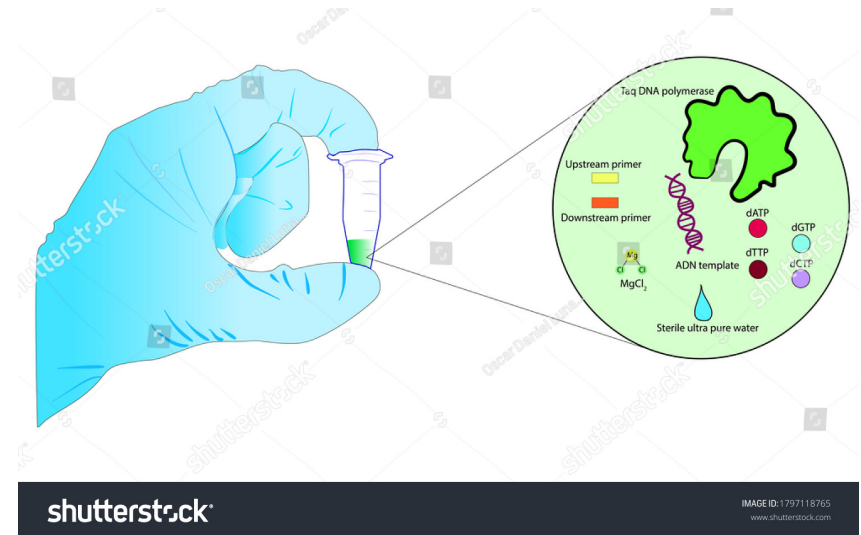


BIO 405L
Cellular and molecular biology laboratory
DNA amplification/Polymerase Chain Reaction



Hugo Castillo, Ph.D.



What is PCR?

What are we copying?



