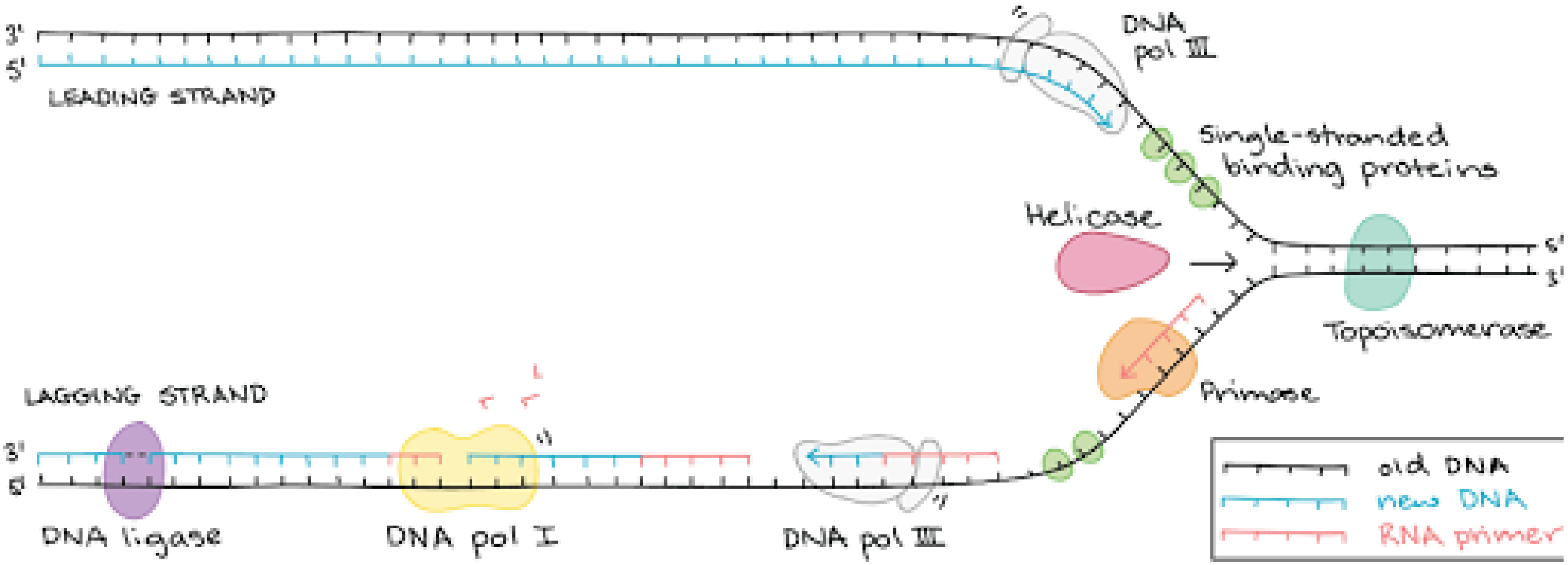
DELTA
BIOTECHNOLOGY

Introduction to PCR

Majid Lotfinia
Pharmaceutical Biotechnology, PhD

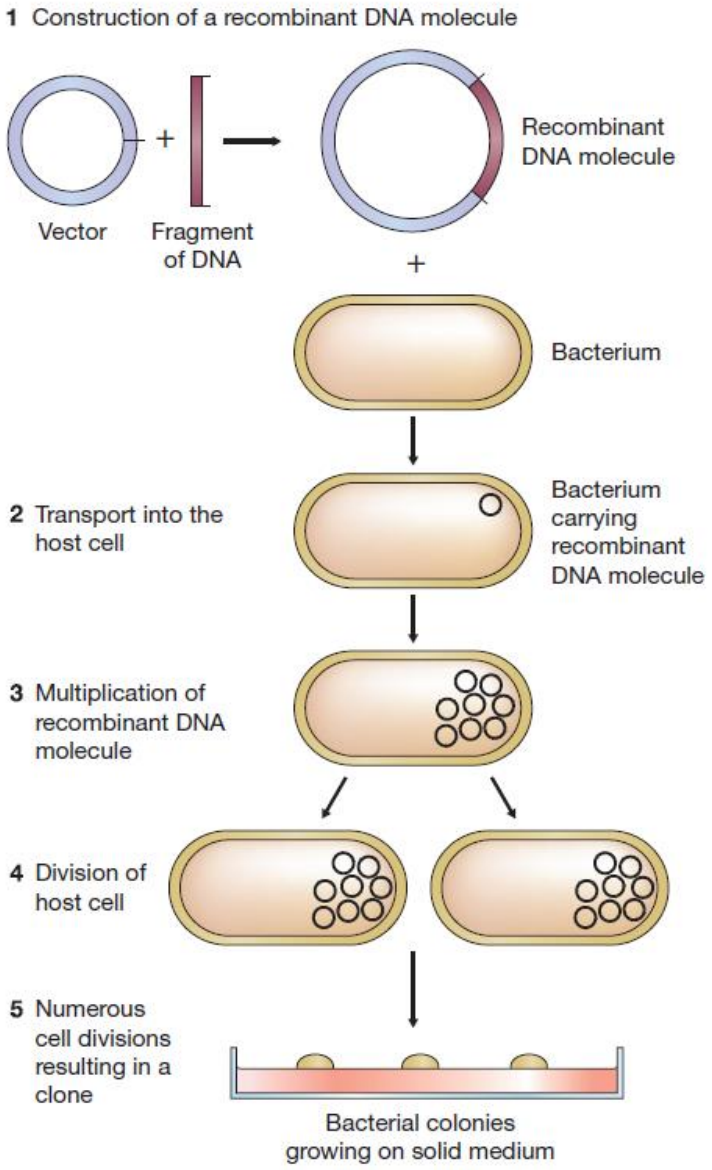
DNA Replication in Cell (In vivo)



https://www.khanacademy.org/science/biology/dna-as-the-genetic-material/dna-replication/a/molecular-mechanism-of-dna-replication

