

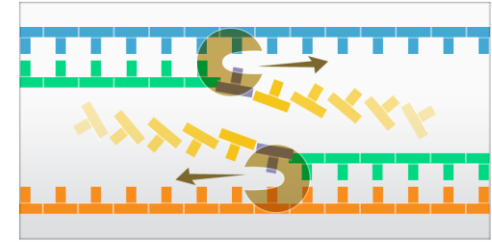
Introduction to applied bioinformatics

PETRA MATOUŠKOVÁ

2025/2026

9/10

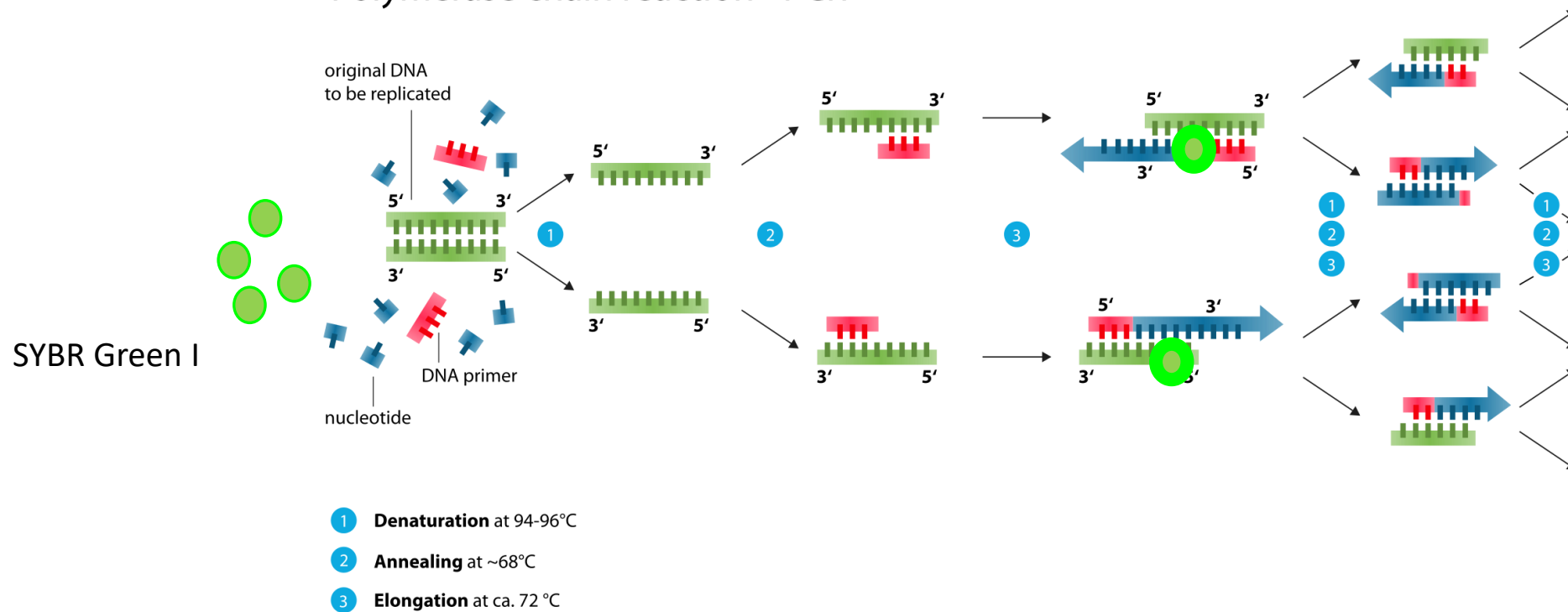
Polymerase chain reaction



- 1) **Amplification** of desired gene / DNA fragment
→ **manual design** (product size depends on the desired amplified fragment)
- 2) Specific gene **detection**
→ **automatic design** (Pick primers) – **specific** primers anywhere along the sequence, product 200-500bp
- 3) **Quantitative** analysis (qPCR)

