

pondělí 22.4.- poslední opakovací lekce

Možné termíny zkoušky:

(ještě v semestru, pak „kdykoliv“)

- 1) STŘEDA 24.4. 17:40 – 2DNY PO OPAKOVÁNÍ
- 2) PONDĚLÍ 29.4. 17:40
- 3) PONDĚLÍ 13.5. 17:40
- 4) PONDĚLÍ 20.5. 17:40 – **PŮVODNÍ PLÁN** -POSLEDNÍ TÝDEN SEMESTRU

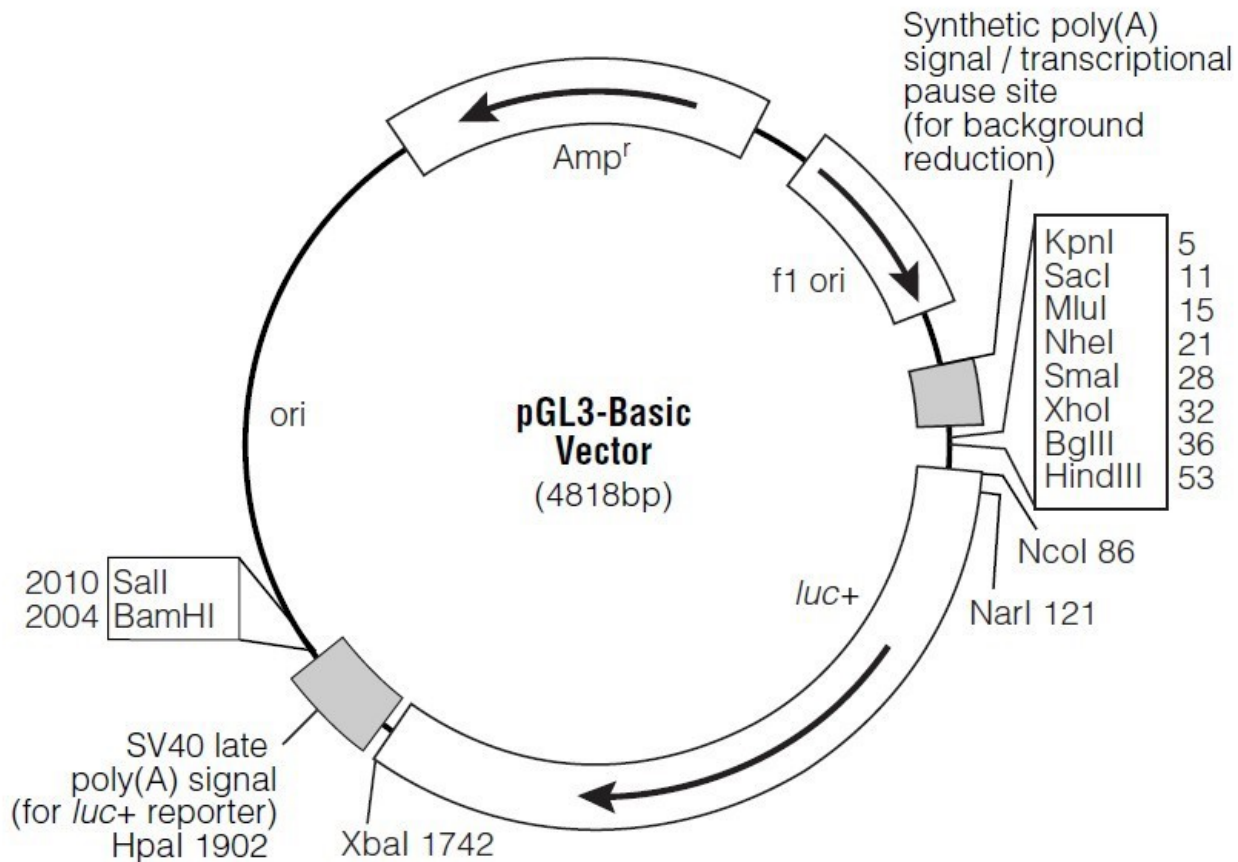
Základy praktické Bioinformatiky

PETRA MATOUŠKOVÁ

2023/2024

9/10

ad DU8



- primery musí obsahovat RE z MCS, ne náhodné RE, které neštěpí fragment.

- nutné hlídat „směr“ klonování:

F primer – KpnI, SacI, ...

R primer- HindII, XhoI..

Nukleotidová bioinformatika V

Cíle:

Student bude schopen navrhnout primery pro **kvantitativní stanovení vybraného genu (qPCR)** a navrhnout primery pro Pfu-mutagenezi .

„Bioinformatika nukleových kyselin IV“

Vyhledávání NK sekvencí

Analýza vlastností sekvencí-složení, reverse complement, identifikace restrikčních míst

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

Klonování, návrh primerů pro PCR, mutační primery, **rt-PCR, kontrola primerů**

Předpověď sekundárních struktur

Porovnávání sekvencí, identifikace neznámé sekvence

Vyhledání SNPs

„čtení“ sekvenačních dat a spojování fragmentů

Vyhledávání hladin expresí jednotlivých genů

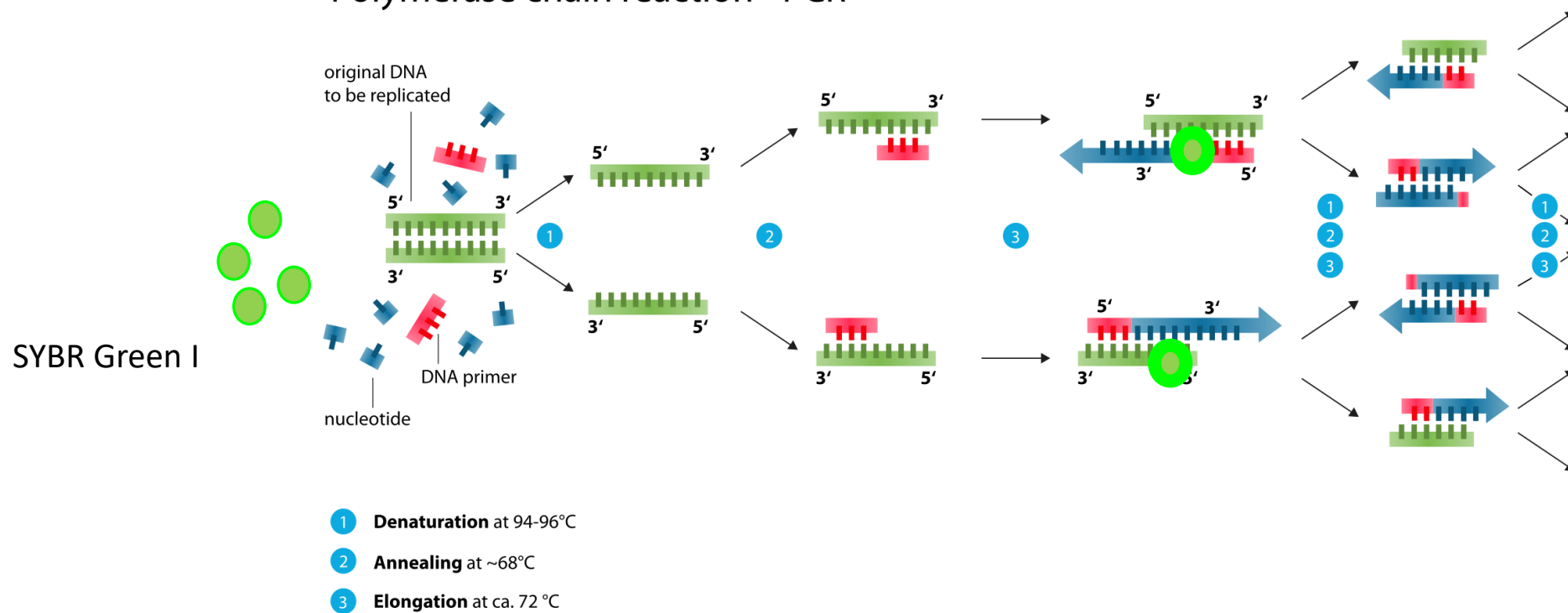
mikroRNA

Celé genomy

....

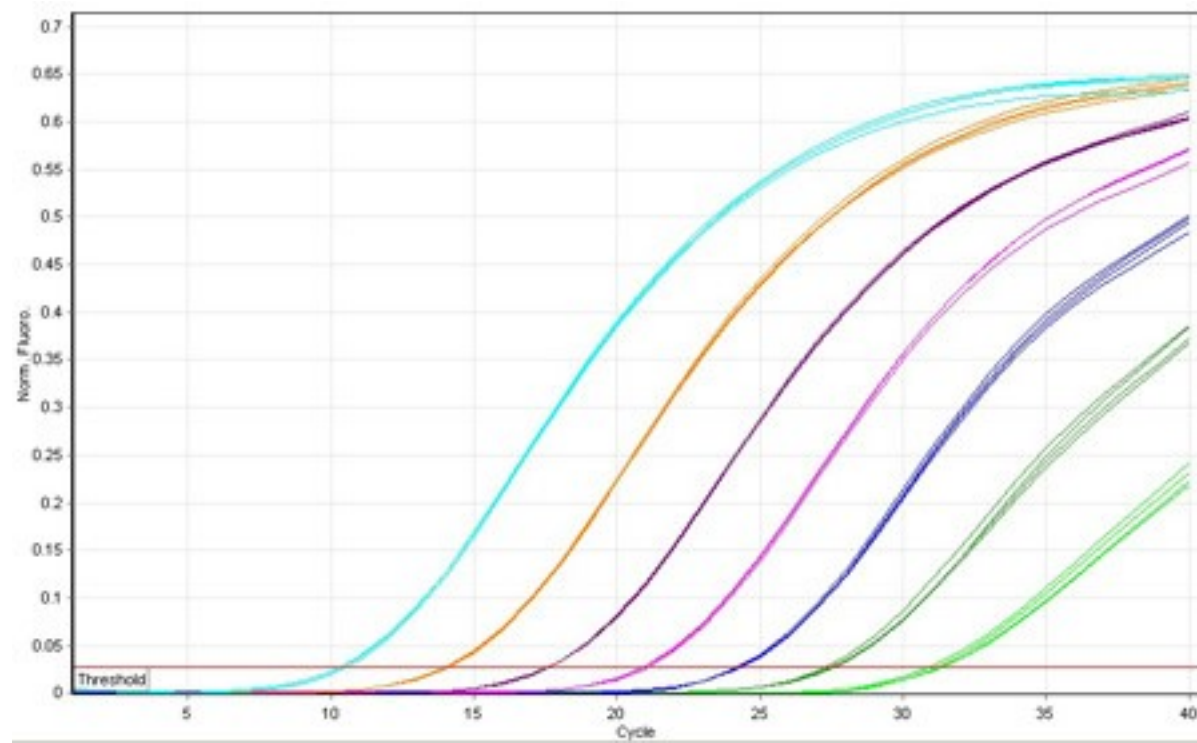
Kvantitativní (q)PCR (real time-PCR)

Polymerase chain reaction - PCR



(wiki)

Kvantitativní (q)PCR (real time - PCR)



Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Primery:

- nezáleží kde v rámci sekvence leží !



