGAACAAAAGAAAGCTGGAAG
CCGACACCTTGTGATATCCAGTTCCTCCCTGCAGTGGTTGGAGTCCCTGCCATTCTGAAAGGCTGGTT
TGAGCGAGTTCATAGGAGAGTTCCTTACACTTACGCTGCCATGTATGACAAAAGGACCCCTCCCGGAGT
AAGAGGCACTGCTTTCATCACCCTGGTGGCACTGGCTCCATGTAATCTCTGCAAGGATCCACGGG
ACATGAATGTCAATCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCGAATTCARATCCTGGAAGGATGGAAGAAA
CGCTGGAGAAATTTGGGATGAGACACCACTGATTTTGGCTCCAAAGCAGCCTCTTGCACCTAAACTCC
AGGCAGGATCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCACTGACAACCAAGATCAAAGCTAGAAAAATGA
```

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

```
CGCTGGAGAATATTTGGGATGAGACACCACTGATATTTGCTCCAAGCAGCTCTTT
GACCTAAACTTC
AGGCAGGATCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTG
GCCTTTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCACTGACAACCAAGCTAGAAAAATGA
```

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
GACCTCTATGCCATGAACCTCAATCCCATCATTTCAGAAAGGACATCACAGSTAAACTG
AAGGACCCCTGCGAACTTTCAGTATCCTGCCAGTCTGTTCTGGCTTATAAAGAAAGGCCAT
CTGAGCCAGATATTGTGGCTGAACAAAAGAGCTGGAAGCCCGACAGCTTGTGATATTC
CAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTTCTGAAAGGCTGGTTTGGCGAGTG
TTCATAGGAGAGTTTGGCTTACACTTACGCTGCCATGTATGACAAGGACCCCTTC
```

```
>411 bp linear fragment from linear parent NM_000903.3:122-946
CGGAGTAAGAAGGCACTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTAATCTGTCG
CAAGGGATCCACGGGGACATGAATGTCAATCTCTGGCCAATTTCAGAGTGGCATTCTGCAT
TTCTGTGGCTTCCAAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCA
GACGCCGAATTCAAATCCTGGAAGGATGGAAAGAAACGCTGGAGAAATATTTGGGATGAG
ACACCCTGATATTTGCTCCAAGCAGCTCTTTCACCTAAACTTCCAGGCAGGATTTCTTA
ATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGGCCAT
CACTTGGGCAAGTCCATCCCACTGACAACCAAGTCAAAGCTAGAAAAATGA
```

A: 414+ 411nt
NQO1:CDS (825nt)



EcoRI

```
>602 bp linear fragment from linear parent NM_000903.3:122-946
ATGGTCGGCAGAAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTAT
GCCATGAAGGAGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGAGTCG
GACCTCTATGCCATGAACCTCAATCCCATCATTTCAGAAAGGACATCACAGSTAAACTG
AAGGACCCCTGCGAACTTTCAGTATCCTGCCAGTCTGTTCTGGCTTATAAAGAAAGGCCAT
CTGAGCCAGATATTGTGGCTGAACAAAAGAGCTGGAAGCCCGACAGCTTGTGATATTC
CAGTTCCTCCCTGCAGTGGTTTGGAGTCCCTGCCATTTCTGAAAGGCTGGTTTGGCGAGTG
TTCATAGGAGAGTTTGGCTTACACTTACGCTGCCATGTATGACAAGGACCCCTCCGGAGT
AAGAAGGCAGTGGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTCAAGGG
ATCCACGGGACATGAATGTCAATCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGT
GGCTTCCAAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCC
CG
```

```
>223 bp linear fragment from linear parent NM_000903.3:122-946
AATTCAAATCCTGGAAGGATGGAAGAAACGCTGGAGAAATTTGGGATGAGACACCCT
GTATTTTGGCTCCAAGCAGCTCTTTGACCTAACTCCAGGCAGGATTTCTTAATGAAAA
AGAGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGGGCCCTCACTTGGG
CAAGTCCATCCCACTGACAACCAAGTCAAAGCTAGAAAAATGA
```