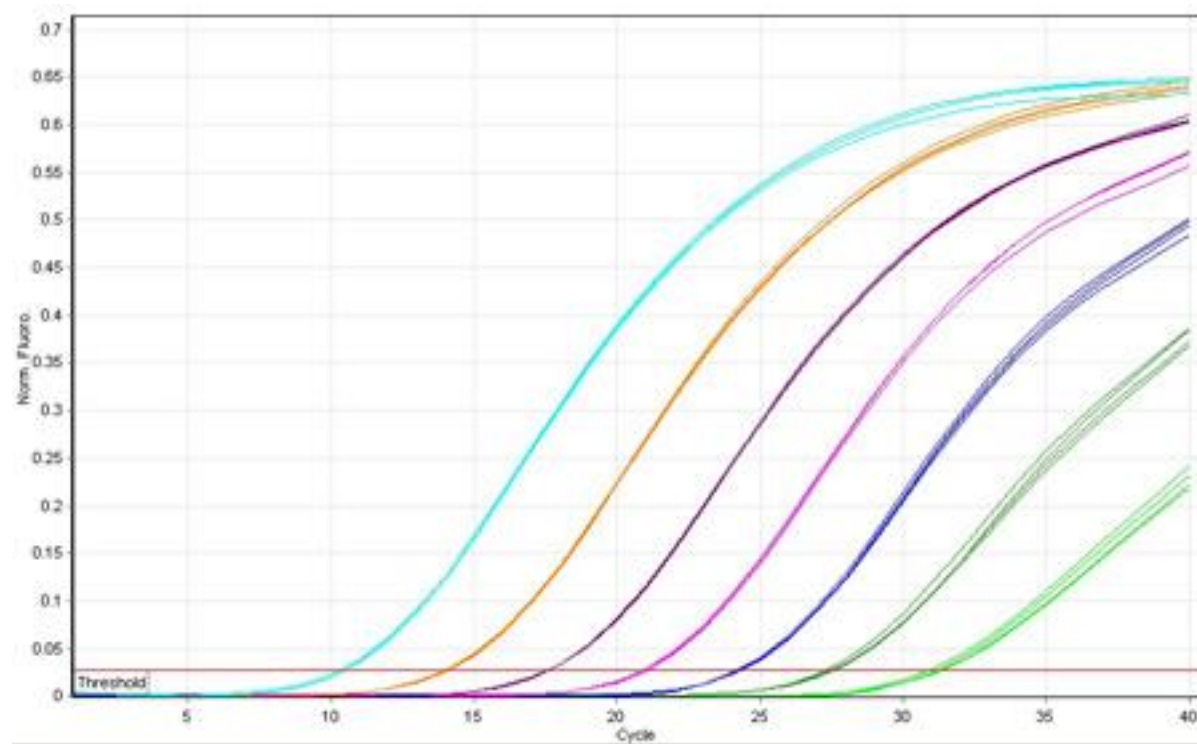
Quantitative (q)PCR (rt-PCR)

Polymerase chain reaction - PCR



(wiki)

Quantitative (q)PCR (rt-PCR)



Quantitative (q)PCR (rt-PCR)

Primers: anywhere along the sequence !



very short amplicon (product): **50-150nt**

but: must work **perfectly** (100% efficiency) → avoid regions in loops (secondary structure)

mFOLD – prediction of DNA secondary structure

DNA secondary structure prediction

The UNAFold Web Server Home DINAMelt ▾ mFold ▾

DNA Folding Form only 2400nt accepts

Users of this service are requested to cite:

M. Zuker

Mfold web server for nucleic acid folding and hybridization prediction.

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

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

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

FASTA format may be used.

```
CTCAGCCTCCCAAAGTGCTGGGATTACAGGCGTGATCCACCACACCTGGCCCTTGCAATCTTCTACTTTA
AGGTTTGCAGAGATAAACCAATAAATCCACACCGTACATCTGCAATATGAATTCAAGAAAGGAAATAGTA
CCTTCAATACTTAAAAATAGTCTTCCACAAAAAATACTTTATTTCTGATCTATACAAATTTTCAGAAGGT
TATTTTCTTTATCATTGCTAAACTGATGACTTACTATGGGATGGGGTCCAGTCCCATGACCTTGGGGTAC
AATTGTAAACCTAGAGTTTTATCAACTTTGGTGAACAGTTTTGGCATAATAGTCAATTTCTACTTCTGGA
AGTCATCTCATTCCACTGTTGGTATTATATAATTCAAGGAGAATATGATAAAACACTGCCCTCTTGTGGT
GCATTGAAAGAAGAGATGAGAAATGATGAAAAGTTGCCTGAAAAATGGGAGACAGCCTCTTACTTGCCA
AGAAAATGAAGGGATTGGACCGAGCTGGAAAACCTCCTTACCAGATGCTGACTGGCACTGGTGGTTTTT
GCTCTCGACAGTATCCACAATAGCTGACGGCTGGGTGTTTCAGTTTGAAAATATTTTGTTCCTTCATCT
TCACTGCAATTTTGTGTAATTTCTCAAAGATCTGAATTAAATAAAATTAATTCATTTCTACAGACCCAC
A
```

