3d molecular designs

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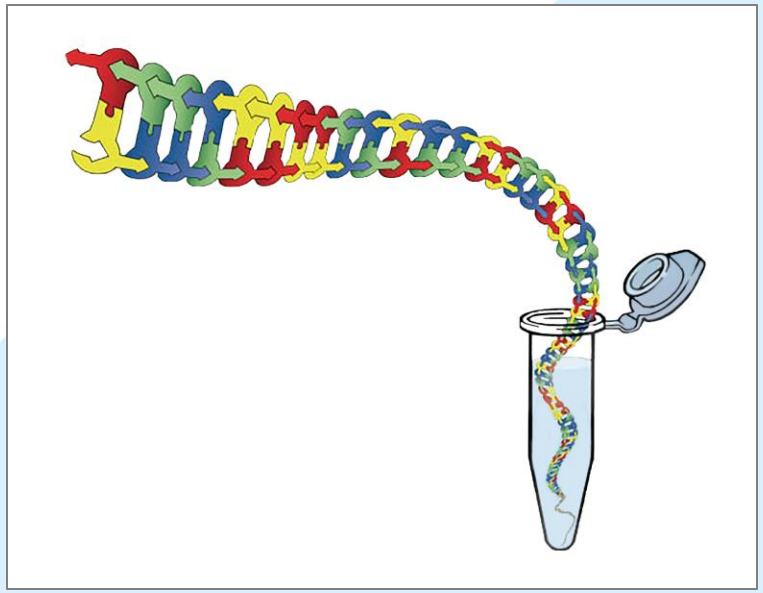

In the Tube Where it Happens:

Using models to support student understanding in biotechnology

Mark Arnholt

Science Educator

SMART Team Coordinator

3D Molecular Designs

Milwaukee, WI

mark.arnholt@3dmoleculardesigns.com

24 years of classroom experience

PLTW BMS Certified (PBS, MI, BI)
Cohort Biology
General Biology (9-10)
Anatomy and Physiology
Biotechnology, Bacteriology, and Genetics
Physical Science(9)
Aviation

After school activities

New Teacher Mentor
SMART team advisor
Wrestling coach
Fishing club advisor

Awards:

Outstanding Science Educator - University of Minnesota – Twin Cities
2014
Educator of the year HUHS
2015
Herb Kohl Fellowship Grant
2016

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Workshop Learning Goals

1. Understand the ability to taste PTC
2. Learn the Process of a Polymerase Chain Reaction
3. Understand the potential applications of the technology
4. See how models build student understanding
5. Have fun!

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Background Knowledge for Students:

Big Concepts:

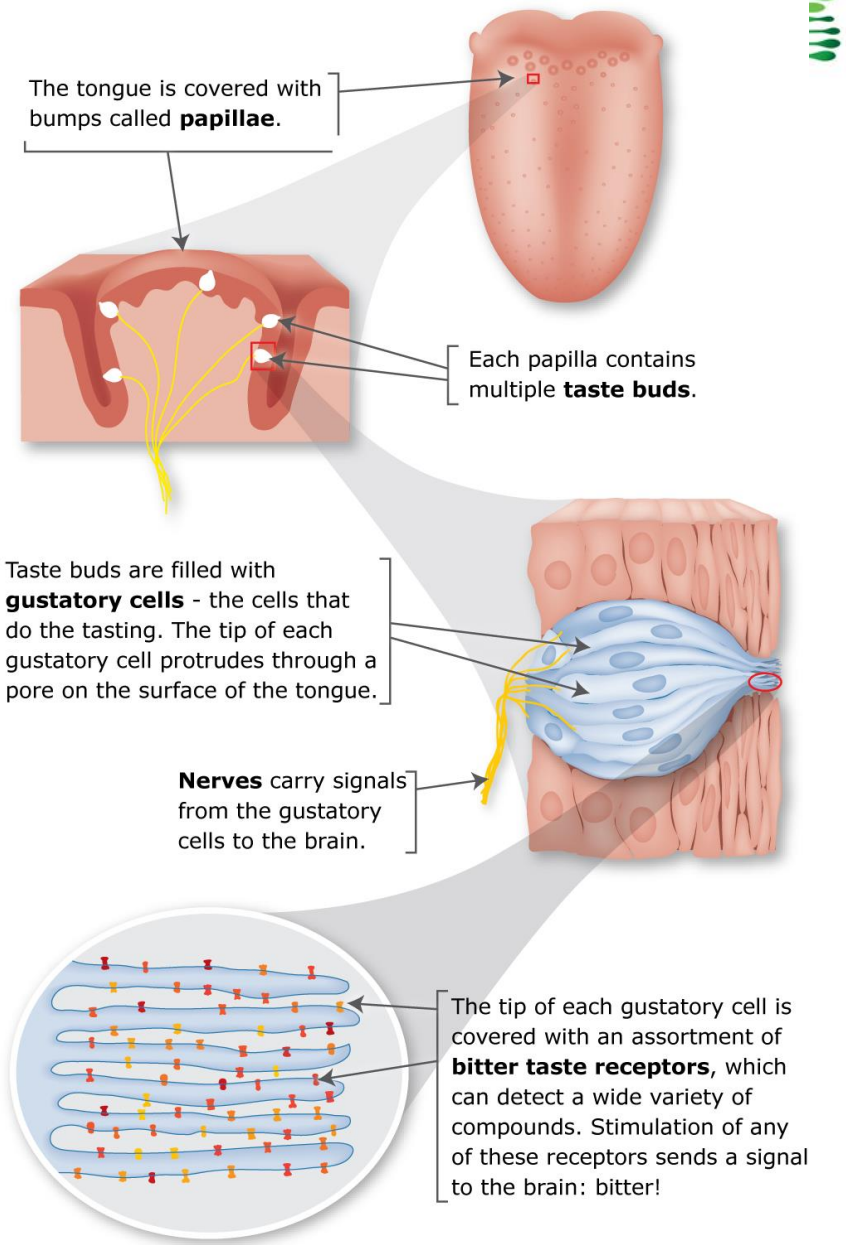
DNA Replication
Rules for Base Pairing
Hydrogen and Covalent bonding
DNA Structure
Point Mutations
Chromosomes, Genes, and Alleles