B: 602+ 223nt
NQO1:CDS (825nt)

Cloning-Fragment analysis

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

NQO1:CDS-restiction digest

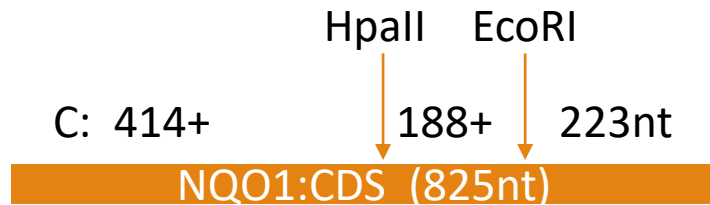
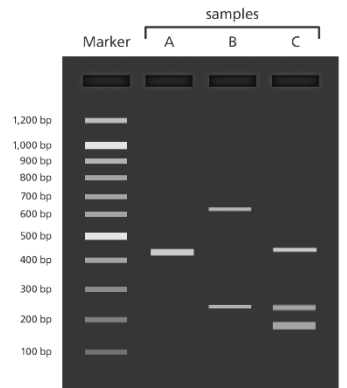
```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),
transcript variant 1, mRNA
ATGGTCGGCAGAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACCTCCTCAACTATGCCATGAAG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAGGATGGGAGGTGGTGGAGTCCGACCTCATGCCATGAAC
CAATCCCATCATTCCAGAAAGGACATCACAGTAACTGAAGGACCTCGCAACTTTCAGTATCCTGCC
GAGTCTGTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAG
CCGACACCTTGTGATATCCAGTCCCGCTGCAGTGGTTGGAGTCCCTGCCATTCTGAAAGGCTGGT
TGAGCGAGTTCATAGGAGAGTTTGCCTTACACTTACGCTGCCATGTATGACAAAAGGACCTTCCGGAGT
AAGAAGGCACTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGATCCACGGG
ACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTCCCAAGTCTTAGAAC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCGAATTCARATCCTGGAAGGATGGAAGAAA
CGCTGGAGATATTGGGATGAGACACCACTGTATTTGCTCCCAAGCAGCCTCTTGCACCTAACTCC
AGGCAGGATCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGG
CCATCACTTGGGCAAGTCCATCCCACTGACCAACAGATCAAAGCTAGAAAAATGA
```

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
GACCTCTATGCCATGAACCTTCAATCCCATCATTTCAGAAAAGGACATCACAGGTAACCTG
AAGGACCCCTGCGAACTTTCAGTATCCTGCCGAGTCTGTTCTGGCTTATAAAGAAGCCAT
CTGAGCCCAGATATTGTGGCTGAACAAAAGAAGCTGGAAGCCGACACCTTGTGATATTC
CAGTTCCCCCTGCAGTGGTGGAGTCCCTGCCATTCTGAAAGGCTGGTGGAGCGAGTG
TTCATAGGAGAGTTTGCTTACACTTACGCTGCCATGTATGACAAAAGGACCCCTTC
```

```
>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
CGGAGTAAGAAGGCAGTGCTTCCATCACCCTGGTGGCAGTGGCTCCATGTACTCTCTG
CAAGGGATCCACGGGGACATGAATGTCATTCTCTGGCCAATTCAGAGTGGCATTCTGCAT
TTCTGTGGCTTCCAAGTCTTAGAACCTCAACTGACATATAGCATTGGGCACACTCCAGCA
GACGCCCC
```



Cloning-Fragment analysis

NQO1:CDS-restriction digest