DNA



Thermal Cycler

DNA replication in lab

In the Cell

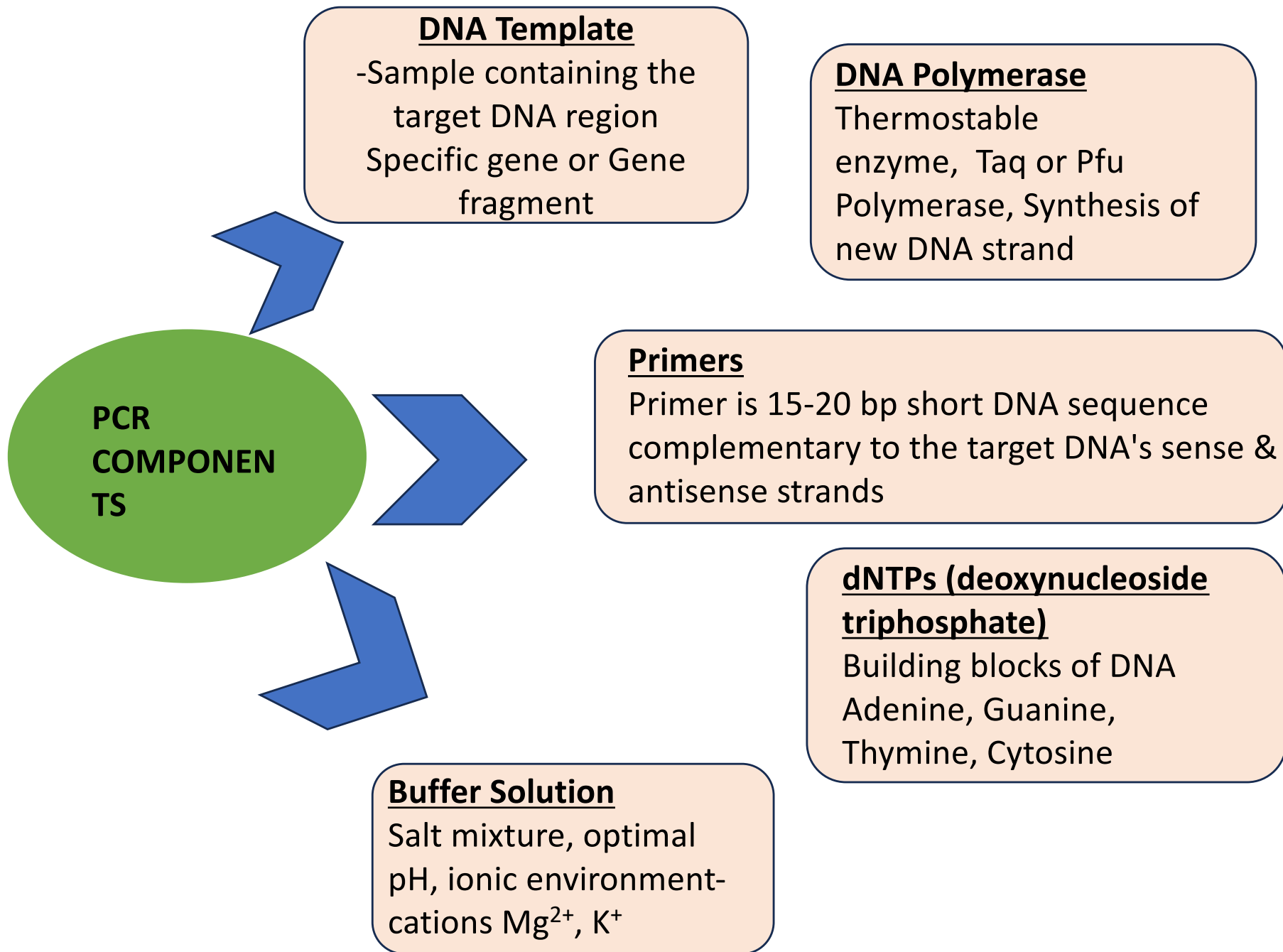


DNA polymerase
copies entire genome

In the Lab (PCR)

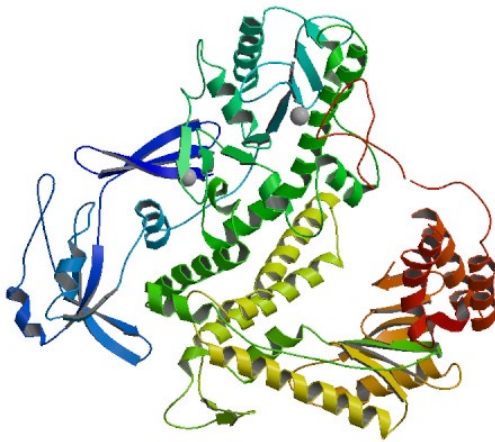


Primers target specific gene
Polymerase copies only that part





Taq polymerase



Pfu polymerase

Polymerases- Essential for synthesis

- **Taq polymerase**

Thermostable enzyme
isolated from bacterium
Thermus aquaticus.