Terminology:

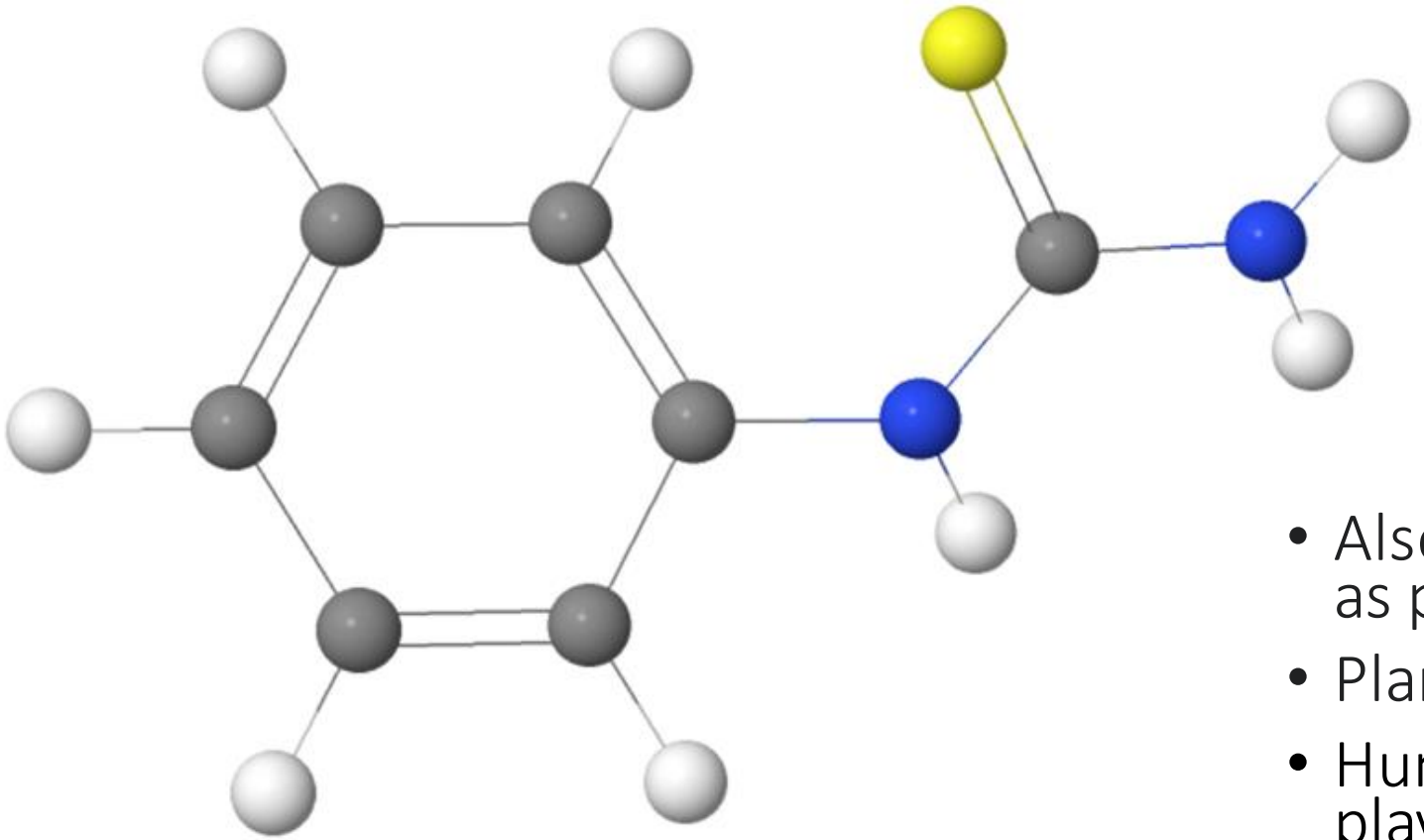
PCR – Polymerase Chain Reaction
Nucleotide (dNTP)
DNA Polymerase
Taq Polymerase
Thermal cycling
Target DNA
Primers
Denature
Anneal
Extend
Electrophoresis