```
>NM_000903.3:122-946 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1),
transcript variant 1, mRNA
ATGGTCGGCAGAGAGCACTGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGG
AGGCTGCTGCAGCGGCTTTGAAGAAGAAAGGATGGGAGGTGGTGGATCGGACCTCTATGCCATGAACCT
CAATCCCATCATTTCCAGAAAGGACATCACAGTAAACTGAAGGACCCCTCGCAACTTTCAGTATCCTGCC
GAGTCTGTTCTGGCTTATAAAGAAGGCCATCTGAGCCAGATATTTGGTGAACAAAGAAAGCTGGAAG
CCGACACCTTGTGATATCCAGTCCCCCTGCAGTGGTTGGAGTCCCTGCCATTTGAAAGGCTGGTT
TGAGCGAGTTCATAGGAGAGTTTGCCTTACACTTACGCTGCCATGTATGACAAAGGACCCCTCCCGAGT
AAGAAGGCACTGCTTCCATCACCACCTGGTGGCAGTGGCTCCATGTACTCTCTGCAAGGATCCACGGG
ACATGAATGTCATTTCTGGCCAATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAGTCTTAGAACC
TCAACTGACATATAGCATTGGGCACACTCCAGCAGACGCCGAAATTCARATCCTGGAAGGATGGAAGAAA
CGCTGGAGAAATTTGGGATGAGACACCACTGTATTTGCTCCCAAGACCCCTCTTGACCTAAACTCC
AGGCAAGGATCTTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCTTCTGTGG
CCATCACTTGGGCAAGTCCATCCCACTGACAAACAGATCAAAGCTAGAAAAATGA
```

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

```
CGCTGGAGAATTTGGGATGAGACCACTGTATTTGCTCCAAGCAGCCTTT
GACCTAAACTTCC
AGGCAGGATTCCTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTG
GCCTTTCTGTTGG
CCATCACTTGGGCAAGTCCATCCCACTGACAACCCAGATCAAAGCTAGAAAAATGA
```

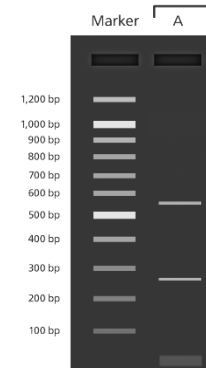
Submit Clear Reset

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