- krátký produkt „amplikon“ = délka sekvence mezi primery (včetně): **50-150nt**
- musí fungovat „**perfektně**“ → vyhnout se místům s vlásenkou (sekundární struktura)
- **mFOLD**-predikce sekundárních struktur DNA

Návrh primerů-predikce sekundárních struktur

The UNAFold Web Server [Home](#) [DINAMelt](#) [mFold](#)

DNA Folding Form

Users of this service are requested to cite:

M. Zuker

mfold web server for nucleic acid folding and hybridization
Nucleic Acids Res. **31 (13)**, 3406-15, (2003)

[\[Abstract\]](#) [\[Full Text\]](#) [\[Supplementary Material\]](#) [\[Additional\]](#)

Enter the sequence to be folded in the box below. All non-FASTA format may be used.

Web www.unafold.org říká

Error! Sequence length = 2521. The maximum length is 2400.

OK

max 2400nt

zkrátit

Enter the sequence to be folded in the box below. All non-alphabet characters will be removed.
FASTA format may be used.

```
2310      2320      2330      2340      2350
T T A C T T G C C A   A G A A A A T G A A   G G G A T T G G A C   C G A G C T G G A A   A A C C T C C T T T
2360      2370      2380      2390      2400
A C C A G A T G C T   G A C T G G C A C T   G G T G G T T T T T   G C T C T C G A C A   G T A T C C A C A A
2410      2420      2430      2440      2450
T A G C T G A C G G   C T G G G T G T T T   C A G T T T G A A A   A T A T T T T G T T   G C C T T C A T C T
2460      2470      2480      2490      2500
T C A C T G C A A T   T T T G T G T A A A   T T T C T C A A A G   A T C T G A A T T A   A A T A A A T A A A
2510      2520      2530      2540      2550
A T T C A T T T C T   A C A G A C C C A C   A
```

Format Sequence

Clear Constraints

Check Constraints

Format Sequence

Clear Constraints

Check Constraints

Návrh primerů-predikce sekundárních struktur

The UNAFold Web Server [Home](#) [DINAMelt](#) [mFold](#)

DNA Folding Form

Users of this service are requested to cite:

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Mfold web server for nucleic acid folding and hybridization prediction.

Nucleic Acids Res. **31 (13)**, 3406-15, (2003)

[\[Abstract\]](#) [\[Full Text\]](#) [\[Supplementary Material\]](#) [\[Additional Information\]](#)

The DNA sequence is

linear

Folding temperature (between 0° and 100° C)

37

60°C

Choose [structure annotation](#):

None: p-num: ss-count: high-light:

Enter high-light region(s):

Fold DNA

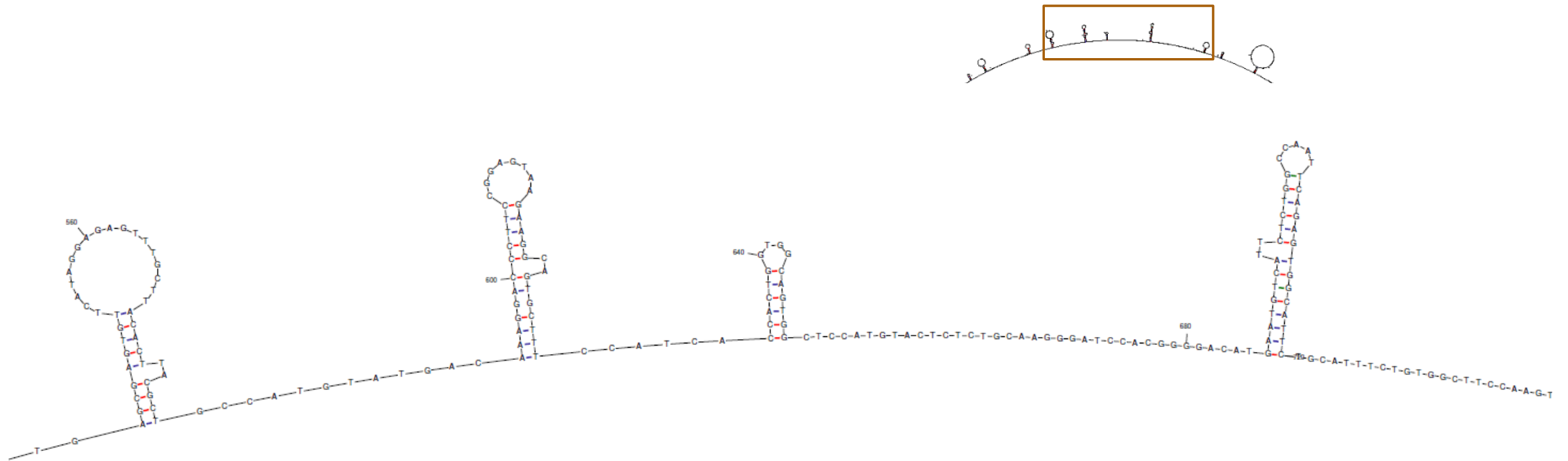
Check Input:

Reset form



Návrh primerů-predikce sekundárních struktur

mFOLD: nastavit 60°C



◆ Structure 1 : $\Delta G = -45.17$ kcal/mol. ([Thermodynamic Details](#)).

Different file formats: [PostScript](#), [pdf](#), [img](#), [jpg](#), [ct file](#), [Vienna](#), [RNAML](#), [RnaViz.ct](#), [Mac.ct](#), [RN](#)

These computations were performed on cc1-03.rit.albany.edu in 1 min. and 1.11 sec. and can be accessed on [17Apr17-14-09-14](#)

The results will be erased in 72 hours. To ask a question or to make a comment, please register and read previously submitted questions and comments.



Návrh primerů-predikce sekundárních struktur

„Folding temperature“: nastavit 60°C

```
1000      1010      1020      1030      1040
.-ATCAAAGCTAGAAAATGAGATTCCTT      TTTCCTTCTAACATGTT
      AGCCTGGA      \
      TCGGACCT      A
\ -----
      TTCTATGGGTCTAAACT
      1070      1060
```

Pro NQO1: 1080-1220

```
1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200      1210      12
.-TCCCTGACTTGCTTTAGTTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAATCC
```

```
1240      1250      1260
.-CTTAGGGAAAGATGTAGAA      -----      AA
      AGATGCT      AGAA      \
      TCTACGG      TCTT      A
\ -----
      AAATT      GT
      1280
```

Případně: až 1440

```
1290      1300      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      :
.-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTG?
```

Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Primery:

- nezáleží kde v rámci sekvence leží !



- krátký produkt „amplikon“ = délka sekvence mezi primery (včetně): **50-150nt**
- musí fungovat „**perfektně**“ → vyhnout se místům s vlásenkou (sekundární struktura)
- **mFOLD**-predikce sekundárních struktur DNA → výběr „vhodné“ oblasti

Home

DINAMelt Application

Mfold Application

For

Applicati

- [RNA Folding I](#)
- [DNA Folding I](#)
- [Structure Dis](#)
- [Determination](#)
- [RNA Folding I](#)
- [energies\)](#)

View Fol

- [Folding Resul](#)

Documen

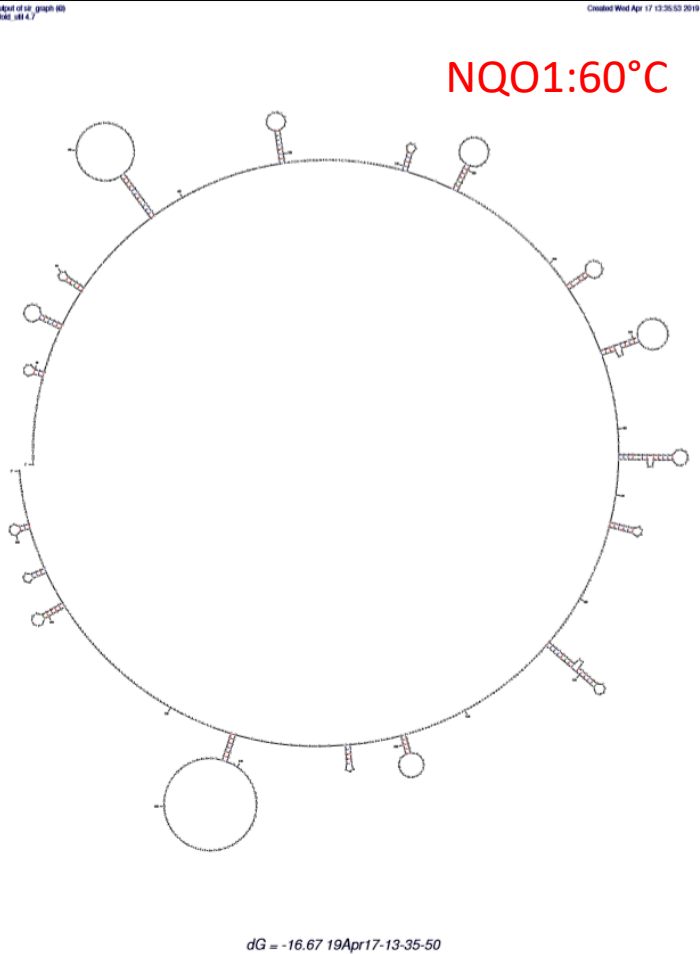
- [Mfold Referen](#)
- [FAQs](#)
- [Folding & outp](#)
- [Folding with c](#)

Software

- [Mfold](#)

About

- [About](#)



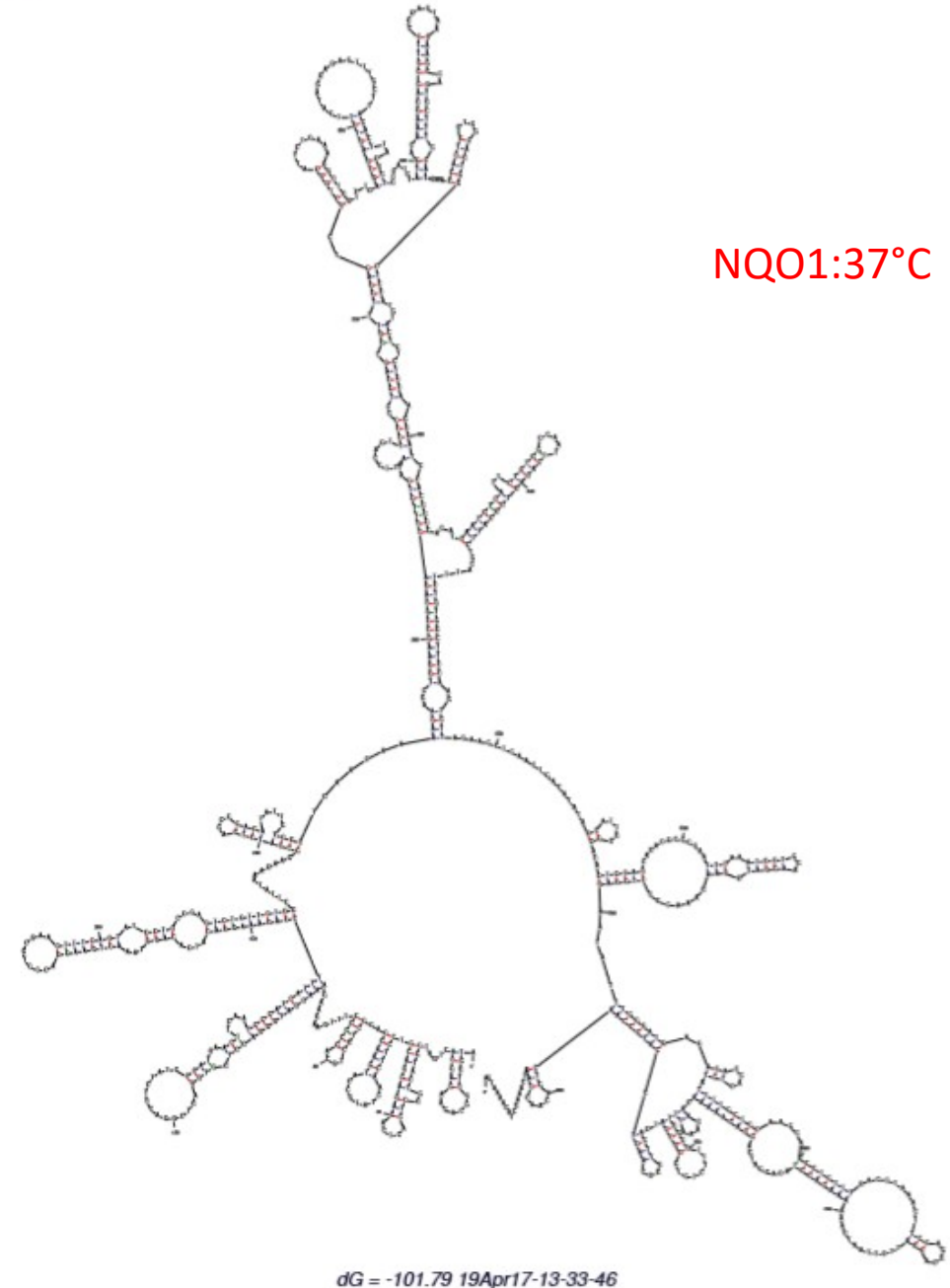
l to cite:

