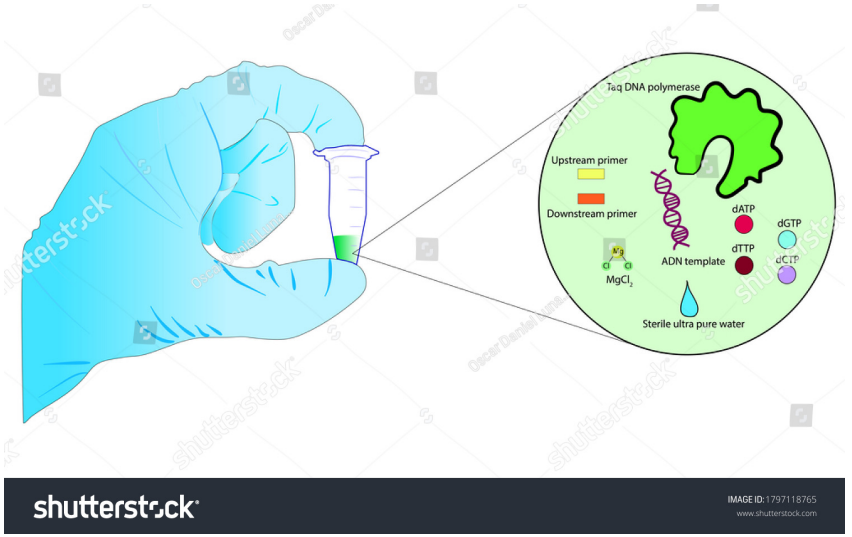
BIO 405L

Cellular and molecular biology laboratory

DNA amplification/Polymerase Chain Reaction

Hugo Castillo, Ph.D.


Taq DNA polymerase
Upstream primer
Downstream primer
dATP
dGTP
dTTP
dCTP
ADN template
$MgCl_2$
Sterile ultra pure water
shutterstock
IMAGE ID: 1797118765
www.shutterstock.com

What is PCR?

What are we copying?

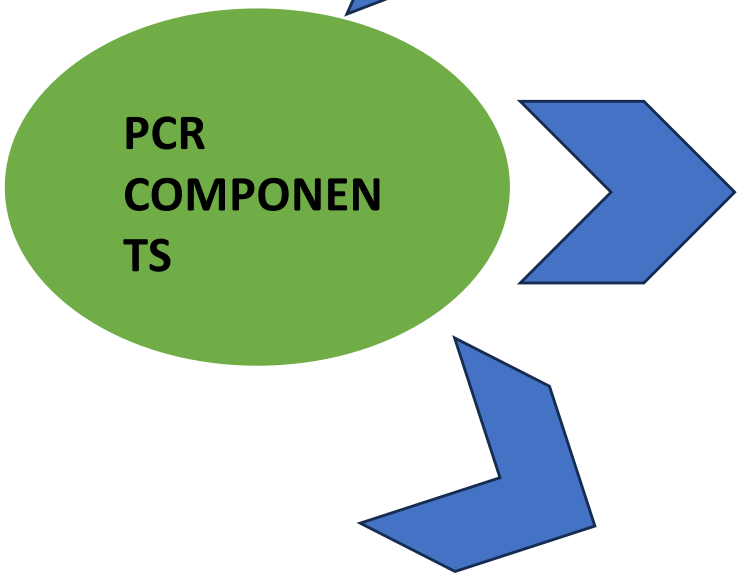
DNA

Thermal Cycler

DNA replication in lab

DNA polymerase
copies entire genome

Primers target specific gene
Polymerase copies only that part



DNA Template

-Sample containing the target DNA region Specific gene or Gene fragment

DNA Polymerase

Thermostable enzyme, Taq or Pfu Polymerase, Synthesis of new DNA strand

Primers

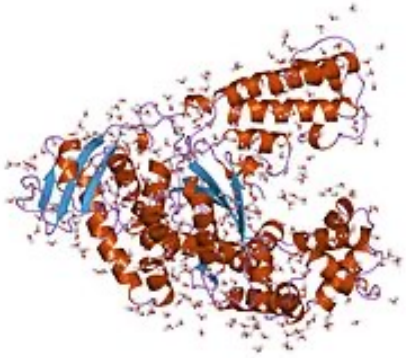
Primer is 15-20 bp short DNA sequence complementary to the target DNA's sense & antisense strands

dNTPs (deoxynucleoside triphosphate)