```
>541 bp linear fragment from linear parent NM_000903.3:122-946
CTGGAAGCCGAGACCTTTGTGATATCCAGTTCGCCCTGCAGTGGTTTGGAGTCCCTGCC
ATTTCTGAAAGGCTGGTTTGGAGGAGTGTTCATAGGAGAGTTTGGCTTACACTTACGCTGCC
ATGTATGACAAAGGACCTTCCGGAGTAAAGAAGGCAGTGCCTTCCATCACCCTGGTGGC
AGTGGCTCCATGTAATCTCTGCAAGGGATCCACGGGGACATGAATGTCATTTCTGGCCA
ATTCAGAGTGGCATTCTGCATTTCTGTGGCTTCCAAGTCTTAGAACCTCAACTGACATAT
AGCATTGGGGCACACTCCAGCAGACGCCCGAATTCAAATCCTGGAAGGATGGAAGAAACGC
CTGGAGAATATTTGGGATGAGACACCACTGTATTTGCTCCCAAGCAGCCTCTTGGACCTA
AACTTCCAGGCAGGATTCCTAATGAAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAA
TTTGGCCTTTCTGTGGGCCATCACTTGGGCAAGTCCATCCCACTGACAACCCAGATCAAAG
G
```

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

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

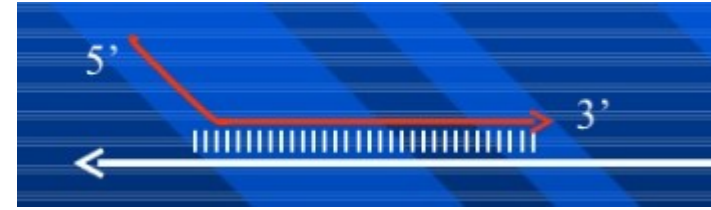


Practical part

- Using **Restriction summary** find RE which cuts you sequence 1x and another that cuts 2x

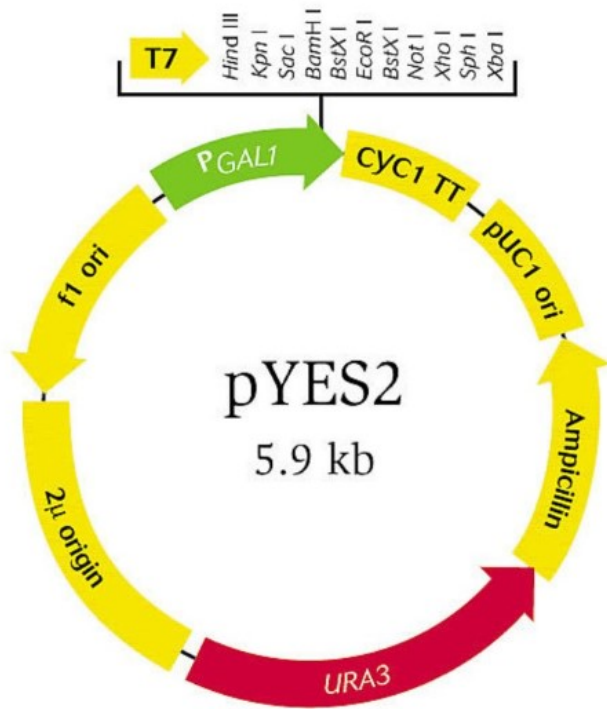
- simulate restriction in **Restriction Digest** what will you see on the gel after such digestion?

cloning - specific



→ primers includes site for REs

1) restriction summary (to avoid enzymes that would cut your sequence)



1) Restriction summary:

HindIII a agctt	none
SacI gagct c	none
EcoRI g aatc	603 X
XhoI c tcgag	none

Specific gene detection

Primers: anywhere along the sequence !



- short amplicon (product): **200 - 500nt**

but: must be **specific** for tested organism → BLAST

Specific gene detection

Gene detection

- Specificity matters! (Primer BLAST!)

NCBI Resources How To Sign in to NCBI

Nucleotide Nucleotide Search Advanced Help

GenBank Send to: Change region shown Customize view

Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

NCBI Reference Sequence: NM_000903.2
[FASTA](#) [Graphics](#)

Go to:

LOCUS NM_000903 2601 bp mRNA linear FRI 29-MAR-2018
DEFINITION Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA.
ACCESSION NM_000903
VERSION NM_000903.2
KEYWORDS RefSeq.
SOURCE Homo sapiens (human)
ORGANISM [Homo sapiens](#)
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 2601)
AUTHORS Cheng X, Liu F, Liu H, Wang G and Hao H.
TITLE Enhanced glycometabolism as a mechanism of NQO1 potentiated growth of NSCLC revealed by metabolomic profiling
JOURNAL Biochem. Biophys. Res. Commun. 496 (1), 31-36 (2018)
PUBMED [29291405](#)
REMARK GeneRIF: Taken together, we proposed that NQO1 could potentiate NSCLC cell proliferation by enhancing cellular glycometabolism, and

Analyze this sequence
Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence
Show in Genome Data Viewer

Articles about the NQO1 gene
Redox modulation of NQO1. [PLoS One. 2018]
Enhanced glycometabolism as a mechanism of NQO1 po [Biochem Biophys Res Commun. 2018]
RNA-binding activity of TRIM25 is mediated by its PRY/SPRY domain and is requi [BMC Biol. 2017]
See all...

Specific gene detection

Gene detection

- Specificity matters! (Primer BLAST!)

Primer-BLAST A tool for finding specific primers

NCBI/ Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST).

[Reset page](#) [Save search parameters](#) [Retrieve recent results](#) [Publication](#) [Tips for finding specific primers](#)

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) [Clear](#)

NM_000903.2

Range

Forward primer From To [Clear](#)

Reverse primer

Or, upload FASTA file

Primer Parameters

Use my own forward primer (5'->3' on plus strand) [Clear](#)

Use my own reverse primer (5'->3' on minus strand) [Clear](#)

PCR product size

Min Max

of primers to return

Primer melting temperatures (T_m)

Min Opt Max Max T_m difference [Clear](#)

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section [Clear](#)

Exon junction span [Clear](#)

Exon junction match

Exon at 5' side Exon at 3' side

Specific gene detection

Gene detection

- Specificity matters! (Primer BLAST!)

Primer-BLAST *A tool for finding specific primers*

► NCBI/ Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST).