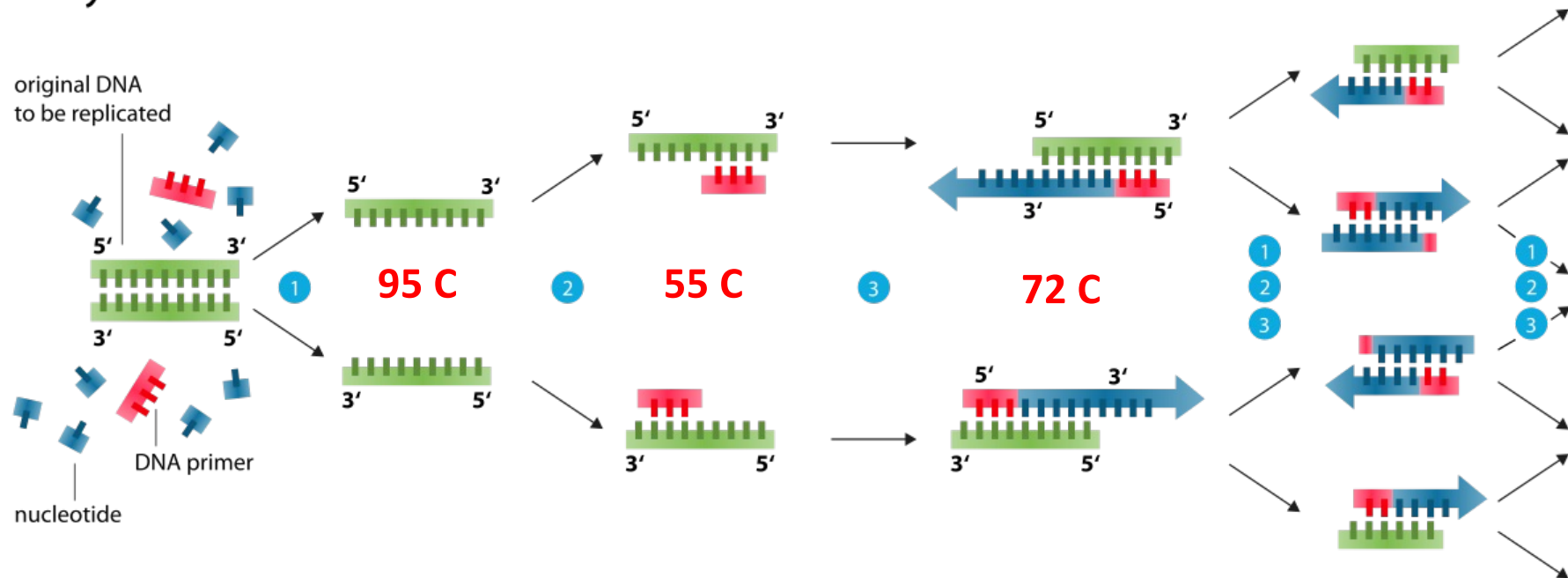
- **Pfu polymerase**

Thermostable enzyme
isolated from the
bacterium *Pyrococcus
furiosus*.

Why? They synthesize new strands. Most importantly, they remain active during repeated high temperature denaturation cycles.

Copying DNA

Polymerase chain reaction - PCR



PCR program

Initial denaturation	95 C	1 min	40 cycles
Denaturation	95 C	30 sec	
Annealing	55 C	30 sec	
Extension	72 C	30 sec	
Final extension	72 C	2 min	
Hold	8 C	infinite	

Amplicons

QUESTION

What happens if the annealing temperature is,

Too low:-

Too high:-

Quick Visualization



PCR Primer Design



Forward primer

5' **GC**TAAATGTT**CAGGCTGTGG** 3'

