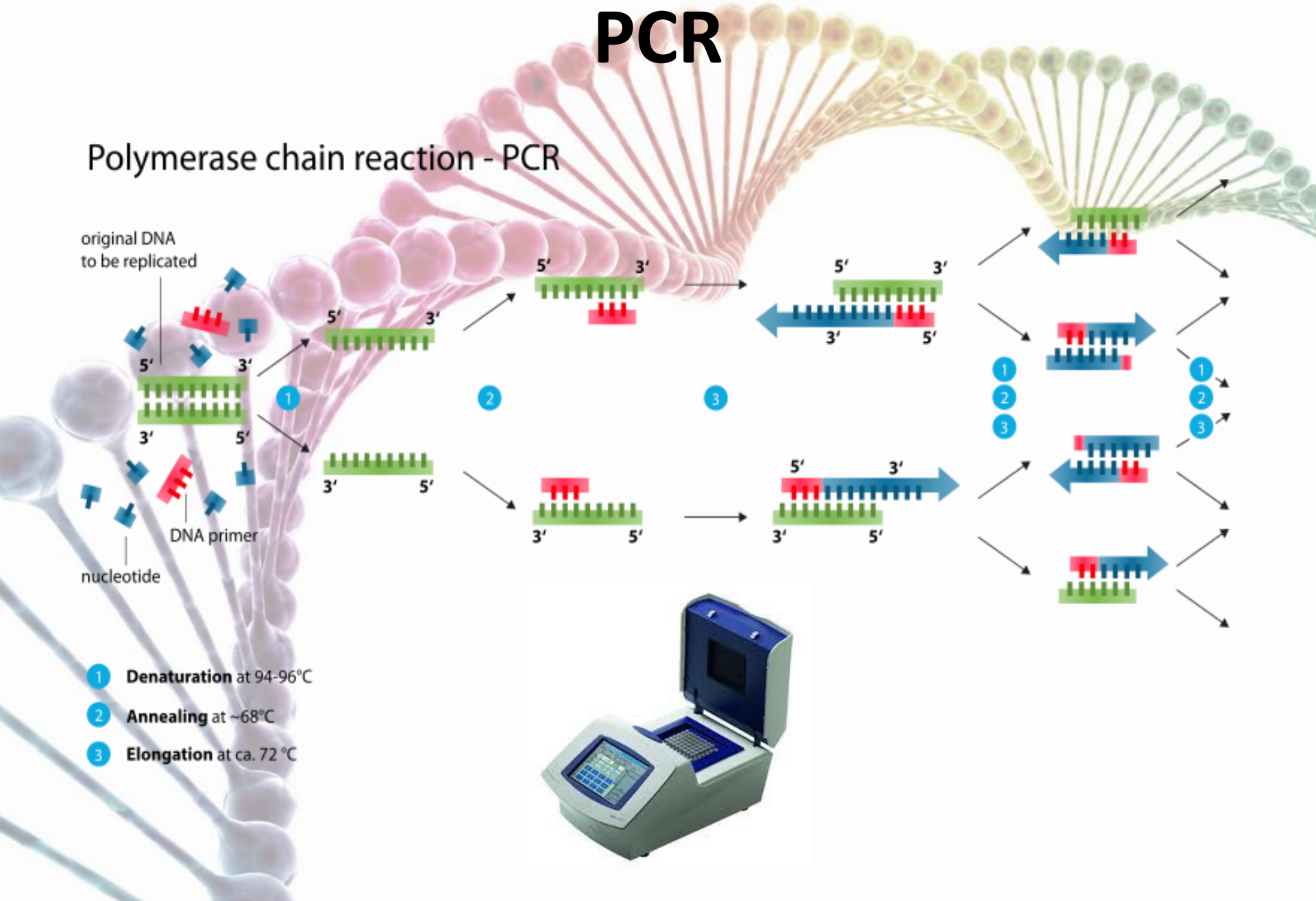
به نام خدا

موضوع: طراحی پرایمر

استاد: دکتر حسامی

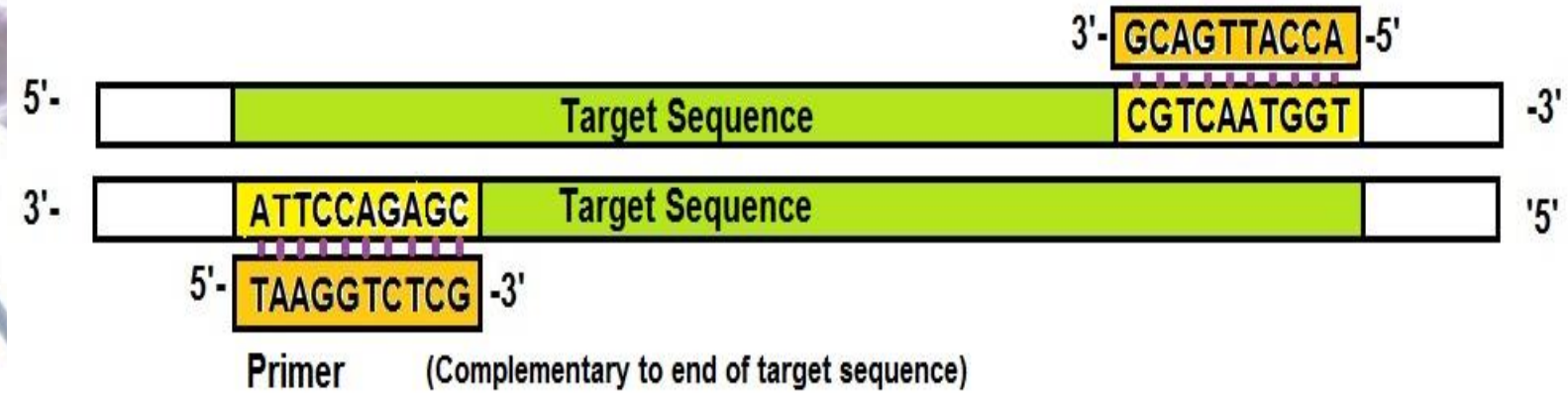
سمانه نعمتی

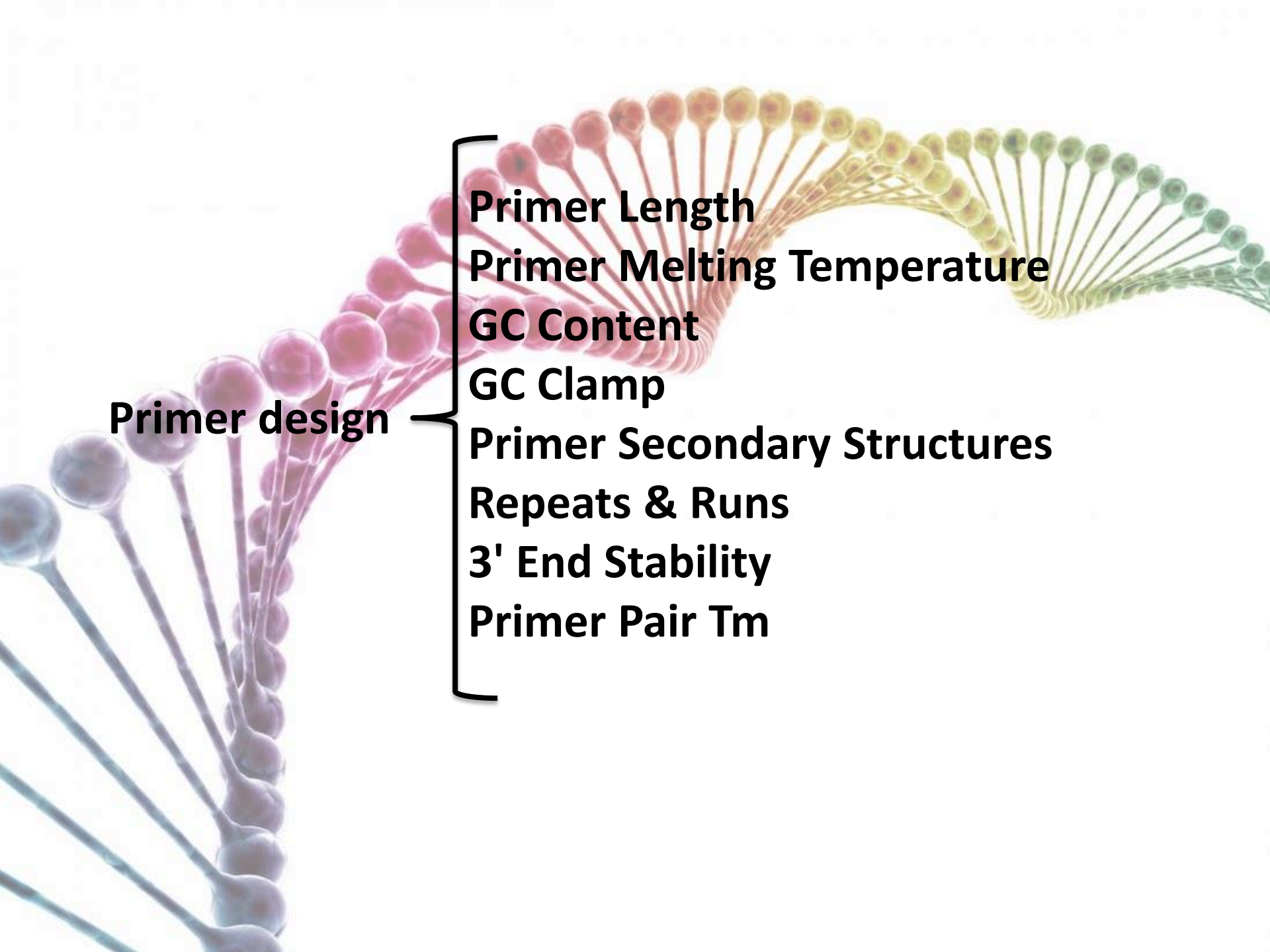
PCR


Polymerase chain reaction - PCR
original DNA
to be replicated
5′
3′
3′
5′
DNA primer
nucleotide
1
2
3
1 Denaturation at 94-96°C
2 Annealing at ~68°C
3 Elongation at ca. 72 °C

Primer

A short synthetic oligonucleotide which is used in many molecular techniques from PCR to DNA sequencing.




Primer design
Primer Length
Primer Melting Temperature
GC Content
GC Clamp
Primer Secondary Structures
Repeats & Runs
3' End Stability
Primer Pair Tm