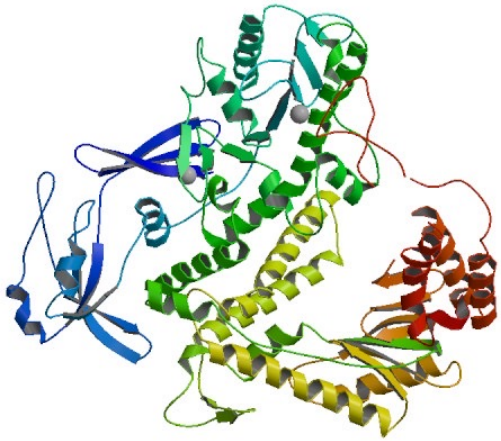
Building blocks of DNA Adenine, Guanine, Thymine, Cytosine

Buffer Solution

Salt mixture, optimal pH, ionic environment- cations Mg^{2+}, K^{+}



Taq polymerase



Pfu polymerase

Polymerases- Essential for synthesis

- **Taq polymerase**

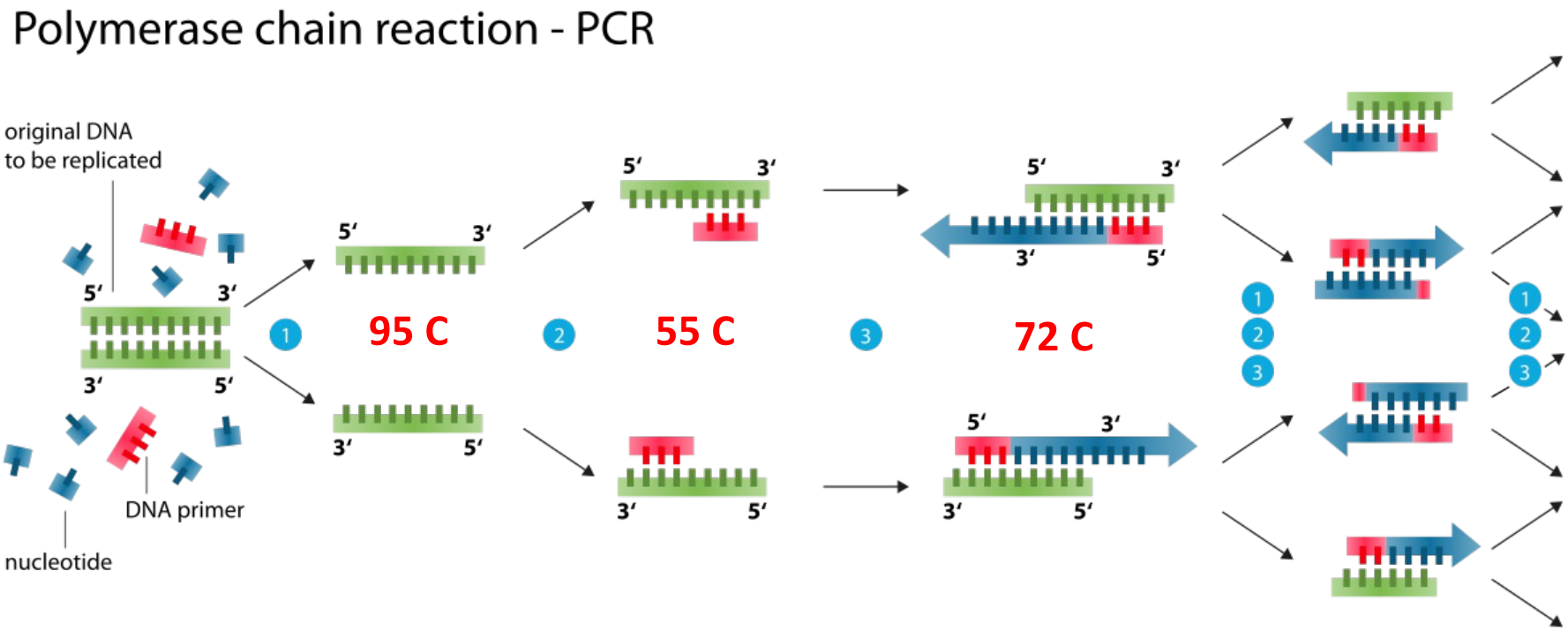
Thermostable enzyme isolated from bacterium Thermus aquaticus.