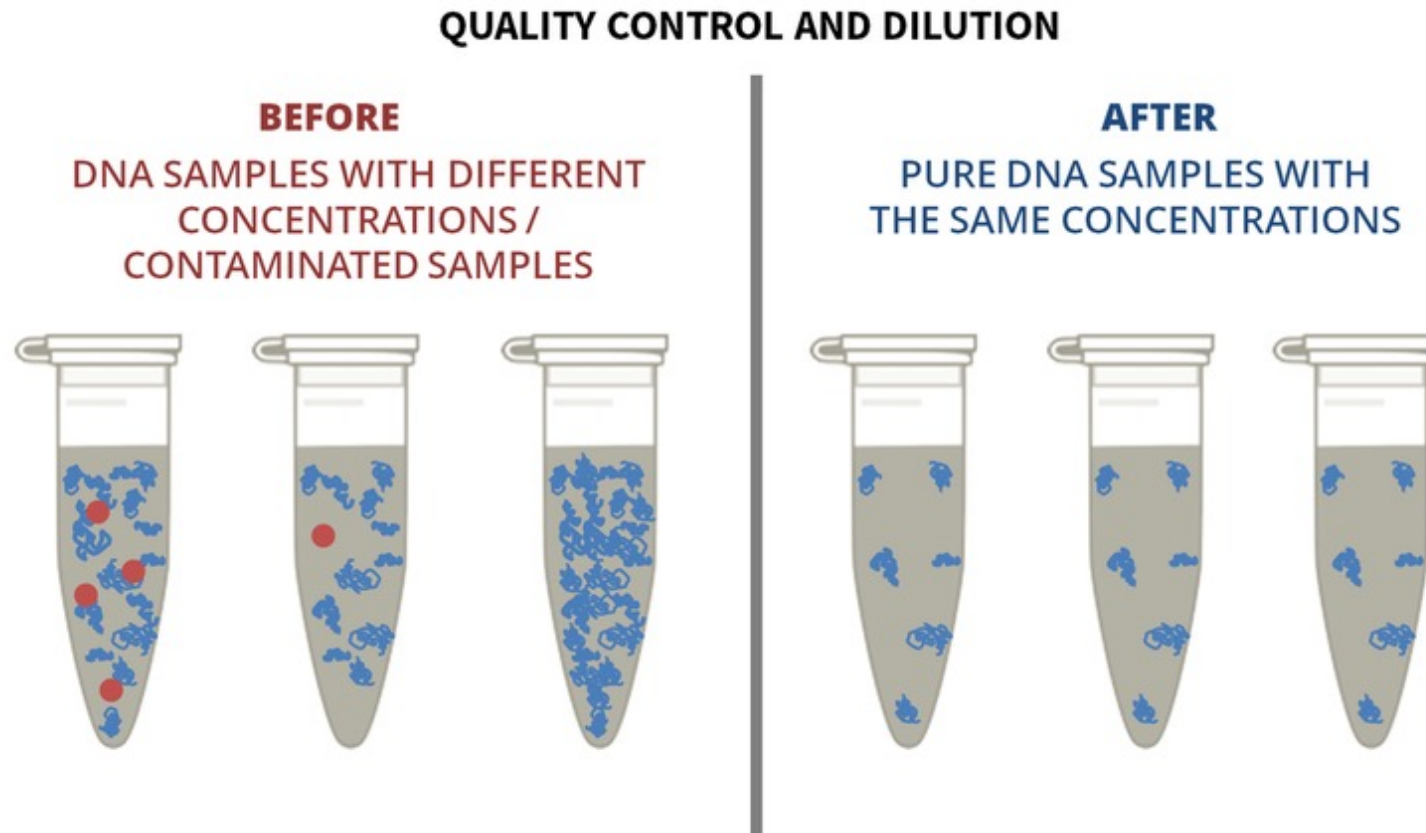
Reverse primer

5' **GG**AATCAA**ACGGAATGACCG** 3'

Top tips for primer design:

- ✓ Length: ~20 nucleotides
- ✓ GC content: ~50%
- ✓ GC clamp: primers end in at least two G or C nucleotides
- ✓ No complementary regions between primer pairs
- ✓ Melting temperature (T_m): ~55-65°C

Template DNA dilution



Prepare 100 uL of DNA at 5 ng/uL

PCR mastermix

Reagent	1X
	uL
10X PCR buffer	5
dNTPs	0.5
Primers (F and R)	2
Taq polymerase	0.5
PCR Water	15
DNA template (10 ng)	2
Final volume	25

Follow the instructor directions to set up the reaction

>*Escherichia coli* 16S rRNA

AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAG
AAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTG
GAAACGGTAGCTAATAACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCAG
ATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACAC
TGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAG
CCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTT
GCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAATACGGAGGGGTGCAAGCGTT
AATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTAAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAC
TGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTG
GAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATAACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAAC
GCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTAAAACCTCAAATGAATTGACGGGGGCCCCGCACAAGCGGTG
GAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCAGAGATGGATTGG
TGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTAAAGTCCCGCAAC
GAGCGCAACCCTTATCTTTTGTGTCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAG
GTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCG
ACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGA
ATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAG
TGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGT
AACAAGGTAACCGTAGGGGAACCTGCGGTTGGATCACCTCCTTA