DNA Amplification

Gene cloning

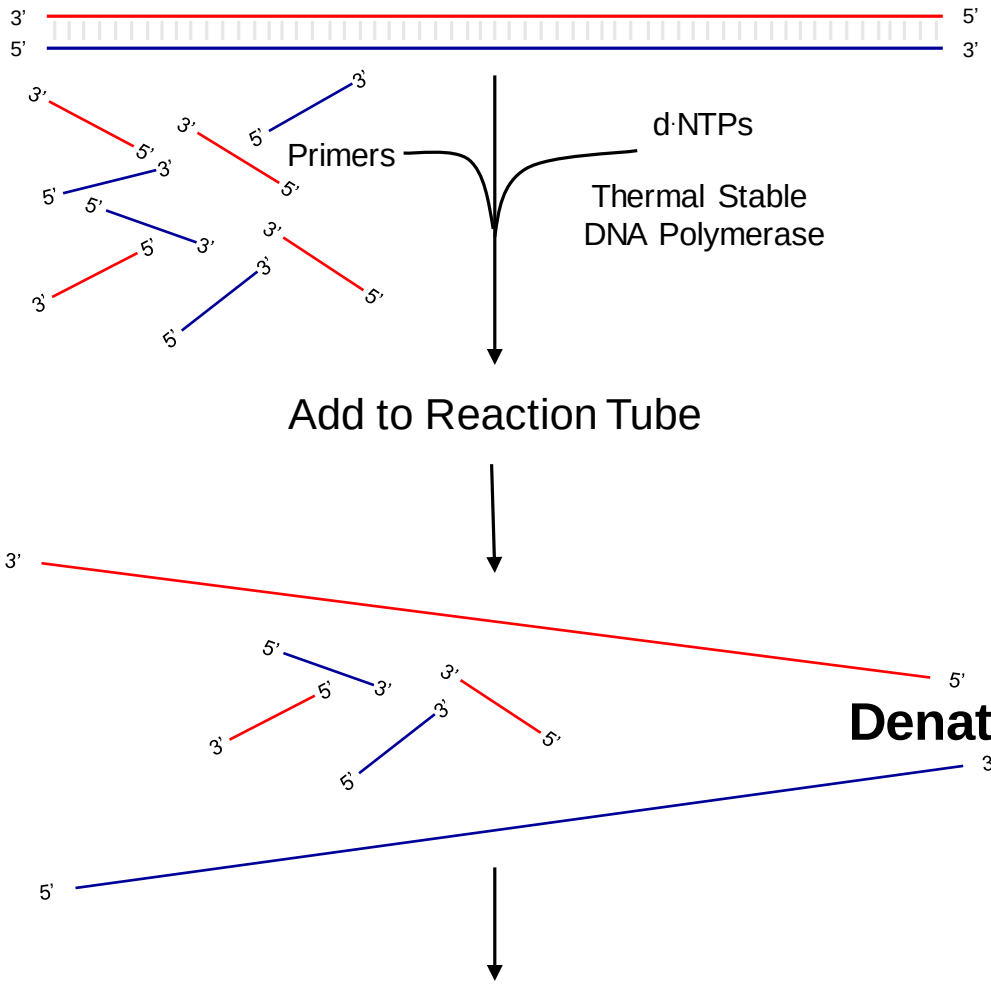


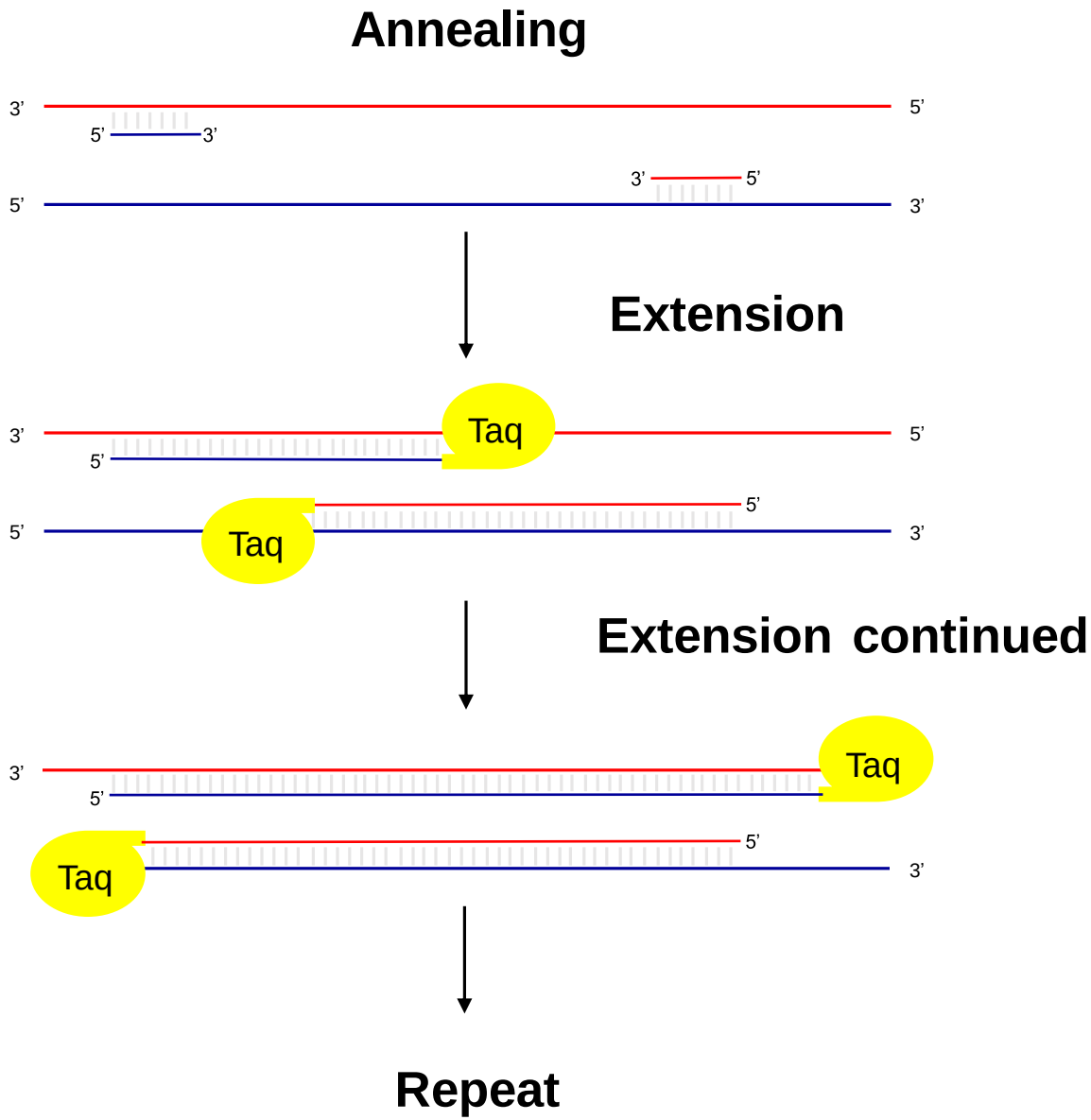
In vitro DNA replication (PCR)

- ✓ **PCR** is a method widely used in molecular biology to make many copies of a specific DNA segment.

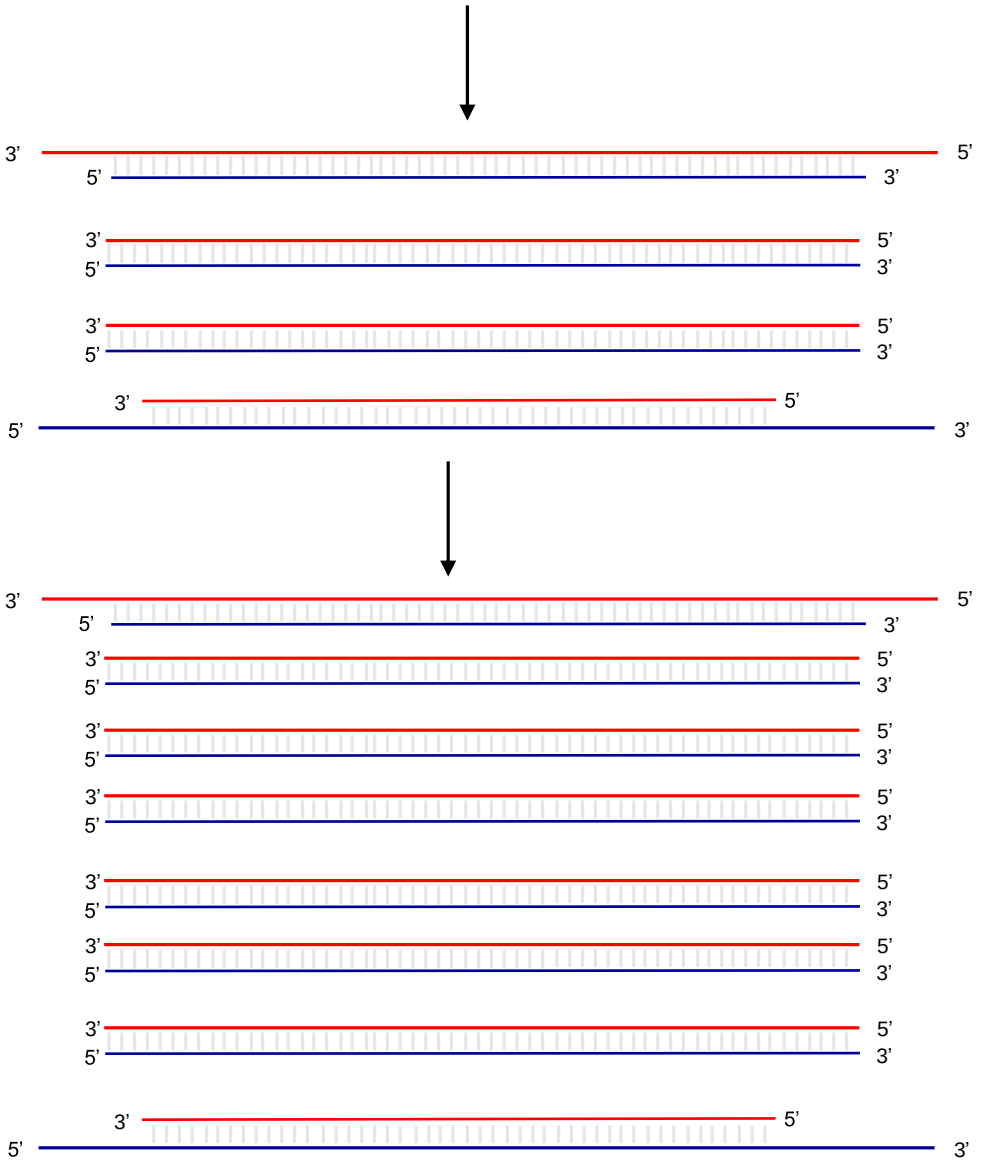
Dr. Kary Banks Mullis

Conventional PCR





Conventional PCR



Cycle 2

4 Copies

Cycle 3

8 Copies

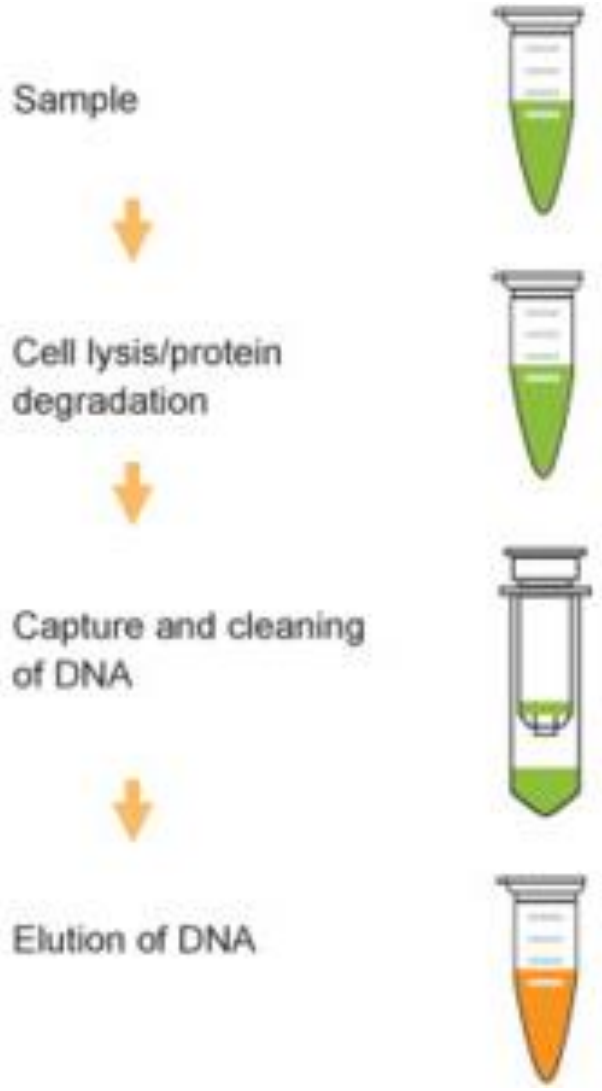
PCR requirements

1) Template (DNA or cDNA)
2) Sequence specific oligonucleotides (primers /probe)
3) Heat stable DNA polymerase
4) Thermal cycling

