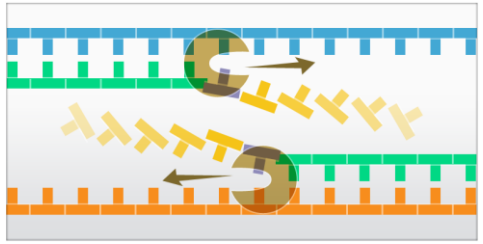
Introduction to applied bioinformatics

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)

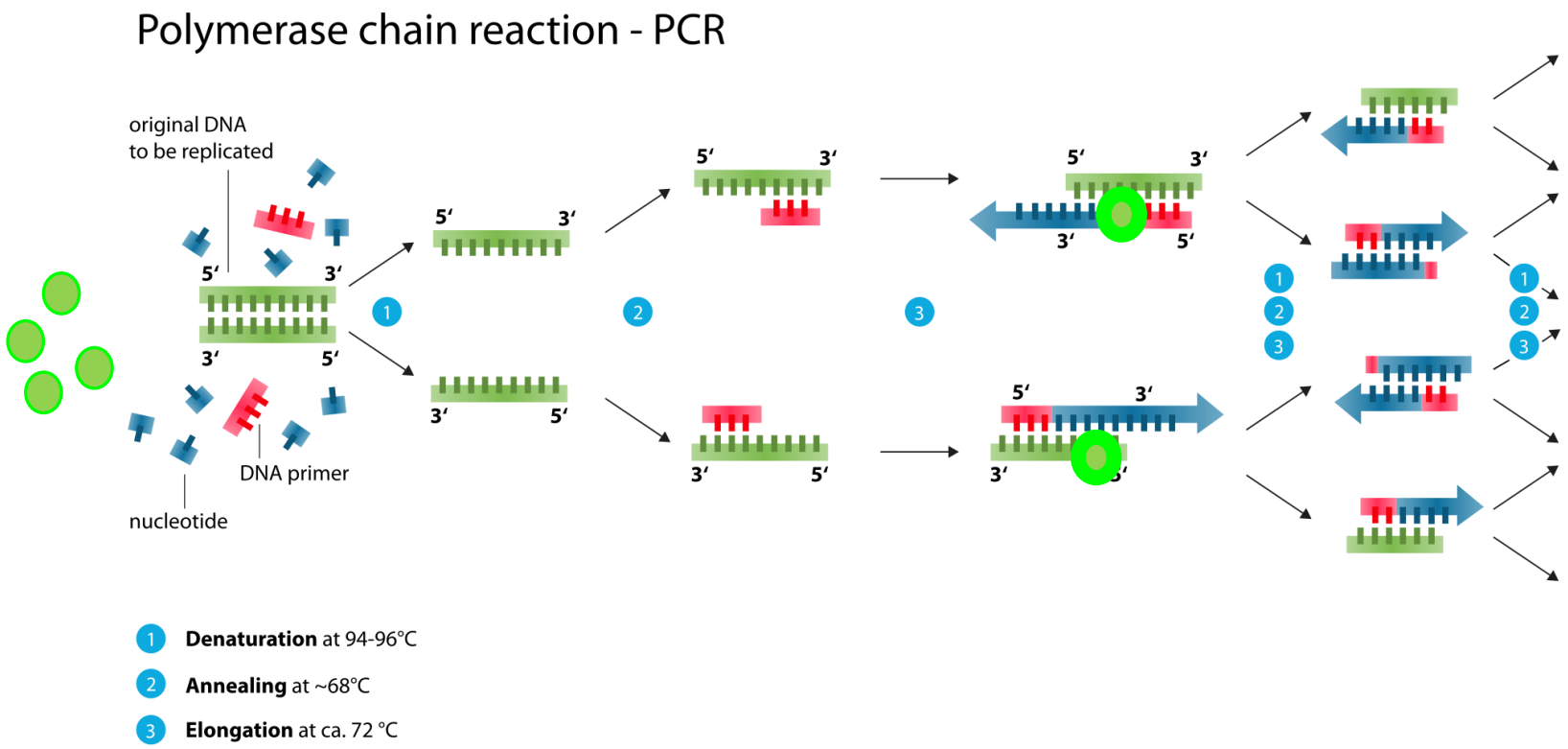
2) Specific gene **detection**

→ **automatic design** (Pick primers) – **specific** primers anywhere along the sequence, product 200-500bp

3) **Quantitative** analysis (qPCR)

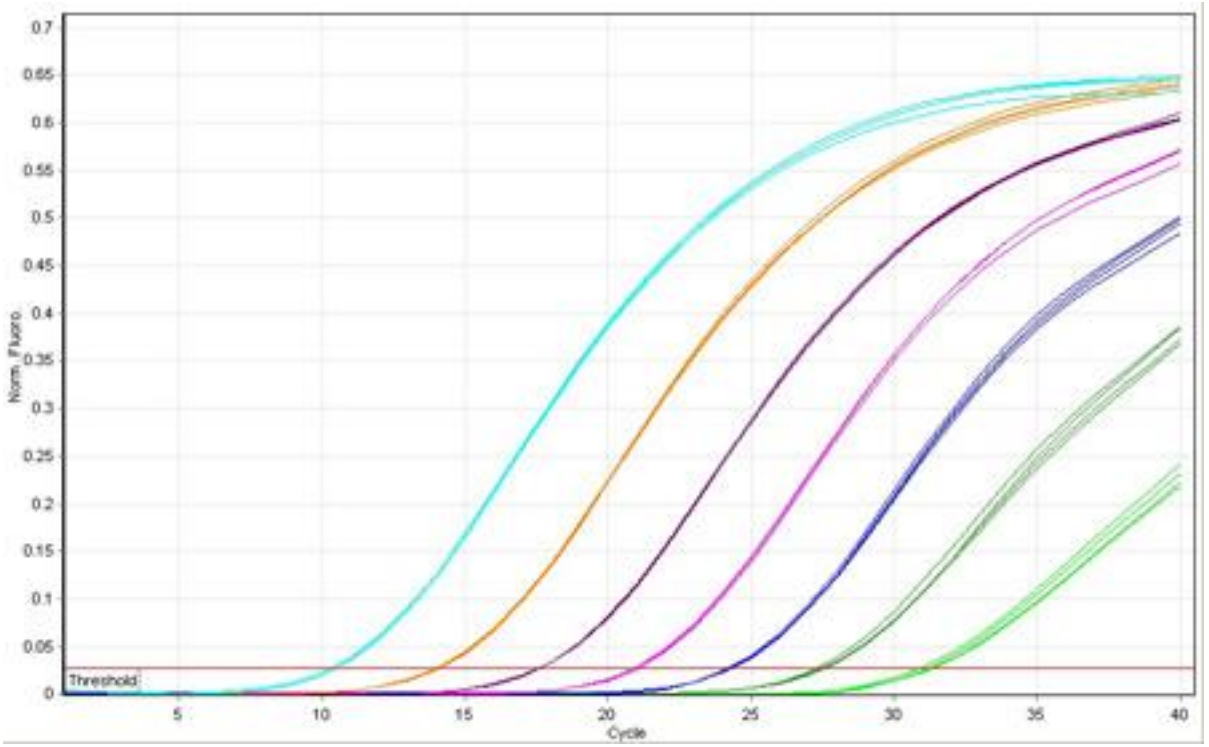
Quantitative (q)PCR (rt-PCR)

SYBR Green I



(wiki)

Quantitative (q)PCR (rt-PCR)



Quantitative (q)PCR (rt-PCR)