The Science of PTC Tasting

Accidental discovery – 1931 – Arthur Fox



Phenylthiocarbamide

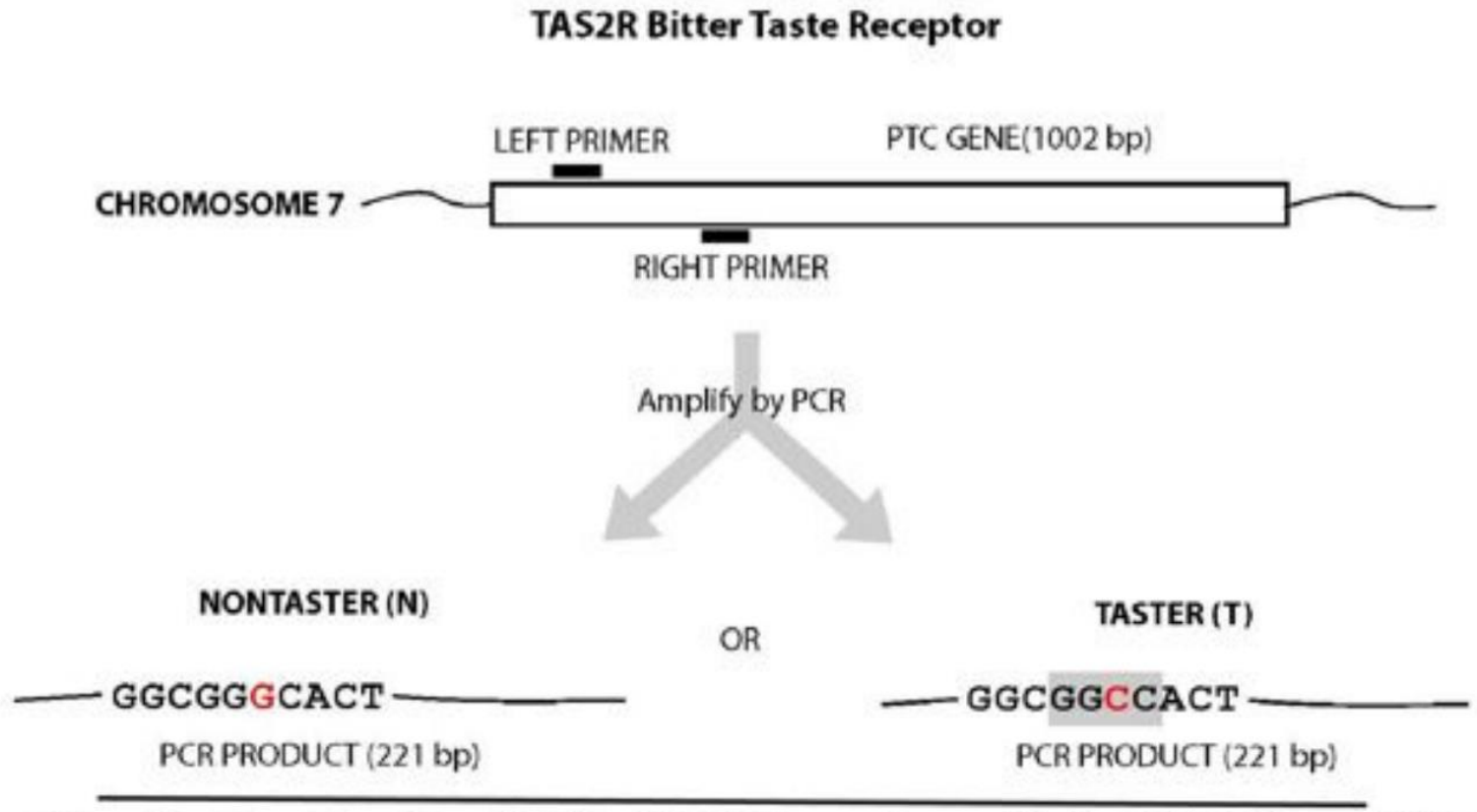


- Also known as phenylthiourea
- Plant Defense Mechanism
- Human ability to taste this plays a role in food choice

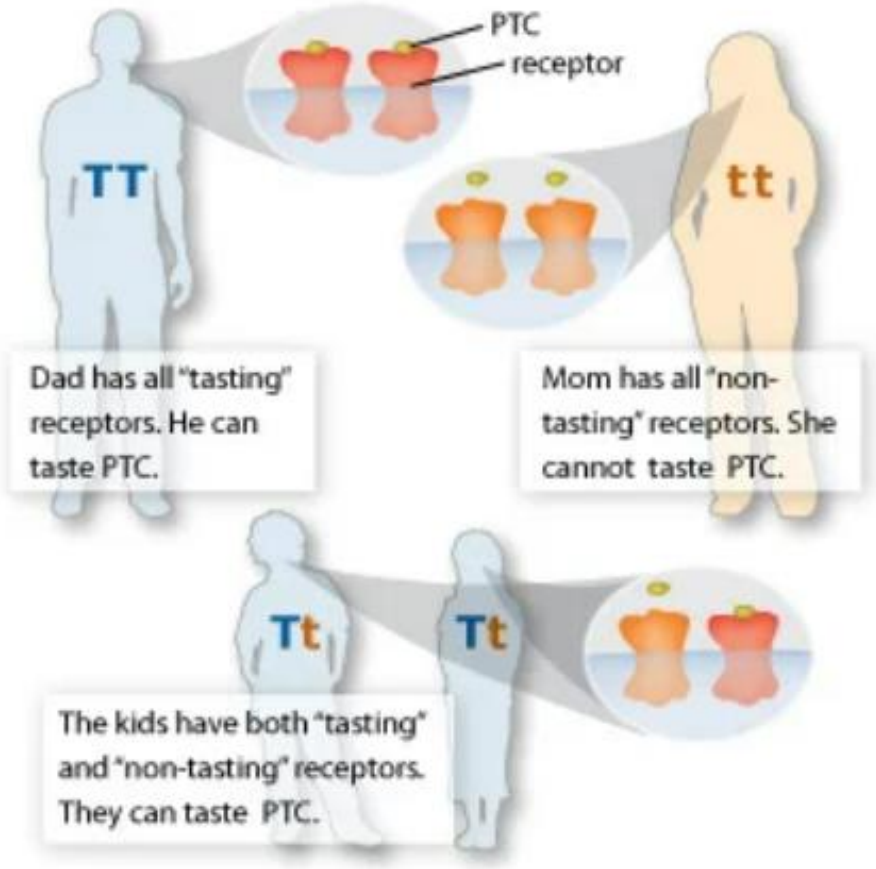
Pull out your DNA Sequence.

What are some initial observations you can make?

The Genetics



The selected region of the 1002 bp TAS2R28 gene is amplified by PCR. The amplified PCR product of tasters of PTC contains the sequence - GGCC -, which is recognized by the restriction enzyme *Hae*III. The amplified PCR product of non-tasters contains a different sequence - GGGC -, which is not recognized by the restriction enzyme.



Genotype	Phenotype
TT	Super Taster
Tt	Taster
tt	Non-taster

The ability to taste PTC shows a dominant pattern of inheritance. A single copy of a tasting allele (T) conveys the ability to taste PTC. Non-tasters have two copies of a non-tasting allele (t).