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5, (2003)
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le [laboratory](#) of Joh
nergy rules is:
w of polymer, dum
io-1465. ([Abstract](#))

cleic Acid Hybridiza

the box below. All

UCR = 24.12 10way0007



Vyzkoušejte si....

-Vložit vaši sekvenci (CDS nebo jen část max 2400nt) do **mFold** a podívat se po „vhodných“ oblastech pro návrh qPCR primerů

Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Primery:

- nezáleží kde v rámci sekvence leží !



- krátký produkt „amplikon“ = délka sekvence mezi primery (včetně): **50-150nt**
- musí fungovat „**perfektně**“ → vyhnout se místům s vlásenkou (sekundární struktura)
- **mFOLD**-predikce sekundárních struktur DNA → výběr „vhodné“ oblasti

Primer3
+omezit na
vybranou oblast

Návrh primerů-predikce sekundárních struktur

```
1000      1010      1020      1030      1040
.-ATCAAAGCTAGAAAATGAGATTCCTT      TTTCCTTCTAACATGTT
      AGCCTGGA      \
      TCGGACCT      A
\ -----
      TTCTATGGGTCTAAACT
      1070      1060
```

Pro NQO1: 1080-1220

```
1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200      1210      1220
.-TCCCTGACTTGCTTTAGTTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAATCC
```

```
1240      1250      1260
.-CTTAGGGAAAGATGTAGAA      -----      AA
      AGATGCT      AGAA      \
      TCTACGG      TCTT      A
\ -----
      AAATT      GT
      1280
```

Případně: až 1440

```
1290      1300      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430
.-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTG
```

Návrh primerů-Primer3-na vybranou oblast

Primer3web version 4.0.0 - Pick primers from a DNA sequence.	disclaimer	code
	cautions	

Select the [Task](#) for primer selection

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a [Mispriming Library \(repeat library\)](#)

```
>NM_000903.2 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
CCGCCCTTGTAGGCTGTCCACCTCAAACGGGCGGACAGGATATATAAGAGAGAATGCACCGTGCACTAC
ACACGCGACTCCCACAAGTTGACAGCCGGAGCCGCCAGCTCACCGAGAGCCTAGTTCCGGCCAGGGTCG
CCCCGGCAACCACGAGCCAGCCAATCAGCGCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
TGATCGTACTGGCTCACTCAGAGAGGCGCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTT
GAAGAAGAAGGATGGGAGGTGGTGGAGTCGGACCTCATGCCATGAAGTTCATCCCATCATTTCCAGA
```

<input checked="" type="checkbox"/> Pick left primer, or use left primer below	<input type="checkbox"/> Pick hybridization probe (internal oligo), or use oligo below	<input checked="" type="checkbox"/> Pick right primer, or use right primer below (5' to 3' on opposite strand)
<input type="text"/>	<input type="text"/>	<input type="text"/>

Sequence Id	<input type="text"/>	A string to identify your output.
Targets	<input type="text"/>	E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and]: e.g. ...ATCT[CCCC]TCAT.. means that primers must flank the central CCCC.
Overlap Junction List	<input type="text"/>	E.g. 27 requires one primer to overlap the junction between positions 27 and 28. Or mark the source sequence with -: e.g. ...ATCTAC-TGTCAT.. means that primers must overlap the junction between the C and T.
Excluded Regions	<input type="text"/>	E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the source sequence with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.
Pair OK Region List	<input type="text"/>	See manual for help.
Included Region	<input type="text" value="1080,140"/>	E.g. 20,400: only pick primers in the 400 base region starting at position 20. Or use { and } in the source sequence to mark the beginning and end of the included region: e.g. in ATC {TTC...TCT}AT the included region is TTC...TCT.
Start Codon Position	<input type="text"/>	
Internal Oligo Excluded Region	<input type="text"/>	<input type="text" value="=1080-1220"/>

Návrh primerů-Primer3-na vybranou oblast

[Pair OK Region List](#) See manual for help.

[Included Region](#) E.g. 20,400: only pick primers in the 400 base region starting at position 20. Or use { and } in the [source sequence](#) to mark the beginning and end of the included region: e.g. in ATC {TTC...TCT}AT the included region is TTC...TCT.

[Start Codon Position](#)

[Internal Oligo Excluded Region](#)

[Force Left Primer Start](#) [Force Right Primer Start](#)

[Force Left Primer End](#) [Force Right Primer End](#)

[Sequence Quality](#)

[Min Sequence Quality](#) [Min End Sequence Quality](#) [Sequence](#)

General Primer Picking Conditions

Upload the settings from a file

[Primer Size](#) Min Opt Max

[Primer Tm](#) Min Opt Max [Max Tm Difference](#)

[Product Tm](#) Min Opt Max

[Primer GC%](#) Min Opt Max

[Product Size Range](#)

[Number To Return](#) [Max 3' Stability](#)

[Max Library Mispriming](#) [Pair Max Library Mispriming](#)

Primer3 Output

```
PRIMER PICKING RESULTS FOR NM_000903.2 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), tra
No mispriming library specified
Using 1-based sequence positions
NO PRIMERS FOUND - Help
```

Primer3 Output

Jiná nebo Větší oblast: 1080,360

```
PRIMER PICKING RESULTS FOR NM_000903.2 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcr
No mispriming library specified
Using 1-based sequence positions
OLIGO
LEFT PRIMER      start  len  tm    gc%  any th  3' th  hairpin  seq
RIGHT PRIMER     start  len  tm    gc%  any th  3' th  hairpin  seq
SEQUENCE SIZE: 2601
INCLUDED REGION SIZE: 340
PRODUCT SIZE: 87, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
```

	start	len	tm	gc%	any th	3' th	hairpin	seq
LEFT PRIMER	1325	20	57.96	50.00	0.00	0.00	0.00	AGGGTACAGTTTGGCTAGGT
RIGHT PRIMER	1411	20	58.90	55.00	0.00	0.00	0.00	CTGAGCAATTCCTTCTGCC

Náv

[Pair OK Region List](#)

[Included Region](#)

[Start Codon Position](#)

[Internal Oligo Excluded Region](#)

[Force Left Primer Start](#)

[Force Left Primer End](#)

[Sequence Quality](#)

[Min Sequence Quality](#)

General Primer Pick

Upload the settings from a

[Primer Size](#) Min

[Primer Tm](#) Min Opt Max [Max Tm Difference](#)

[Product Tm](#) Min Opt Max

[Primer GC%](#) Min Opt Max

[Product Size Ranges](#)

[Number To Return](#)

[Max 3' Stability](#)

[Max Library Mispriming](#)

[Pair Max Library Mispriming](#)

```
1000      1010      1020      1030      1040
.-ATCAAAGCTAGAAAATGAGATTCCTT          TTTCCTTCTAACATGTT
          AGCCTGGA          \
          TCGGACCT          A
\-----
          1070      1060

1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200      1210      12
.-TCCCTGACTTGCTTTAGTTTTAAGATTTGTGTTTTCTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTTCGTGCATTTTTGGATCATTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAATCC
\-----

1240      1250      1260
.-CTTAGGAAAGATGTAGAA          -----          AA
          AGATGCT          AGAA          \
          TCTACGG          TCTT          A
\-----
          AAATT          GT
          1280

1290      1300      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420
.-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTG
\-----

left →
← right
```

No mispriming library specified

Using 1-based sequence positions

OLIGO	start	len	tm	gc%	any th	3' th	hairpin	seq
LEFT PRIMER	1325	20	57.96	50.00	0.00	0.00	0.00	AGGGTACAGTTTGGCTAGGT
RIGHT PRIMER	1411	20	58.90	55.00	0.00	0.00	0.00	CTGAGCAATTCCTTCTGCC

SEQUENCE SIZE: 2661

INCLUDED REGION SIZE: 340

PRODUCT SIZE: 87, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00



Vyzkoušejte si....

Vložit vaši sekvenci do mFold a podívat se po „vhodných“ oblastech pro návrh qPCR primerů

Najít primery v těchto „vhodných“ oblastech

```

1000      1010      1020      1030      1040
.-ATCAAAGCTAGAAAATGAGATTCCTT      TTCCTTCTAACATGTT
      AGCCTGGA      \
      TCGGACCT      A
---      TTCTATGGGTCTAAACT
1070      1060

```

Vhodná oblast:1080,360

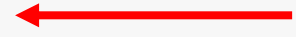
left



```

1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200      1210      1220
.-TCCCTGACTTGCTTTAGTTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCCAAAATCG

```



right

\ -----

```

1240      1250      1260
.-CTTAGGGAAAGATGTAGAA      -----      AA
      AGATGCT      AGAA      \
      TCTACGG      TCTT      A
\ -----      AAATT      GT
1280

```

```

1290      1300      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420
.-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTG

```

\ -----

Vhodná oblast:1080,360

```
1000      1010      1020      1030      1040
.-ATCAAAGCTAGAAAATGAGATTCCTT      TTCCTTCTAACATGTT
      AGCCTGGA      \
      TCGGACCT      A
-----
      TTCTATGGGTCTAAACT
1070      1060
```

```
1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200      1210      1220
.-TCCCTGACTTGCTTTAGTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAATCG
```

\ -----

```
1240      1250      1260
.-CTTAGGGAAAGATGTAGAA      -----      AA
      AGATGCT      AGAA      \
      TCTACGG      TCTT      A
\ -----
      AAATT      GT
1280
```

left



```
1290      1300      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430
.-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTG
```

right

\ -----

```

1000      1010      1020      1030      1040
.-ATCAAAGCTAGAAAATGAGATTCCTT      TTCCTTCTAACATGTT
      AGCCTGGA      \
      TCGGACCT      A
-----
      TTCTATGGGTCTAAACT
1070      1060