erase everything longer than 2400nt

Format Sequence

Clear Constraints

Check Constraints

DNA secondary structure prediction

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 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:

Fold DNA

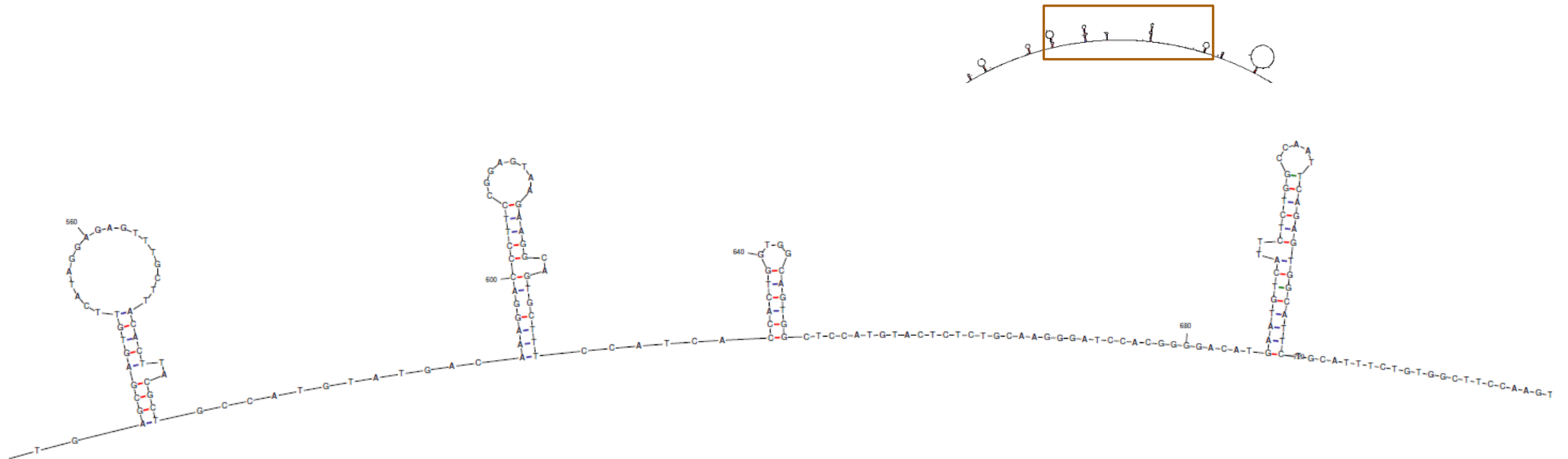
Check Input: ☐

Reset form

Enter high-light region(s):

DNA secondary structure prediction

mFOLD: nastavit 60°C

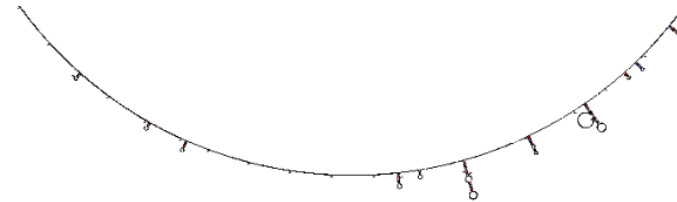


◆ **Structure 1** : $\Delta G = -45.17$ kcal/mol. ([Thermodynamic Details](#)).

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 at [17Apr17-14-09-14](#).