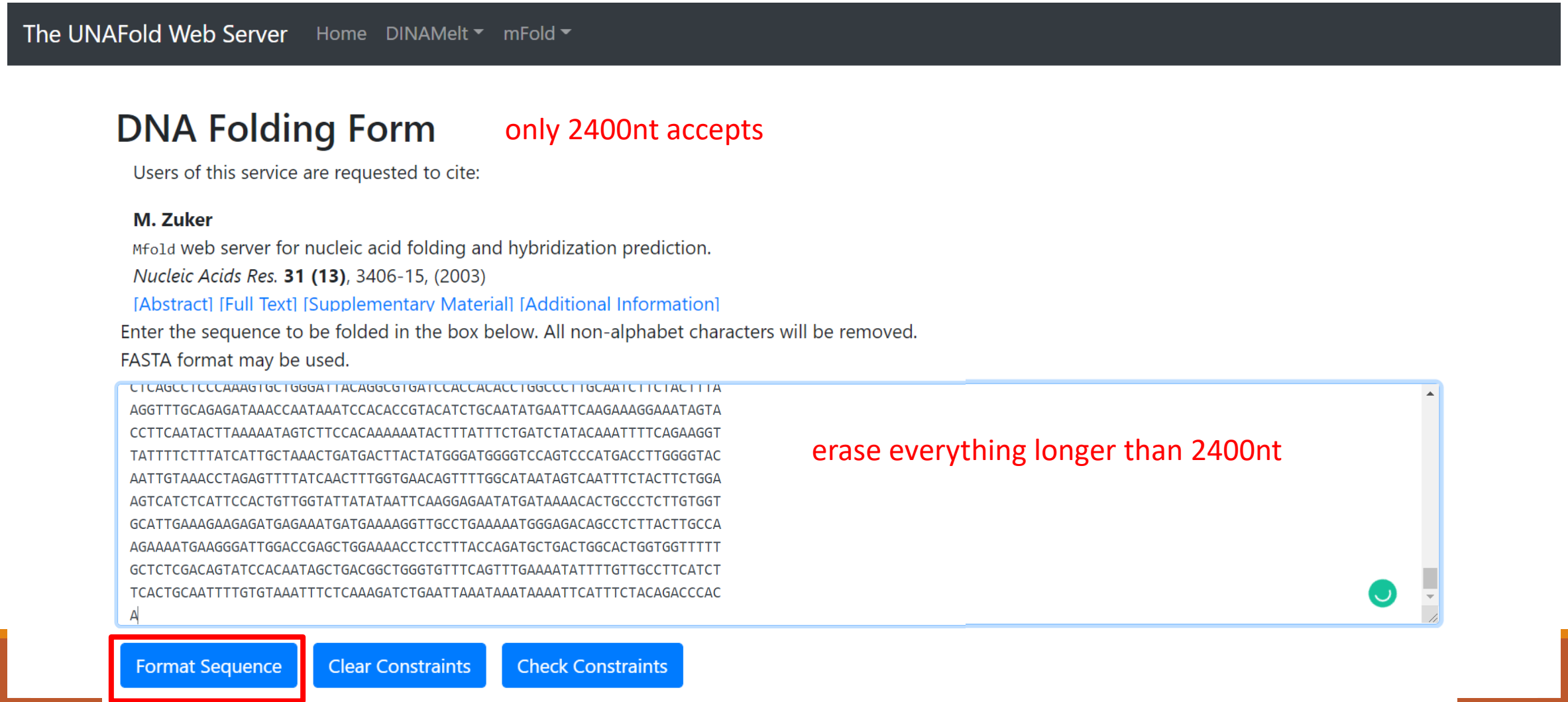
Primers: anywhere along the sequence !

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

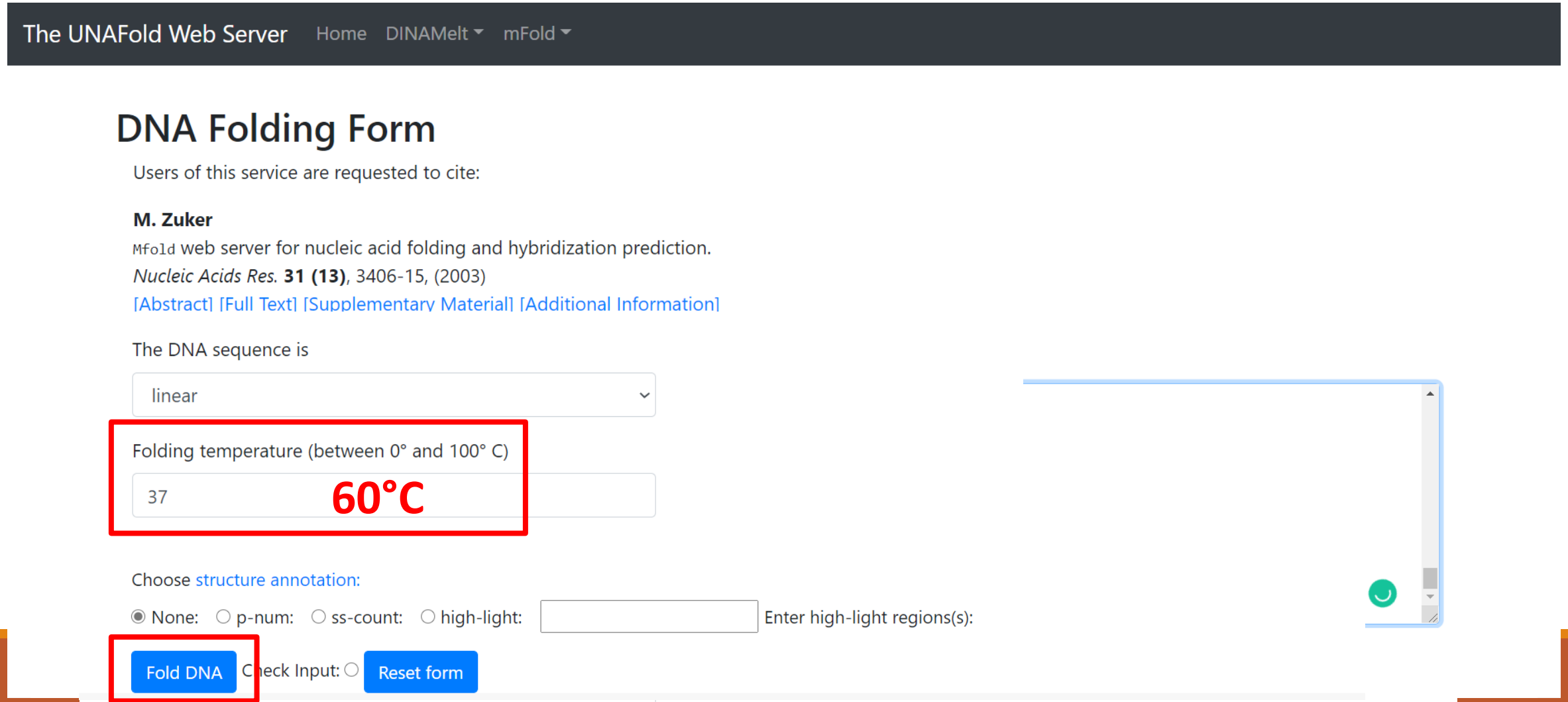
but: must work **perfectly** (100% efficiency) → avoid regions in loops (secondary structure)