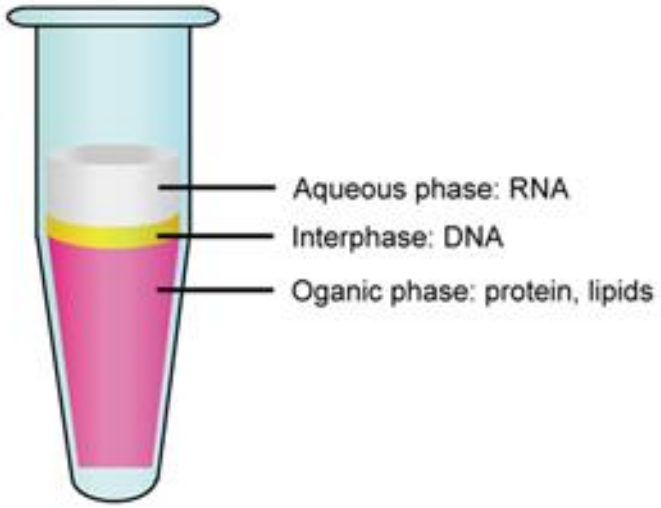
Template (genomic DNA)

DNA Extraction

Kit (silica-based)



Organic (TRIzol)

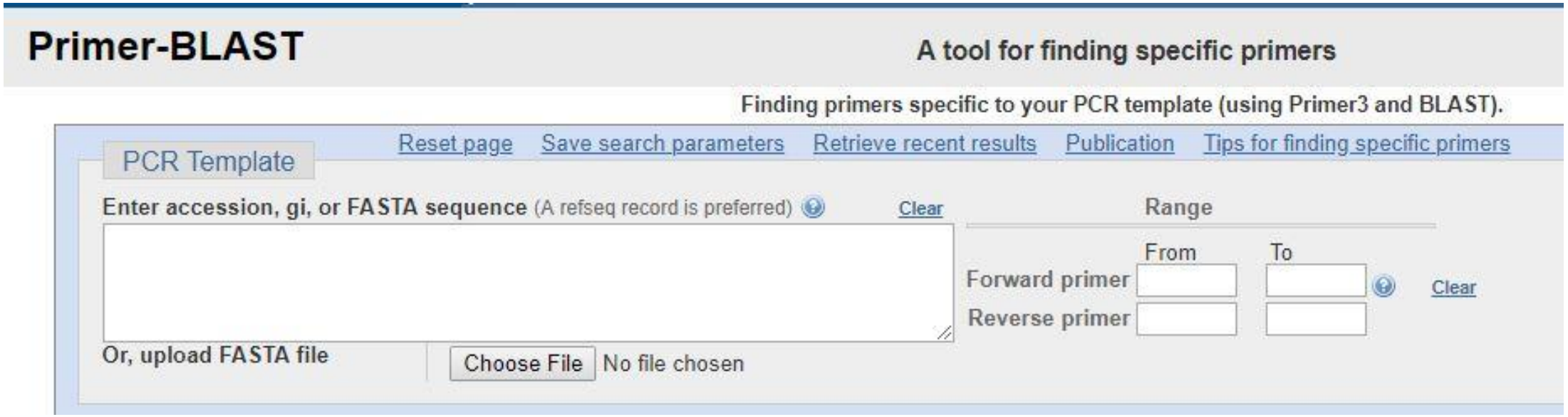


Primer selection or design

❖ Literatures & databases

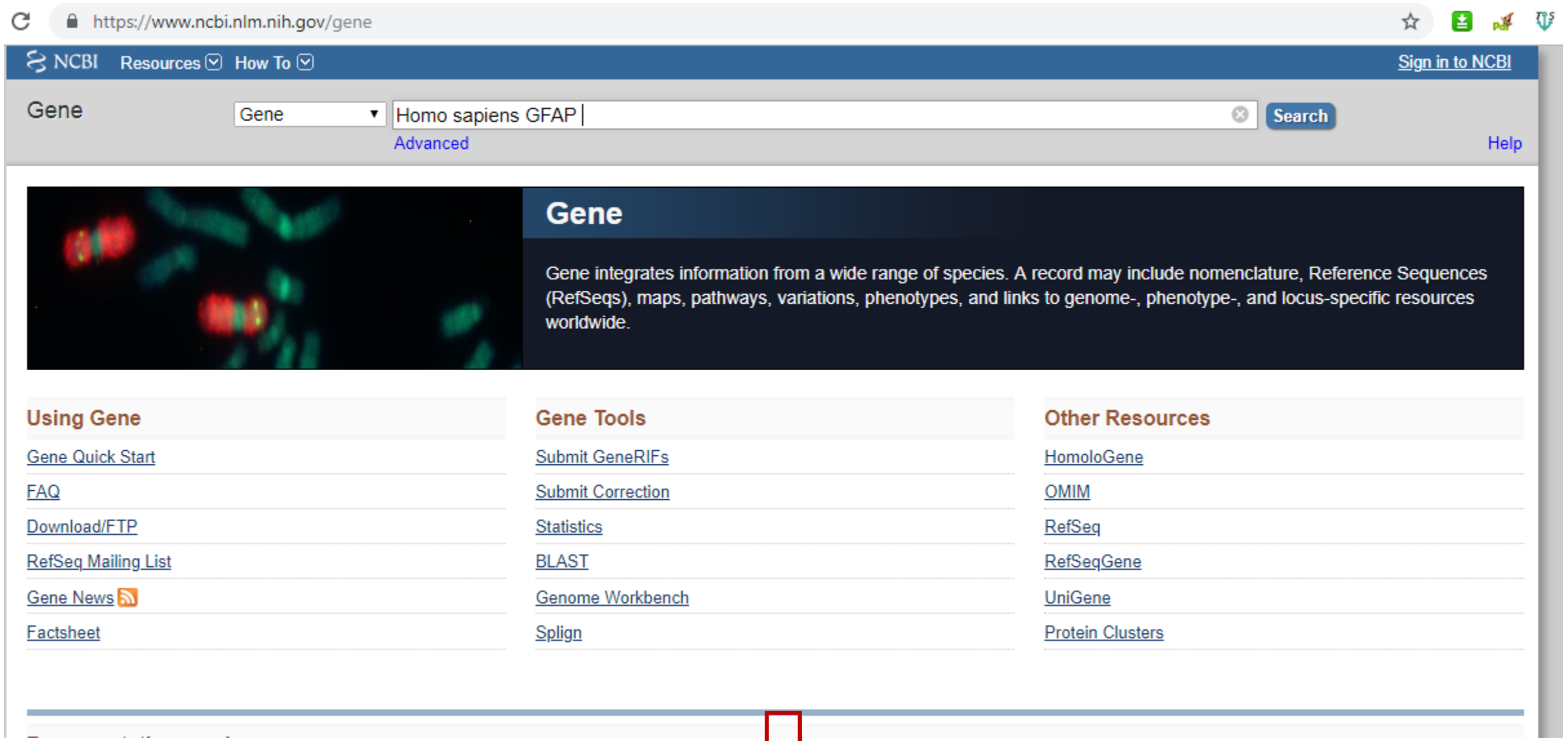
- PrimerBank (http://pga.mgh.harvard.edu/primerbank/index.html)
- GetPrime (https://gecftools.epfl.ch/getprime)

❖ Primer design



https://www.ncbi.nlm.nih.gov/tools/primer-blast/

Primer selection



Gene sources
Genomic

Categories
Alternatively spliced
Annotated genes
Protein-coding
Pseudogene

Sequence content
CCDS
Ensembl
RefSeq
RefSeqGene

Status clear
✓ Current

Clear all

Show additional filters

Tabular 20 per page Sort by Relevance Send to:

GENE

Was this helpful?

GFAP – glial fibrillary acidic protein

Homo sapiens (human)

Also known as: ALXDRD

GeneID: 2670

RefSeq transcripts (8) RefSeq proteins (8) RefSeqGene (1) PubMed (256)

Genome Browser BLAST Download

RefSeq transcripts +

RefSeq proteins +

Search results

Items: 1 to 20 of 162

See also 3 discontinued or replaced items.

<< First < Prev Page 1 of 9 Next > Last >>

Name/Gene ID	Description	Location	Aliases	MIM
GFAP ID: 2670	glial fibrillary acidic protein [*Homo sapiens* (human)]	Chromosome 17, NC_000017.11 (44903159..44915572, complement)	ALXDRD	137780

Hide sidebar >>

Filters: Manage Filters

Results by taxon

Top Organisms [Tree]
Homo sapiens *(161)*
Human immunodeficiency virus 1 *(1)*

Find related data

Database: Select

Find items

Search details

(("Homo sapiens"[Organism] OR Homo sapiens[All Fields]) AND GFAP[All Fields]) AND alive[prop]

Search See more...