- **Pfu polymerase**

Thermostable enzyme isolated from the bacterium Pyrococcus furiousus.

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

Copying DNA



PCR program

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

QUESTION

What happens if the annealing temperature is,

Too low:-

Too high:-

Quick Visualization

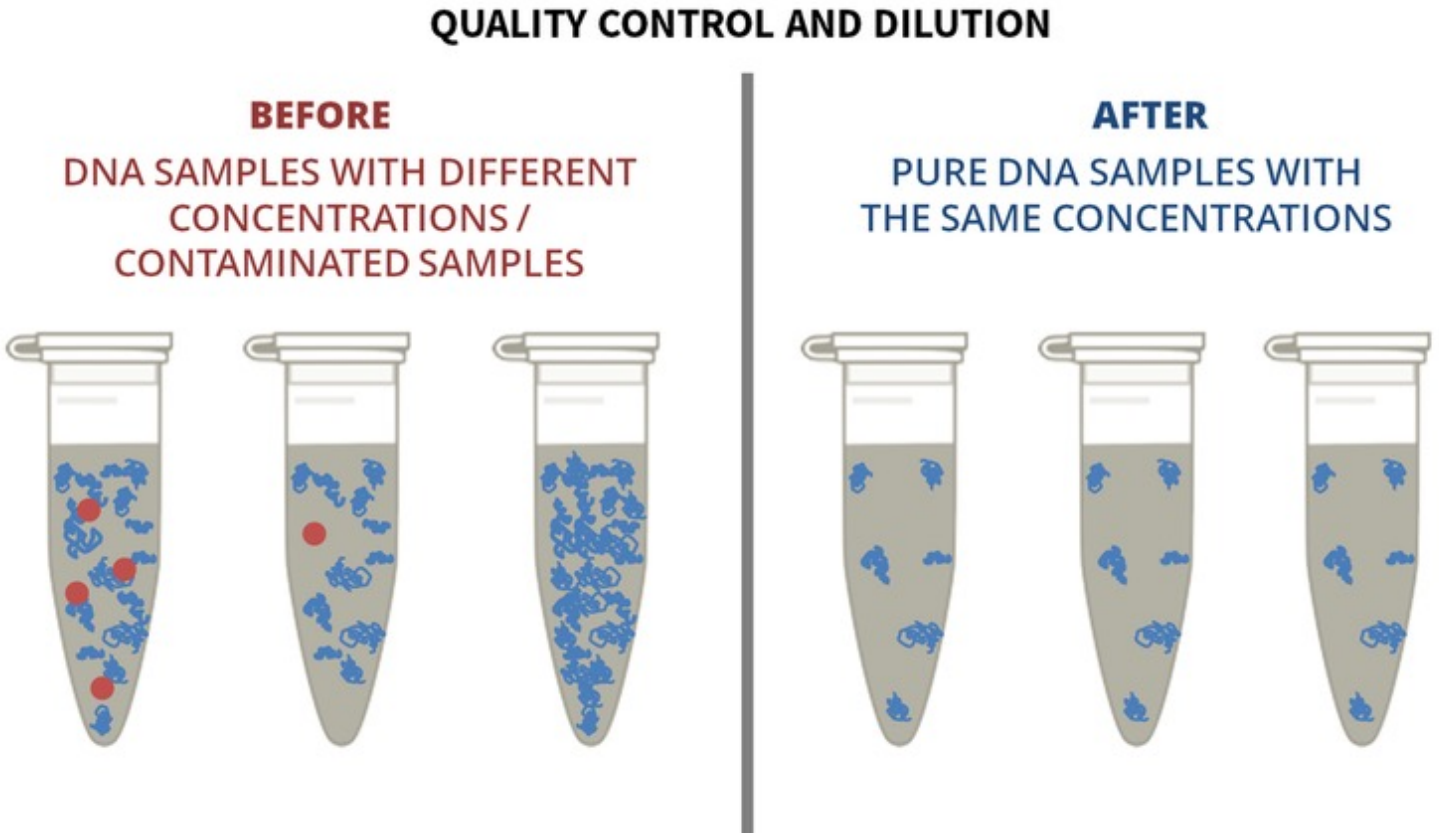
PCR Primer Design

Forward primer	5' GCTAAATGTTCAGGCTGTGG 3'
Reverse primer	5' GGAATCAAACGGAATGACCG 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

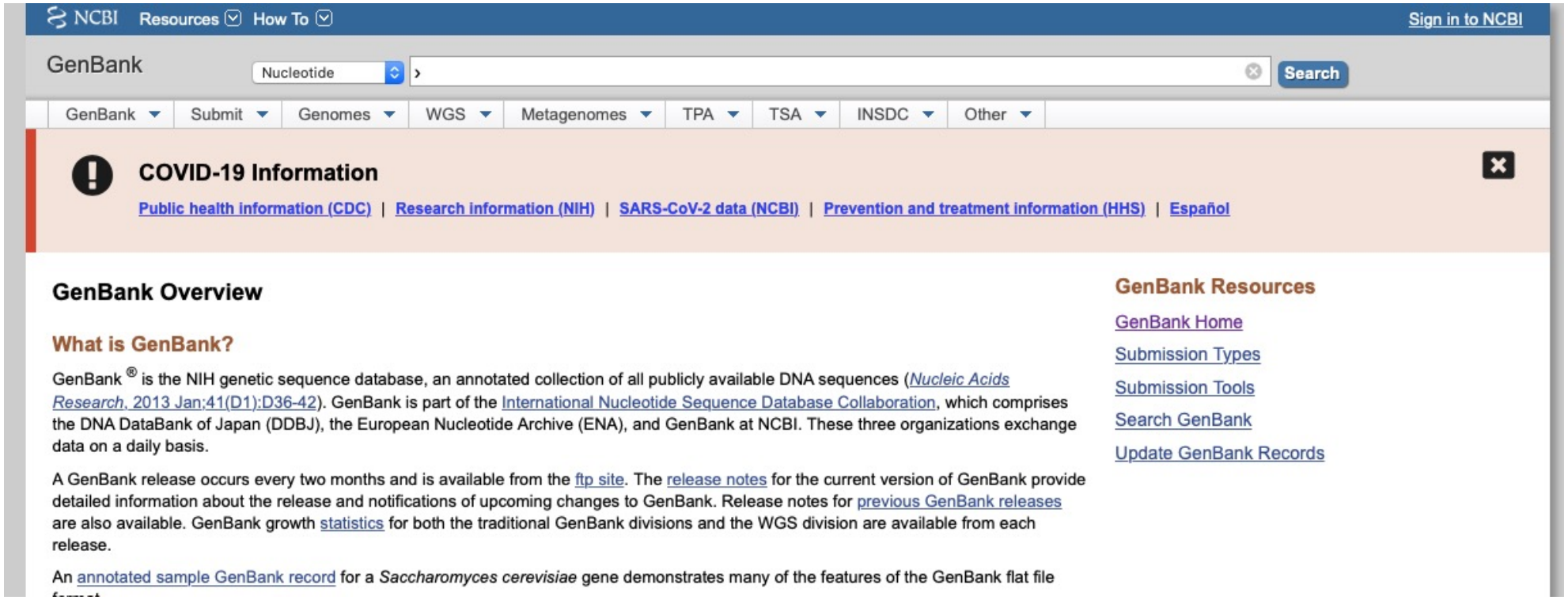
>*Escherichia coli* 16S rRNA

AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAG
AAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTG
GAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAG
ATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACAC
TGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAG
CCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTT
GCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTT
AATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAC
TGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTG
GAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAAC
GCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTG
GAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGG
TGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTATCTTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAG
GTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCG
ACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGA
ATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAG
TGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGT
AACAAGGTAACCGTAGGGGAACCTGCGGTTGGATCACCTCCTTA