mFOLD – prediction of DNA secondary structue

DNA secondary structure prediction

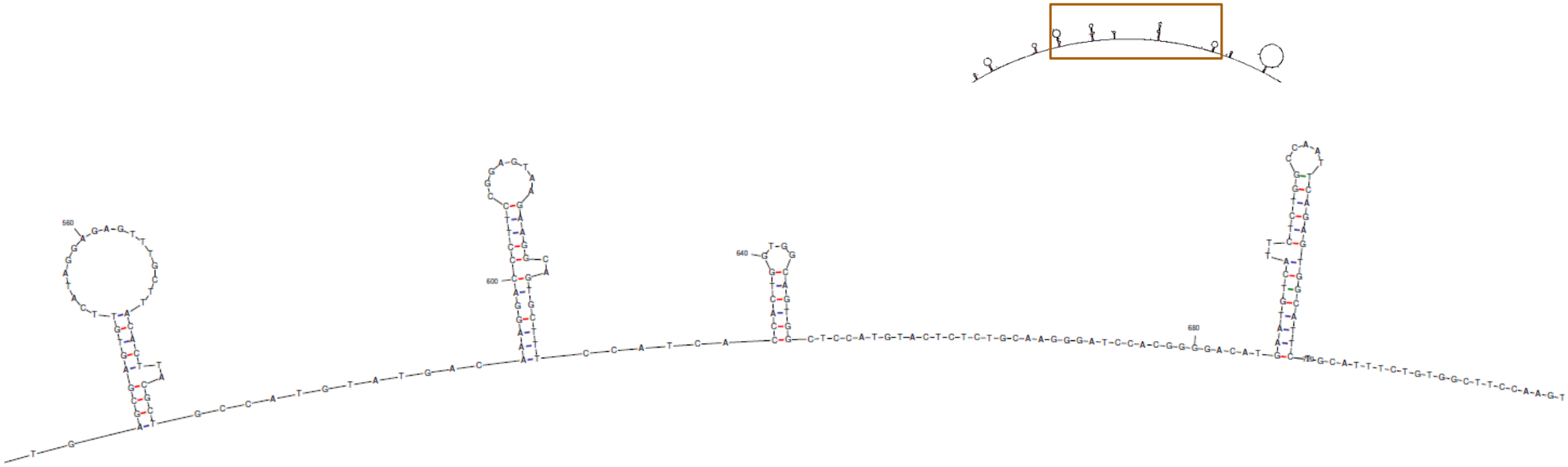


DNA secondary structure prediction



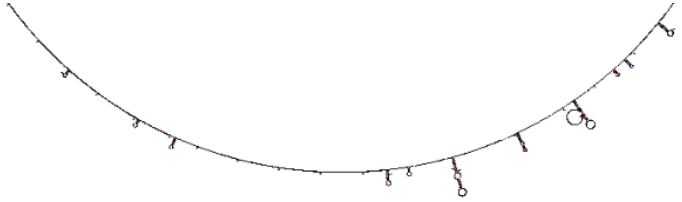
DNA secondary structure prediction

mFOLD: nastavit 60°C



Structure 1 : ΔG = -45.17 kcal/mol (Thermodynamic Details).
Different file formats: PostScript, pdf, png, 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
17Apr17-14-09-14
The results will be erased in 72 hours. To ask a question or to make a comment, please regist
reading previously submitted questions and comments.



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The mfold Web Server

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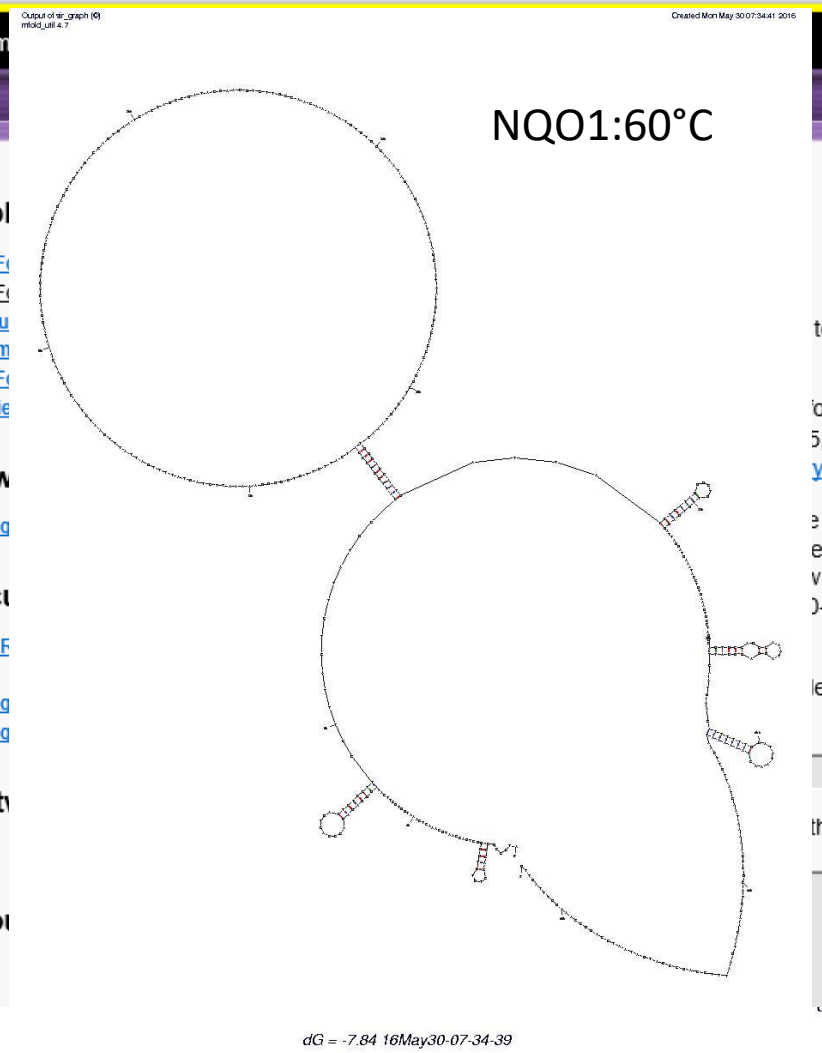
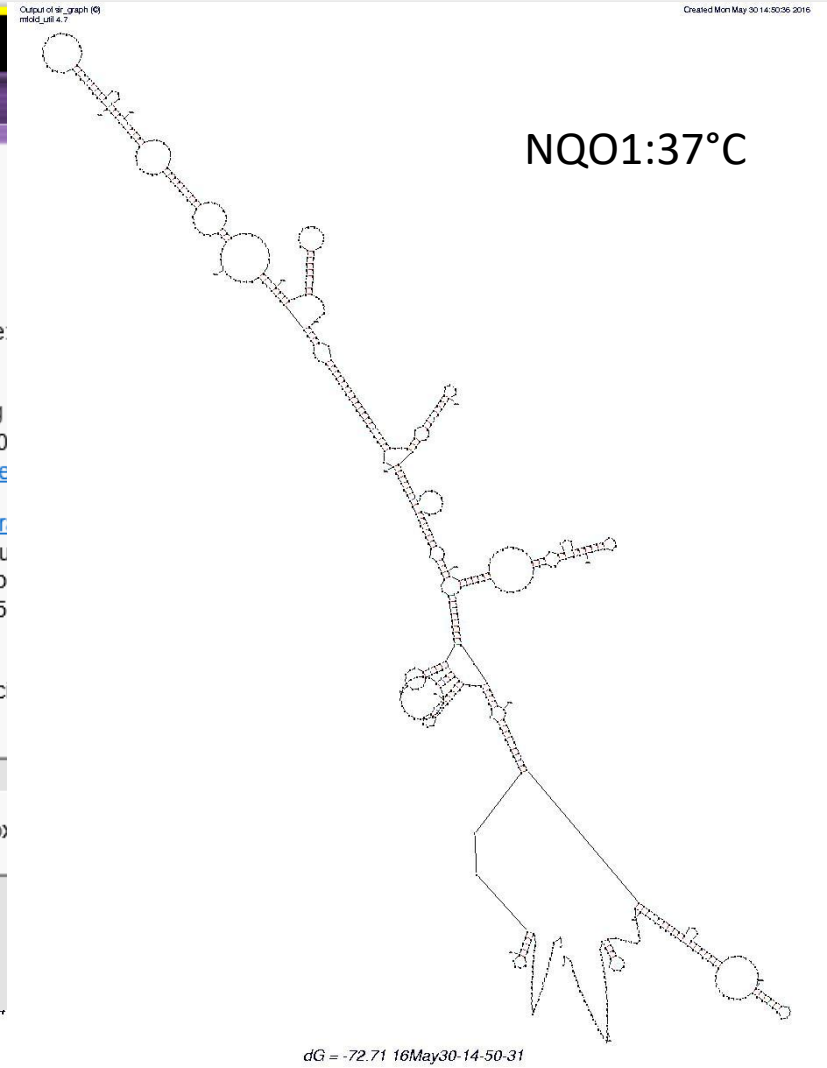


NQO1:60°C



NQO1:37°C

DNA secondary structure prediction

mFOLD: 60°C

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The UNAFold Web Server

SEARCH

NQO1: 1080-1220

```
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      12
  .-TCCCTGACTTGCTTTAGTTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAAATCC

   \ ----------------------------------------------------------------------------------------------------------------------------------------------------

     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      1
     .-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTGA

      \ ----------------------------------------------------------------------------------------------------------------------------------------------------
```