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



The mfold Web Server

Home

Applications

- RNA Folding
- DNA Folding
- Structural Determination
- RNA Folding Energies

View

- Folding

Documentation

- Mfold FAQs
- FAQs
- Folding
- Folding

Software

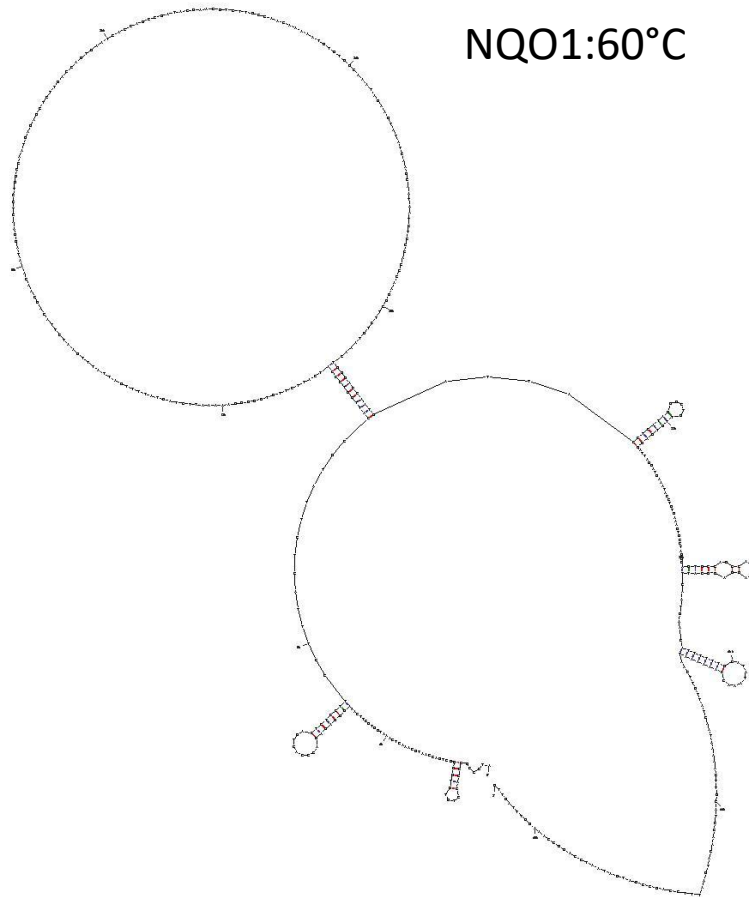
- Mfold

About

- About

Output of mfold graph (6)
mfold_v4.7

Created Mon May 30 07:34:41 2016



dG = -7.84 16May30-07-34-39

to cite:

Folding
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v of po
0-1465

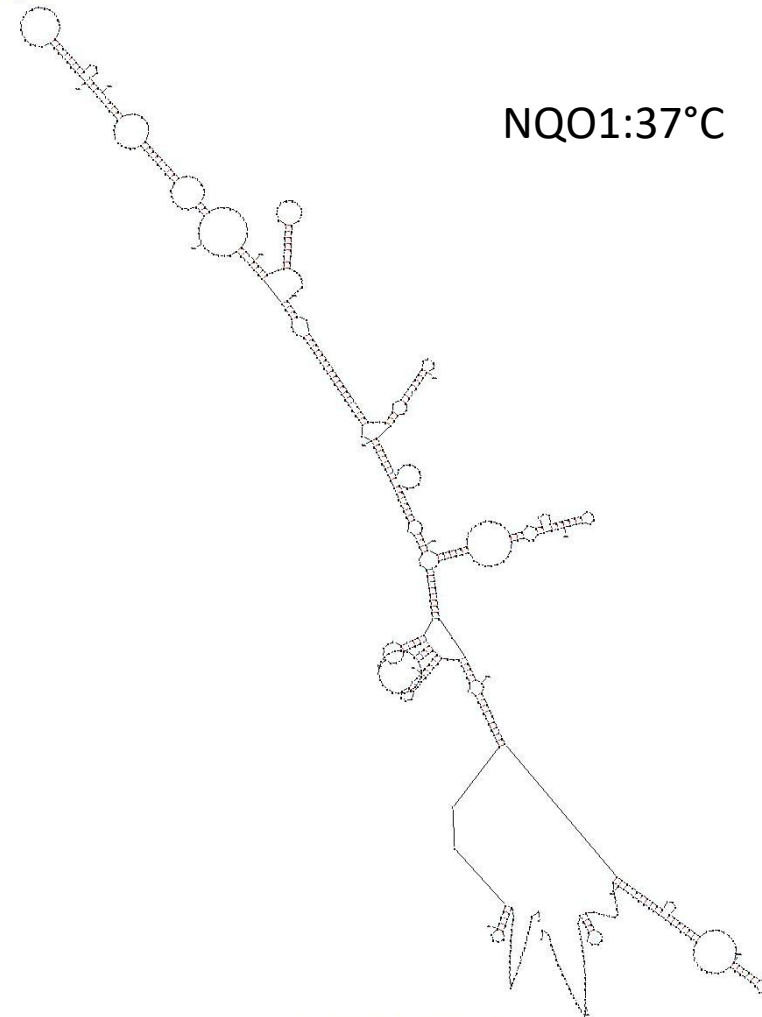
leic Ac

the box

UCR = 254

Output of mfold graph (6)
mfold_v4.7

Created Mon May 30 14:50:36 2016



dG = -72.71 16May30-14-50-31

rsity, Department of Chemistry, Detroit,

ween 0° and 100° C) 60

DNA secondary structure prediction

mFOLD: 60°C

The UNAFold Web Server

SEARCH

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

NQO1: 1080-1220



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

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

Until 1440

```
1290      1300      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1
.-ACACAATTTAATTCCTCTTTTATAGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTG?
\ -----
```

Quantitative (q)PCR (rt-PCR)

Primers: anywhere along the sequence !

very short amplicon (product): **50-150nt**

but: must work **perfectly** (100% efficiency) → avoid regions in loops (secondary structure)

mFOLD – prediction of DNA secondary structure

→ selection of suitable region

Practical part....

-Analyze 2400nt of your CDS sequence to find „good“ region for qPCR primers

Quantitative (q)PCR (rt-PCR)

Primers: anywhere along the sequence !

very short amplicon (product): **50-150nt**

but: must work **perfectly** (100% efficiency) → avoid regions in loops (secondary structure)

mFOLD – prediction of DNA secondary structure

Primer3 – tool for primer design -

DNA secondary structure prediction

mFOLD: 60°C

The UNAFold Web Server

SEARCH

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

NQO1: 1080-1220



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

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

Until 1440

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

Primer design-Primer3-area selection

Primer3web version 4.0.0 - Pick primers from a DNA sequence.

[disclaimer](#)[code](#)[cautions](#)

Select the [Task](#) for primer selection

generic

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 \(repeat library\)](#)

NONE

>NM_000903.2 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA
CCGCCCTTGTAGGCTGTCCACCTCAAACGGGCGGACAGGATATATAAGAGAGAATGCACCGTGCACTAC
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCTCACCAGAGCCTAGTTCCGGCCAGGGTCG
CCCCGGCAACCAACGAGCCAGCCCAATCAGCGCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
TGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTT
GAAGAAGAAGGATGGGAGGTGGTGGAGTCGACCTCTATGCCATGAAGTTCATCCCATCATTTCCAGA

NQO1: 1080-1220

1080-1220=140nt

☒ Pick left primer, or use left primer below

☐ Pick hybridization probe (internal oligo), or use oligo below

☒ Pick right primer, or use right primer below (5' to 3' on opposite strand)

Pick PrimersDownload SettingsReset Form

- [Sequence Id](#)

A string to identify your output.

[Targets](#)

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](#)

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](#)

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](#)

See manual for help.

[Included Region](#)

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](#)

[Internal Oligo Excluded Region](#)

selected area (by mfold) – from, how many bases allowed

primer design-Primer3-area selection

Pair OK Region List

Included Region

Start Codon Position

Internal Oligo Excluded Region

1080,140

Force Left Primer Start

Force Right Primer Start

-100000

-100000

Force Left Primer End

Force Right Primer End

-100000

-100000

Sequence Quality

Min Sequence Quality

Min End Sequence Quality

Sequence

0

0

Pick Primers

Download Settings

Reset Form

General Primer Picking Conditions

Upload the settings from a file

Procházet...

Primer Size

Primer Tm

Product Tm

Primer GC%

Min

Opt

Max

Min

Opt

Max

Min

Opt

Max

Min

Opt

Max

18

20

23

57.0

59.0

62.0

-100000

0.0

100000

30.0

50.0

70.0

Product Size Range

Number To Return

Max 3' Stability

50-150

5

9.0

Max Library Mispriming

Pair Max Library Mispriming

12.00

20.00

See manual for help.