***E. coli* primers**

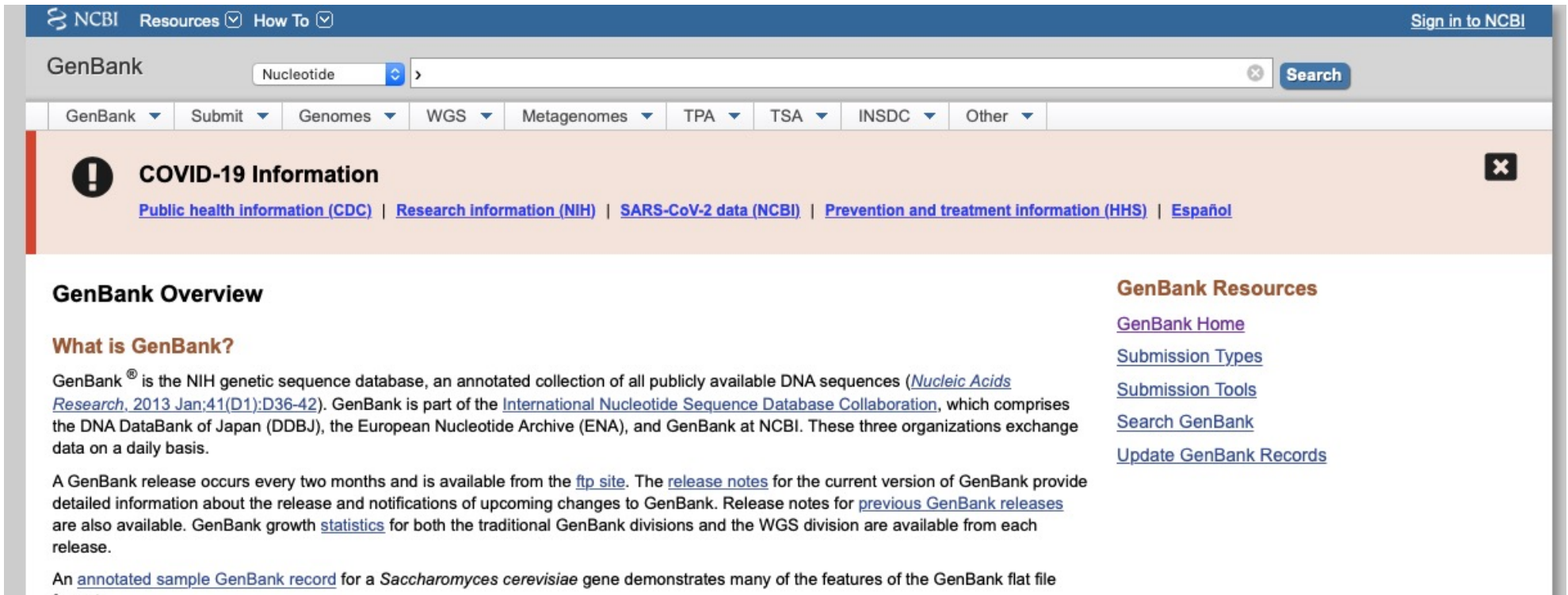
Forward (27F)

5'-AGAGTTTGATCATGGCTCAG -3'

Reverse (1392R)

5'-GGTTACCTTGTTACGACTT-3'