Until 1440

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 structue

→ 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 structue

Primer3 – tool for primer design -

DNA secondary structure prediction

mFOLD: 60°C

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The UNAFold Web Server

SEARCH

NQO1: 1080-1220

```
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      12
  .-TCCCTGACTTGCTTTAGTTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAAATCC

   \ ----------------------------------------------------------------------------------------------------------------------------------------------------

     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      1
     .-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTGA

      \ ----------------------------------------------------------------------------------------------------------------------------------------------------
```

Until 1440

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
CCGCCCTTGTAGGCTGTCCACCTCAAACGGGCCGGACAGGATATATAAGAGAGAATGCACCGTGCACTAC
ACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCCAGCTCACCGAGAGCCTAGTTCCGGCCAGGGTCG
CCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACTGCACCAGAGCCATGGTCGGCAGAAGAGCAC
TGATCGTACTGGCTCACTCAGAGAGGACGTCCTTCAACTATGCCATGAAGGAGGCTGCTGCAGCGGCTTT
GAAGAAGAAAGGATGGGAGGTGGTGGAGTCGGACCTCTATGCCATGAACTTCAATCCCATCATTTCCAGA
```

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 Primers | Download Settings | Reset 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 1080,140

Start Codon Position

Internal Oligo Excluded Region

Force Left Primer Start -10000 Force Right Primer Start -10000

Force Left Primer End -10000 Force Right Primer End -10000

Sequence Quality

Min Sequence Quality 0 Min End Sequence Quality 0 Sequen

Pick Primers | Download Settings | Reset Form

General Primer Picking Conditions

Upload the settings from a file Procházet...

Primer Size	Min	18	Opt	20	Max	23	
Primer Tm	Min	57.0	Opt	59.0	Max	62.0	Max Tm Difference