Let’s review DNA replication in a cell

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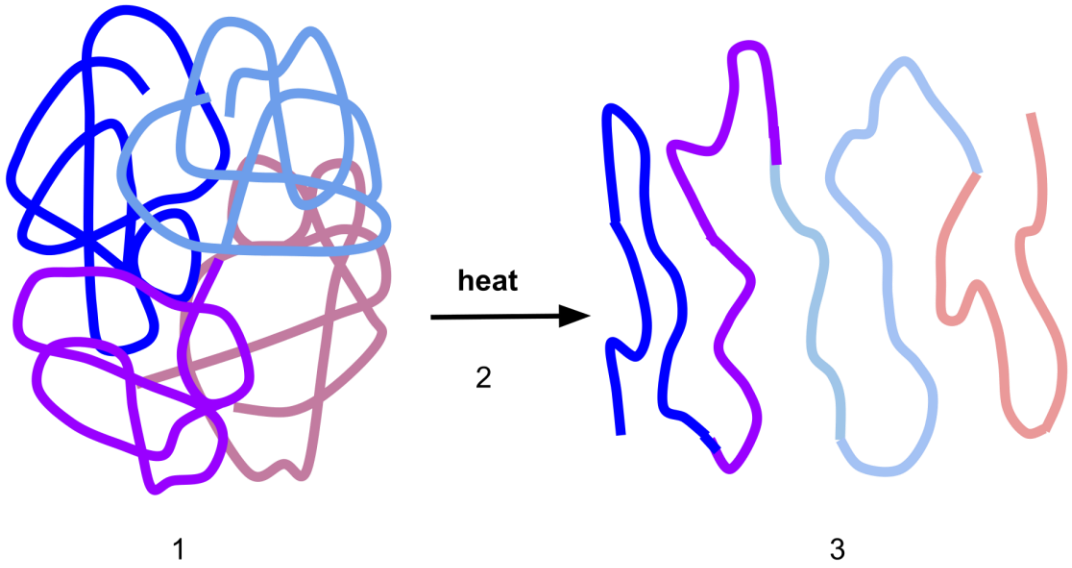
So many enzymes!

What needs to be done	Enzyme
Separate Strands	Helicase / Topoisomerase
Add Primers to DNA	Primase
Extend DNA	DNA Polymerase-ð
Remove RNA / Replace with DNA	DNA Polymerase-α
Ligation of Okazaki fragments	Ligase

Working with enzymes is difficult!

- Problems include:
  - CO$T
  - Stability
  - Temperature
  - pH
  - Salt (cations)
  - Buffers

- Denatured enzymes won't work!



The Process

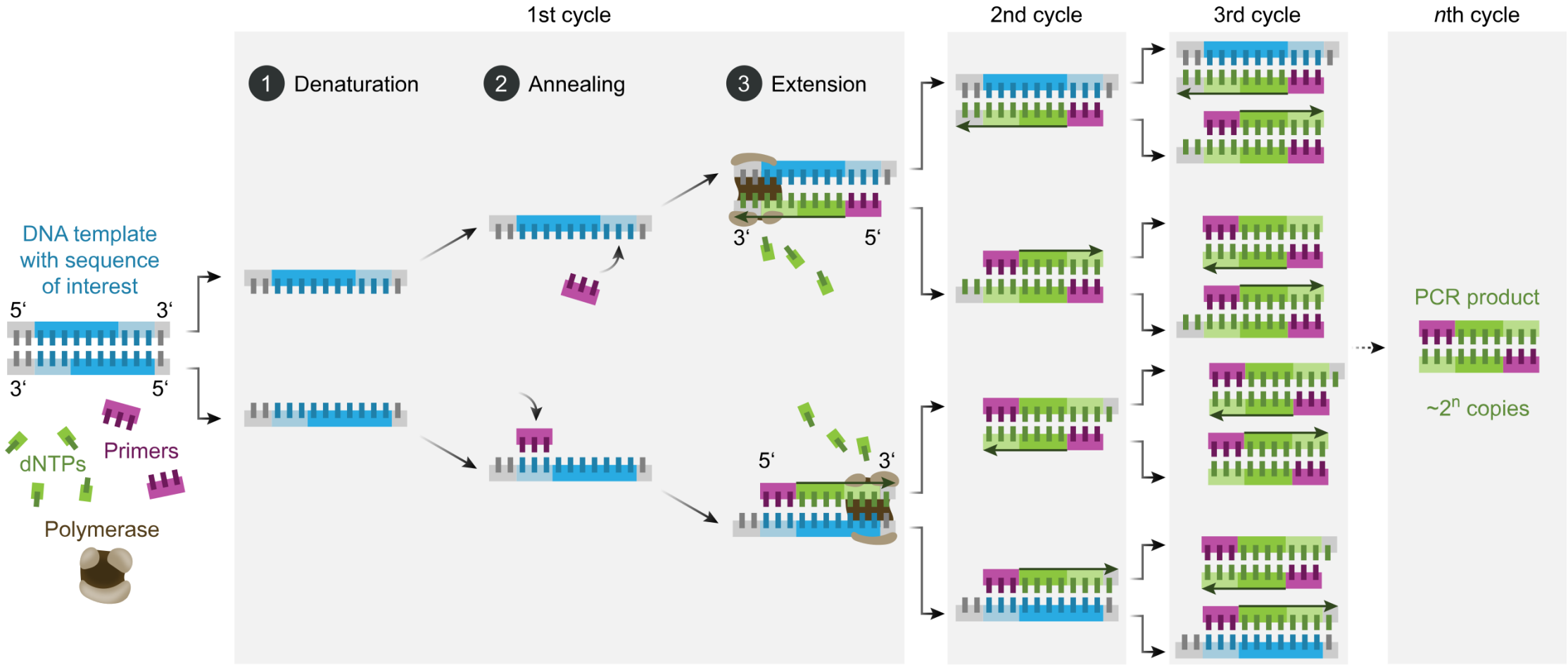
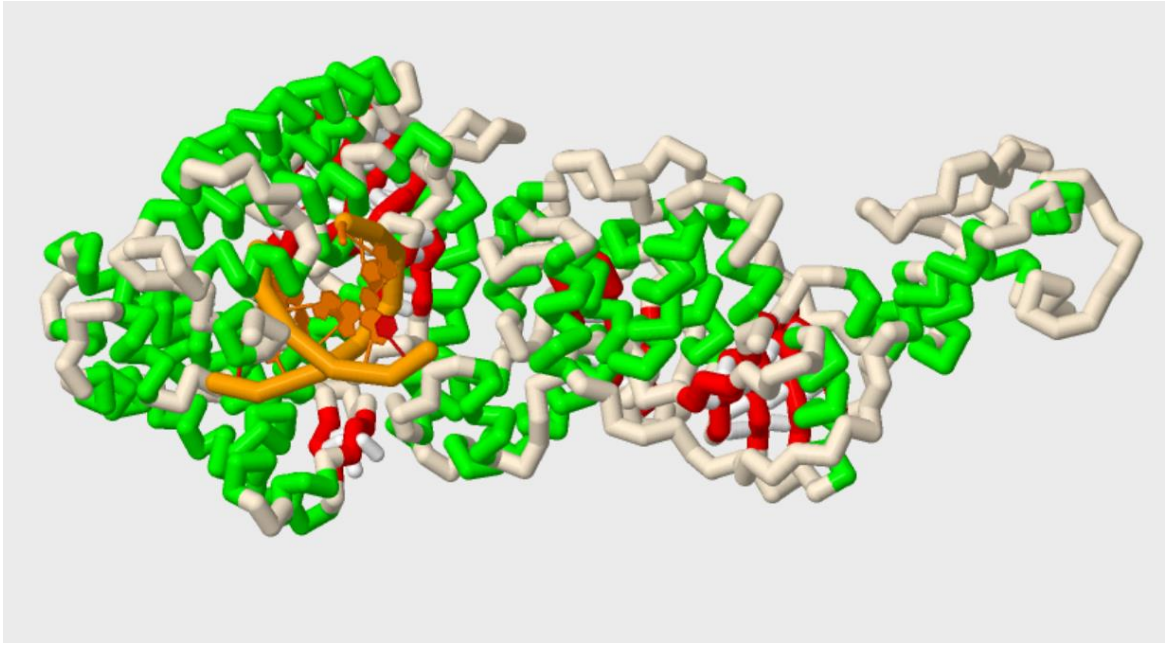