1. Obtain the gene sequence



The screenshot shows the NCBI GenBank homepage. At the top, there is a navigation bar with 'NCBI', 'Resources', and 'How To' links, along with a 'Sign in to NCBI' button. Below this is a search bar with a dropdown menu set to 'Nucleotide' and a 'Search' button. A horizontal menu contains links to 'GenBank', 'Submit', 'Genomes', 'WGS', 'Metagenomes', 'TPA', 'TSA', 'INSDC', and 'Other'. A prominent orange banner for 'COVID-19 Information' is displayed, featuring a warning icon and links to 'Public health information (CDC)', 'Research information (NIH)', 'SARS-CoV-2 data (NCBI)', 'Prevention and treatment information (HHS)', and 'Español'. The main content area is divided into two columns. The left column, titled 'GenBank Overview', includes a section 'What is GenBank?' which describes the database as an annotated collection of publicly available DNA sequences, part of the International Nucleotide Sequence Database Collaboration (INSDC). It mentions the DNA DataBank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at NCBI. It also notes that releases occur every two months and provides links to 'ftp site', 'release notes', and 'previous GenBank releases'. The right column, titled 'GenBank Resources', lists links for 'GenBank Home', 'Submission Types', 'Submission Tools', 'Search GenBank', and 'Update GenBank Records'.

<https://www.ncbi.nlm.nih.gov/genbank/>

NOTE: You will often get multiple hits when you look for a specific gene. In this case, make sure you select the one that gives you the complete sequence (e.g the 16S rRNA gene is ~1600).



KEGG

Escherichia coli

Search Help

» Japanese

KEGG Home

- Release notes
- Current statistics

KEGG Database

- KEGG overview
- Searching KEGG
- KEGG mapping
- Color codes

KEGG Objects

- Pathway maps
- Brite hierarchies
- KEGG DB links

KEGG Software

- KEGG API
- KGML

- KEGG FTP
- Subscription
- Background info

- GenomeNet
- DBGET/LinkDB
- Feedback
- Copyright request
- Kanehisa Labs

KEGG: Kyoto Encyclopedia of Genes and Genomes

KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies. See [Release notes](#) (August 1, 2021) for new and updated features.

New article [KEGG mapping tools for uncovering hidden features in biological data](#)

Main entry point to the KEGG web service

KEGG2 [KEGG Table of Contents](#) [[Update notes](#) | [Release history](#)]

Data-oriented entry points

KEGG PATHWAY	KEGG pathway maps	Pathway Brite Brite table Module Network KO (Function) Organism Virus Compound Disease (ICD) Drug (ATC) Drug (Target) Antimicrobials
KEGG BRITE	BRITE hierarchies and tables	
KEGG MODULE	KEGG modules	
KEGG ORTHOLOGY	KO functional orthologs [Annotation]	
KEGG GENES	Genes and proteins [SeqData]	
KEGG GENOME	Genomes [KEGG Virus Taxonomy]	
KEGG COMPOUND	Small molecules	
KEGG GLYCAN	Glycans	
KEGG REACTION	Biochemical reactions [RModule]	
KEGG ENZYME	Enzyme nomenclature	
KEGG NETWORK	Disease-related network variations	
KEGG DISEASE	Human diseases	
KEGG DRUG	Drugs [New drug approvals]	
KEGG MEDICUS	Health information resource [Drug labels search]	

Organism-specific entry points

KEGG Organisms Enter org code(s) [hsa](#) [hsa eco](#)

This is a good source to obtain the full length of a gene.

2. Paste the sequence on a primer design website and set the desired parameters.

News: Try out our new tool: [Wiley-DNA-Editor](#) - A DNA/Plasmid editor running in your browser!

Primer3Plus

pick primers from a DNA sequence

More...	Source Code
Help	About

Load server settings: Default *Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.*

Task: generic

Main	General Settings	Advanced Settings	Internal Oligo	Penalty Weights	Advanced Sequence
------	------------------	-------------------	----------------	-----------------	-------------------

Sequence Id:

[Paste template sequence below](#) Or upload sequence file: no file selected

Mark selected region:

[Excluded Regions:](#) < >

[Targets:](#) []

[Included Region:](#) { }

[Primer overlap positions:](#) -

[Pair OK Region List:](#)

☒ Pick left primer	☐ Pick hybridization probe	☒ Pick right primer
or use left primer below.	(internal oligo) or use oligo below.	or use right primer below (5'→3' on opposite strand).