Product Tm	Min	-10000	Opt	0.0	Max	100000	
Primer GC%	Min	30.0	Opt	50.0	Max	70.0	

Product Size Ranges 50-150

Number To Return 5 Max 3' Stability 9.0

Max Library Mispriming 12.00 Pair Max Library Mispriming 20.00

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

other or longer region needed: 1080,340

Primer3 Output

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

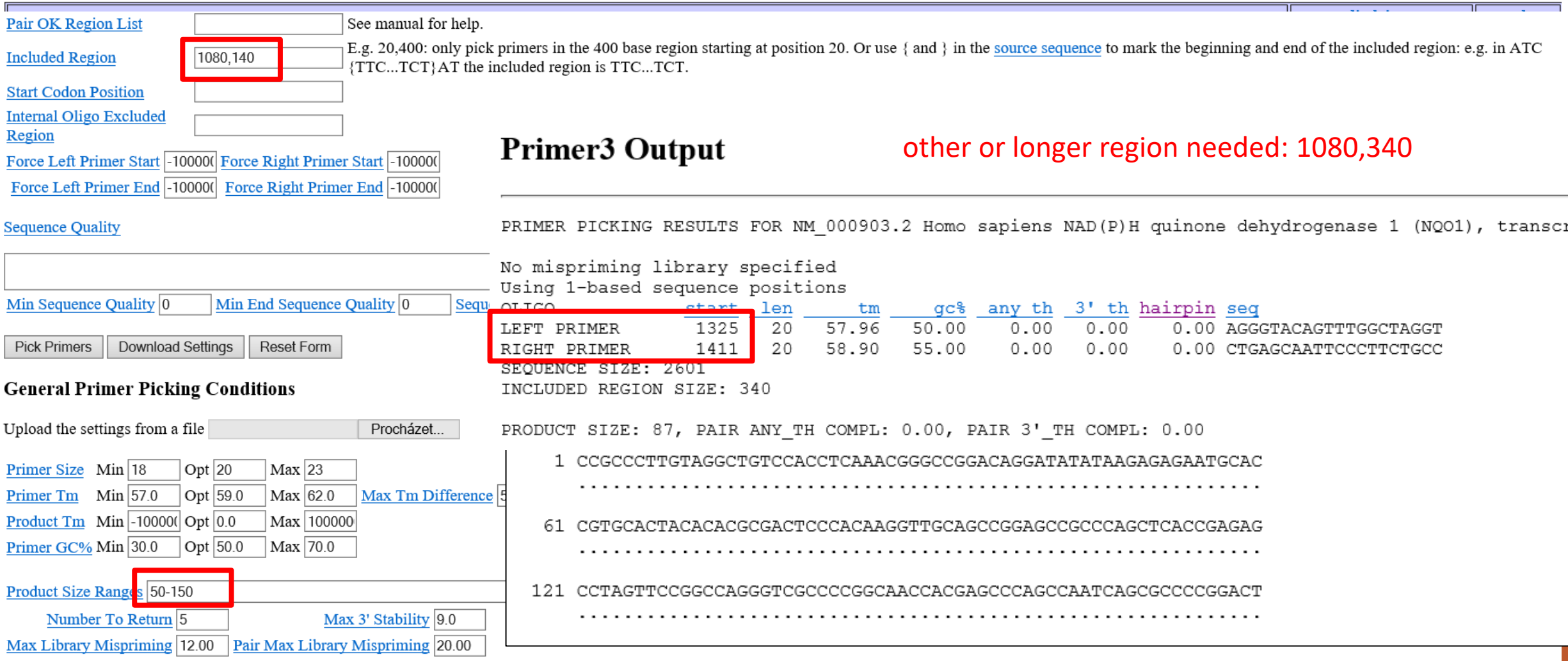
No mispriming library specified
Using 1-based sequence positions

OLIGO	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	CTGAGCAATTCCCTTCTGCC

SEQUENCE SIZE: 2601
INCLUDED REGION SIZE: 340

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

primer design-Primer3-area selection



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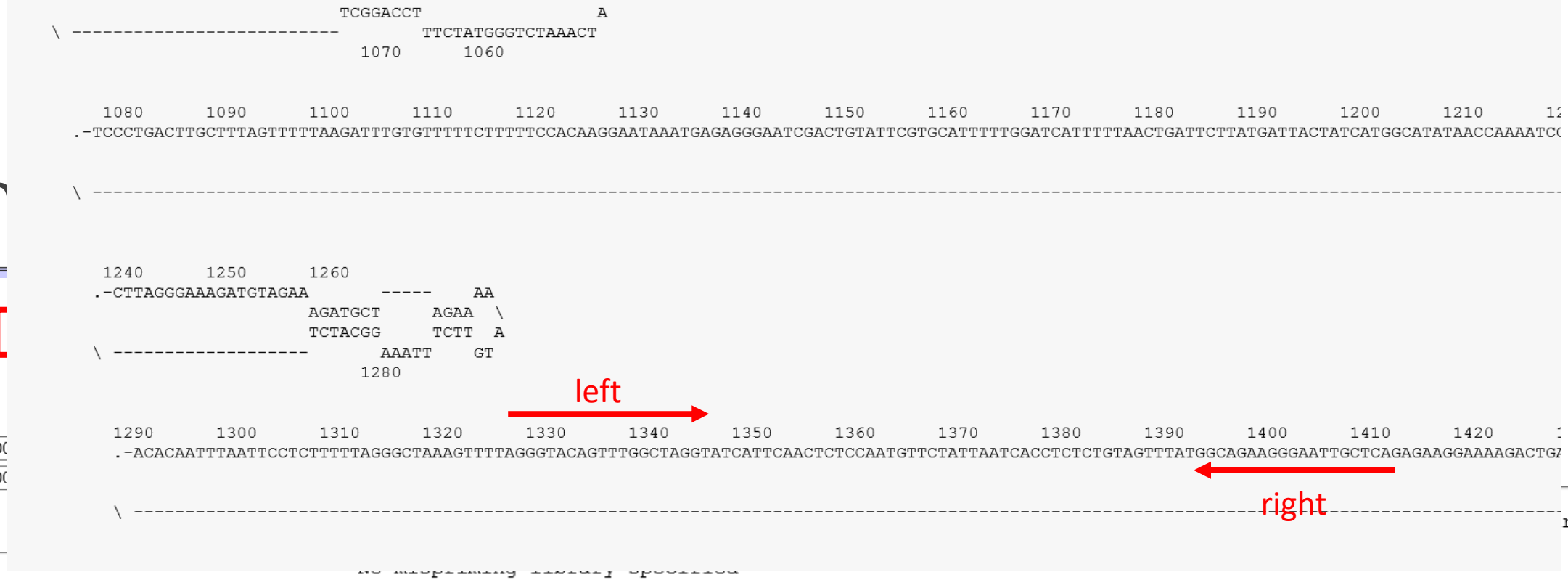


Pair OK Region List

Included Region

Start Codon Position

Internal Oligo Excluded Region

Force Left Primer Start -10

Force Left Primer End -10

Sequence Quality

Min Sequence Quality 0 Min End Sequence Quality 0 Sequ

Pick Primers | Download Settings | Reset Form

General Primer Picking Conditions

Upload the settings from a file Procházet...

Primer Size Min 18 Opt 20 Max 23

Primer Tm Min 57.0 Opt 59.0 Max 62.0 Max Tm Difference

Product Tm Min -10000 Opt 0.0 Max 100000

Primer GC% Min 30.0 Opt 50.0 Max 70.0

Product Size Ranges 50-150

Number To Return 5 Max 3' Stability 9.0

Max Library Mispriming 12.00 Pair Max Library Mispriming 20.00

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	CTGAGCAATTCCCTTCTGCC

√

SEQUENCE SIZE: 2601

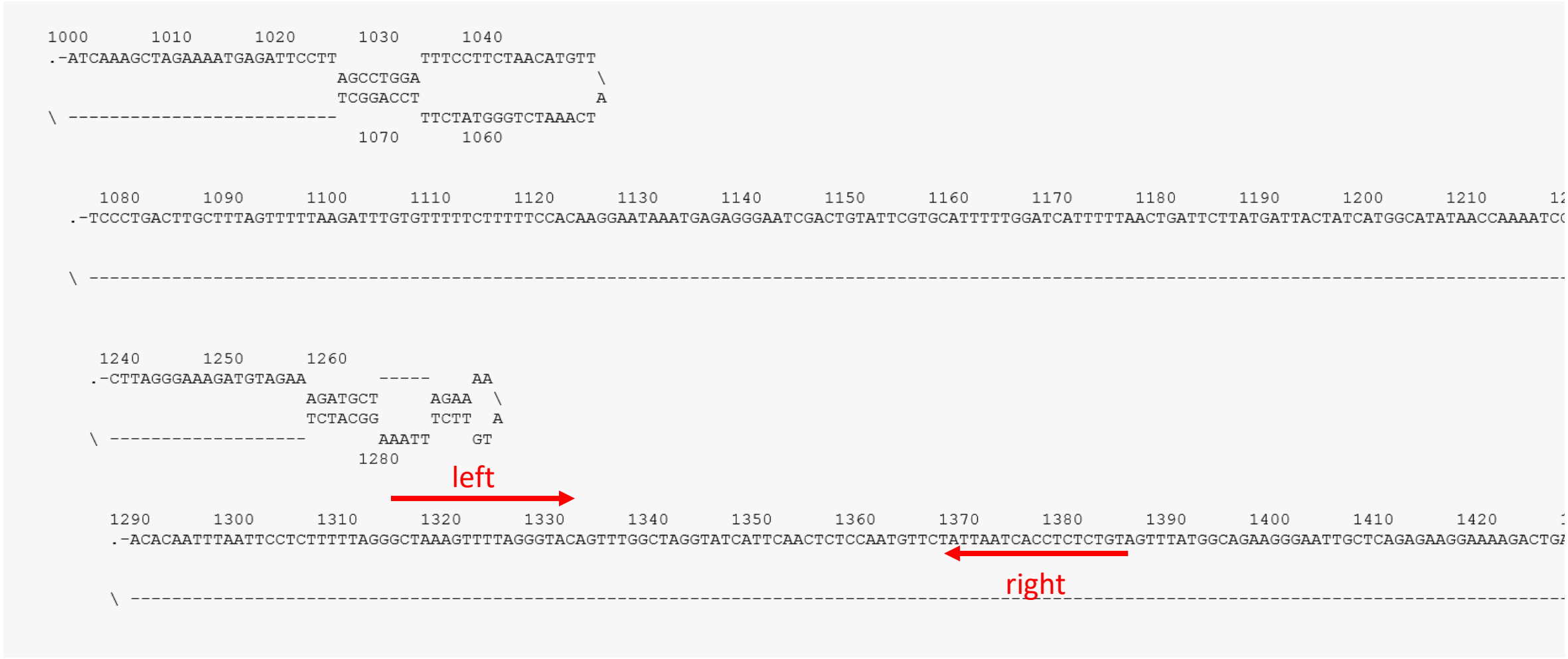
INCLUDED REGION SIZE: 340

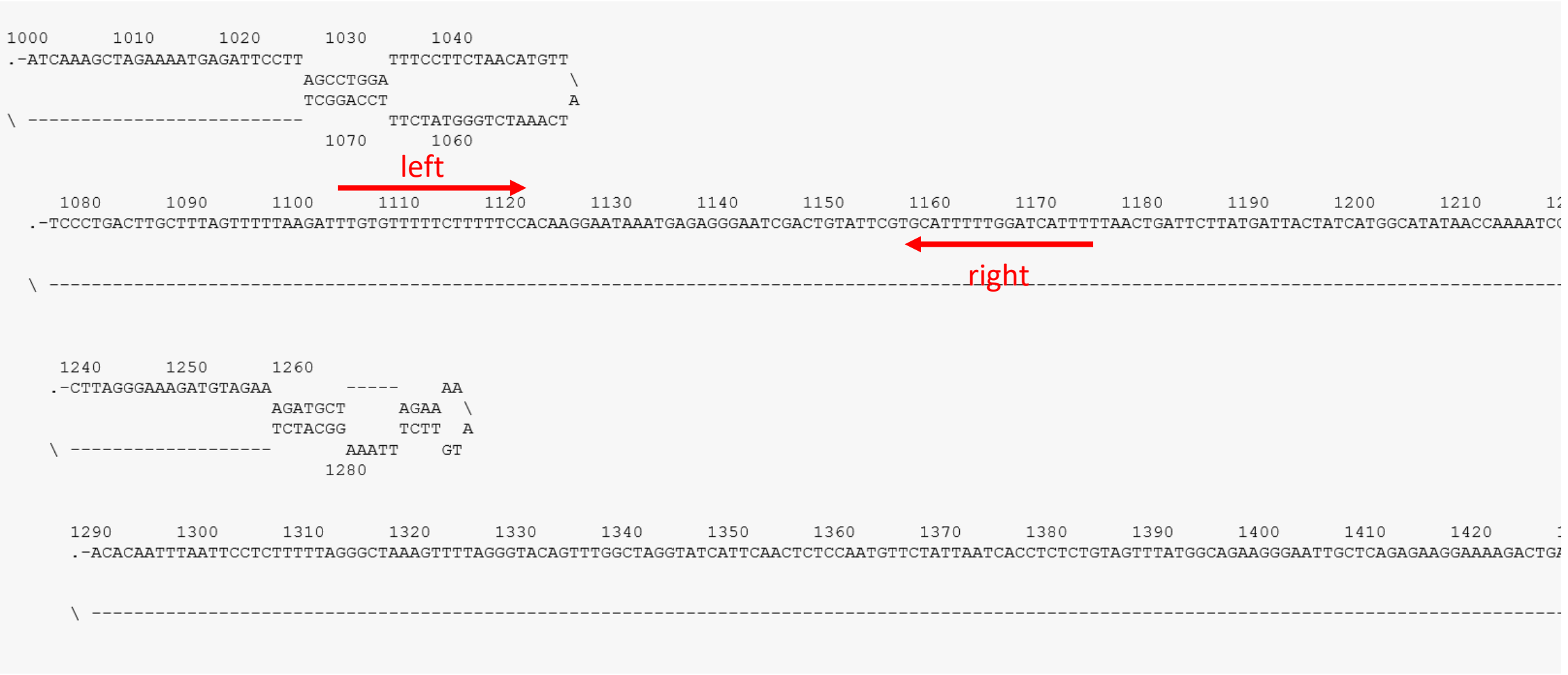
PRODUCT SIZE: 87, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

```
   1 CCGCCCTTGTAGGCTGTCCACCTCAAACGGGCCGGACAGGATATATAAGAGAGAATGCAC
     ............................................................

  61 CGTGCACTACACACGCGACTCCCACAAGGTTGCAGCCGGAGCCGCCCAGCTCACCGAGAG
     ............................................................