```

Vhodná oblast:1080,340

```

1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200      1210      1220
.-TCCCTGACTTGCTTTAGTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAATCG

```



\ -----

```

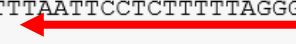
1240      1250      1260
.-CTTAGGGAAAGATGTAGAA      -----      AA
      AGATGCT      AGAA      \
      TCTACGG      TCTT      A
\ -----
      AAATT      GT
1280

```

```

1290      1300      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420
.-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTG

```



right

\ -----


```

1000      1010      1020      1030      1040
.-ATCAAAGCTAGAAAATGAGATTCCTT      TTCCTTCTAACATGTT
      AGCCTGGA      \
      TCGGACCT      A
-----
1070      1060      TTCTATGGGTCTAAACT

```

Vhodná oblast:1080,340

```

1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200      1210      12
.-TCCCTGACTTGCTTTTAGTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAATC
\ -----
1240      1250      1260      AA
.-CTTAGGGAAAGATGTAGAA      -----      AA
      AGATGCT      AGAA      \
      TCTACGG      TCTT      A
\ -----
      AAATT      GT
      1280

```

left

```

1290      1300      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1
.-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTG
\ -----
      right

```

→ Někdy nutné „maskování“ oblastí vlásenek: < > (Alt gr „<“, Alt gr „>“)

```

1000      1010      1020      1030      1040
.-ATCAAAGCTAGAAAATGAGATTCCTT      TTCCTTCTAACATGTT
      AGCCTGGA      \
      TCGGACCT      A
-----
      TTCTATGGGTCTAAACT
1070      1060

```

Vhodná oblast:1080,340

```

1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200      1210      12
.-TCCCTGACTTGCTTTAGTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAATC
\ -----

1240      1250      1260
<-CTTAGGGAAAGATGTAGAA      -----      AA
      AGATGCT      AGAA      \
      TCTACGG      TCTT      A
\ -----
      AAATT      GT
      1280

1290      1300      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420
.->CACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTG
\ -----

```

→ Někdy nutné „maskování“ oblastí vlásenek: < > (Alt gr „<“, Alt gr „>“)

Nebo: Excluded region: 1240,50 (odkud kolik nt zakážeme)

Návrh primerů:

Primer3web version 4.0.0 - Pick primers from a DNA

 Pick left primer, or use left primer below Pick hybridization pro

Pick Primers Download Settings Reset Form

[Sequence Id](#) A string to identify your output.

[Targets](#) E.g. 50,2 requires primers to surround the 2 bas that primers must flank the central CCCC.

[Overlap Junction List](#) E.g. 27 requires one primer to overlap the junct that primers must overlap the junction between

[Excluded Regions](#) E.g. 401,7 68,3 forbids selection of primers in t ..ATCT<CCCC>TCAT.. forbids primers in the

[Pair OK Region List](#) See manual for help.

[Included Region](#) E.g. 20,400: only pick primers in the 400 base 1 included region: e.g. in ATC{TTC...TCT}AT th

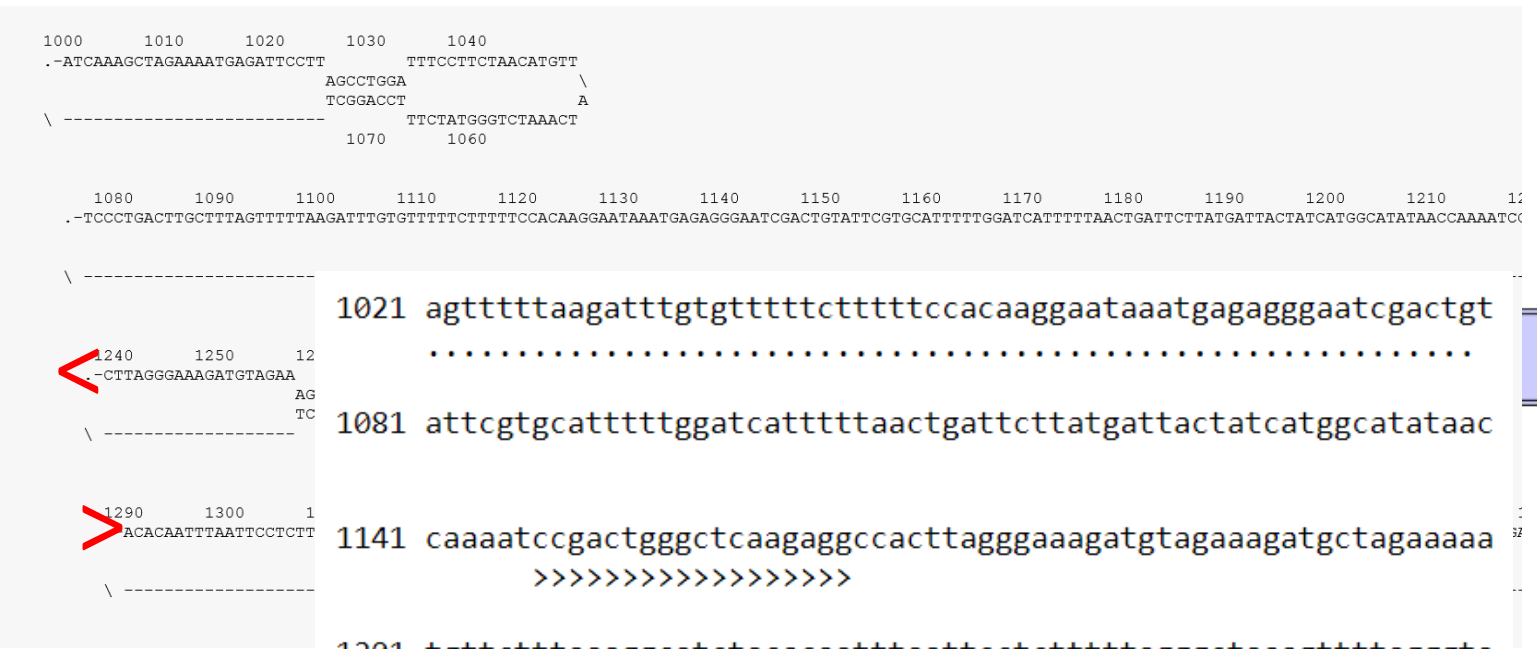
[Start Codon Position](#)

[Internal Oligo](#)

[Excluded Region](#)

[Force Left Primer Start](#) [Force Right Primer Start](#)

[Force Left Primer End](#) [Force Right Primer End](#)



The screenshot shows a DNA sequence on a grey background with coordinates 1000 to 1210. Below the sequence, several primers are listed with their corresponding sequence motifs and quality scores. Red arrows point to the start and end of the sequence. The primer list on the right includes:

- 1021 agtttttaagatttgtgttttctttttccacaaggaataaatgagagggaatcgactgt
- 1081 attcgtgcatttttggatcatttttaactgattcttatgattactatcatggcatataac
- 1141 caaatccgactgggctcaagaggccacttagggaaagatgtagaagatgctagaaaa
- 1201 tgttctttaaggcatctacacaatttaattcctcttttttagggctaaagtttttagggt
- 1261 cagtttggttaggtatcattcaactctccaatgttctattaatcacctctctgtagttta
- 1321 tggcagaaggaattgctcagagaaggaaaagactgaatctacctgcctaaggactta
- 1381 acttgtttggtagtttagccatctaagtctgtttatgatattttcttgctttcaattacaa
- 1441 agcagttactaatatgcctagcacaagtaccactcttggtcagcttttgttattatata
- 1501 cagtacacagataccttgaaaggaagagctaataaatctcttctttgctgcagtcattc

Vyzkoušejte si....

Vložit vaši sekvenci do mFold a podívat se po „vhodných“ oblastech pro návrh qPCR primerů

Najít primery v těchto „vhodných“ oblastech – když to nejde...< > maskovat úseky vlásenek

Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Primery: nezáleží kde v rámci sekvence leží !

krátký amplicon = délka sekvence mezi primery (včetně): **50-150nt**

ale: musí fungovat „perfektně“ → vyhnout se místům s vlásenkou

mFOLD-predikce sekundárních struktur DNA

→ výběr „vhodné“ oblasti

Primer3-omezit na vybranou oblast

→ Někdy nutné „maskování“ oblastí vlásenek:

Excluded region nebo < > (Alt gr „<“, Alt gr „>“)

→ **Primer BLAST**-kontrola specificity

Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Kontrola specifiity: NCBI/Pick Primers (Primer BLAST)

NCBI Resources How To jostovap My NCBI Sign Out

Nucleotide Nucleotide Search Advanced Help

FASTA Send: Change region shown Customize view Analyze this sequence Run BLAST Pick Primers Highlight Sequence Features Find in this Sequence

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

NCBI Reference Sequence: NM_000903.2

[GenBank](#) [Graphics](#)

```
>NM_000903.2 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
CCGCCCTTGTTAGGCTGTCCACCTCAAACGGGCGGACAGGATATATAAGAGAGAATGCACCGTGCCTACTAC
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCTCACCGAGAGCCTAGTTCCGGCCAGGGTTCG
CCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
TGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTT
GAAGAAGAAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTTCAATCCCATCATTTCAGAG
```

Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Kontrola specifity: NCBI/Pick Primers (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

Or, upload FASTA file

Range

Forward primer From To [Clear](#)

Reverse primer

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

of primers to return

Zkopírovat nalezené primery

Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Kontrola specificity: NCBI/Pick Primers (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

Splice variant handling 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

Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Kontrola specifiity: NCBI/Pick Primers (Primer BLAST)

Primer-BLAST *Primer-Blast results*

▶ **NCBI/ Primer-BLAST** : results: Job id=3tQBxuvt5kXBe_x-8R7YTIsFyX6mFtJjpw [more...](#)

Input PCR template [NM_000903.2](#) Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
Range 1 - 2601
Specificity of primers primers may **not** be specific to the input PCR template as targets were found in selected database:Nucleotide collection (nt) (Organism limited to Homo sapiens)...[help on specific primers](#)
Other reports ▶ [Search Summary](#)

Graphical view of primer pairs

Ready Tracks shown: 4/13

Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Kontrola specificity: NCBI/Pick Primers (Primer BLAST)

Primer-BLAST *Primer-Blast results*

▶ NCBI/ Primer-BLAST : results: Job id=3tQBxuvt5kXBe_x-8R7YTIsFyX6mFtJjpw [more...](#)

Input PCR template [NM_000903.2](#) Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
Range 1 - 2601
Specificity of primers primers may **not** be specific to the input PCR template as targets were found in selected database:Nucleotide collection (nt) (Organism limited to Homo sapiens)...[help on specific primers](#)
Other reports ▶ [Search](#) [Summary](#)

Detailed primer reports

Primer pair 1

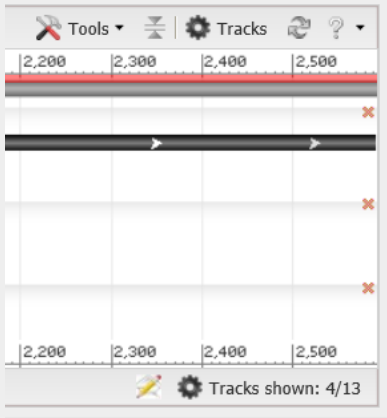
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGGGTACAGTTTGGCTAGGT	Plus	20	1325	1344	57.96	50.00	4.00	0.00
Reverse primer	CTGAGCAATTCCTTCTGCC	Minus	20	1411	1392	58.90	55.00	4.00	1.00
Product length	87								