Image: File:Polymerase chain reaction-en.svg - Wikimedia Commons

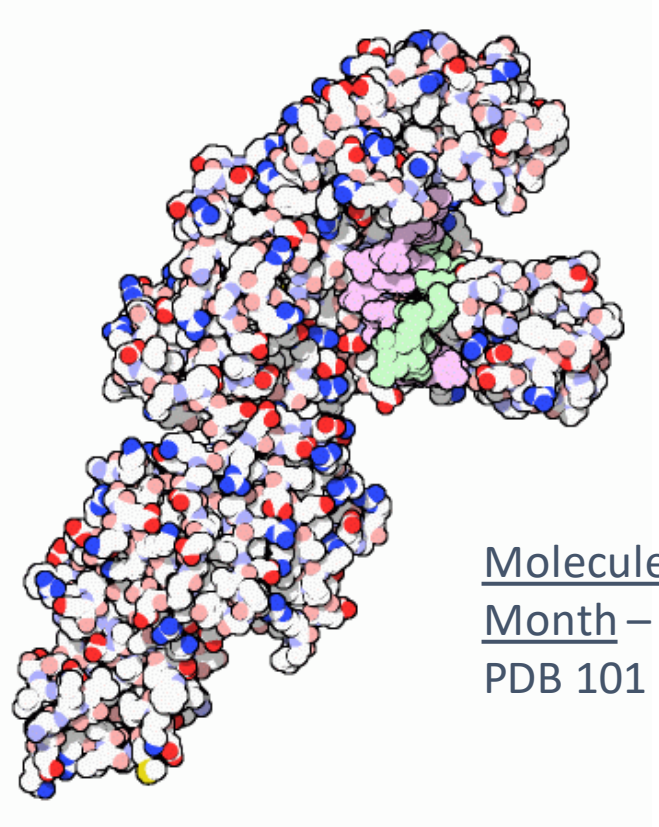
In the tube

What needs to be done	Enzyme or process?
Separate Strands	Temperature
Add Primers to DNA	Process - Annealing
Extend DNA	*Taq* Polymerase*
Remove RNA / Replace with DNA	Not required
Ligation of Okazaki fragments	Not required

Why do we need to use *Taq* Polymerase?