 121 CCTAGTTCCGGCCAGGGTCGCCCCGGCAACCACGAGCCCAGCCAATCAGCGCCCCGGACT
     ............................................................
```


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
.-TCCCTGACTTGCTTTAGTTTTTAAGATTTGTGTTTTTCTTTTTCCACAAGGAATAAATGAGAGGGAATCGACTGTATTCGTGCATTTTTGGATCATTTTTAACTGATTCTTATGATTACTATCATGGCATATAACCAAAATCC
\ ----------
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
.-ACACAATTTAATTCCTCTTTTTAGGGCTAAAGTTTTAGGGTACAGTTTGGCTAGGTATCATTCAACTCTCCAATGTTCTATTAATCACCTCTCTGTAGTTTATGGCAGAAGGGAATTGCTCAGAGAAGGAAAAGACTGA
right
\ ----------


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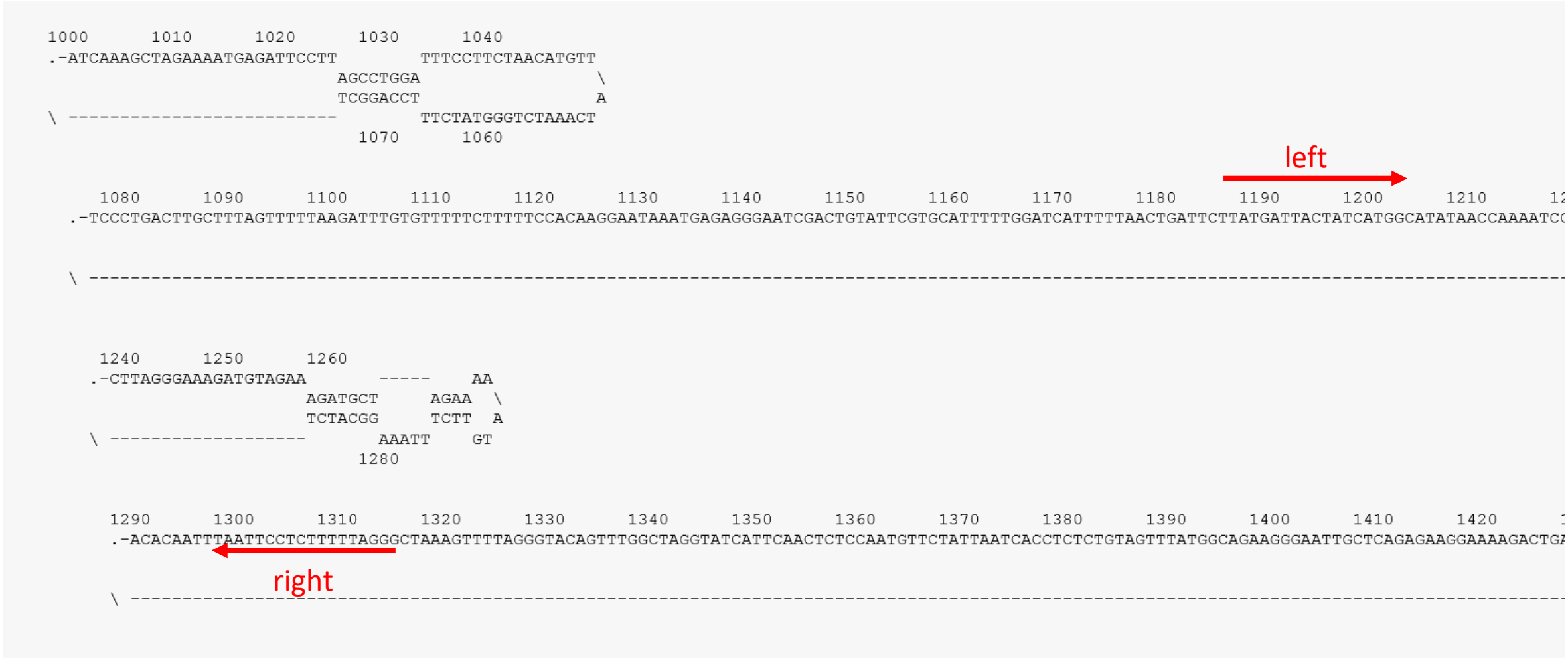
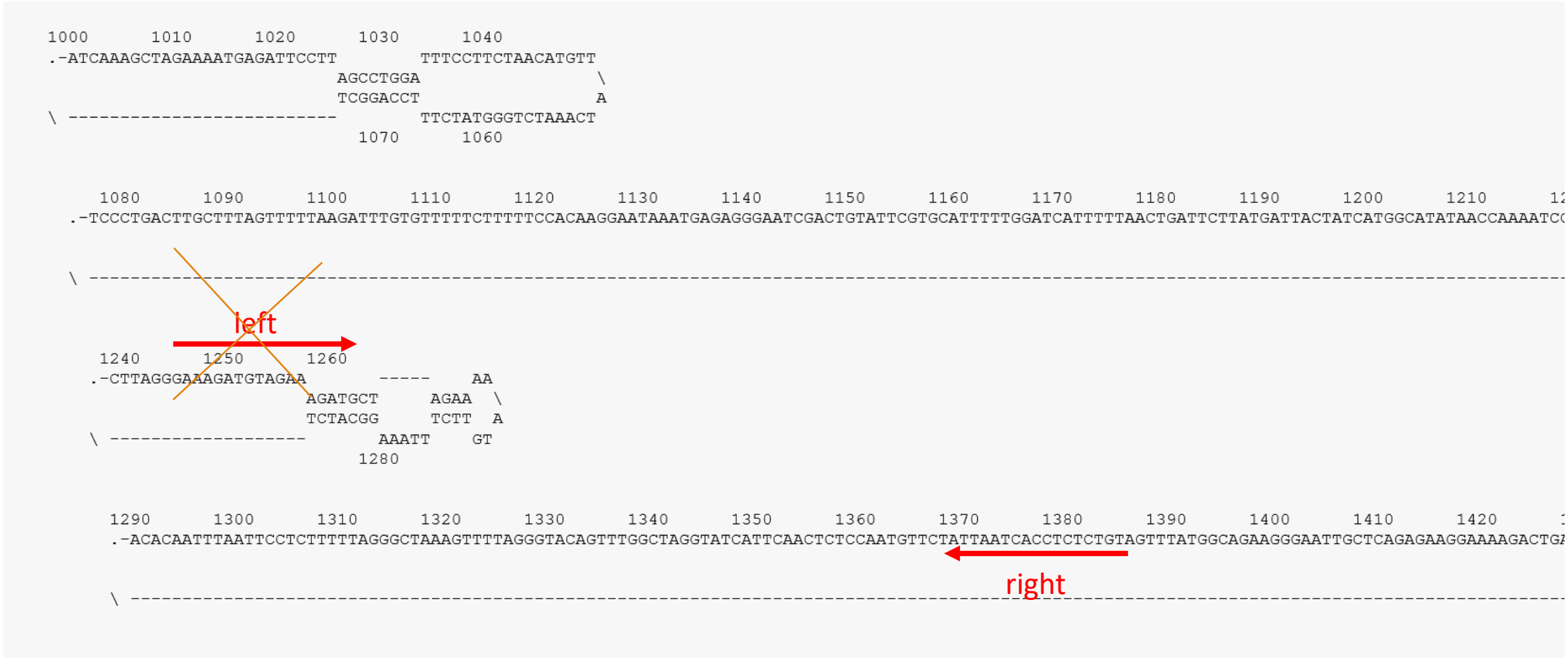

left
right



→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)