Primer Length

optimal length of PCR primers: 18-22 nucleotide

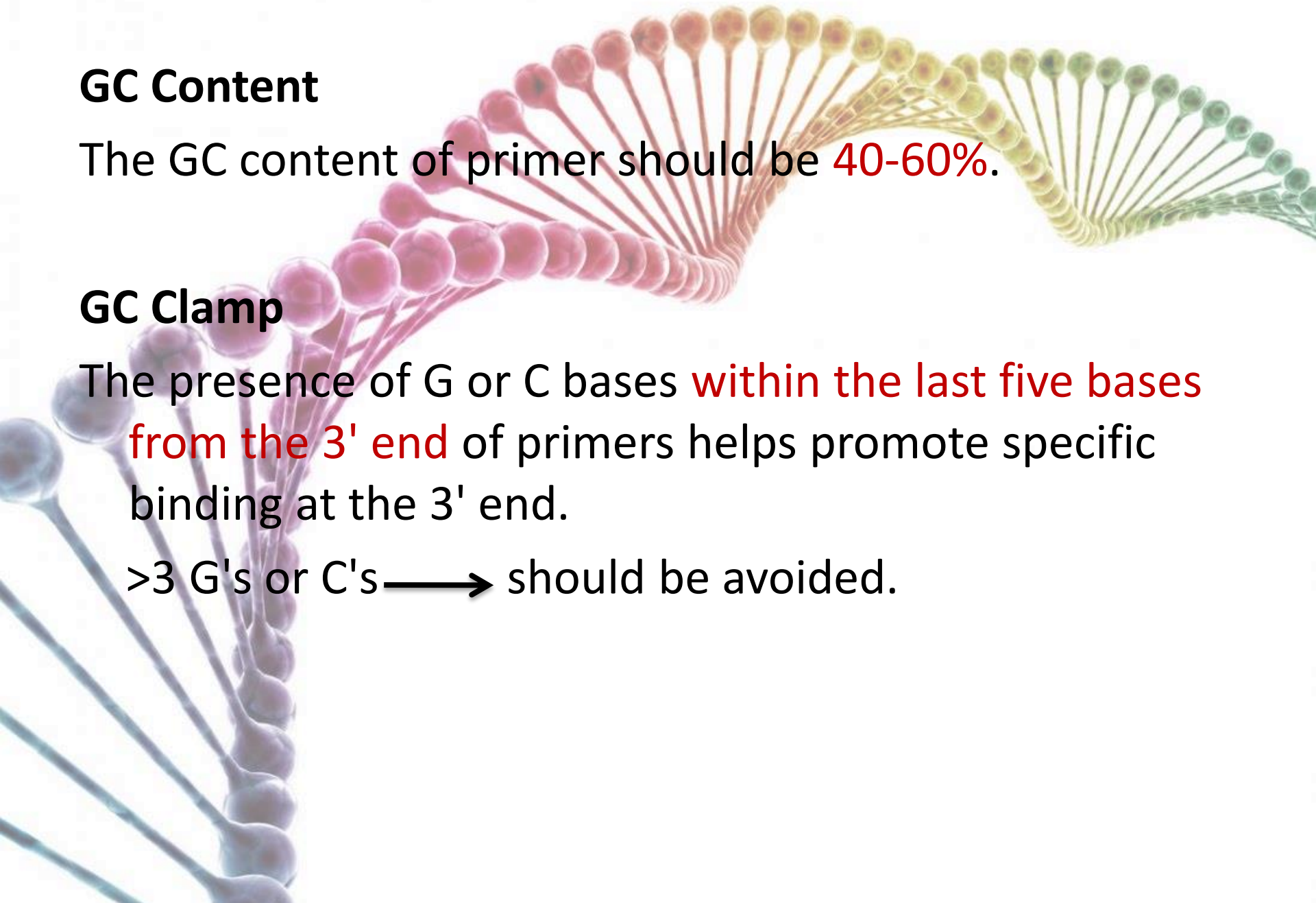
<18 ⟶ increase nonspecific connections

>22 ⟶ less hybridization

Primer Melting Temperature(Tm)

Optimal Tm: in the range of 52-58 ^{o}C

$$Tm=4(C-G)+2(A-T)$$



GC Content

The GC content of primer should be 40-60%.

GC Clamp

The presence of G or C bases within the last five bases from the 3' end of primers helps promote specific binding at the 3' end.

>3 G's or C's ⟶ should be avoided.

Primer Secondary Structures

1. Hairpins

```
C T A C G C A G T A C ⟶  C T A C G ┐
                        I I I     A
                        C A T G C ┘
```

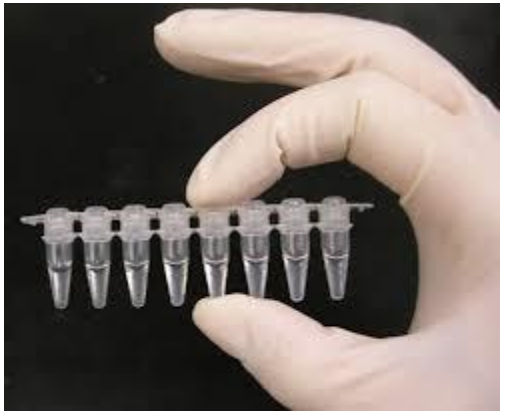
2. Self Dimer

3. Cross(hetero) Dimer

```
C T A C G C A G ⟶ F.P
          I I I
    C  T A G T C C T G A ⟶ R.P
```

4.Palindromic sequences

```
G A A T T C
```



1.Template
2.primers
3.DNA polynerase
4.dNTPs
5.Buffer & H2O

Repeats

A di-nucleotide occurring many times consecutively.

Like:(ATATATAT) MAX: 4 di-nucleotides

Runs

Primers with long runs of a single base.

Like:(ATCCCCCGT) MAX: 4 nucleotides

3' End Stability

The maximum ΔG value of the five bases from the 3' end.

Primer Pair Tm

The two primers of a primer pair should have closely matched melting temperatures for maximizing PCR product yield.

At most ⟶ 3°C

Ideal ⟶ 0°C

Tools

All tools

BioMart >

Export custom datasets from Ensembl with this data-mining tool

BLAST/BLAT >

Search our genomes for your DNA or protein sequence

Variant Effect Predictor >

Analyse your own variants and predict the functional consequences of known and unknown variants

Ensembl is a genome browser for vertebrate genomes that supports research in comparative genomics, evolution, sequence variation and transcriptional regulation. Ensembl annotate genes, computes multiple alignments, predicts regulatory function and collects disease data. Ensembl tools include BLAST, BLAT, BioMart and the Variant Effect Predictor (VEP) for all supported species.

Search

Human for

fumarase Go

e.g. BRCA2 or rat 5:62797383-63627669 or rs699 or coronary heart disease

Ensembl Release 92 (April 2018)

- New goat annotation on the ARS1 assembly
- Update of Marmoset assembly and genebuild
- Mouse: update to Ensembl-Havana GENCODE gene set
- Update to Ensembl-Havana human GENCODE gene set (release 28)
- New command line tool for LD

Full details | All web updates, by release | More news on our blog

- 02 May 2018: Eukaryotic Annotation Team Leader
- 02 May 2018: Ensembl Outreach Officer
- 27 Apr 2018: Cool stuff the VEP can do: Filtering

Go to Ensembl blog

All genomes

-- Select a species --

- View full list of all Ensembl species
- Edit your favourites

Favourite genomes

Human

GRCh38.p12

Still using GRCh37?

Mouse

GRCm38.p6

Current selection:

< all Species

Only searching Human

Restrict category to:

Gene 1

Phenotype 1

Protein Domain 2

Variant 162

Per page:

10 25 50 100

Layout:

Standard Table

Tip:

You can choose which results appear near the top of your search by updating your favourite species.

Only searching Human ▼ fumarase

166 results match **fumarase** when restricted to species: Human ✘

Did you mean... ▼

FUMARASE DEFICIENCY (Human Phenotype)

Human Phenotype

Fumarase deficiency.