Products on intended target **ok**
>[NM_000903.2](#) Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

```
product length = 87
Forward primer 1   AGGGTACAGTTTGGCTAGGT  20
Template         1325 ..... 1344

Reverse primer 1   CTGAGCAATTCCTTCTGCC  20
Template         1411 ..... 1392
```

Products on allowed transcript variants **ok**
>[NM_001286137.1](#) Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 4, mRNA



Návrh primerů: kvantitativní (q)PCR (rt-PCR)

Kontrola specifiity: NCBI/Pick Primers (Primer BLAST)

Primer-BLAST *Primer-Blast results*

► NCBI/ Primer-BLAST : results: Job id=3tQBxuvt5kXBe_x-8R7YTI5FyX6mFtJjpw [more...](#)

Input PCR template [NM_000903.2](#) Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
Range 1 - 2601
Specificity of primers primers may **not** be specific to the input PCR template as targets were found in selected database:Nucleotide collection (nt) (Organism limited to Homo sapiens)...[help on specific primers](#)
Other reports ► [Search Summary](#)

Detailed primer reports

Products on potentially unintended templates

>[XM_017028426.1](#) PREDICTED: Homo sapiens PR domain 15 (PRDM15), transcript variant X12, mRNA

product length = 1268	Dlouhý vedlejší produkt nevadí		
Reverse primer	1	CTGAGCAATTCCTTCTGCC	20
Template	2047	..CT.G..A.....	2028
Reverse primer	1	CTGAGCAATTCCTTCTGCC	20
Template	780	G...C...GC.....T	799

GC%	Self complementarity	Self 3' complementarity
50.00	4.00	0.00
55.00	4.00	1.00

>[XM_011529676.2](#) PREDICTED: Homo sapiens PR domain 15 (PRDM15), transcript variant X6, mRNA

product length = 1268			
Reverse primer	1	CTGAGCAATTCCTTCTGCC	20
Template	2048	..CT.G..A.....	2029
Reverse primer	1	CTGAGCAATTCCTTCTGCC	20
Template	781	G...C...GC.....T	800

>[XM_017017253.1](#) PREDICTED: Homo sapiens NLR family, pyrin domain containing 6 (NLRP6), transcript variant X3, mRNA

product length = 1836			
Reverse primer	1	CTGAGCAATTCCTTCTGCC	20

Vyzkoušejte si....

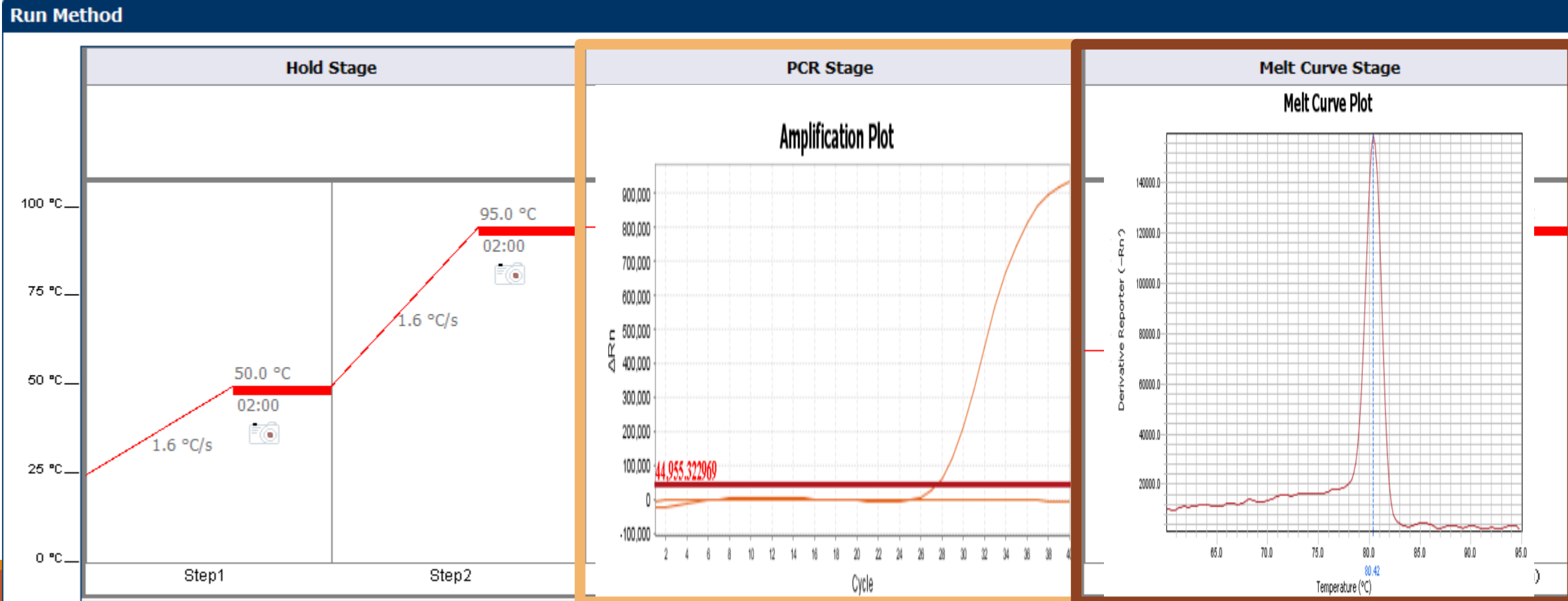
Vložit vaši sekvenci do mFold a podívat se po „vhodných“ oblastech pro návrh qPCR primerů

Najít primery v těchto „vhodných“ oblastech

Zkontrolujte specifitu nalezených primerů

Ověření qPCR primerů – v laborce

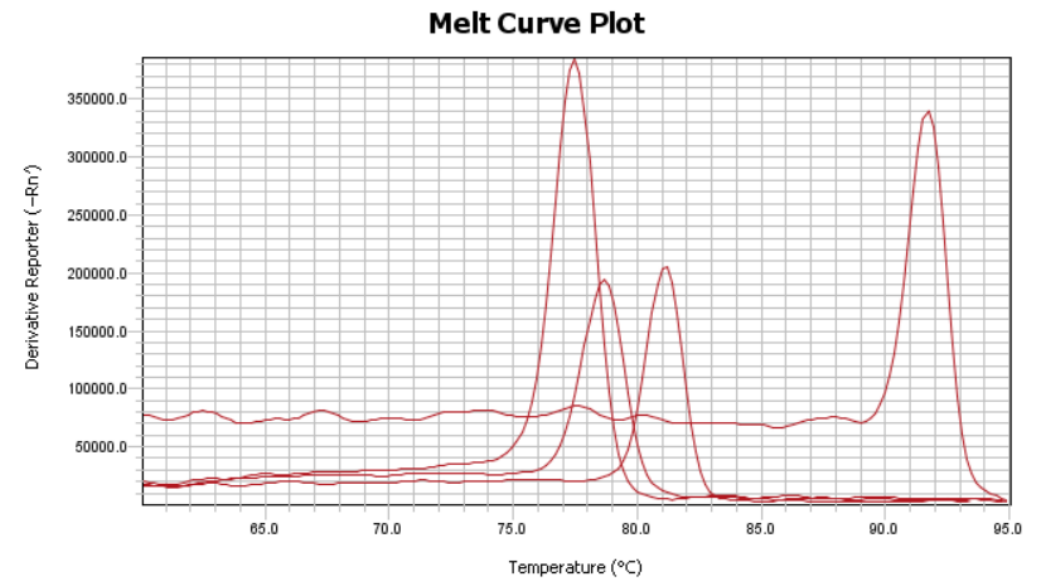
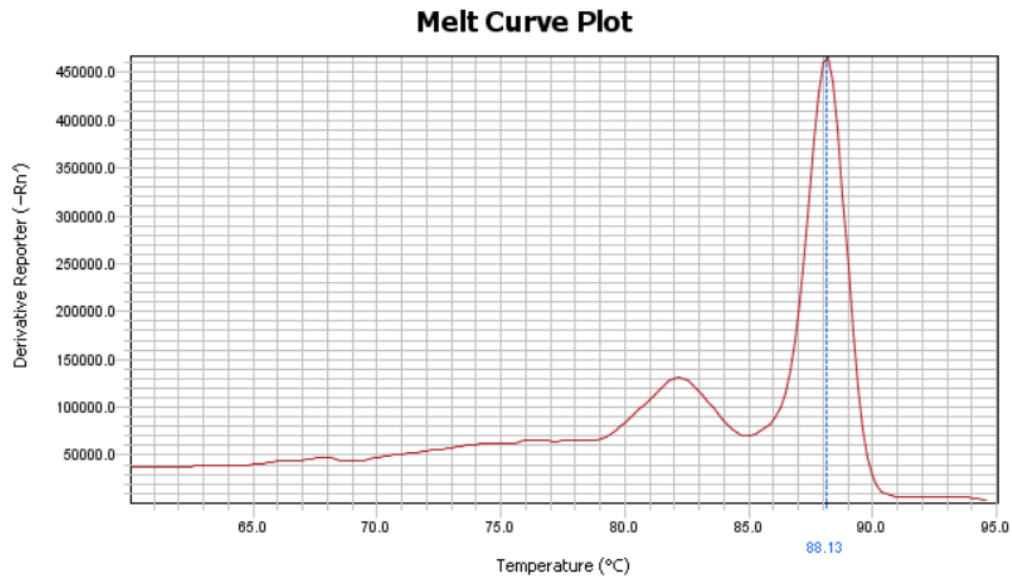
Funkce: cDNA (+NTC)