***E. coli* primers**

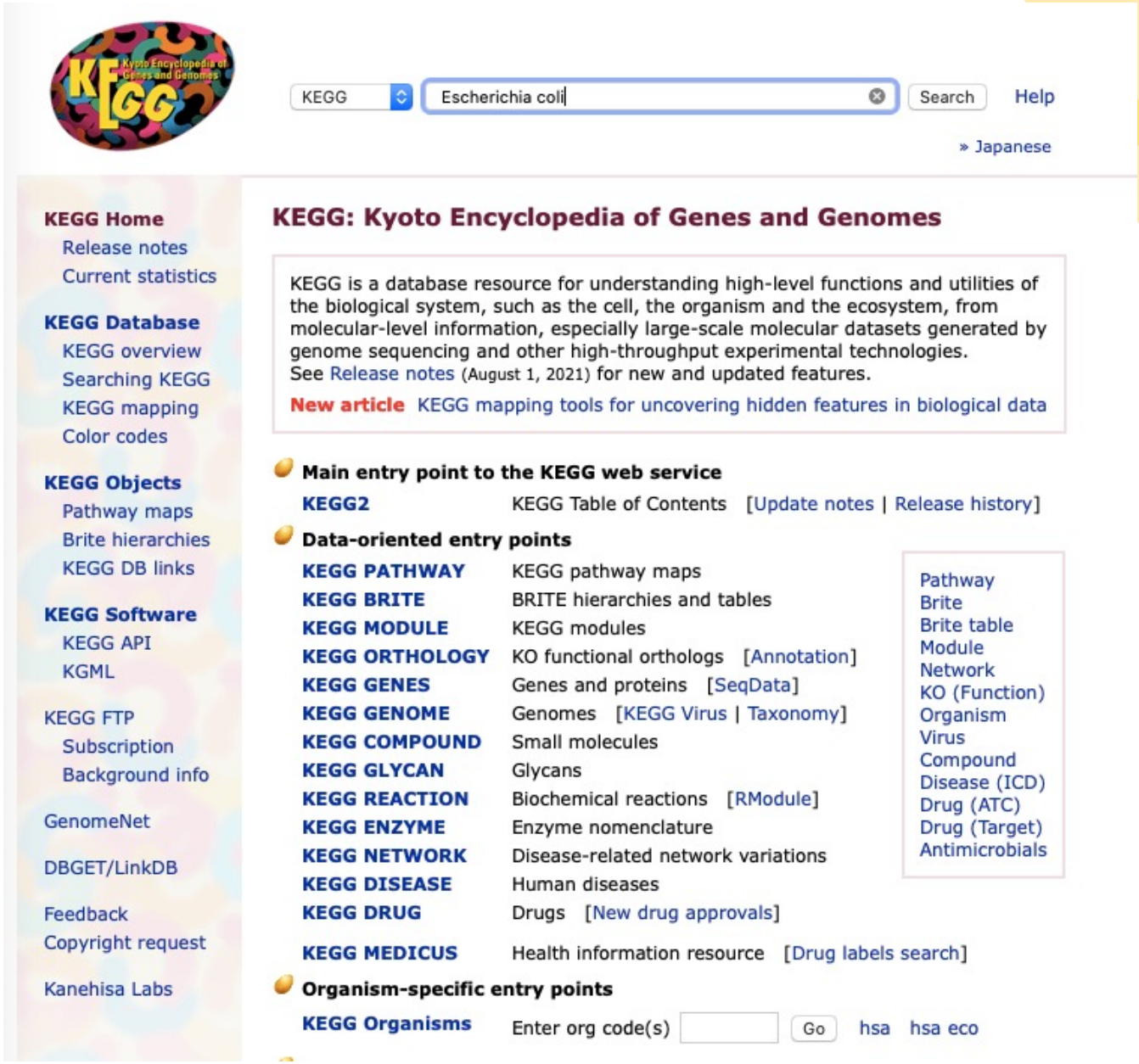
Forward (27F)	Reverse (1392R)
5’-AGAGTTTGATCATGGCTCAG -3’	5’-GGTTACCTTGTTACGACTT-3’