FH (Human Gene) ←

ENSG00000091483 1:241497603-241519761:-1

Fumarate hydratase [Source:HGNC Symbol;Acc:HGNC:3700]

FUMARASE DEFICIENCY; FMRD [#606812] (MIM morbid record; description: *FUMARASE* DEFICIENCY; FMRD;;FUMARIC ACIDURIA,) is an external reference matched to Gene ENSG00000091483

Variant table • Phenotypes • Location • External Refs. • Regulation • Orthologues • Gene tree

IPR018951 (Protein Domain in Human)

IPR018951

Interpro domain IPR018951 [Fumarase C, C-terminal] is found in 1 gene in Human and has 1 record from signature databases: PF10415.

IPR024083 (Protein Domain in Human)

IPR024083

Interpro domain IPR024083 [Fumarase/histidase, N-terminal] is found in 4 genes in Human and has 1 record from signature databases: 1.10.275.10.

Transcript-based displays
- Summary
- Sequence
  - Exons
  - cDNA
  - Protein
- Protein Information
  - Protein summary
  - Domains & features
  - Variants
- Genetic Variation
  - Variant table
  - Variant image
  - Haplotypes
  - Population comparison
  - Comparison image
- External References
  - General identifiers
  - Oligo probes
- Supporting evidence
- ID History
  - Transcript history
  - Protein history

Configure this page

Custom tracks

Export data

Transcript: FH-201 ENST00000366560.3

Description	fumarate hydratase [Source:HGNC Symbol;Acc:HGNC:3700]
Synonyms	fumarase
Location	Chromosome 1: 241,497,603-241,519,761 reverse strand.
About this transcript	This transcript has 10 exons, is annotated with 18 domains and features, is associated with 716 variations and maps to 482 oligo probes.
Gene	This transcript is a product of gene ENSG00000091483 Hide transcript table

Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
FH-201	ENST00000366560.3	1797	510aa	Protein coding	CCDS1617	A0A0S2Z4C3 P07954	NM_000143 NP_000134	TSL:1 GENCODE basic APPRIS P1
FH-202	ENST00000493477.1	414	No protein	Processed transcript	-	-	-	TSL:3
FH-203	ENST00000497042.1	351	No protein	Processed transcript	-	-	-	TSL:2

Exons

Download sequence

Exons/ Introns Translated sequence Flanking sequence Intron sequence UTR

Bookmark this page

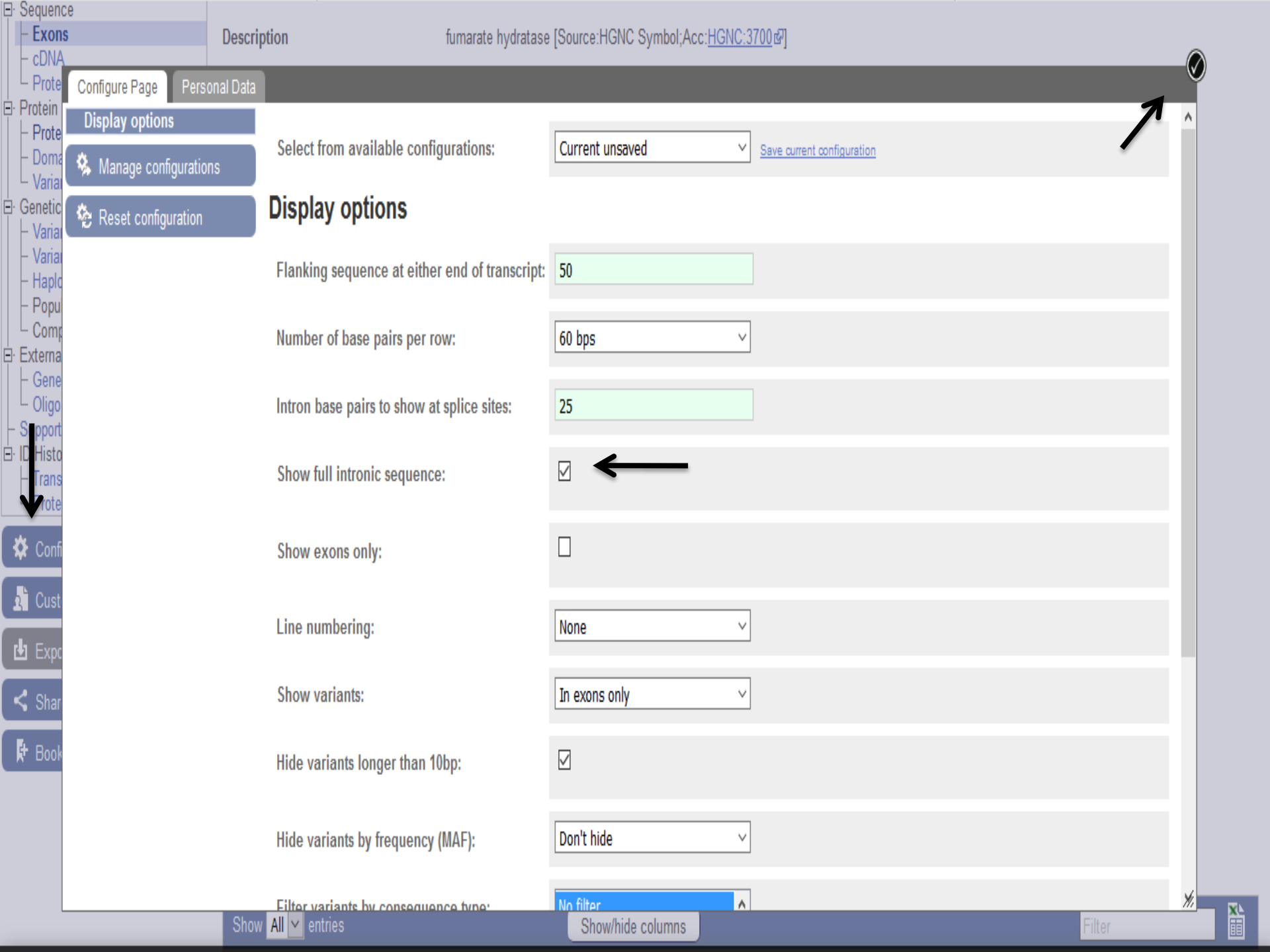
Exons/ Introns Translated sequence Flanking sequence Intron sequence UTR