PDB file: 1TAU



Molecule of the Month – PDB 101

Let’s break this down

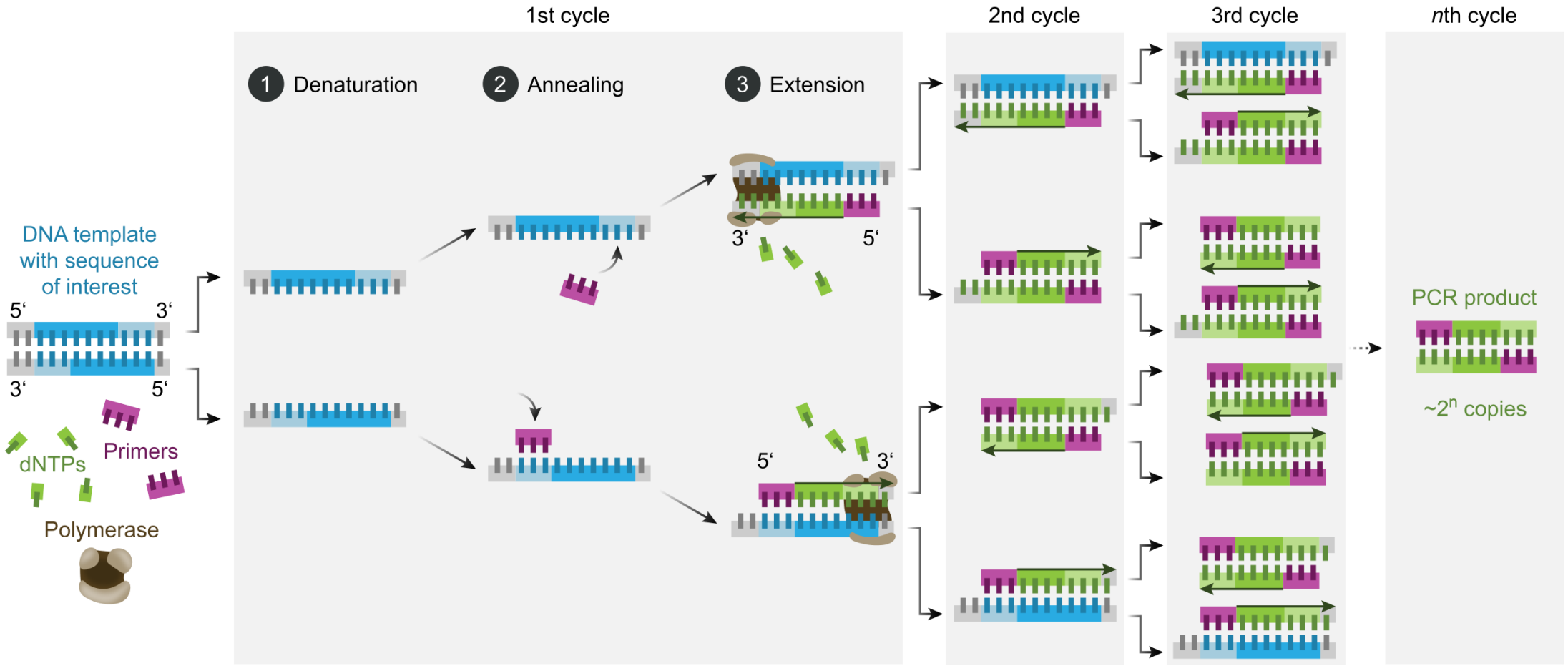
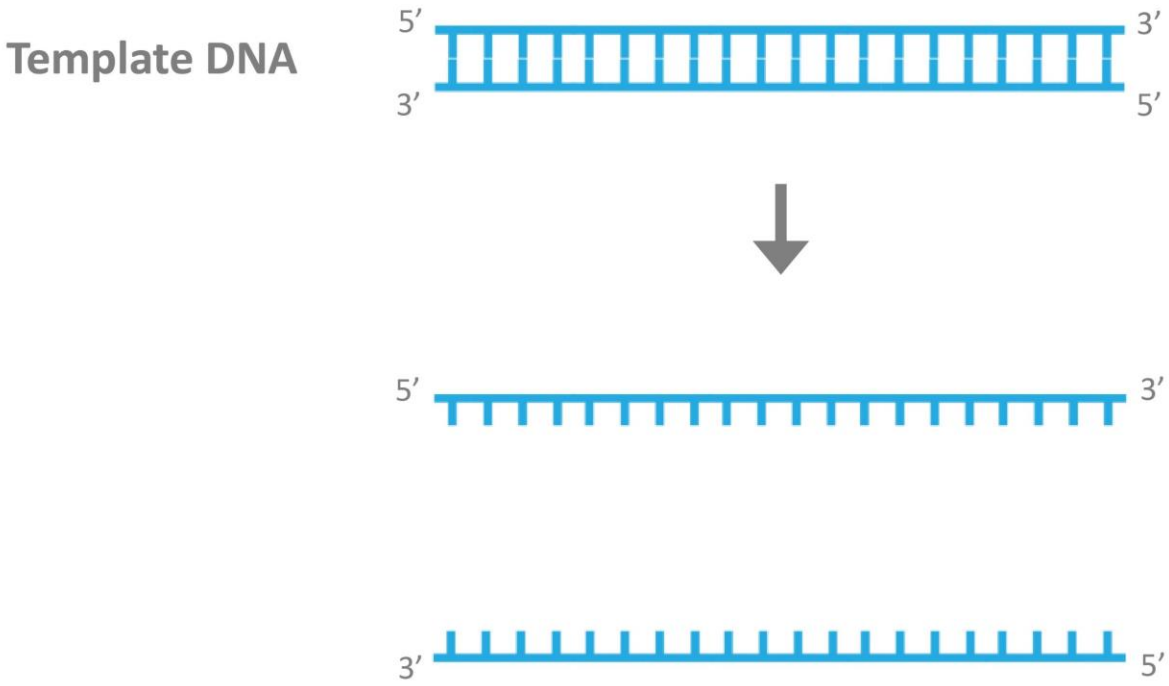


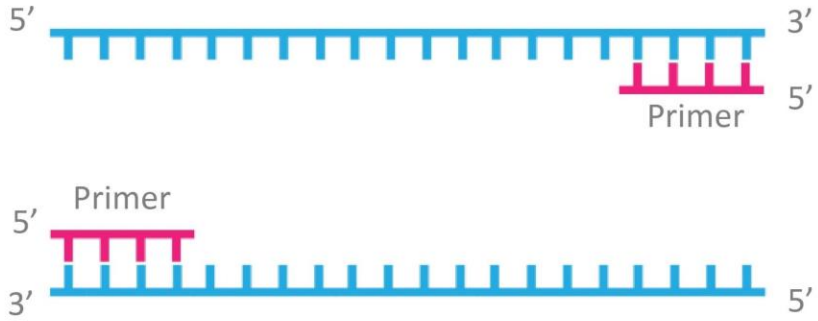
Image: File:Polymerase chain reaction-en.svg - Wikimedia Commons