1. Obtain the gene sequence



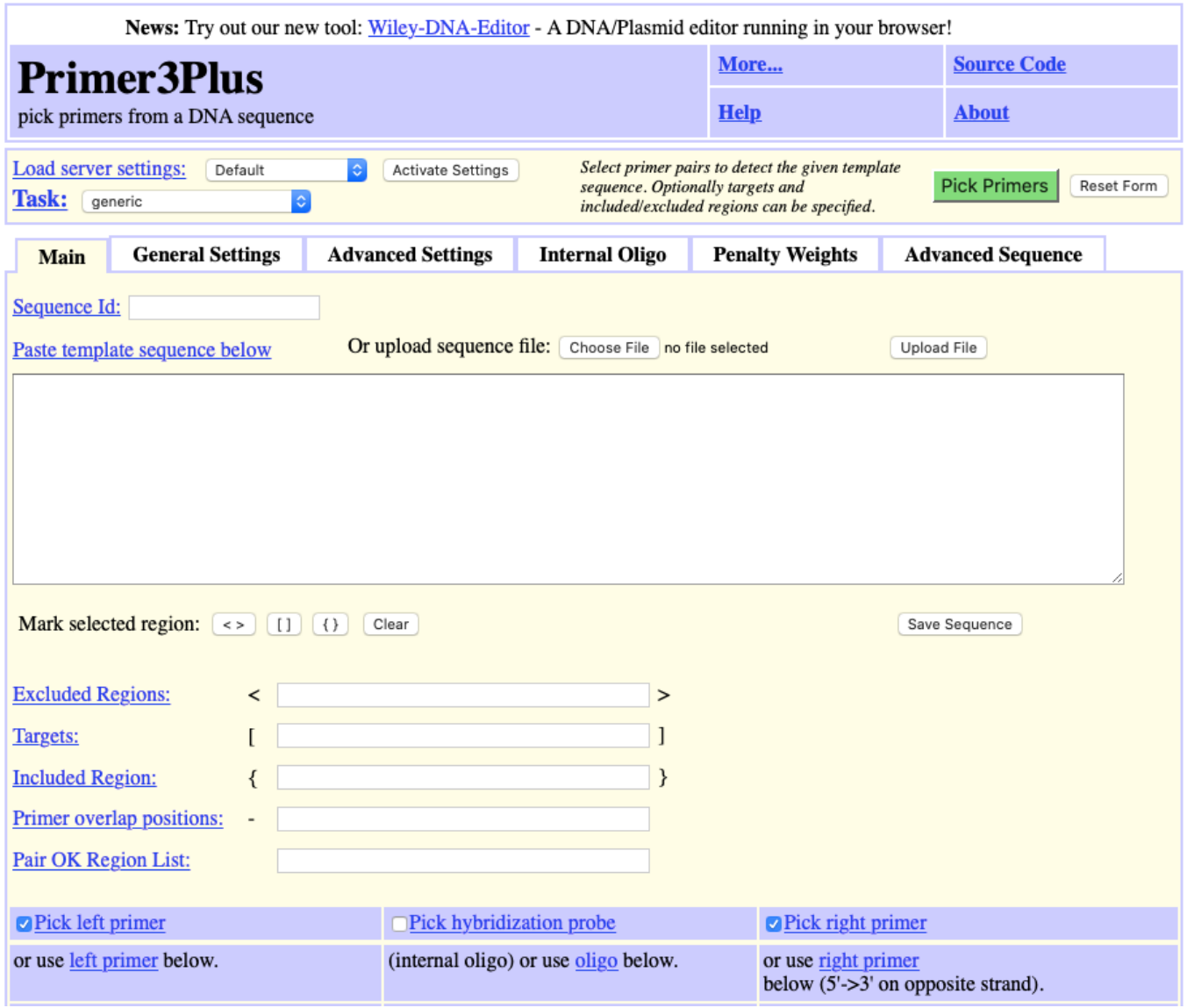
https://www.ncbi.nlm.nih.gov/genbank/

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).