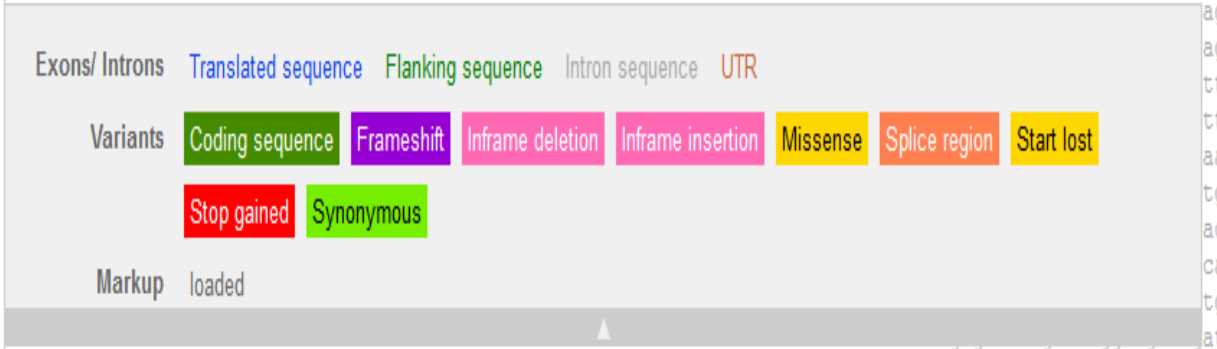
Variants Coding sequence Frameshift Inframe deletion Inframe insertion Missense Splice region Start lost Stop gained Synonymous

Markup loaded

Show All entries Show/hide columns Filter

No.	Exon / Intron	Start	End	Start Phase	End Phase	Length	Sequence
	5' upstream sequence						.....ggaggccggtgggcggaacggtttctgacaaccggcgtggaggcgtggcc
1	ENSE00001442032	241,519,761	241,519,591	-	0	171	ACAGCCGCCCAGAAATTCTACCCAAGCTCCCTCAGCACCATGTACCGAGCACTTCGGCTC CTCGCGCGCTCGCGTCCCCTCGTGCGGGGCTCCAGCCGCAGCCTTAGCTTCGGCTCCGGC TTGGGTGGCGCGGCCGTGCCCTCGTTTTGGCCTCCGAACGCGGCTCGAATG
	Intron 1-2	241,519,590	241,517,317			2,274	gtgagcgcaggccgccatcccccgg.....aatagtgttgtattcttgtctttag
2	ENSE00003687860	241,517,316	241,517,182	0	0	135	GCAAGCCAAAATTCCTTCCGGATAGAATATGATACCTTTGGTGAACTAAAGGTGCCAAAT GATAAGTATTATTGCGCCCAGACCGTGAGATCTACGATGAACTTTAAGATTGGAGGTGTG ACAGAACGCATGCCA
	Intron 2-3	241,517,181	241,513,714			3,468	gtaagtggcatttgtggaaatgttg.....gaaatatttttctgattaatttag
3	ENSE00000961688	241,513,713	241,513,603	0	0	111	ACCCCAGTTATTAAAGCTTTTGGCATCTTGAAGCGGAGCGGCCGCTGAAGTAAACCAGGAT TATGGTCTTGATCCAAAGATTGCTAATGCAATAATGAAGGCAGCAGATGAG
	Intron 3-4	241,513,602	241,512,144			1,459	gtaggaggtgataagtgtgtttgct.....tacatttttaacatttttttctgcag
4	ENSE00000961689	241,512,143	241,511,967	0	0	177	GTAGCTGAAGGTAAATTTAAATTGATCATTTTCCTCTCGTGGTATGGCAGACTGGATCAGGA ACTCAGACAAATATGAATGTAAATGAAGTCATTAGCAATAGAGCAATTGAAATGTTAGGA GGTGAACTTGGCAGCAAGATACCTGTGCATCCCAACGATCATGTTAATAAAAGCCAG
	Intron 4-5	241,511,966	241,508,786			3,181	gtcagtatgtgagctttgctgtttt.....atttcttttgaaaaatttccaacag
5	ENSE00000961690	241,508,785	241,508,603	0	0	183	AGCTCAAATGATAGTTTTCGCACAGCAATGCACATTGCTGCTGCAATAGAAGTTCATGAA


Sequence
Exons
cDNA
Protein
Genetic
External
Description
fumarate hydratase [Source:HGNC Symbol;Acc:HGNC:3700]
Configure Page
Personal Data
Display options
Manage configurations
Reset configuration
Select from available configurations:
Current unsaved
Save current configuration
Display options
Flanking sequence at either end of transcript:
50
Number of base pairs per row:
60 bps
Intron base pairs to show at splice sites:
25
Show full intronic sequence:
Show exons only:
Line numbering:
None
Show variants:
In exons only
Hide variants longer than 10bp:
Hide variants by frequency (MAF):
Don't hide
Filter variants by consequence type:
No filter
Show
All
entries
Show/hide columns
Filter



2	ENSE00003687860	241,517,316	241,517,182	0	0	135	GCAAGCCAAAATTCCTTCCGGATAGAATATGATACCTTTGGTGAACTAAAGGTGCCAAATGATAAGTATTATGGCGCCCAGACCGTGAGATCTACGATGAACTTTAAGATTGGAGGTGTGACAGAACGCATGCCA
	Intron 2-3	241,517,181	241,513,714			3,468	gtaagtggcatttgtggaaatgttggctattttggatgaagtaggctgtattcatgagtcactttacttttataaatatttttcagagttaaaatgtaaagttattagaaaagatggcttcgtgagtaataagaggttctctttcactaaaatgttaaagaacttttgaagttttagcatgctattattttgagccatcgctttgaatattttggatttttaaaatttaaggccgggcacggtggcttatgcctgtaatcccagaactttgggaggccaaggtgggcagatcacgaggtcaagagatcgagaccatcctggccaacatggtgaaactctgtctctactaaaaatacaaaaattagccgggcacggtggcgcgcgcctgtagtcccaattactctggaggctgagacaggagaattgcttgaacccgggaggcggaggttgcagtgagctgagatcgcaccactgcactccagcctggcgacagagtgagactccgtctcaaaaaaaaaaaaattaatttaatttaagttccgggatacacatgcaggatatgcagttttgttgcataggtaaatgtgtgccatggtggtttgctgcacctctcaacccatcacctagatattaaaacccggcattagctatttttttctgctgctccccctccgcccacaggccctgtgtgctttgttcccctccttgtgtccatatgttctcattgttcagctcccacttacaagtgagaacatgcgggtttggttttctgttcctctgttagtatgctgagggtcacagcttctagctccatccatatctctgcaaaggacatgatctcattctttttttatggttgcataatattccatggtggatatatacaacttttttctttatccctttattgatggacatttgggttgatttcatgtctttgctattgtgaatagttatttgggatttttatttggcagaactaaatgattgtctttttcactcttgctgggtatttacagatt