Ověření qPCR primerů – v laborce

x Více produktů v jedné reakci

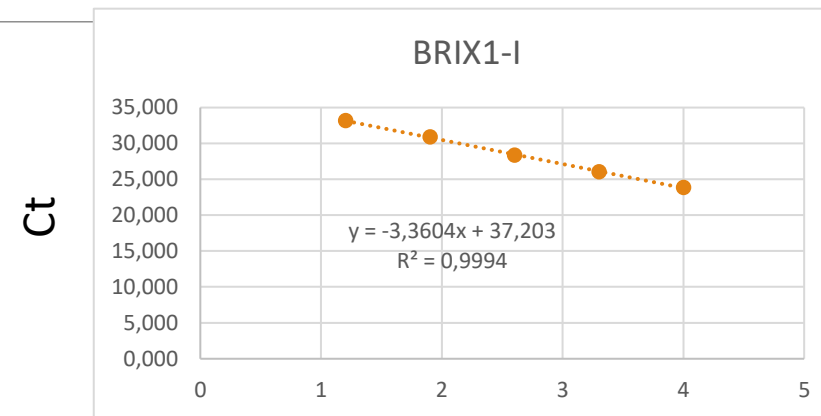
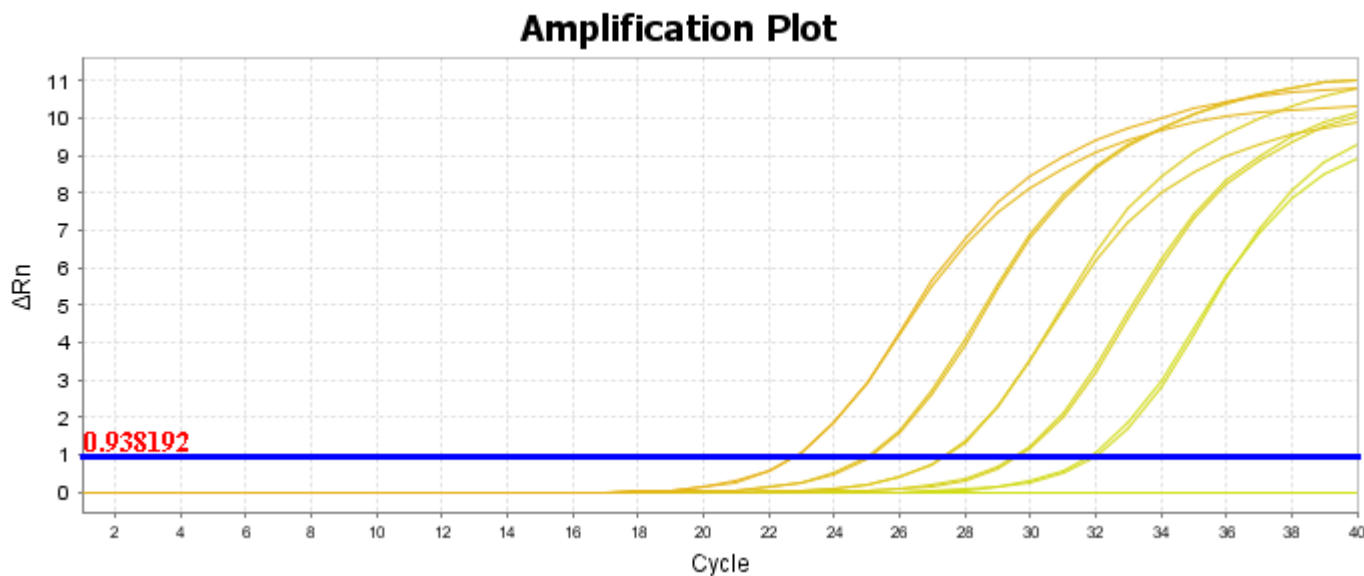
různé produkty stanovené různými primery



Ověření qPCR primerů – v laborce

✓ Funkce: cDNA (+NTC)

Účinnost: ředící řada cDNA



Log quantity

$$E = 10^{-(1/\text{slope})} - 1$$

$$E = 10^{-(1/-3,3604)} - 1 = 0,98... \mathbf{98\%}$$

(90-110%)

Nukleotidová bioinformatika V

Cíle:

Student bude schopen navrhnout primery pro Pfu-mutagenezi a predikovat vazbu mikroRNA na vybraný gen.

„Bioinformatika nukleových kyselin V“

Vyhledávání NK sekvencí

Analýza vlastností sekvencí-složení, reverse complement, identifikace restrikčních míst

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

Klonování, návrh primerů pro PCR, **mutační primery**, rt-PCR, kontrola primerů

Předpověď sekundárních struktur

Porovnávání sekvencí, identifikace neznámé sekvence

Vyhledání SNPs

„čtení“ sekvenačních dat a spojování fragmentů

Vyhledávání hladin expresí jednotlivých genů

mikroRNA

Celé genomy

....

Klonování

Sekvenování-nezávislé na čtecím rámci

Specifické (např. analýza promotorové oblasti, 3' UTR...)

Exprese-příprava rekombinantního proteinu-nutné dodržet čtecí rámec

Mutageneze

Klonování-Návrh primerů – Pfu mutageneze

Bodová záměna kódující sekvence

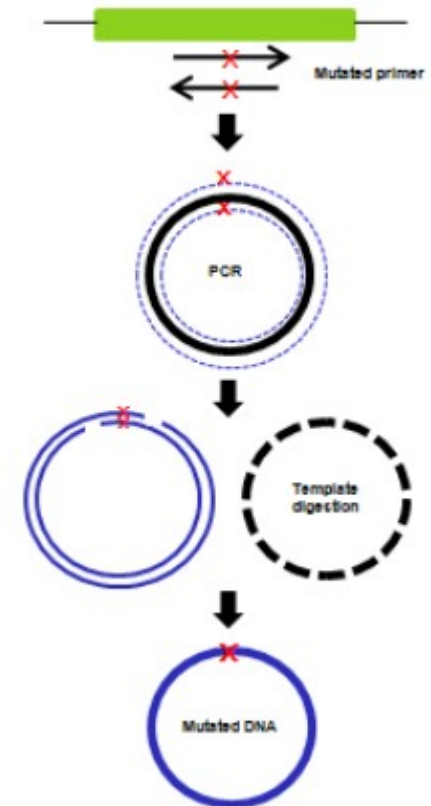
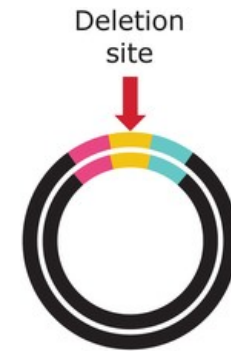
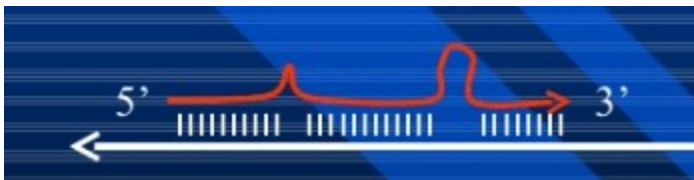
Mutace cílového místa pro RE, mikroRNA....

(site-directed mutagenesis)

- Sekvenci máme v plasmidu (<10kB)

→ návrh mutačních primerů (F+R)

=téměř komplementární sekvence s mutací „uprostřed“ a stejnými úseky na obě strany...cca 15nt



Klonování-Návrh primerů

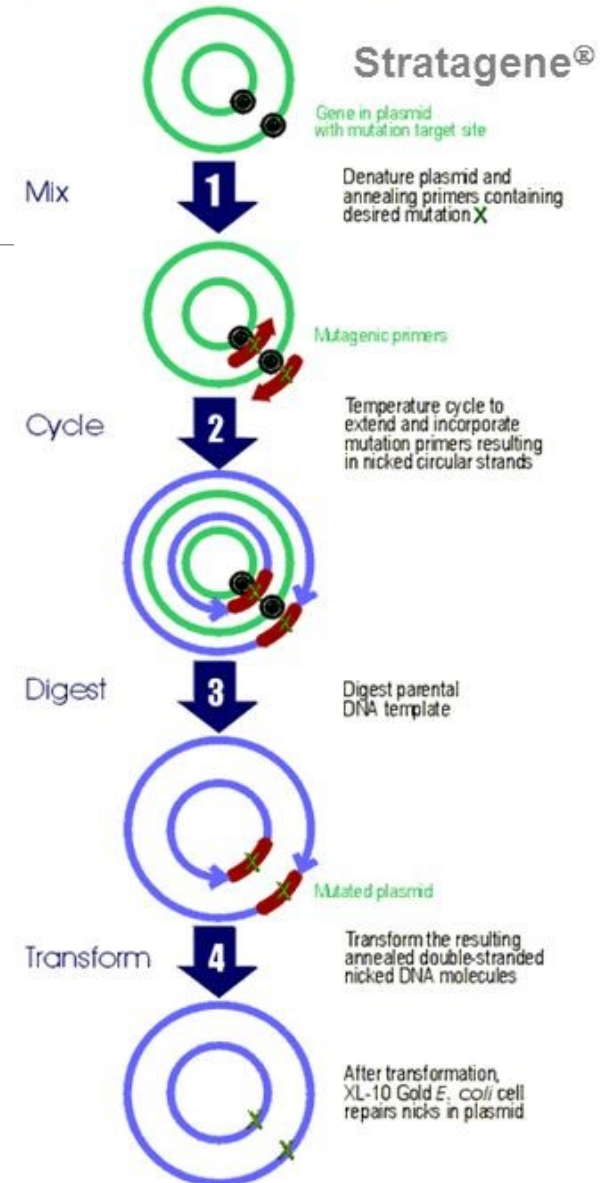
Bodová záměna kódující sekvence

Mutace cílového místa pro RE, mikroRNA....

(site-directed mutagenesis)

- Postup:**
- 1) Sekvenci naklonujeme do vhodného plasmidu (<10kb)
→ Návrh dvou mutačních primerů
 - 2) PCR s těmito primery a plasmidem izolovaným z *E.coli* (templát)-po kontrole sekvence
→ vytvoří se nové „cirkulární“ DNA k sobě komplementární, nesoucí mutaci v požadovaném místě, přerušené na „začátku“ primerů (tzv nick)
 - 3) ošetření restrikční endonukleázou DpnI (štěpí pouze metylovanou DNA, tedy templátový plasmid)
 - 4) transformace do bakterií (dokážou propojit jednořetězcové zlomy) a namnožení mutovaného cirkulárního plasmidu (→kontrola mutace sekvencí)

QuikChange™ XL 1-Day Mutagenesis Method



Klonování-Návrh primerů

POZOR: Při vkládání sekvence ve FASTA formátu je lepší za názvem vložit „enter“. Někdy nerozpozná program nový řádek.

Zadání: „mutujte“ 100.aminokyselinu na Glycin (G)

-identifikace 100.AMK (SMS/ range extractor Protein...“100“)

-zápis příslušné mutace: →NQO1: mutace: „**F100G**“ = mutace fenylalaninu na glycin

SMS Sequence Manipulation Suite:
Range Extractor Protein

Range Extractor Protein accepts a protein sequence along with a set of positions or ranges. The residues corresponding to the position sequence, a set of FASTA records, as uppercase text, or as lowercase text. Use Range Extractor Protein to obtain subsequences using the following syntax:

Paste a raw sequence or one or more FASTA sequences into the text area below. Input limit is 500000 characters.

```
MVGRRALIVLAHSERTSPNYAMKEAAAAALKKGGWEVVEDLYAMNENPIISRKIDITGKLDKDFANFQYPAESVLAHKEGHLSPDIVAEQKKLEADLVIQFPFLQWFGVPAILKGFWRVFIQGEFAYTYAAMYDKGPFRSKKAIVLSITGGSGSMYSLQGIHGDMMVILWPIQSGILHPCGFQVLEPQLTYISIGHTPADARIQILEGKKRLENIWDETPLYFAPSSLPDLNFAAGFLMKKEVQDEBKNKPFGLSVGHHLGKSIPTDNQIKARK
```

Enter the residue positions or ranges to be extracted. Use ".." to represent a range, and use a comma to separate entries. The words place of digits, to represent the beginning, end, middle, and length of the sequence. Arithmetic expressions can be included in the range of a sequence, the range 'end - 2)..end' can be used. To obtain the 30 bases on either side of the center residue along with (center - 1), center, (center + 1)..(center + 30)' can be used.

100

Please check the browser compatibility page before using this program.

Submit Clear Reset

• Sequence segments should be returned as

*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

Sequence Manipulation Suite - Internet ...

about:blank

Range Extractor Protein results

>results for 274 residue sequence "Untitled" starting "MV
F

129%

Klonování-Návrh primerů

POZOR: Při vkládání sekvence ve FASTA formátu je lepší za názvem vložit „enter“. Někdy nerozpozná program nový řádek.