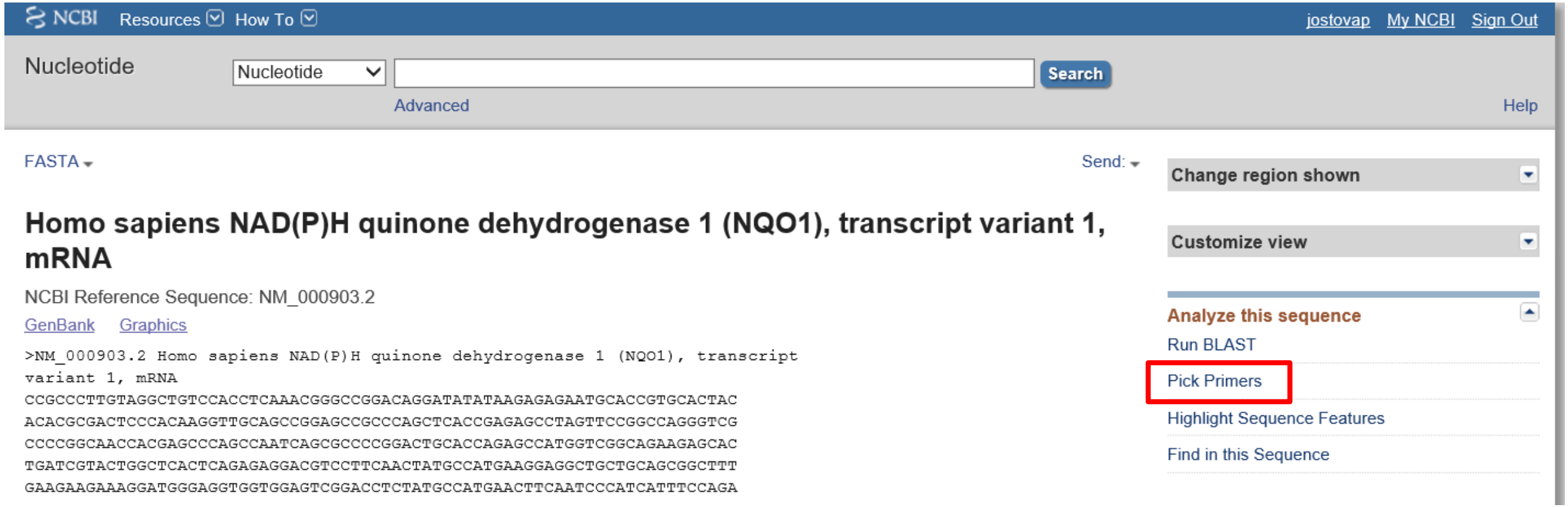
mFOLD – prediction of DNA secondary structue

Primer3 – tool for primer design

Check for specificity – qPCR primers should be specific

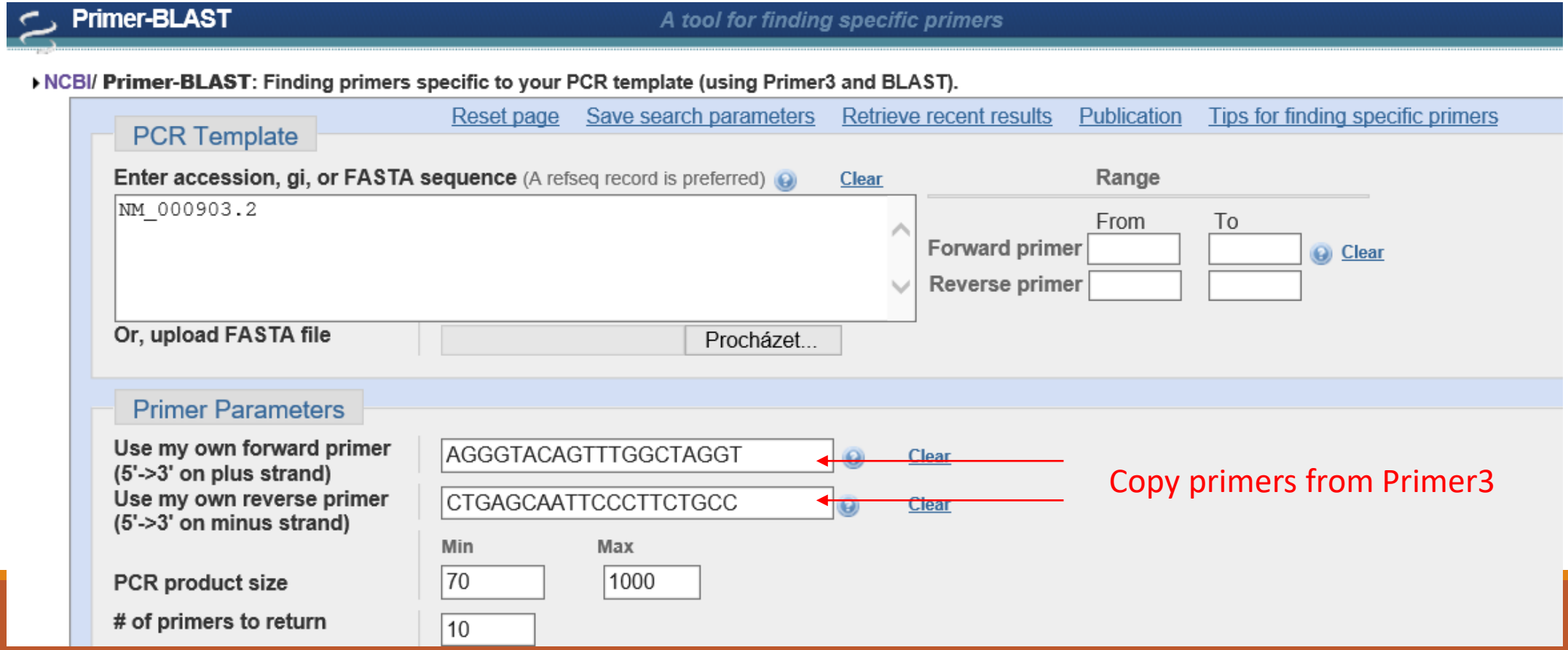
primers: (q)PCR (rt-PCR) - specificity

NCBI/Pick Primers (Primer BLAST)



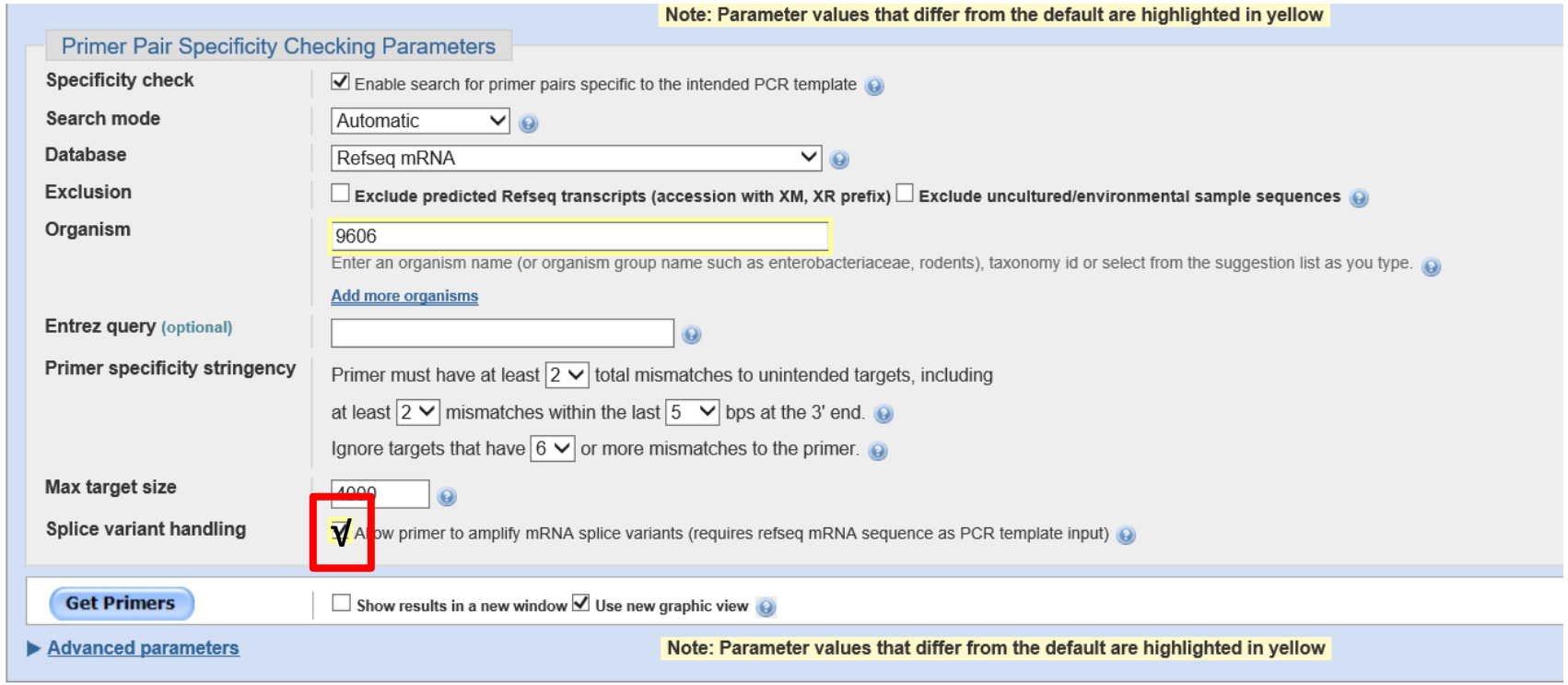
primers: (q)PCR (rt-PCR) - specificity

NCBI/Pick Primers (Primer BLAST)



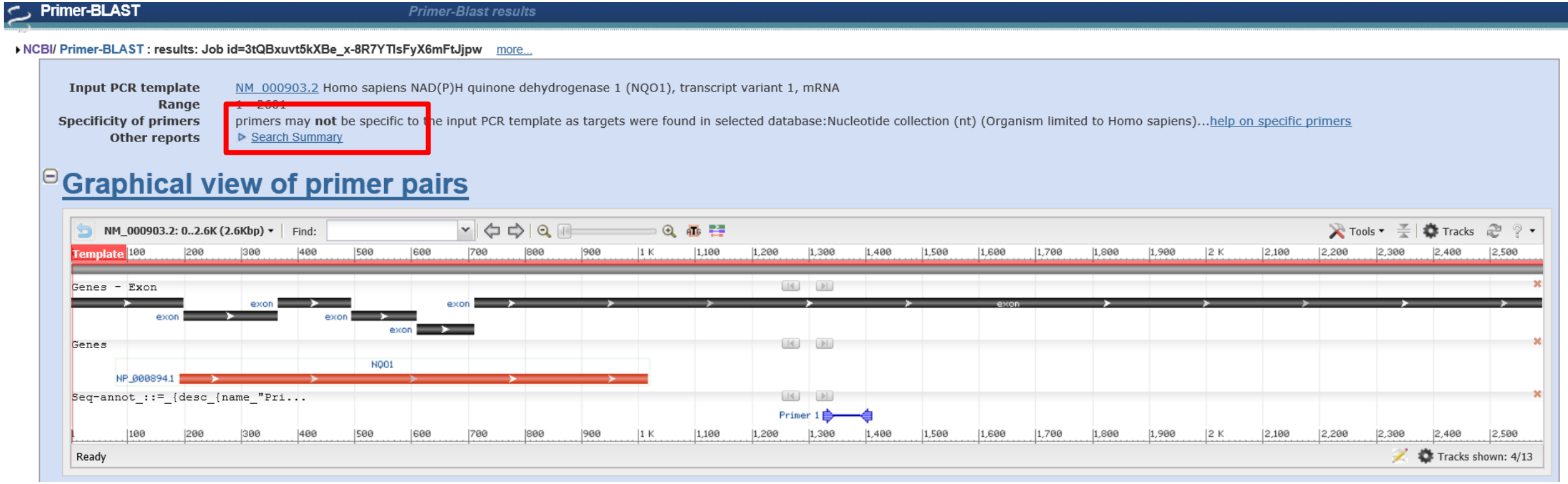
primers: (q)PCR (rt-PCR) - specificity

NCBI/Pick Primers (Primer BLAST)



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

NCBI/Pick Primers (Primer BLAST)



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

NCBI/Pick Primers (Primer BLAST)

Primer-BLAST *Primer-Blast results*