agggaagcagactggagaacttctgaatctat
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catagtatattagttctacagtctgtctttaaagcctgaattaataagtttgtgtgacaa
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ttgtctttgtgatacttattcaggtttctttctctttaacagctgataagatgcgattac
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Atactcttgaaatggaaaagattactatttgtatttcaaagtctttatgtttatcaccagcatagtatattagttctaca
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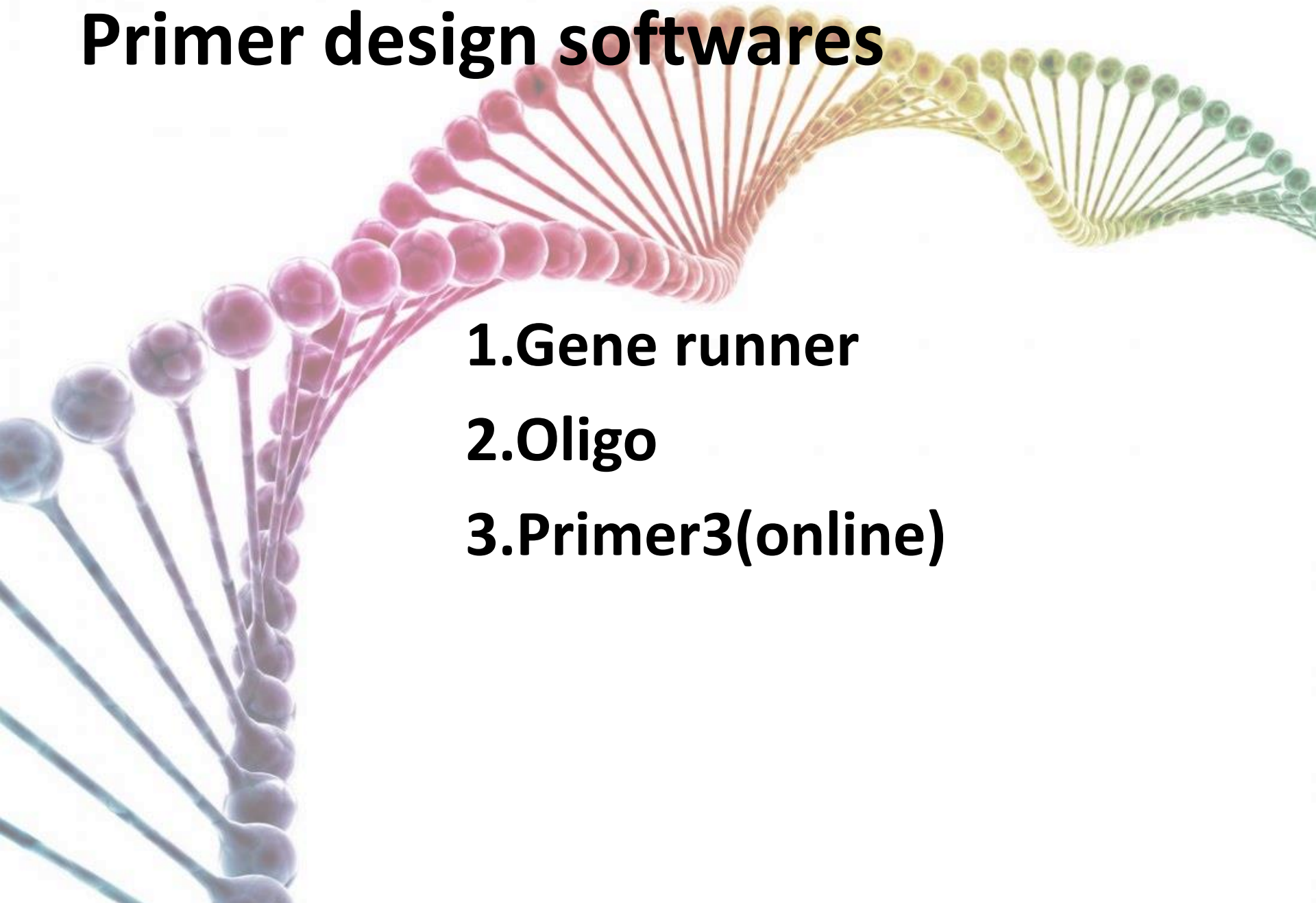
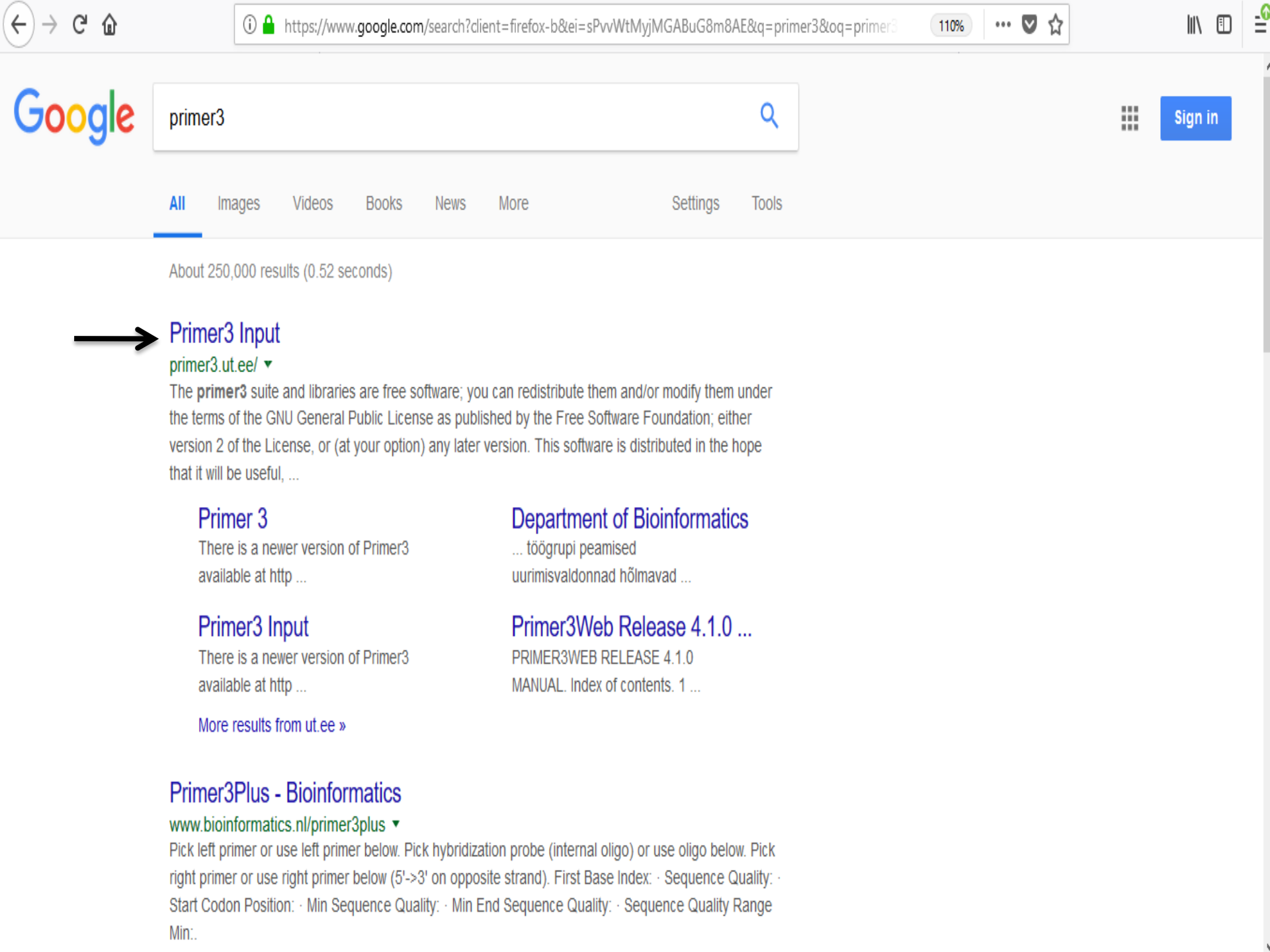
atgagaatccaaatagtatttttttgtctttgtgatacttattcaggtttctttctctttaacagctgataagatgcgatta
ctttt[gatcctgggtttctttttcaacttgtaatagtgttgtattcttgtctttagGCAAGCCAAAATTCCTTCCG
GATAGAATATGATACCTTTGGTGAACTAAAGGTGCCAAATGATAAGTATTATGGCGCCC
AGACCGTGAGATCTACGATGAACTTTAAGATTGGAGGTGTGACAGAACGCATGCCAgta
agtggcatttgtggaaatgttggctattttggatgaagtaggctgta]ttcatgagtcactttactttataaatatttttc
agagttaaaatgtaaagttattagaaaagatggcttcgtgagtaataagaggttctctttcactaaaatgttaaaga
acttttgaagttttagcatgctattattttgagccatcgctttgaatattttggatttttaaaatttaaggccgggcac
ggtggcttatgcctgtaatcccagaactttgggaggccaaggtgggcagatcacgaggt