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.

Primer3 Output

PRIMER PICKING RESULTS FOR NM_000903.2 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcribed from the minus strand

No mispriming library specified

Using 1-based sequence positions

NO PRIMERS FOUND - [Help](#)

Primer3 Output

PRIMER PICKING RESULTS FOR NM_000903.2 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcribed from the minus strand

No mispriming library specified

Using 1-based sequence positions

OLIGO	start	len	tm	gc%	any th	3' th	hairpin	seg
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	CTGAGCAATTCCCTTCTGCC

SEQUENCE SIZE: 2601

INCLUDED REGION SIZE: 340

PRODUCT SIZE: 87, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00

other or longer region needed: 1080,340

primer design-Primer3-area selection

[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](#)

Pick Primers

Download Settings

Reset Form

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

other or longer region needed: 1080,340

PRIMER PICKING RESULTS FOR NM_000903.2 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcrip

No mispriming library specified
Using 1-based sequence positions

OLIGO	start	len	tm	gc%	any th	3' th	hairpin	seg
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	CTGAGCAATTCCCTTCTGCC

SEQUENCE SIZE: 2601
INCLUDED REGION SIZE: 340

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

```
1 CCGCCCTTGTTAGGCTGTCCACCTCAAACGGGCCGGACAGGATATATAAGAGAGAATGCAC
.....
61 CGTGCACTACACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCTCACCGAGAG
.....
121 CCTAGTTCCGGCCAGGGTCGCCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACT
.....
```

prim

[Pair OK Region List](#)

[Included Region](#)

[Start Codon Position](#)

[Internal Oligo Excluded Region](#)

[Force Left Primer Start](#) -100

[Force Left Primer End](#) -100

[Sequence Quality](#)

[Min Sequence Quality](#) 0

[Min End Sequence Quality](#) 0

[Sequ](#)

[OLIGO](#)

[start](#)

[len](#)

[tm](#)

[gc%](#)

[any th](#)

[3' th](#)

[hairpin](#)

[seg](#)

[LEFT PRIMER](#)

[RIGHT PRIMER](#)

[SEQUENCE SIZE: 2601](#)

[INCLUDED REGION SIZE: 340](#)

[PRODUCT SIZE: 87, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00](#)

[1 CCGCCCTTGTAGGCTGTCCACCTCAAACGGGCCGGACAGGATATATAAGAGAGAATGCAC](#)

[61 CGTGCACTACACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCTCACCGAGAG](#)

[121 CCTAGTTCCGGCCAGGGTCGCCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACT](#)

[5](#)

[Max Tm Difference](#)

[Product Tm](#)

[Primer GC%](#)

[Primer Size](#)

[Primer Tm](#)

[Product Tm](#)

[Primer GC%](#)

[Primer Size](#)

[Primer Tm](#)

[Product Tm](#)

[Primer GC%](#)

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[Primer Size](#)

[Primer Tm](#)

[Product Tm](#)

[Primer GC%](#)

[Primer Size](#)

[Primer Tm](#)

[Product Tm](#)

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

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

      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      1430
.-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTGGA
\ -----

      left
      right
```

```
1000      1010      1020      1030      1040
.-ATCAAAGCTAGAAAATGAGATTCCTT      TTTCCTTCTAACATGTT
      AGCCTGGA      \
      TCGGACCT      A
\ -----
      1070      1060
      left
      1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200      1210      1220
.-TCCCTGACTTGCTTTAGTTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAAATCG
      right
\ -----

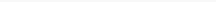
1240      1250      1260
.-CTTAGGGAAAGATGTAGAA      -----      AA
      AGATGCT      AGAA      \
      TCTACGG      TCTT      A
\ -----
      AAATT      GT
      1280

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

```

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

```



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

```

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

.-ACACAATTTAAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAAGACTG

← TAAATCCCTCTTTTAGGC