Denature



95°C – This will break the hydrogen bonds between base pairs

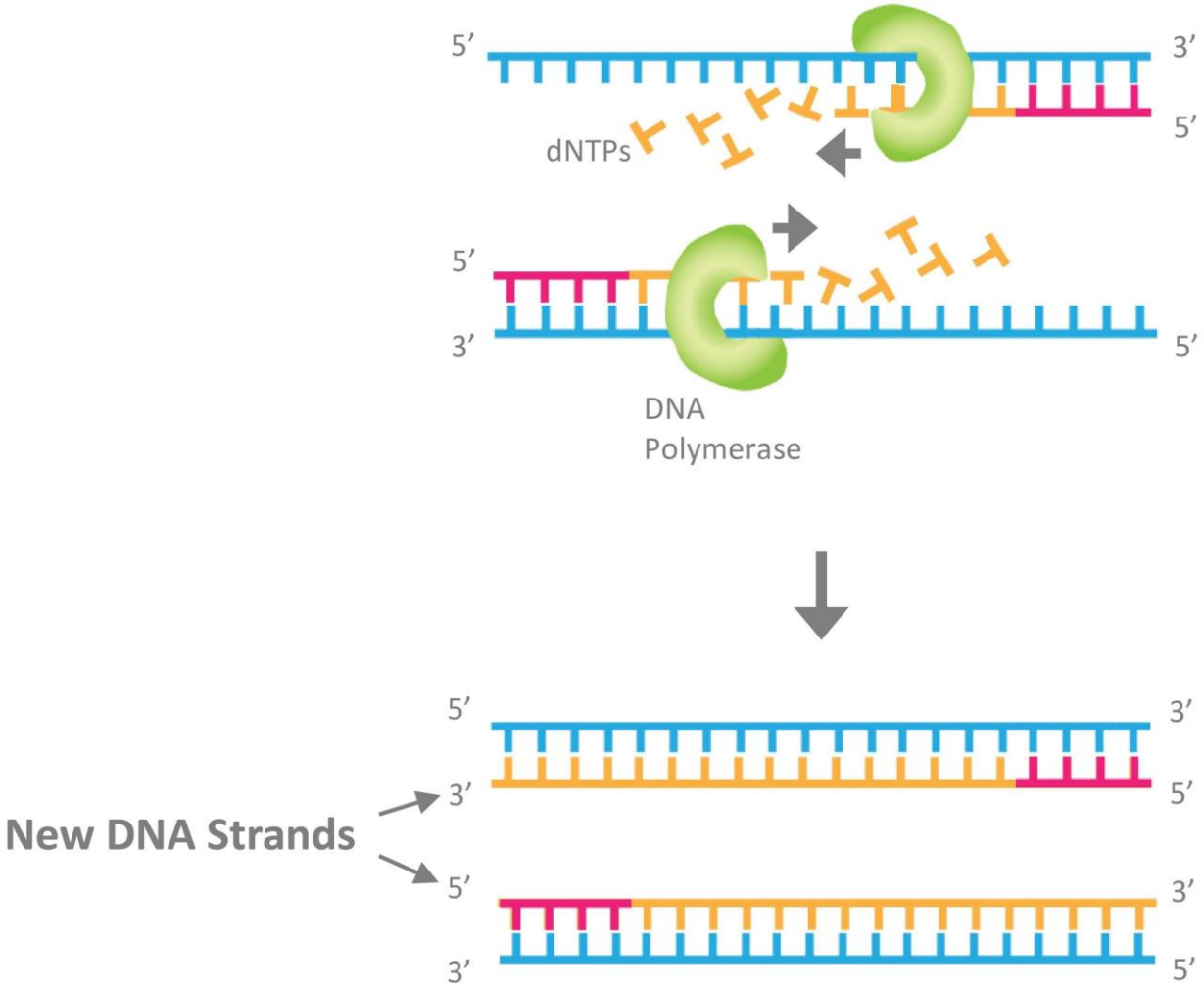
Anneal



Annealing

50(ish*)^{o}C – Cooling it down will allow the primers to hydrogen bond to the appropriate targeted sequence

Extend



Extension

72°C – Activates the Taq Polymerase to complete the extension of the target sequence

Hurry up…

How do you fill "down time" when you run a Biotechnology or Microbiology lab?

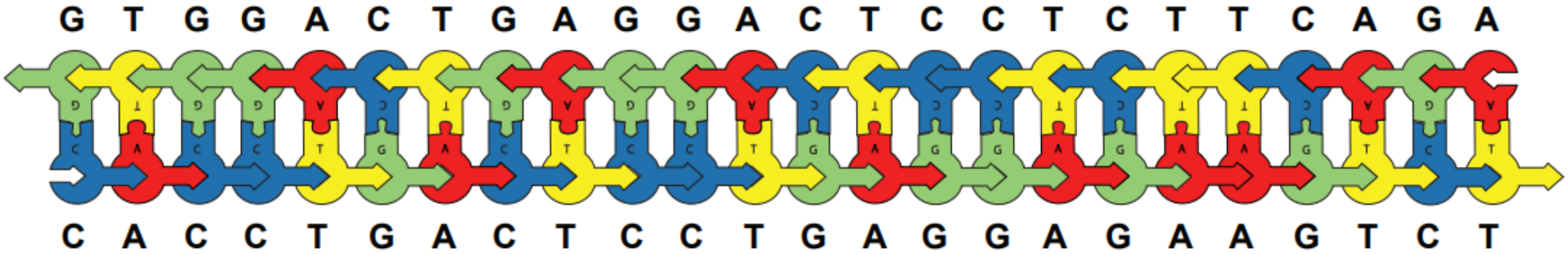
- … and wait

Your table is now a 0.2 mL PCR tube.

- **<u>We need to add:</u>**
- Template DNA
- 2 different Primers
- Nucleotides (dNTPs)
- *Taq* Polymerase

- We also need 3 temperature regions. 95°C, 52°C, and 72°C

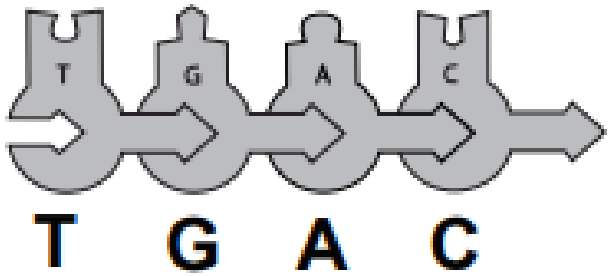
Time to build our Template DNA



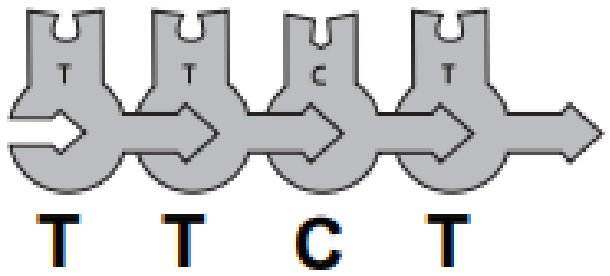
* These should be pre-built in your kit. In your classroom, you may have your students assemble them.