Zadání: „mutujte“ 100.aminokyselinu na Glycin (G)

→ **mutace fenylalaninu na glycin**
F(TTC) na G(GGC)

-Identifikace příslušného kodonu (SMS/DNA range extractor...“298..300 “)

F(TTC) na G(GGC)

1..3 1. kodon (ATG)

4..6 2. kodon

7..9 3. kodon

..

298..300 100.kodon

vložit CDS

SMS Sequence Manipulation Suite:
Range Extractor DNA

Range Extractor DNA accepts a DNA sequence along with a set of positions or ranges. The bases corresponding to the positions or ranges are returned, either as lowercase text. Use Range Extractor DNA to obtain subsequences using position information.

Paste a raw sequence or one or more FASTA sequences into the text area below. Input limit is 500000 characters.

```
CGCCTGGAGAATAITTTGGGATGAGACACCACCTGTATTTTGTCCCAAGCAGCCTCTTTGACC
TAAACTTCC
AGGCAGGATTCTTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCT
TTCTGIGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Enter the base positions or ranges to be extracted. Use "." to represent a range, and use a comma to separate entries. The words 'start', 'end', 'center', and 'length' of the sequence. Arithmetic expressions can be included in the ranges. For example, to obtain the last three bases of a sequence, the range '(end - 2)..end' can be used. **the center base, the ranges '(center - 30)..(center - 1), center, (center + 1)..(center + 30)' can be used.**

298..300

Please check the browser compatibility page before using this program.

Submit Clear Reset

- Obtain bases from the strand.
- Sequence segments should be returned as

*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

Sequence Manipulation Suite - Internet Explorer
about:blank

Range Extractor DNA results

>results for 825 residue sequence "Untitled" starting "ATGGTCGGCA"
TTC

		SECOND BASE				
FIRST BASE	UUU } Phenylalanine F	UCU } Serine S	UAU } Tyrosine Y	UGU } Cysteine C	THIRD BASE	
	UUC } Leucine L	UCC } Serine S	UAC } Tyrosine Y	UGC } Cysteine C		
	UUA } Leucine L	UCA } Serine S	UAA } Stop codon	UGA } Stop codon		
	UUG } Leucine L	UCG } Serine S	UAG } Stop codon	UGG } Tryptophan W		
FIRST BASE	CUU } Leucine L	CCU } Proline P	CAU } Histidine H	CGU } Arginine R	THIRD BASE	
	CUC } Leucine L	CCC } Proline P	CAC } Histidine H	CGC } Arginine R		
	CUA } Leucine L	CCA } Proline P	CAA } Glutamate Q	CGA } Arginine R		
	CUG } Leucine L	CCG } Proline P	CAG } Glutamate Q	CGG } Arginine R		
FIRST BASE	AUU } Isoleucine I	ACU } Threonine T	AAU } Asparagine N	AGU } Serine S	THIRD BASE	
	AUC } Isoleucine I	ACC } Threonine T	AAC } Asparagine N	AGC } Serine S		
	AUA } Methionine start codon M	ACA } Threonine T	AAA } Lysine K	AGA } Arginine R		
	AUG } Methionine start codon M	ACG } Threonine T	AAG } Lysine K	AGG } Arginine R		
FIRST BASE	GUU } Valine V	GCU } Alanine A	GAU } Aspartic acid D	GGU } Glycine G	THIRD BASE	
	GUC } Valine V	GCC } Alanine A	GAC } Aspartic acid D	GGC } Glycine G		
	GUA } Valine V	GCA } Alanine A	GAA } Glutamic acid E	GGA } Glycine G		
	GUG } Valine V	GCG } Alanine A	GAG } Glutamic acid E	GGG } Glycine G		

Klonování-Návrh primerů

POZOR: Při vkládání sekvence ve FASTA formátu je lepší za názvem vložit „enter“. Někdy nerozpozná program nový řádek.

Zadání: „mutujte“ 100.aminokyselinu na Glycin (G)

→ mutace fenylalaninu na glycin

-Identifikace příslušného kodonu (SMS/DNA range extractor...“298..300 “)

SMS Sequence Manipulation Suite: **vložit CDS**

Range Extractor DNA

Range Extractor DNA accepts a DNA sequence along with a set of positions or ranges. The bases corresponding to the positions or ranges are returned, either as lowercase text. Use Range Extractor DNA to obtain subsequences using position information.

Paste a raw sequence or one or more FASTA sequences into the text area below. Input limit is 500000 characters.

```
CGCCTGGAGAATAITTTGGGATGAGACACCACCTGTATTTTGTCCCAAGCAGCCTCTTTTACC
TAAACTTCC
AGGCAGGATTCTTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCT
TTCTGIGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAAATGA
```

Enter the base positions or ranges to be extracted. Use ".." to represent a range, and use a comma to separate entries. The words 'start', 'end', 'center', and 'length' length of the sequence. Arithmetic expressions can be included in the ranges. For example, to obtain the last three bases of a sequence, the range '(end - 2)..end' can be used.

the center base, the ranges '(center - 30)..(center - 1), center, (center + 1)..(center + 30)' can be used.

298..300

Please check the browser compatibility page before using this program.

Submit Clear Reset

- Obtain bases from the strand.
- Sequence segments should be returned as

*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

→Pro NQO1: mutace: „F100G“

1..3 1. kodon (ATG)

4..6 2. kodon

7..9 3. kodon

..

298..300 100.kodon

Vyzkoušejte si....

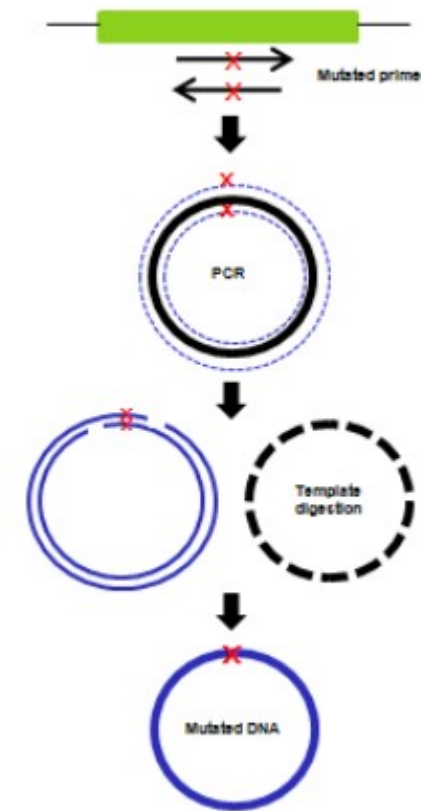
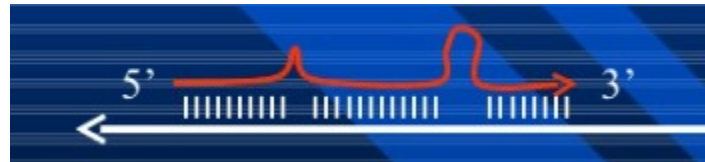
- identifikujte 100. aminokyselinu ve vašem proteinu
- jaký triplet jí kóduje

Klonování-Návrh primerů – Pfu mutageneze

Zadání: „mutujte“ 100.aminokyselinu na Glycin (G)

-identifikace 100.AMK (SMS/protein range extractor...“100“)

-kontrola v proteinové sekvenci: (př. F100G)



Klonování-Návrh primerů

Zadání: „mutujte“ 100.aminokyselinu na Glycin (G)

-“vytažení“ potřebného úseku pro návrh primerů:

SMS/DNA range extractor...“ **283..315** „ (při vložení CDS)

5 kodonů **mutace** 5kodonů
283..297 **298..300** 301..315

SMS Sequence Manipulation Suite:
Range Extractor DNA

Range Extractor DNA accepts a DNA sequence along with a set of positions or ranges. The bases corresponding to the positions or ranges are returned, either lowercase text. Use Range Extractor DNA to obtain subsequences using position information.

Paste a raw sequence or one or more FASTA sequences into the text area below. Input limit is 500000 characters.