Recent activity

Turn Off Clear

Homo sapiens GFAP AND (alive[prop]) (162) Gene

Homo sapiens GAPDH OR ACTB AND (alive[prop]) (1222) Gene

Homo sapiens GAPDH OR act AND (alive[prop]) (99436) Gene

MASSACHUSETTS GENERAL HOSPITAL | The Center for Computational and Integrative Biology | HARVARD MEDICAL SCHOOL

PrimerBank

PCR Primers for Gene Expression Detection and Quantification

Primer Search

Search for PCR Primers

Search by NCBI Gene ID

Species All Species

For text 2670 Submit

You can blast your sequence against the primerbank sequence DB **here.**

Order Oligos

You can have primers synthesized and PCR reaction products sequenced at:

DNA Core Facility
Center for Computational and Integrative Biology

PrimerBank is a public resource for PCR primers. These primers are designed for gene expression detection or quantification (real-time PCR). PrimerBank contains over 306,800 primers covering most known human and mouse genes. There are several ways to search for primers: GenBank Accession, NCBI protein accession, NCBI Gene ID, Gene Symbol[New!], PrimerBank ID or Keyword (gene description) or you can blast your gene sequence against the primerbank Sequence DB[New!].

The primer design algorithm has been extensively tested by real-time PCR experiments for PCR specificity and efficiency. We have tested 26,855 primer pairs that correspond to 27,681 mouse genes by Real Time PCR followed by agarose gel electrophoresis and sequencing of the PCR products. The design success rate is 82.6% (22,187 successful primer pairs) based on agarose gel electrophoresis.

All experimental validation data for mouse primers are available from PrimerBank. In order to view, please follow the appropriate links seen on the primer information page.

PrimerBank

The following matches are found for NCBI Gene ID (All Species): "2670"

Gene Descriptions:

NCBI GeneID	2670
GenBank Accession	NM_001242376
NCBI Protein Accession	NP_001229305
Species	Human
Coding DNA Length	1317
Gene Description	Homo sapiens glial fibrillary acidic protein (GFAP), transcript variant 3, mRNA.

Primer Pair 1 (Click here for cDNA and amplicon sequence):

PrimerBank ID 334688843c1

Amplicon Size 209

	Sequence (5' –> 3')	Length	Tm	Location
Forward Primer	CTGCGGCTCGATCAACTCA	19	62.1	373-391
Reverse Primer	TCCAGCGACTCAATCTTCCTC	21	61.3	581-561

Primer Pair 2 (Click here for cDNA and amplicon sequence):

PrimerBank ID 334688843c2

Amplicon Size 82

	Sequence (5' –> 3')	Length	Tm	Location
Forward Primer	AGGTCCATGTGGAGCTTGAC	20	61.5	656-675
Reverse Primer	GCCATTGCCTCATACTGCGT	20	62.9	737-718

Confirmation by in silico PCR

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UCSC In-Silico PCR

Genome: Human | Assembly: Dec. 2013 (GRCh38/hg38) | Target: genome assembly | Forward Primer: CTGCGGCTCGATCAACTCA | Reverse Primer: TCCAGCGACTCAATCTTCC | submit

Max Product Size: 4000 | Min Perfect Match: 15 | Min Good Match: 15 | Flip Reverse Primer:

About In-Silico PCR

In-Silico PCR searches a sequence database with a pair of PCR primers, using an indexing strategy for fast performance. See an example video on our YouTube channel.

Configuration Options

Genome and Assembly - The sequence database to search.
Target - If available, choose to query transcribed sequences.
Forward Primer - Must be at least 15 bases in length.
Reverse Primer - On the opposite strand from the forward primer. Minimum length of 15 bases.
Max Product Size - Maximum size of amplified region.
Min Perfect Match - Number of bases that match exactly on 3' end of primers. Minimum match size is 15.
Min Good Match - Number of bases on 3' end of primers where at least 2 out of 3 bases match.
Flip Reverse Primer - Invert the sequence order of the reverse primer and complement it.

Output

When successful, the search returns a sequence output file in fasta format containing all sequence in the database that lie between and include the primer pair. The fasta header describes the region in the database and the primers. The fasta body is capitalized in areas where the primer sequence matches the database sequence and in lower-case elsewhere. Here is an example from human:

```
>chr22:31000551+31001000  TAACAGATTGATGATGCATGAAATGGG CCCATGAGTGGCTCCTAAAGCAGCTGC
TtACAGATTGATGATGCATGAAATGGGgggtggccaggggtgggggtga
gactgcagagaaaggcagggctggttcataacaagctttgtgcgtcccaa
tatgacagctgaagttttccaggggctgatggtgagccagtgagggtaag
tacacagaacatcctagagaaaccctcattccttaaagattaaaaataaa
gacttgctgtctgtaagggattggattatcctatttgagaaattctgtta
tccagaatggcttaccccacaatgctgaaaagtgtgtaccgtaatctcaa
agcaagctcctcctcagacagagaaacaccagccgtcacaggaagcaaag
aaattggcttcacttttaaggtgaatccagaacccagatgtcagagctcc
aagcactttgctctcagctccacGCAGCTGCTTTAGGAGCCACTCATGaG
```

The + between the coordinates in the fasta header indicates this is on the positive strand.

Author

In-Silico PCR was written by Jim Kent. Interactive use on this web server is free to all. Sources and executables to run batch jobs on your own server are available free for academic, personal, and non-profit purposes. Non-exclusive commercial licenses are also available. Contact Jim for details.

https://genome.ucsc.edu/cgi-bin/hgPcr?hgsid=732790353_MxkMKQqxWwVs9SsBSkVUZamZ9IVi&org=Human&db=hg38

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UCSC In-Silico PCR

>chr17:44913765-44915114 1350bp CTGCGGCTCGATCAACTCA TCCAGCGACTCAATCTTCCTC
CTGCGGCTCGATCAACTCAccgccaacagcgcccggctggaggttgagag
ggacaatctggcacaggacctggccactgtgaggcagaagtgaggagggg
atggggaaggggggccttgtgagcagaaggggctgaatccccaagaagga
gtgcccgagaagtctcagggaggggccgaacctccctgctccctgggcct
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accagaaacaggcccagggccccggggatctgatgcccgcatgccttctg
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ggcagcgagctcacctattctgagcctgtgtccatataaaggaggagttg
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acagcccccaagctcagcccgccagagcaatgtgctgctccccactgccc
cagaaggaggggtaaggcaaggctgaggaatggggagaaggagcctgggg
ccccagtggccctctactcctcacccctcctgccactccaggaagcagat
gaagccaccctggcccgtctggatctggaGAGGAAGATTGAGTCGCTGGA

Primer Melting Temperatures

Forward: 63.7 C ctgcggctcgatcaactca
Reverse: 61.8 C tccagcgactcaatcttcctc
The temperature calculations are done assuming 50 mM salt and 50 nM annealing oligo concentration. The code to calculate the melting temp comes from Primer3.

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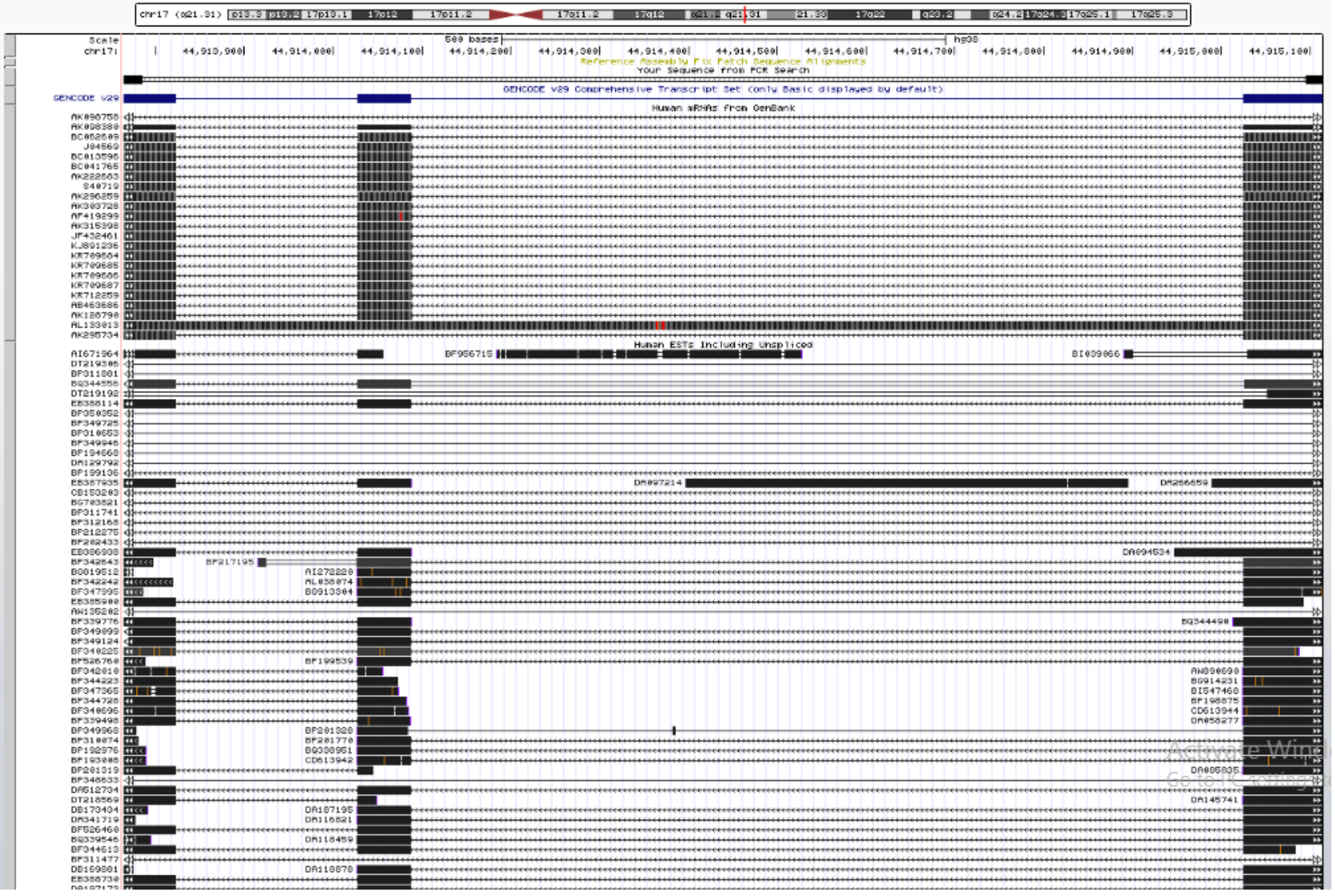
UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x