Primer design softwares



1. Gene runner
2. Oligo
3. Primer3(online)


https://www.google.com/search?client=firefox-b&ei=sPvvWtMyjMGABuG8m8AE&q=primer3&oq=primer3
110%
Google
primer3
Sign in
All
Images
Videos
Books
News
More
Settings
Tools
About 250,000 results (0.52 seconds)
Primer3 Input
primer3.ut.ee/
The primer3 suite and libraries are free software; you can redistribute them and/or modify them under the terms of the GNU General Public License as published by the Free Software Foundation; either version 2 of the License, or (at your option) any later version. This software is distributed in the hope that it will be useful, ...
Primer 3
There is a newer version of Primer3 available at http ...
Department of Bioinformatics
... töögrupi peamised uurimisvaldonnad hõlmavad ...
Primer3 Input
There is a newer version of Primer3 available at http ...
Primer3Web Release 4.1.0 ...
PRIMER3WEB RELEASE 4.1.0 MANUAL. Index of contents. 1 ...
More results from ut.ee »
Primer3Plus - Bioinformatics
www.bioinformatics.nl/primer3plus
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'->3' on opposite strand). First Base Index: · Sequence Quality: · Start Codon Position: · Min Sequence Quality: · Min End Sequence Quality: · Sequence Quality Range Min:.

Primer3web version 4.1.0 - Pick primers from a DNA sequence.

disclaimer | code | cautions

Select the Task for primer selection: generic

Template masking before primer design (available species)

Select species: Example: Mus musculus

Primer failure rate cutoff: < 0.1

Nucleotides to mask in 5' direction: 1

Nucleotides to mask in 3' direction: 0

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): HUMAN

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.

Pick Primers | Download Settings | Reset Form

General Primer Picking Conditions

Upload the settings from a file Browse... No file selected.

Primer Size	Min	18	Opt	20	Max	23			
Primer Tm	Min	57.0	Opt	59.0	Max	62.0	Max Tm Difference	1	Table of thermodynamic parameters: SantaLucia 1998
Product Tm	Min	-100000	Opt	0.0	Max	1000000			
Primer GC%	Min	40.0	Opt	50.0	Max	60.0			

Product Size Ranges: 200_600

Number To Return: 5 | Max 3' Stability: 9.0

Max Library Mispriming: 12.00 | Pair Max Library Mispriming: 20.00

Thermodynamic Secondary Structure Alignments		Old Secondary Structure Alignments	
☑ Use Thermodynamic Oligo Alignment			
TH: Max Self Complementarity	45.0	Max Self Complementarity	8.00
TH: Max 3' Self Complementarity	35.0	Max 3' Self Complementarity	3.00
TH: Max Pair Complementarity	45.0	Max Pair Complementarity	8.00
TH: Max 3' Pair Complementarity	35.0	Max 3' Pair Complementarity	3.00
TH: Max Primer Hairpin	24.0		