NCBI/ Primer-BLAST : results: Job id=3tQBxuvt5kXBe_x-8R7YTlsFyX6mFtJjpw 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	CTGAGCAATTCCCTTCTGCC	Minus	20	1411	1392	58.90	55.00	4.00	1.00
Product length	87								

Products on intended target ok

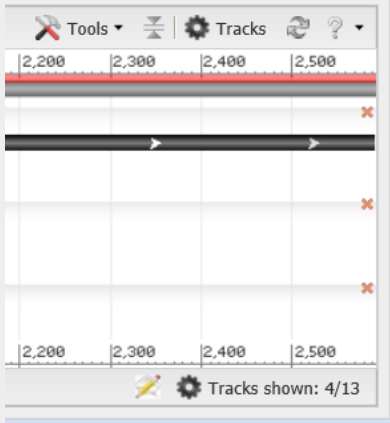
>NM_000903.2 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 1, mRNA

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

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

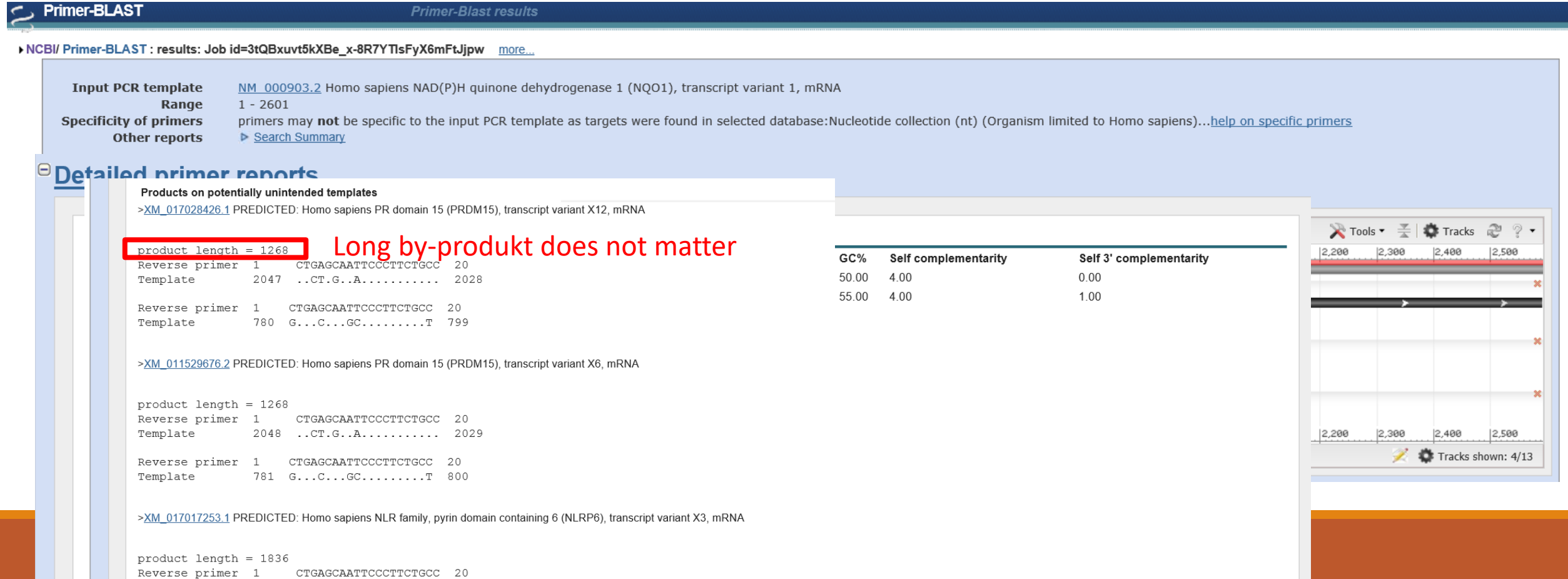
Products on allowed transcript variants ok

>NM_001286137.1 Homo sapiens NAD(P)H quinone dehydrogenase 1 (NQO1), transcript variant 4, mRNA



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

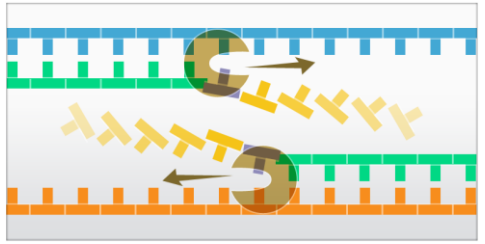
NCBI/Pick Primers (Primer BLAST)



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