```

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

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

\ -----

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

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

\ -----

```

Diagram illustrating sequence alignment with annotations:

- A red arrow labeled "left" points from position 1250 to 1240.
- A red arrow labeled "right" points from position 1370 to 1360.
- An orange 'X' is drawn over the alignment between positions 1240 and 1260, indicating a potential loop or misalignment.

→ loops have to be avoided

Practical part....

- Analyze 2400nt of your sequence to find „good“ region for qPCR primers
- Try to design primers in this selected area

Quantitative (q)PCR (rt-PCR)

Primers: anywhere along the sequence !

very short amplicon (product): **50-150nt**

but: must work **perfectly** (100% efficiency) → avoid regions in loops (secondary structure)

mFOLD – prediction of DNA secondary structure

Primer3 – tool for primer design

Check for specificity – qPCR primers should be specific

primers: (q)PCR (rt-PCR) - specificity

NCBI/Pick Primers (Primer BLAST)

 [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

CCGCCCTTGTAGGCTGTCCACCTCAAACGGGCGGACAGGATATATAAGAGAGAATGCACCGTGCACTAC
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCAGCTCACCGAGAGCCTAGTTCCGGCCAGGGTCG
CCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
TGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTT
GAAGAAGAAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTTCAATCCCATCATTTCAGA

primers: (q)PCR (rt-PCR) - specificity

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).

PCR Template

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

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

Range

Forward primer

Reverse primer

From

To

[Clear](#)

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

Primer Parameters

Use my own forward primer
(5'->3' on plus strand)

Use my own reverse primer
(5'->3' on minus strand)

PCR product size

of primers to return

Min

Max

[Clear](#)

[Clear](#)

Copy primers from Primer3

primers: (q)PCR (rt-PCR) - 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

primers: (q)PCR (rt-PCR) - 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 - 2661

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

The graphical view displays the genomic context of the primer pair. The top track shows the input template (NM_000903.2) with a range of 1 to 2661. Below this, the gene structure is shown with exons and introns. The primer pair is highlighted in blue, and its position is indicated by a blue bar. The primer pair is labeled 'Primer 1'.

Ready

Tracks shown: 4/13

primers: (q)PCR (rt-PCR) - specificity

NCBI/Pick Primers (Primer BLAST)

Primer-BLAST

Primer-Blast results

► [NCBI/ Primer-BLAST](#) : results: Job id=3tQBxuv5kXBe_x-8R7YTIsFyX6mFtJjpw [more...](#)

Input PCR template

Range

Specificity of primers

Other reports

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

1 - 2601

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](#)

► [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

Tools

Tracks

2,200 2,300 2,400 2,500

Tracks shown: 4/13

primers: (q)PCR (rt-PCR) - specificity

NCBI/Pick Primers (Primer BLAST)

Primer-BLAST

Primer-Blast results

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

Input PCR template

Range

Specificity of primers

Other reports

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

1 - 2601

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](#)

► [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

Reverse primer 1 CTGAGCAATTCCTTCTGCC 20

Template 2047 ..CT.G..A..... 2028

Reverse primer 1 CTGAGCAATTCCTTCTGCC 20

Template 780 G...C...GC.....T 799

>[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

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

Tools

Tracks

2,200 2,300 2,400 2,500

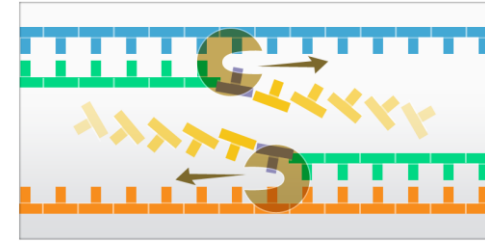
2,200 2,300 2,400 2,500

Tracks shown: 4/13

Practical part....

- Analyze 2400nt of your sequence to find „good“ region for qPCR primers
- Try to design primers in this selected area
- Check the specificity of found qPCR primers

Polymerase chain reaction



- 1) **Amplification** of desired gene / DNA fragment
→ **manual design** (product size depends on the desired amplified fragment)
- 2) Specific gene **detection**
→ **automatic design** (Pick primers) – **specific** primers anywhere along the sequence, product 200-500bp
- 3) **Quantitative** analysis (qPCR)
→ **automatic design** (Primer3, mfold) – **specific** primers anywhere along the sequence, **short** product 50-150bp

Homework 9

Work with „your“ nucleotide sequence.

- 1) Design primers for quantitative detections outside folded area
and check for primers specificity