chr17:44,913,765-44,915,114 1,350 bp. enter position, gene symbol, HGVS or search terms go



Last update finished on 2015 November 12 using Ensembl release 81. More species are available (13, see below).

Comma or space-separated list of identifiers:

Ensembl release: 81

Organism: Homo sapiens

Limit: 100

Search

562645 primer pairs found. Only 100 first displayed. Download the full dataset.

Search all columns:

ID	Gene	Transcripts	Rank	# Transcripts	Amplicon length	Forward primer	Tm fwd	Reverse primer	Tm rev	Ensemb status
1772453	ENSG00000277212 AC116311.1	ENST00000610309	★★★ (1)	1/1	66	5:101288737-101288755 GTGCAAATGTAATTGCGGT 1 SNPs	58	5:101288785-101288803 CTGGTGCAAACGTAATTGC 3 SNPs	58	Novel
1772454	ENSG00000277212 AC116311.1	ENST00000610309	★★☆ (2)	1/1	67	5:101288736-101288754 AGTGCAAATGTAATTGCGG 1 SNPs	57	5:101288785-101288803 CTGGTGCAAACGTAATTGC 3 SNPs	58	Novel
1772455	ENSG00000277212 AC116311.1	ENST00000610309	★☆☆ (3)	1/1	67	5:101288736-101288755 AGTGCAAATGTAATTGCGGT 1 SNPs	59	5:101288785-101288803 CTGGTGCAAACGTAATTGC 3 SNPs	58	Novel
1772456	ENSG00000212517 SNORA26	ENST00000391215	★★★ (1)	1/1	60	20:5121490-5121508 GAGGCTGATACAGAAGGGT 0 SNPs	59	20:5121448-5121467 GTTTCACAGTCTCTTCCCTG 0 SNPs	58	Novel
1772457	ENSG00000212517 SNORA26	ENST00000391215	★★☆ (2)	1/1	60	20:5121474-5121495 AAGGGTAAAGTTAAGTCTCCAC 0 SNPs	58	20:5121435-5121458 GGTTCTAAGATGAGTTTCACAGTC 0 SNPs	60	Novel
						20:5121491-5121509		20:5121449-5121468		

Primer design roles

Below is a list of characteristics that should be considered when designing primers:

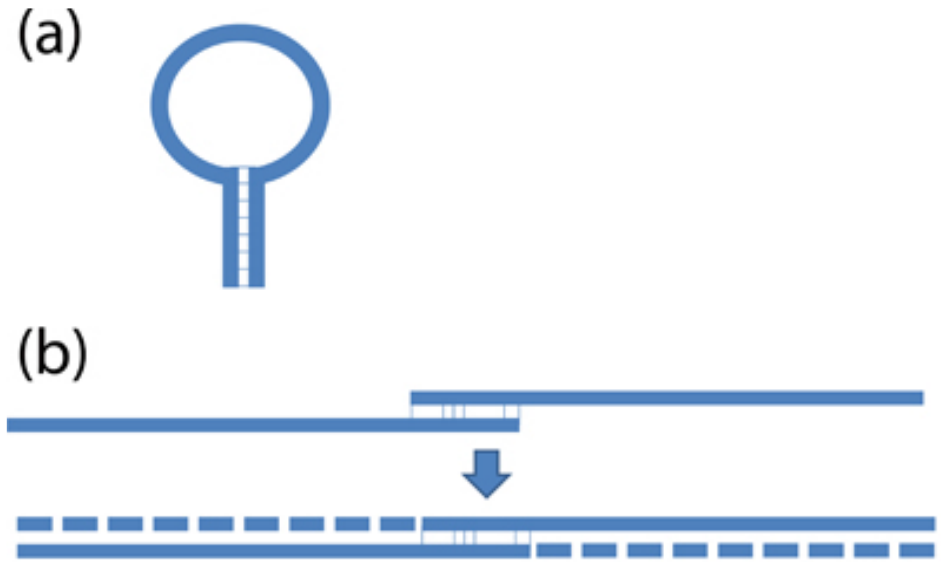
1. Primer length should be 15-30 nucleotide residues (bases).
2. Optimal G-C content should range between 40-60%.
3. The 3' end of primers should contain a G or C in order to clamp the primer and prevent "breathing" of ends, increasing priming efficiency. DNA "breathing" occurs when ends do not stay annealed but fray or split apart. The three hydrogen bonds in GC pairs help prevent breathing but also increase the melting temperature of the primers.
4. The 3' ends of a primer set, which includes a plus strand primer and a minus strand primer, should not be complementary to each other, nor can the 3' end of a single primer be complementary to other sequences in the primer. These two scenarios result in formation of primer dimers and hairpin loop structures, respectively.
5. Optimal melting temperatures (Tm) for primers range between 52-58 °C, although the range can be expanded to 45-65 °C. The final Tm for both primers should differ by no more than 5 °C.
6. Di-nucleotide repeats (e.g., GCGCGCGCGC or ATATATATAT) or single base runs (e.g., AAAAA or CCCCC) should be avoided as they can cause slipping along the primed segment of DNA and or hairpin loop structures to form. If unavoidable due to nature of the DNA template, then only include repeats or single base runs with a maximum of 4 bases.

Lorenz, T.C. Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. J. Vis. Exp. (63), e3998, doi:10.3791/3998 (2012).

Primer design limitations

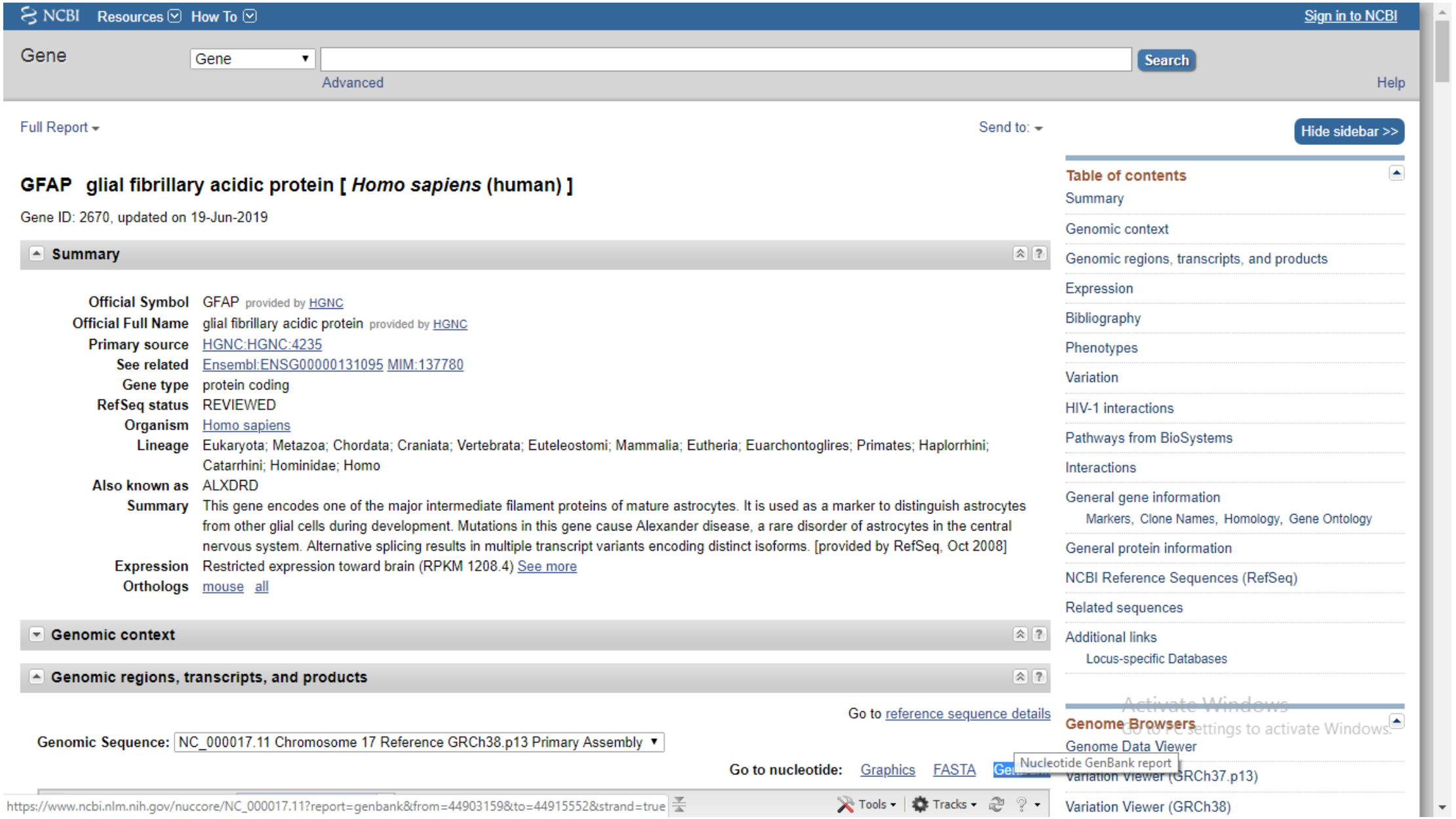
There are a few common problems that arise when designing primers:

1) self-annealing of primers resulting in formation of secondary structures such as hairpin loops
2) primer annealing to each other, rather than the DNA template, creating primer dimers
3) drastically different melting temperatures (Tm) for each primer, making it difficult to select an annealing temperature that will allow both primers to efficiently bind to their target sequence during thermal cycling.



Lorenz, T.C. Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. J. Vis. Exp. (63), e3998, doi:10.3791/3998 (2012).

Primer design



The Nucleotide database will include EST and GSS sequences in early 2019. Read more.

GenBank Send to:

Homo sapiens chromosome 17, GRCh38.p13 Primary Assembly

NCBI Reference Sequence: NC_000017.11

FASTA Graphics

```
LOCUS       NC_000017              12394 bp    DNA     linear   CON 14-JUN-2019
DEFINITION  Homo sapiens chromosome 17, GRCh38.p13 Primary Assembly.
ACCESSION   NC_000017 REGION: complement(44903159..44915552)
VERSION     NC_000017.11
DBLINK      BioProject: PRJNA168
            Assembly: GCF_000001405.39
KEYWORDS    RefSeq.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 12394)
  AUTHORS   Zody,M.C., Garber,M., Adams,D.J., Sharpe,T., Harrow,J.,
            Lupski,J.R., Nicholson,C., Searle,S.M., Wilming,L., Young,S.K.,
            Abouelleil,A., Allen,N.R., Bi,W., Bloom,T., Borowsky,M.L.,
            Bugalter,B.E., Butler,J., Chang,J.L., Chen,C.K., Cook,A., Corum,B.,
            Cuomo,C.A., de Jong,P.J., DeCaprio,D., Dewar,K., FitzGerald,M.,
            Gilbert,J., Gibson,R., Gnerre,S., Goldstein,S., Grafham,D.V.,
            Grocock,R., Hafez,N., Hagopian,D.S., Hart,E., Norman,C.H.,
            Humphray,S., Jaffe,D.B., Jones,M., Kamal,M., Khodiyar,V.K.,
            LaButti,K., Laird,G., Lehoczky,J., Liu,X., Lokyitsang,T.,
            Loveland,J., Lui,A., Macdonald,P., Major,J.E., Matthews,
            Mauceli,E., McCarroll,S.A., Mihalev,A.H., Mudge,J., Nguy
            Nicol,R., O'Leary,S.B., Osoegawa,K., Schwartz,D.C., Shaw
            Stankiewicz,P., Steward,C., Swarbreck,D., Venkataraman,V
            Whittaker,C.A., Yang,X., Zimmer,A.R., Bradley,A., Hubbard,T.,
            Birren,B.W., Rogers,J., Lander,E.S. and Nusbaum,C.
  TITLE     DNA sequence of human chromosome 17 and analysis of rearrangement
```

Design and test primers for this sequence using Primer-BLAST.

Change region shown

- Whole sequence (abbreviated view)
- Selected region

from: 44903159 to: 44915552

Update View

Customize view

- Abbreviated view
- Customize

Basic Features

- All features
- Gene, RNA, and CDS features only

Display options

- Show sequence
- Show reverse complement

Update View

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?ORGANISM=9606&INPUT_SEQUENCE=NC_000017.11&LINK_LOC=nuccore&PRIMER5_START=44903159&PRIMER3_END=44915552

Primer-BLAST

A tool for finding specific primers

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

NC_000017.11

Or, upload FASTA file: Choose File | No file chosen

Range

	From	To
Forward primer	44903159	
Reverse primer		44915552

Clear

Primer Parameters

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

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

	Min	Max
PCR product size	70	1000

# of primers to return: 10

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

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

Organism: 9606

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

Add more organisms

Entrez query (optional):

Primer specificity stringency:

Primer must have at least 2 total mismatches to unintended targets, including

at least 2 mismatches within the last 5 bps at the 3' end.

Ignore targets that have 6 or more mismatches to the primer.

Max target size: 4000

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

Get Primers

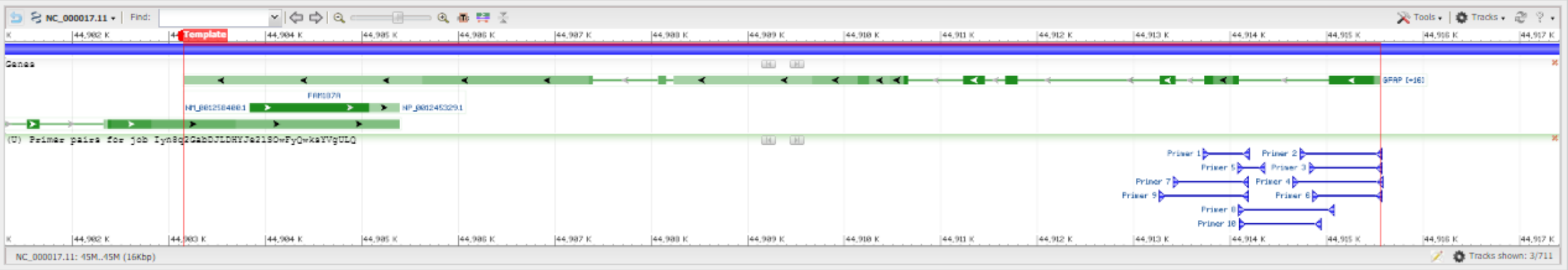
Show results in a new window Use new graphic view

Primer-BLAST» JOB ID:Iyn8q2GabDJLDHYJe2ISOwFyQwksYVgULQ

Primer-BLAST Results

Input PCR template NC_000017.11 Homo sapiens chromosome 17, GRCh38.p13 Primary Assembly
Range 44903159 - 44915552
Specificity of primers Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)
Other reports ▷Search Summary

Graphical view of primer pairs



Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCTCCTCCAGCGACTCAATC	Plus	20	44913760	44913779	59.90	60.00	3.00	3.00
Reverse primer	AGGGAGACTGAGGCAGGTATT	Minus	21	44914159	44914139	59.99	52.38	3.00	1.00
Product length	400								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTGCACTGCTTTCCCCAGTA	Plus	20	44914764	44914783	59.96	55.00	5.00	2.00
Reverse primer	CTCCTTCATAAAGCCCTCGCA	Minus	21	44915535	44915515	60.13	52.38	6.00	0.00
Product length	772								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TCTGGTCCCTCCCATCATGT	Plus	20	44914859	44914878	59.96	55.00	5.00	3.00
Reverse primer	TCCTTCATAAAGCCCTCGCAT	Minus	21	44915534	44915514	59.51	47.62	6.00	2.00
Product length	676								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGAATAGGTGAGCTCGCTGC	Plus	20	44914694	44914713	59.90	55.00	6.00	3.00
Reverse primer	CCCACTCCTTCATAAAGCCCT	Minus	21	44915539	44915519	59.43	52.38	6.00	2.00
Product length	846								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity

Heat stable DNA polymerase

Table 1.2 Commonly used DNA polymerases for PCR

	Taq DNA polymerase	*Tth* DNA polymerase	Stoffel fragment	KlenTaq fragment	*Pfu* DNA polymerase
Organism	*Thermus aquaticus* YT1	*Thermus thermophilus* HB8	*Thermus aquaticus* YT1	*Thermus aquaticus* YT1	*Pyrococcus furiosus*
Molecular weight	94 kDa	94 kDa	61 kDa	63 kDa	90 kDa
Number of amino acids	832	832	544	555	775
Single chain or subunits	Single	Single	Single	Single	Single
Extension rate	2 kb–4 kb/min	2 kb–4 kb/min	2 kb–4 kb/min	2 kb–4 kb/min	1 kb–2 kb/min
Reverse transcriptase activity	Minimal/low	Yes, Mn2+ dependent	Minimal/low	Minimal/low	No
Half life @ 95°C	40 mins	20 mins	80 mins	80 mins	>4 hrs
Processivity	50–60 bases	30–40 bases	5–10 bases	5–10 bases	15–25 bases
5′–3′ exonuclease activity	Yes	Yes	No	No	No
3′–5′ exonuclease activity	No	No	No	No	Yes
Incorporates dUTP	Yes	Yes	Yes	Yes	No
Extra A addition	Yes	Yes	Yes	Yes	No