```
CGCCTGGAGAATATTTGGGATGAGACACCACTGTATTTGCTCCAAGCAGCCTCTTTGACC
TAAACTTCC
AGGCAGGATTCTTAATGAAAAAGAGGTACAGGATGAGGAGAAAAACAAGAAATTTGGCCT
TTCTGTGGG
CCATCACTTGGGCAAGTCCATCCCAACTGACAACCAGATCAAAGCTAGAAAATGA
```

Enter the base positions or ranges to be extracted. Use ".." to represent a range, and use a comma to separate entries. The words 'start', 'end', 'center', and 'length' can be used. Arithmetic expressions can be included in the ranges. For example, to obtain the last three bases of a sequence, the range '(end - 2)..(end - 1)..(end)' can be used. The ranges '(center - 30)..(center - 1), center, (center + 1)..(center + 30)' can be used.

283..315

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

Submit Clear Reset

- Obtain bases from the strand.
- Sequence segments should be returned as

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

Fri Jun 17 16:17:06 2010
Valid XHTML 1.0; Valid CSS

Sequence Manipulation Suite - Internet Explorer

about:blank

Range Extractor DNA results

>results for 825 residue sequence "Untitled" starting "ATGGTCGGCA"
GACCTTGTGATATTCAGTCCCCCTGCAG

100%

Klonování-Návrh primerů

Zadání: „mutujte“ 100.aminokyselinu na Glycin (G)

Kontrola: „náhled“ sekvence: NCBI Graphic

Graphics ▾

Send to: ▾

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

NCBI Reference Sequence: NM_000903.2

[GenBank](#) [FASTA](#)

Vložit „vytažený kus sekvence“

Link To This Page | Feedback

1 100 200 300 400 500 600 700 800 900 1,000 1,100 1,200 1,300 1,400 1,500 1,600 1,700 1,800 1,900 2 K 2,100 2,200 2,300 2,400 2,500

200 250 300 350 400 450 500 550

Genes - Exon

exon

Genes

NQO1

NP_000894.1

Flavodoxin 2

phosphorylation

Substrate binding

STS Markers

NQO1

misc_feature Features

polyA_site Features

Search Results

Features Components **Sequence** Tracks

Label	From	To	Strand
No Search Results To Display			

Page 0 of 0

No Search Results To Display

Close

3871

Vybrat položku sequence a dvojklik na sekvenci

Klonování-Návrh primerů

Zadání: „mutujte“ 100.aminokyselinu na Glycin (G)

Kontrola: „náhled“ sekvence: NCBI Graphic

Graphics ▾

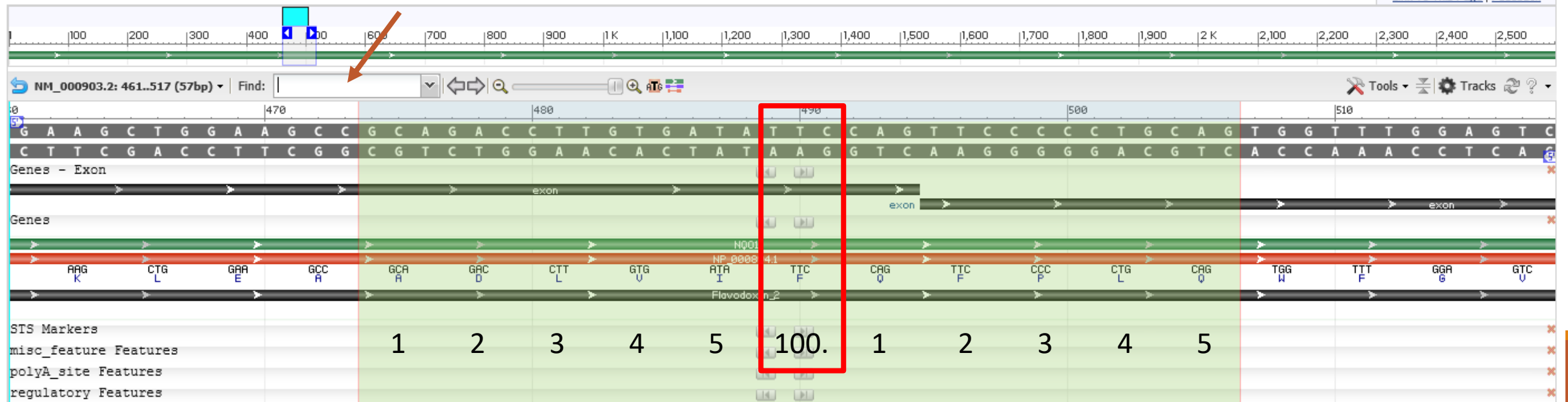
Send to: ▾

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

NCBI Reference Sequence: NM_000903.2

[GenBank](#) [FASTA](#)

[Link To This Page](#) [Feedback](#)



Vyzkoušejte si....

....navrhnout primery pro mutaci 100.AMK vašeho proteinu

- identifikujte, o kterou AMK se jedná (a jakým triplet jí přísluší) v
- vyberte úsek DNA sekvence pro návrh primerů

Klonování-Návrh primerů

Zadání: „mutujte“ 100.aminokyselinu na Glycin (G)

Rozdělení na kodony a identifikace potřebného kodonu(OligoCalc)

→ manuálně mutovat F(TTC) na G(GGC)

Oligo Calc: Oligonucleotide Properties Calculator

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

Nucleotide base codes

GCA GAC CTT GTG ATA **ggc** CAG TTC CCC CTG CAG

Reverse Complement Strand(5' to 3') is:

CTG CAG GGG GAA CTG GCC TAT CAC AAG GTC TGC

5' modification (if any) 3' modification (if any) Select molecule

50 nM Primer 1 Measured Absorbance at 260 nanometers

50 mM Salt (Na⁺)

Calculate Swap Strands BLAST mfold

Physical Constants

Length: 33 Molecular Weight: 10090.64 GC content: 61%

1 ml of a sol'n with an Absorbance of 1 at 260 nm is 2.979 microMolar and contains 30.1 micrograms.

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

RlnK 33.404 cal/(°K*mol) deltaH 279.4 Kcal/mol

deltaG 49 Kcal/mol deltaS 726.8 cal/(°K*mol)

Deprecated Hairpin/self dimerization calculations

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

4 (Minimum base pairs required for a hairpin)

Check Self-Complementarity

= forward horní primer
= reverse (dolní) primer

Codon usage (NQO1)

Gly	GGG	3.00
Gly	GGA	6.00
Gly	GGT	2.00
Gly	GGC	10.00

		Second nucleotide				
		U	C	A	G	
First nucleotide	U	UUU Phe	UCU	UAU Tyr	UGU Cys	U
	C	UUC	UCC Ser	UAC	UGC	C
	A	UUA Leu	UCA	UAA STOP	UGA STOP	A
	G	UUG	UCG	UAG STOP	UGG Trp	G
C	U	CUU Leu	CCU	CAU His	CGU	U
	C	CUC	CCC Pro	CAC	CCG	C
	A	CUA	CCA	CAA Gln	CGA Arg	A
	G	CUG	CCG	CAG	CGG	G
A	U	AUU Ile	ACU	AAU Asn	AGU Ser	U
	C	AUC	ACC Thr	AAC	AGC	C
	A	AUA	ACA	AAA Lys	AGA Arg	A
	G	AUG Met	ACG	AAG	AGG	G
G	U	GUU Val	GCU	GAU Asp	GGU Gly	U
	C	GUC	GCC Ala	GAC	GGC	C
	A	GUA	GCA	GAA Glu	GGA	A
	G	GUG	GCG	GAG	GGG	G

Klonování-Návrh primerů

„mutujte“ 100.aminokyselinu na Glycin (G)

Rozdělení na kodony a identifikace potřebného kodonu(OligoCalc)

Oligo Calc: Oligonucleotide Properties Calculator

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

Nucleotide base codes

CTG CAG GGG GAA CTG GCC TAT CAC AAG GTC TGC

= reverse (dolní) primer

Reverse Complement Strand(5' to 3') is:

GCA GAC CTT GTG ATA GGC CAG TTC CCC CTG CAG

5' modification (if any) 3' modification (if any) Select molecule

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

Calculate **Swap Strands** BLAST mfold

Physical Constants

Length: 33 Molecular Weight: 10179.6⁴ GC content: 61%

1 ml of a sol'n with an Absorbance of 1 at 260 nm is 2.838⁵ microMolar and contains 28.9⁵ micrograms.

Melting Temperature (T_M) Calculations

1 69.4 °C (Basic)
2 79.1 °C (Salt Adjusted)
3 68.56 °C (Nearest Neighbor)

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

RlnK 33.404 cal/(°K²mol) deltaH 279.4 Kcal/mol
deltaG 49 Kcal/mol deltaS 726.8 cal/(°K²mol)

Deprecated Hairpin/self dimerization calculations

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

Check Self-Complementarity

→ manuálně mutovat F(TTC) na G(GGC)
→ **SWAP STRANDS** (výměna řetězců mezi okny)

Codon usage (NQO1)

Gly	GGG	3.00
Gly	GGA	6.00
Gly	GGT	2.00
Gly	GGC	10.00

		Second nucleotide				
		U	C	A	G	
U	U	UUU Phe	UCU	UAU Tyr	UGU Cys	U
	C	UUC	UCC Ser	UAC	UGC	C
	A	UUA Leu	UCA	UAA STOP	UGA STOP	A
	G	UUG	UCG	UAG STOP	UGG Trp	G
C	U	CUU	CCU	CAU His	CGU	U
	C	CUC Leu	CCC Pro	CAC	CCG	C
	A	CUA	CCA	CAA Gln	CGA Arg	A
	G	CUG	CCG	CAG	CGG	G
A	U	AUU Ile	ACU	AAU Asn	AGU Ser	U
	C	AUC	ACC Thr	AAC	AGC	C
	A	AUA	ACA	AAA Lys	AGA Arg	A
	G	AUG Met	ACG	AAG	AGG	G
G	U	GUU Val	GCU	GAU Asp	GGU Gly	U
	C	GUC	GCC Ala	GAC	GGC	C
	A	GUA	GCA	GAA Glu	GGA	A
	G	GUG	GCG	GAG	GGG	G

Klonování-Návrh primerů

„mutujte“ 100.aminokyselinu na Glycin (G)

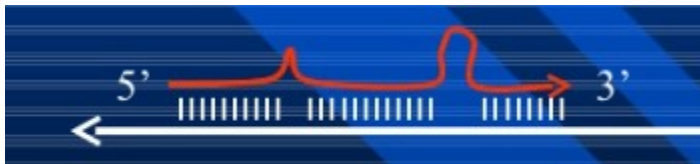
→ manuálně mutovat F(TTC) na G(GGC)

Pro NQO1: mutace: „F100G“

GCA GAC CTT GTG ATA **TTC** CAG TTC CCC CTG CAG

GCA GAC CTT GTG ATA **GGC** CAG TTC CCC CTG CAG

F primer: GCA GAC CTT GTG ATA **GGC** CAG TTC CCC CTG CAG



		Second nucleotide				
		U	C	A	G	
U	UUU	Phe	UCU	UAU Tyr	UGU Cys	U
	UUC		UCC Ser	UAC Tyr	UGC Cys	C
	UUA	Leu	UCA	UAA STOP	UGA STOP	A
	UUG		UCG	UAG STOP	UGG Trp	G
C	CUU		CCU	CAU His	CGU	U
	CUC	Leu	CCC Pro	CAC His	CGC Arg	C
	CUA		CCA	CAA Gln	CGA Arg	A
	CUG		CCG	CAG Gln	CGG	G
A	AUU	Ile	ACU	AAU Asn	AGU Ser	U
	AUC		ACC Thr	AAC Asn	AGC Ser	C
	AUA		ACA Thr	AAA Lys	AGA Arg	A
	AUG	Met	ACG	AAG Lys	AGG Arg	G
G	GUU		GCU	GAU Asp	GGU Gly	U
	GUC	Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA		GCA Ala	GAA Glu	GGA Gly	A
	GUG		GCG	GAG Glu	GGG Gly	G

Klonování-Návrh primerů

„mutujte“ 100.aminokyselinu na Glycin (G)

→ manuálně mutovat F(TTC) na G(GGC)

Pro NQO1: mutace: „F100G“

GCA GAC CTT GTG ATA **TTC** CAG TTC CCC CTG CAG

F primer: GCA GAC CTT GTG ATA **GGC** CAG TTC CCC CTG CAG

R primer: **CTG CAG GGG GAA CTG GCC TAT CAC AAG GTC TGC**

Oligo Calc: Oligonucleotide Properties Calculator

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

Nucleotide base codes

CTG CAG GGG GAA CTG GCC TAT CAC AAG GTC TGC

Reverse Complement Strand(5' to 3') is:

GCA GAC CTT GTG ATA GGC CAG TTC CCC CTG CAG

Pro opačný řetězec
(reverse complement
/swap strands)

		Second nucleotide				
		U	C	A	G	
U	UUU	Phe	UCU	UAU Tyr	UGU Cys	U
	UUC		UCC Ser	UAC Tyr	UGC Cys	C
	UUA	Leu	UCA Ser	UAA STOP	UGA STOP	A
C	CUU		CCU	CAU His	CGU	U
	CUC		CCC Pro	CAC His	CGC Arg	C
	CUA		CCA Pro	CAA Gln	CGA Arg	A
A	AUU	Ile	ACU	AAU Asn	AGU Ser	U
	AUC		ACC Thr	AAC Asn	AGC Ser	C
	AUA		ACA Thr	AAA Lys	AGA Arg	A
G	AUG	Met	ACG	AAG Lys	AGG Arg	G
	GUU		GCU	GAU Asp	GGU Gly	U
	GUC		GCC Ala	GAC Asp	GGC Gly	C
	GUA	Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG		GCG	GAG Glu	GGG Gly	G

Vyzkoušejte si....

....navrhnout primery pro mutaci 100.AMK vašeho proteinu

- identifikujte, o kterou AMK se jedná (a jakým triplet jí přísluší) ✓
- vyberte úsek DNA sekvence pro návrh primerů ✓
- navrhněte F i R mutační primer

DÚ9

- 1) Navrhňte primery programem Primer3 na „váš“ gen, tak aby nebyly ve vlásenkových oblastech (mfold) a zkontrolujte specifitu (specifické být nutně nemusí) – запиšte výsledek
- 2) Navrhňte mutační primery pro mutaci 100. aminokyseliny "vašeho" proteinu:
 - identifikujte 100. AMK (X), запиšte plánovanou mutaci ve tvaru: X100G
 - identifikujte příslušný kodon
 - navrhňte mutační primery se záměnou v glycin (GGC)