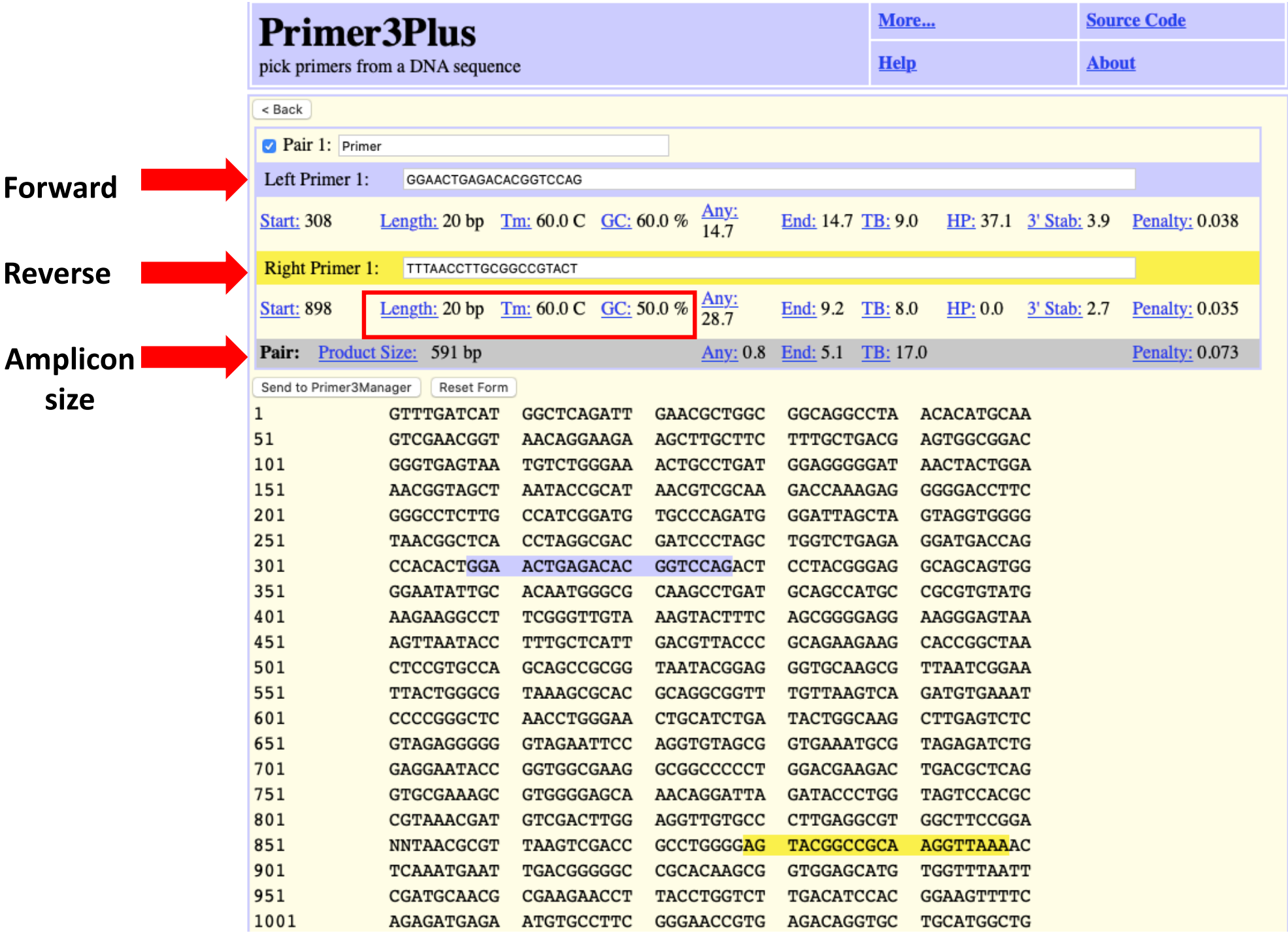


This is a good source to obtain the full length of a gene.

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



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


Forward
Reverse
Amplicon size
Primer3Plus
pick primers from a DNA sequence
More...
Source Code
Help
About
< Back
Pair 1: Primer
Left Primer 1: GGAACTGAGACACGGTCCAG
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: TTTAACCTTGCGGCCGTACT
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 TCGGGTTGTA 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