PCR Mix

- Tris-HCl pH 8.5, (NH4)2S04, 3 mM MgCl2, 0.2% Tween 20
- 0.4 mM of each dNTP
- 0.2 units/µl Ampliqon Taq DNA polymerase

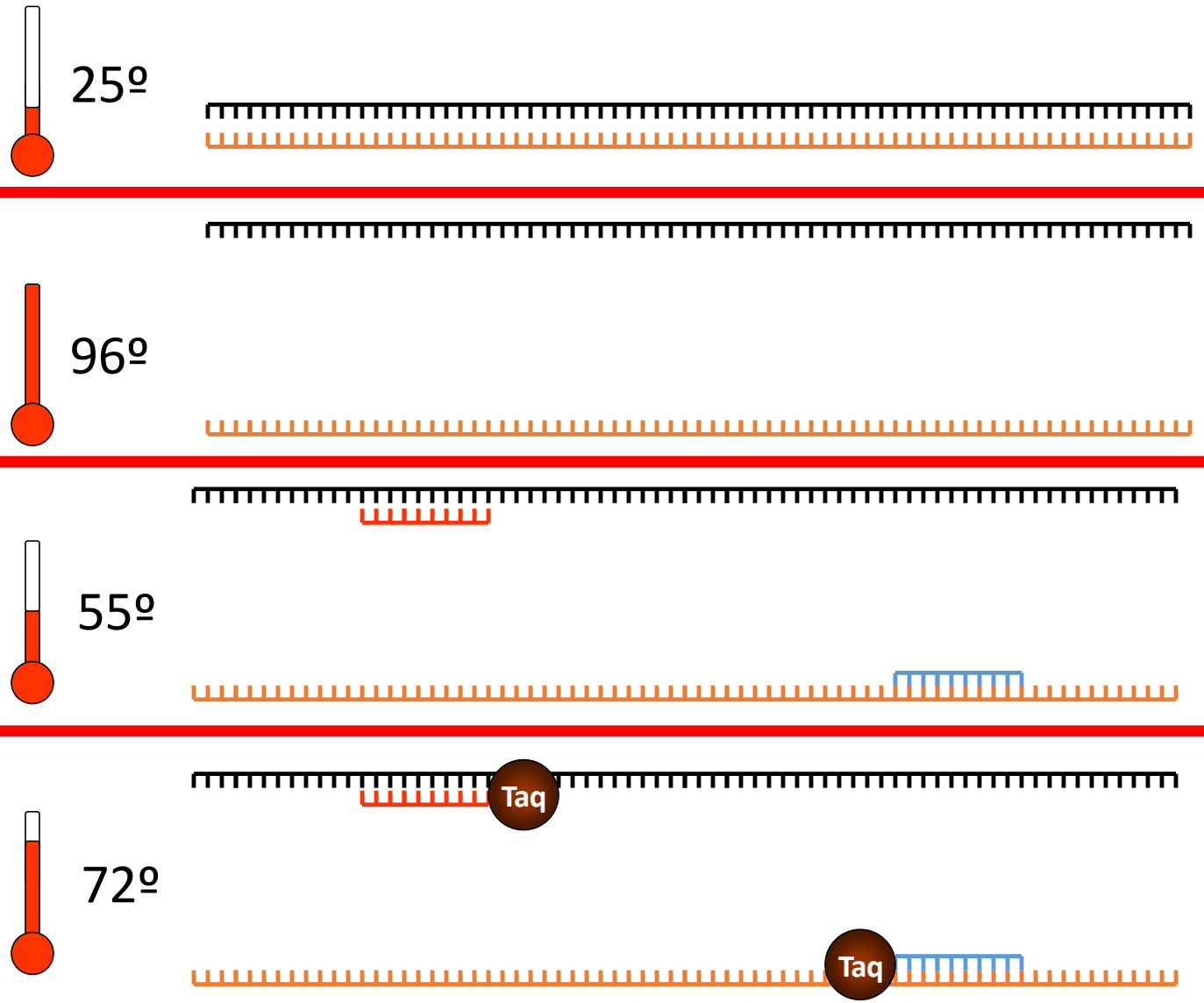
Table 2. Reaction components (reaction mix and template DNA)

Component	Vol./reaction*	Final concentration*
Taq 2x Master Mix	25 µl	1x
25 mM $MgCl_2$	X µl	1.5 mM (0.5 – 5 mM)
Primer A (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
Primer B (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
PCR-grade H_2O	X µl	-
Template DNA	X µl	genomic DNA: 50 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	50 µl	-

Table 3. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	2 – 5 minutes	95 °C
25 - 35	20 – 30 seconds[a] 20 – 40 seconds[b] 30 seconds[c]	95 °C 50 – 65 °C 72 °C
1	5 minutes[d]	72 °C

Thermal cycles



A. Double strand DNA

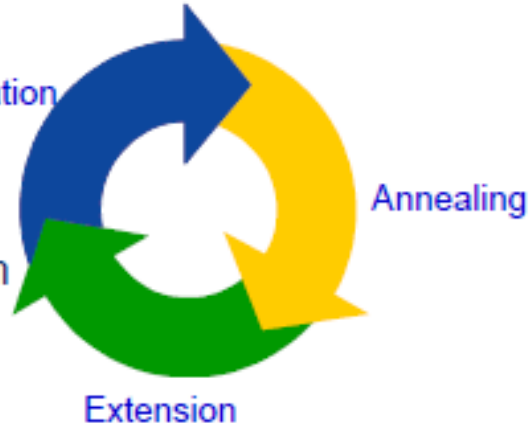
B. Denature

C. Anneal primers

D. Polymerase binds & extends

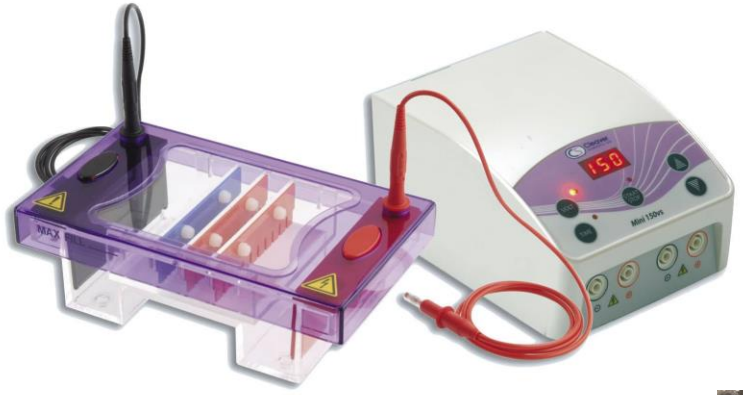
Thermocycler

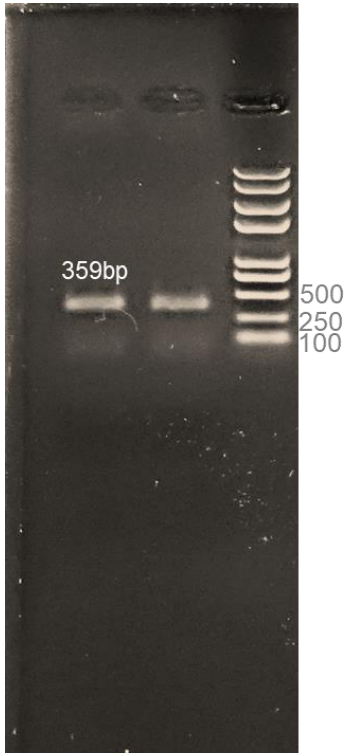
Cycling:
Exponential amplification
of PCR products



PCR product visualization

Agarose gel electrophoresis

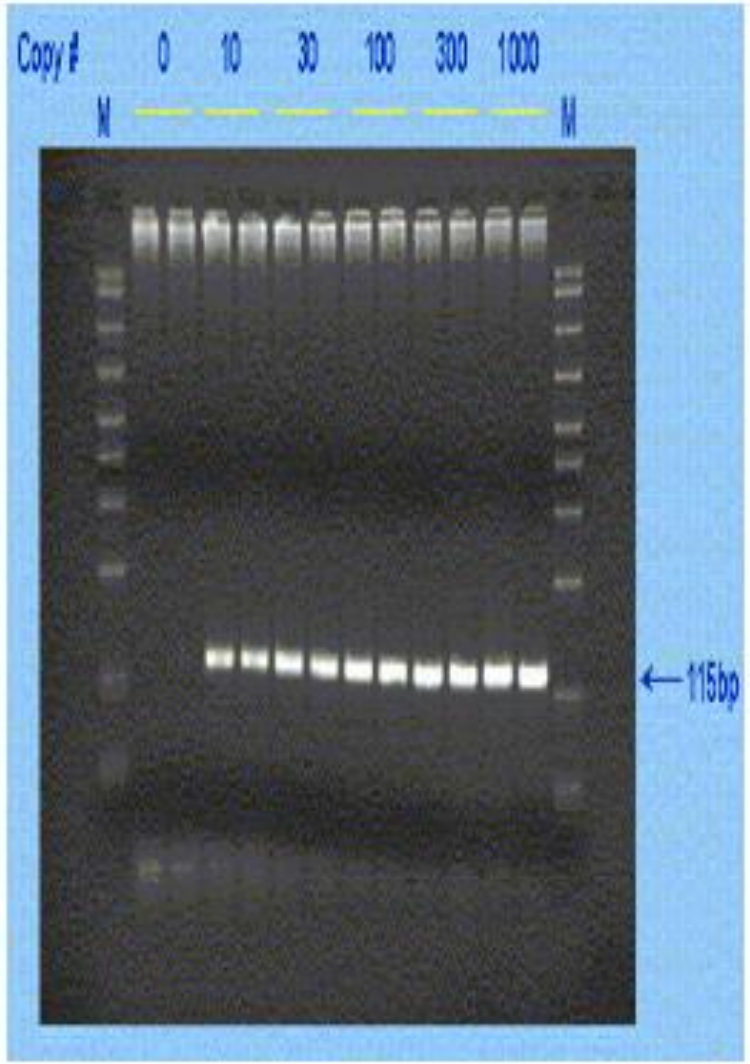




PCR limitations

Limitations of Traditional End-Point PCR

- Low sensitivity
- Poor precision
- Results are not expressed as numbers
- Ethidium bromide staining is not quantitative
- Post-PCR processing required