<http://primer3plus.com/cgi-bin/dev/primer3plus.cgi>

Primer3Plus

pick primers from a DNA sequence

[More...](#)[Source Code](#)[Help](#)[About](#)[< Back](#)☒ Pair 1: Left Primer 1:

[Start:](#) 308 [Length:](#) 20 bp [Tm:](#) 60.0 C [GC:](#) 60.0 % [Any:](#) 14.7 [End:](#) 14.7 [TB:](#) 9.0 [HP:](#) 37.1 [3' Stab:](#) 3.9 [Penalty:](#) 0.038

Right Primer 1:

[Start:](#) 898 [Length:](#) 20 bp [Tm:](#) 60.0 C [GC:](#) 50.0 % [Any:](#) 28.7 [End:](#) 9.2 [TB:](#) 8.0 [HP:](#) 0.0 [3' Stab:](#) 2.7 [Penalty:](#) 0.035

Pair: [Product Size:](#) 591 bp [Any:](#) 0.8 [End:](#) 5.1 [TB:](#) 17.0 [Penalty:](#) 0.073

[Send to Primer3Manager](#)[Reset Form](#)

1	GTTTGATCAT	GGCTCAGATT	GAACGCTGGC	GGCAGGCCTA	ACACATGCAA
51	GTCGAACGGT	AACAGGAAGA	AGCTTGCTTC	TTTGCTGACG	AGTGGCGGAC
101	GGGTGAGTAA	TGTCTGGGAA	ACTGCCTGAT	GGAGGGGGAT	AACTACTGGA
151	AACGGTAGCT	AATACCGCAT	AACGTCGCAA	GACCAAAGAG	GGGGACCTTC
201	GGGCCTCTTG	CCATCGGATG	TGCCCAGATG	GGATTAGCTA	GTAGGTGGGG
251	TAACGGCTCA	CCTAGGCGAC	GATCCCTAGC	TGGTCTGAGA	GGATGACCAG
301	CCACACTGGA	ACTGAGACAC	GGTCCAGACT	CCTACGGGAG	GCAGCAGTGG
351	GGAATATTGC	ACAATGGGCG	CAAGCCTGAT	GCAGCCATGC	CGCGTGTATG
401	AAGAAGGCCT	TCGGGTGTGA	AAGTACTTTC	AGCGGGGAGG	AAGGGAGTAA
451	AGTTAATACC	TTTGCTCATT	GACGTTACCC	GCAGAAGAAG	CACCGGCTAA
501	CTCCGTGCCA	GCAGCCGCGG	TAATACGGAG	GGTGCAAGCG	TTAATCGGAA
551	TTACTGGGCG	TAAAGCGCAC	GCAGGCGGTT	TGTTAAGTCA	GATGTGAAAT
601	CCCCGGGCTC	AACCTGGGAA	CTGCATCTGA	TACTGGCAAG	CTTGAGTCTC
651	GTAGAGGGGG	GTAGAATTCC	AGGTGTAGCG	GTGAAATGCG	TAGAGATCTG
701	GAGGAATACC	GGTGGCGAAG	GCGGCCCCCT	GGACGAAGAC	TGACGCTCAG
751	GTGCGAAAGC	GTGGGGAGCA	AACAGGATTA	GATACCCTGG	TAGTCCACGC
801	CGTAAACGAT	GTCGACTTGG	AGGTTGTGCC	CTTGAGGCGT	GGCTTCCGGA
851	NNTAACGCGT	TAAGTCGACC	GCCTGGGGAG	TACGGCCGCA	AGGTTAAAAC
901	TCAAATGAAT	TGACGGGGGC	CGCACAAGCG	GTGGAGCATG	TGGTTTAATT
951	CGATGCAACG	CGAAGAACCT	TACCTGGTCT	TGACATCCAC	GGAAGTTTTC
1001	AGAGATGAGA	ATGTGCCTTC	GGGAACCGTG	AGACAGGTGC	TGCATGGCTG

Forward

Reverse

Amplicon
size