Thermodynamic Template Alignments	Old Template Alignments
☐ Use Thermodynamic Template Alignment	

Primer3 Output

PRIMER PICKING RESULTS FOR

Template masking not selected
Using mispriming library humrep_and_simple.txt
Using 1-based sequence positions

OLIGO	start	len	tm	gc%	any th	3' th	hairpin	rep	seq
LEFT PRIMER	113	21	57.88	47.62	18.55	9.36	0.00	11.00	tgtgacaaagagctctcaagg
RIGHT PRIMER	567	23	57.05	43.48	0.00	0.00	0.00	11.75	gaacctcttattactcacgaagc

F.P

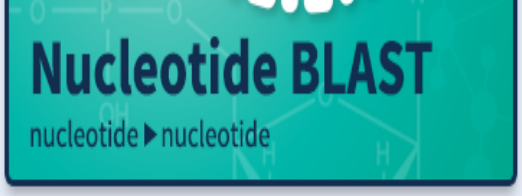
R.P

SEQUENCE SIZE: 729
INCLUDED REGION SIZE: 729

PRODUCT SIZE: 455, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.11
TARGETS (start len

```
    1 Atactcttgaaatggaaaagattactatttgtatttcaaagtctttatgtttatcaccag


   61 catagtatattagttctacagtctgtctttaaagcctgaattaataagtttgtgtgacaa
                                                       >>>>>>>>

  121 agagctctcaaggaaagacctgaaagtgaggaagagaatgagaatccaaatagtatttttt
      >>>>>>>>>>>>>

  181 ttgtctttgtgatacttattcaggtttctttctctttaacagctgataagatgcgattac


  241 ttttgatcctgggtttcttttcaacttgtaatagtgttgtattcttgtctttagGCAAGC
          ************************************************************

  301 CAAAATTCCTTCCGGATAGAATATGATACCTTTGGTGAACTAAAGGTGCCAAATGATAAG
      ************************************************************

  361 TATTATGGCGCCCAGACCGTGAGATCTACGATGAACTTTAAGATTGGAGGTGTGACAGAA
      ************************************************************

  421 CGCATGCCAgtaagtggcatttgtggaaatgttggctattttggatgaagtaggctgtat
      ************************************************************
```

```
  541 gatggcttcgtgagtaataagaggttctctttcactaaaatgttaaagaacttttgaag
         <<<<<<<<<<<<<<<<<<<<<<

  601 ttttagcatgctattattttgagccatcgctttgaatattttggatttttaaaatttaa


  661 ggccgggcacggtggcttatgcctgtaatcccagaactttgggaggccaaggtgggcaga


  721 tcacgaggt


KEYS (in order of precedence):
****** target
>>>>>> left primer
<<<<<< right primer

ADDITIONAL OLIGOS
                  start  len      tm     gc%  any th  3' th hairpin   rep seq

 1 LEFT PRIMER      114   23   58.87   43.48   18.55   0.00    0.00 12.00 gtgacaaagagctctcaaggaaa
   RIGHT PRIMER     671   19   59.11   52.63    7.03   0.00    0.00 10.00 cgtgcccggccttaaattt
   PRODUCT SIZE: 558, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

 2 LEFT PRIMER       78   22   57.31   40.91   10.51   6.05    0.00 11.00 acagtctgtctttaaagcctga
   RIGHT PRIMER     670   21   57.40   42.86    7.03   1.85    0.00 11.00 gtgcccggccttaaattttaa
   PRODUCT SIZE: 593, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

Statistics
        con   too    in    in   not         no    tm    tm   high  high  high  high        high
        sid  many   tar  excl    ok   bad    GC   too   too any_th 3'_th hair-   lib  poly   end
       ered    Ns   get   reg   reg   GC% clamp   low  high  compl compl   pin   sim     X  stab    ok
Left    1347     0     0     0     0  1075     0   202     0      0     0    49     0     0     0    21
Right   1383     0     0     0     0  1085     0   153    49      0     0    42     5     0     0    49
Pair Stats:
considered 26349, unacceptable product size 26139, tm diff too large 202, high mispriming library similarity 5, primer in pair overlaps a primer in a better pair 3246,
libprimer3 release 2.4.0



(primer3_results.cgi release 4.1.0)
```



BLAST Genomes

Enter organism common name, scientific name, or tax id

Search

Human Mouse Rat Microbes

Standalone and API BLAST

Download BLAST
Get BLAST databases and executables

Use BLAST API
Call BLAST from your application

Use BLAST in the cloud
Start an instance at a cloud provider

Specialized searches

SmartBLAST

Find proteins highly similar to your query

Primer-BLAST

Design primers specific to your PCR template

Global Align

Compare two sequences across their entire span

CD-search

Find conserved domains in your sequence

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

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

PCR product size: Min 70 Max 1000

# of primers to return: 10

Primer melting temperatures (Tm): Min 57.0 Opt 60.0 Max 63.0 Max Tm difference 3

Exon/intron selection

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

Exon junction span: No preference

Exon junction match: Exon at 5' side 7 Exon at 3' side 4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: ☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range: Min 1000 Max 1000000

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: Genomes for selected organisms (primary reference assembly only)

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

Organism: Homo sapiens

Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type.

NCBI/ Primer-BLAST : results: Job id=wsgdaw4LA6MknQaYC_giqnHjM5hc8CiFXQ more...

Input PCR template none
Specificity of primers Target templates were found in selected database: Genome database (reference assembly only) for selected species (Organism limited to Homo sapiens)
Other reports Search Summary

Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGTGACAAAGAGCTCTCAAGG	21	57.88	47.62	8.00	0.00
Reverse primer	GAACCTCTTATTACTCACGAAGC	23	57.05	43.48	3.00	2.00

Products on target templates

>NC_000001.11 Homo sapiens chromosome 1, GRCh38.p12 Primary Assembly

product length = 455
Features associated with this product:
fumarate hydratase, mitochondrial

fumarate hydratase, mitochondrial isoform X1

```
Forward primer  1          TGTGACAAAGAGCTCTCAAGG  21
Template        241517498  .....................  241517478

Reverse primer  1          GAACCTCTTATTACTCACGAAGC  23
Template        241517044  .......................  241517066
```

THANKS