[Reset page](#) [Save search parameters](#) [Retrieve recent results](#) [Publication](#) [Tips for finding specific primers](#)

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) [Clear](#)

	From	To
Forward primer	196	<input type="text"/>
Reverse primer	<input type="text"/>	1016

[Clear](#)

Or, upload FASTA file [Procházet...](#)

Primer Parameters

Use my own forward primer (5'→3' on plus strand) [Clear](#)

Use my own reverse primer (5'→3' on minus strand) [Clear](#)

PCR product size	Min <input type="text" value="200"/>	Max <input type="text" value="500"/>		
# of primers to return	<input type="text" value="10"/>			
Primer melting temperatures (T _m)	Min <input type="text" value="57.0"/>	Opt <input type="text" value="60.0"/>	Max <input type="text" value="63.0"/>	Max T _m difference <input type="text" value="3"/>

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span

Exon junction match

when CDS displayed

Specific gene detection

Gene detection

- Specificity matters! (Primer BLAST!)

Note: Parameter values that differ from the default are highlighted in yellow

Primer Pair Specificity Checking Parameters

Specificity check Enable search for primer pairs specific to the intended PCR template

Search mode Automatic

Database Refseq mRNA

Exclusion Exclude predicted Refseq transcripts (accession with XM, XR prefix) Exclude uncultured/environmental sample sequences

Organism 9606
Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type.
[Add more organisms](#)

Entrez query (optional)

Primer specificity stringency Primer must have at least 2 total mismatches to unintended targets, including at least 2 mismatches within the last 5 bps at the 3' end. Ignore targets that have 6 or more mismatches to the primer.

Max target size 4000

Allow splice variants Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)

Get Primers Show results in a new window Use new graphic view

[Advanced parameters](#) Note: Parameter values that differ from the default are highlighted in yellow

Only human sequences

Specific gene detection

Gene detection

- Specificity matters! (Primer BLAST!)

Primer-BLAST *Primer-Blast results*

▶ NCBJ/ Primer-BLAST : results: Job id=DwXQRHukdgxRNmAzbVNEARdIVTM6W04uOw [more...](#)

Input PCR template [NM_000903.2](#) Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
Range 196 - 1016
Specificity of primers Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)
Other reports [▶ Search Summary](#)

Graphical view of primer pairs

Genes

Primer pairs for job DwXQRHukdgxRNmAzbVNEARdIVTM6W04uOw

Tracks shown: 3/12

Specific gene detection

Gene detection

- Specificity matters! (Primer BLAST!)

Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCCGAGTCTGTTCTGGCTTA	Plus	20	399	418	59.75	55.00	3.00	2.00
Reverse primer	GTGGATCCCTTGCAGAGAGT	Minus	20	677	658	59.09	55.00	6.00	2.00
Product length	279								

Products on intended target

>[NM_000903.2](#) Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

product length = 279

```
Forward primer 1  GCCGAGTCTGTTCTGGCTTA  20
Template       399  ..... 418

Reverse primer 1  GTGGATCCCTTGCAGAGAGT  20
Template       677  ..... 658
```

Products on allowed transcript variants

>[NM_001025434.1](#) Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 3, mRNA

product length = 165

```
Forward primer 1  GCCGAGTCTGTTCTGGCTTA  20
Template       399  ..... 418

Reverse primer 1  GTGGATCCCTTGCAGAGAGT  20
Template       563  ..... 544
```

Practical part....

Design primers **specific** to your sequence using „pick primers“ (and BLAST).

Verify primers positions

(Hw) Align the primers designed by the program (Pick primers) with your CDS and mRNA

Homework 8

Work with „your“ nucleotide sequence.

- 1) Find primers for the detection of your gene that would be specific (other transcript variants allowed)
- 2) Align them in multalin (CDS,mRNA, F and R)
- 3) Find out if and how many times cut your CDS following REs: EcoRI, XbaI, NcoI
- 4) Find out a RE that cuts just once and simulate the restriction digest of your CDS, what will be the products.