Reverse Transcription (cDNA synthesis)

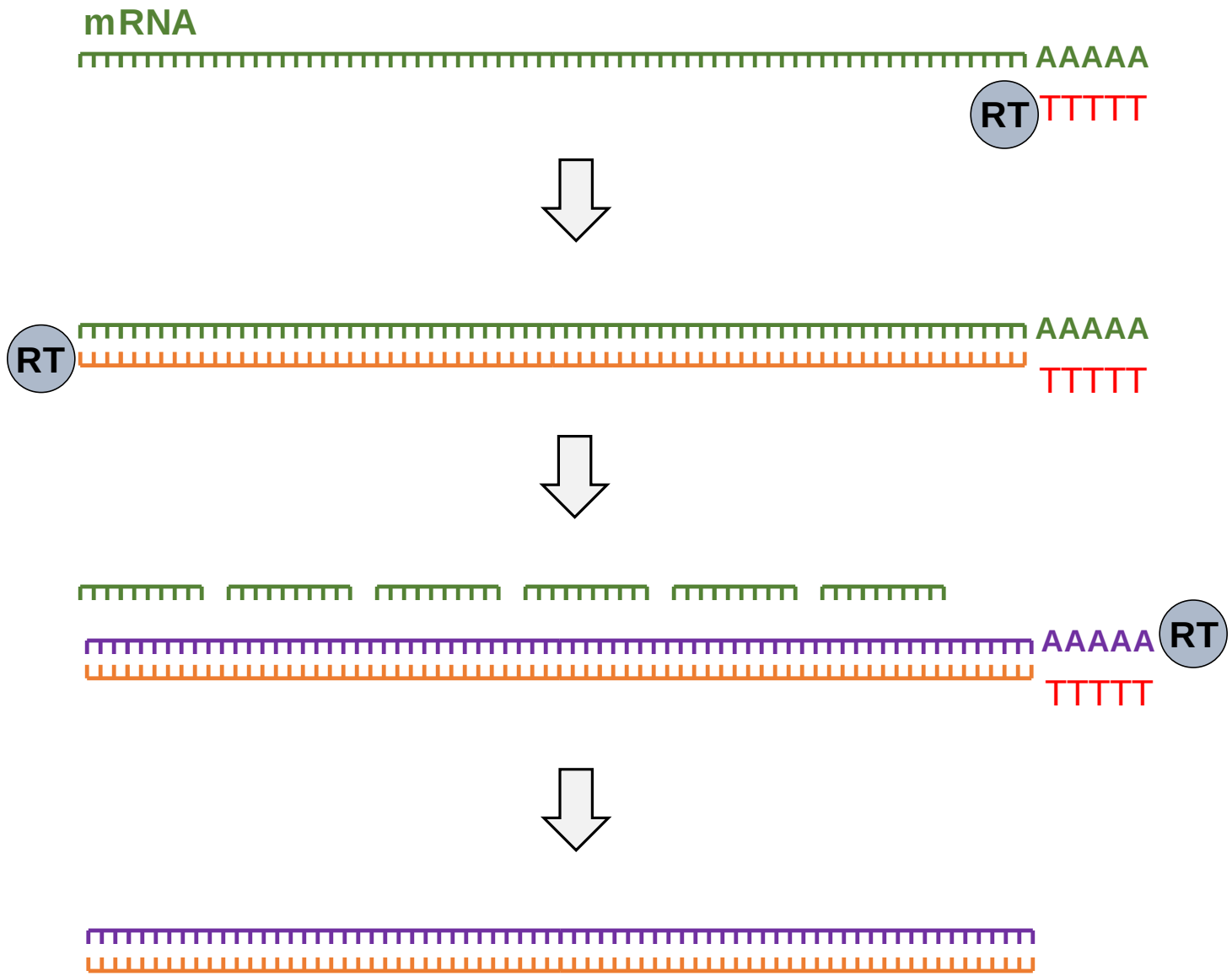
OligodT primer is bound to mRNA

Reverse transcriptase (RT) is bound to oligodT primer

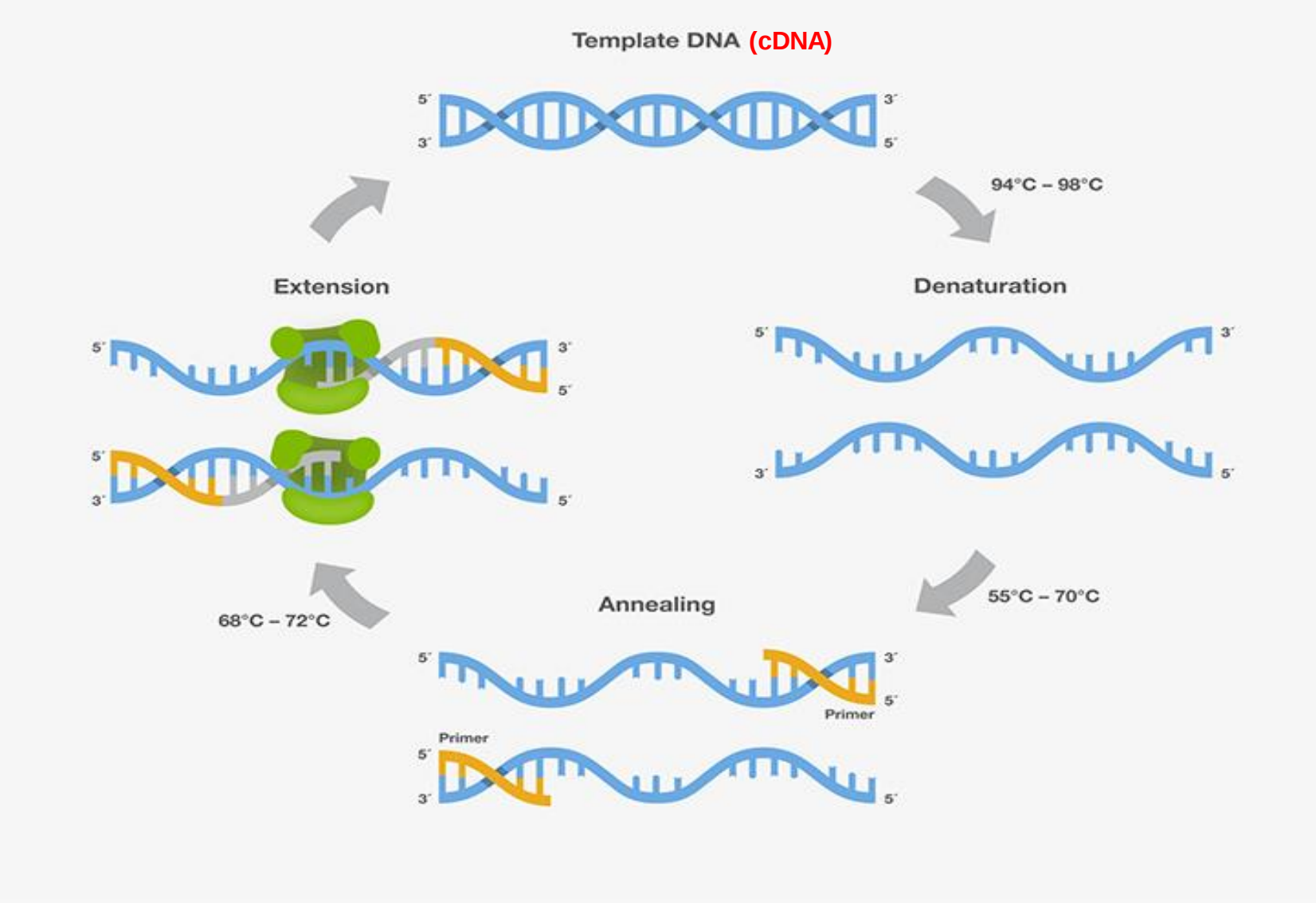
Reverse transcriptase copies first cDNA strand

Reverse transcriptase digests and displaces mRNA and copies second strand of cDNA

Double strand cDNA



RT-PCR


Template DNA (cDNA)
5′
3′
3′
5′
94°C – 98°C
Denaturation
5′
3′
3′
5′
55°C – 70°C
Annealing
5′
3′
Primer
Primer
5′
3′
5′
68°C – 72°C
Extension
5′
3′
5′
5′
3′
5′

Many Thanks