Next, build our Primers. We need 7 of each.

Primer Sequence I

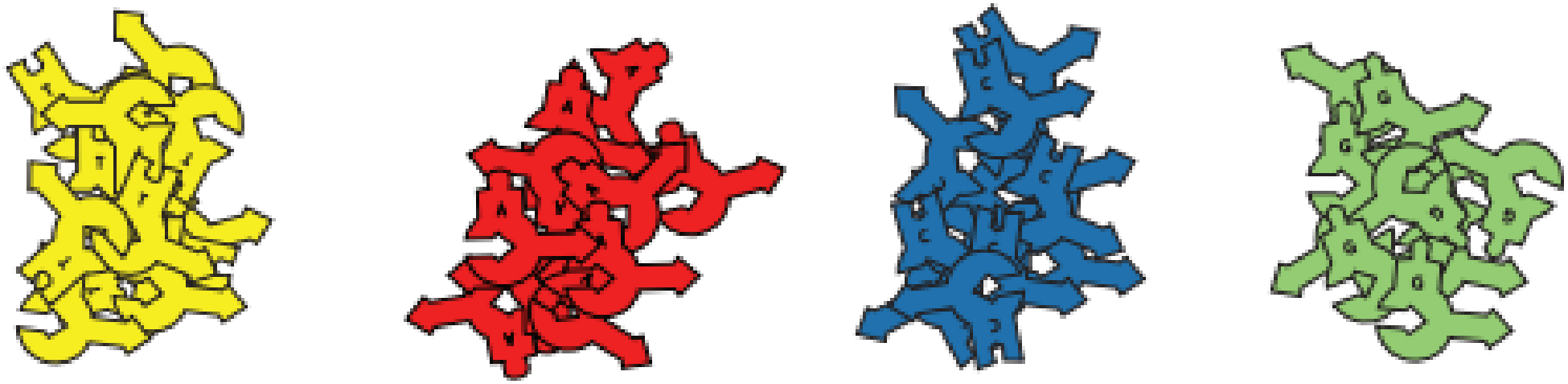


Primer Sequence II



Why do we need 2?

Add a “big pile” of nucleotides



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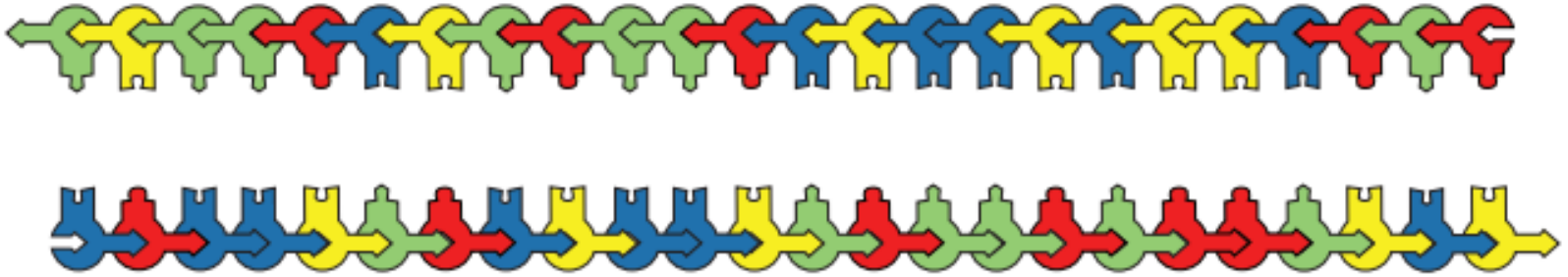
And finally, the *Taq* Polymerase

If you have our Flow of Genetics Information Kit, pull the DNA Polymerase.

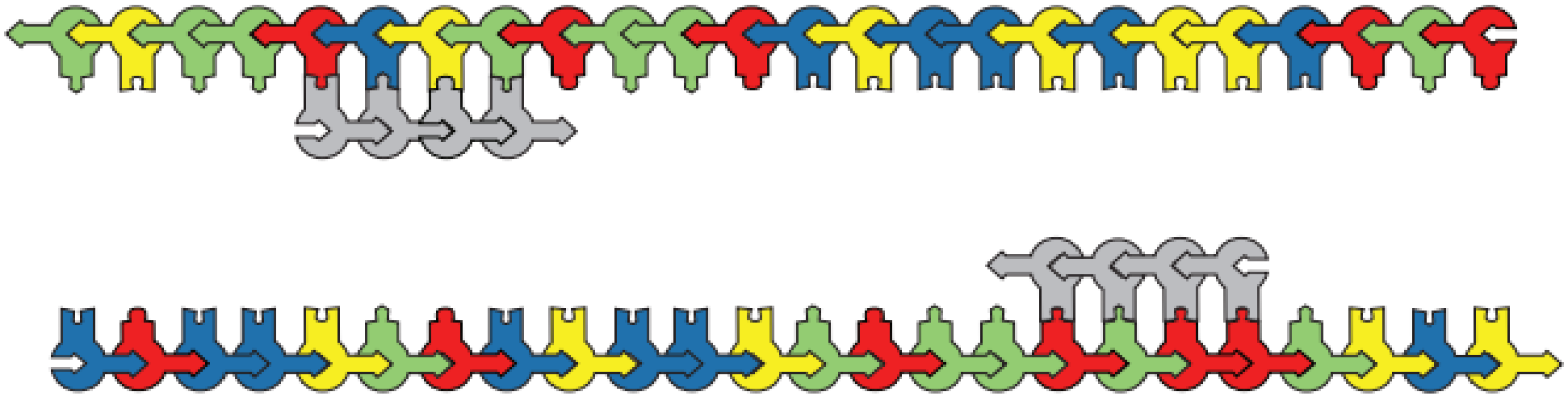
Why do we use *Taq* Polymerase?

Into the Thermal Cycler - Denature

Separate the two template DNA strands.

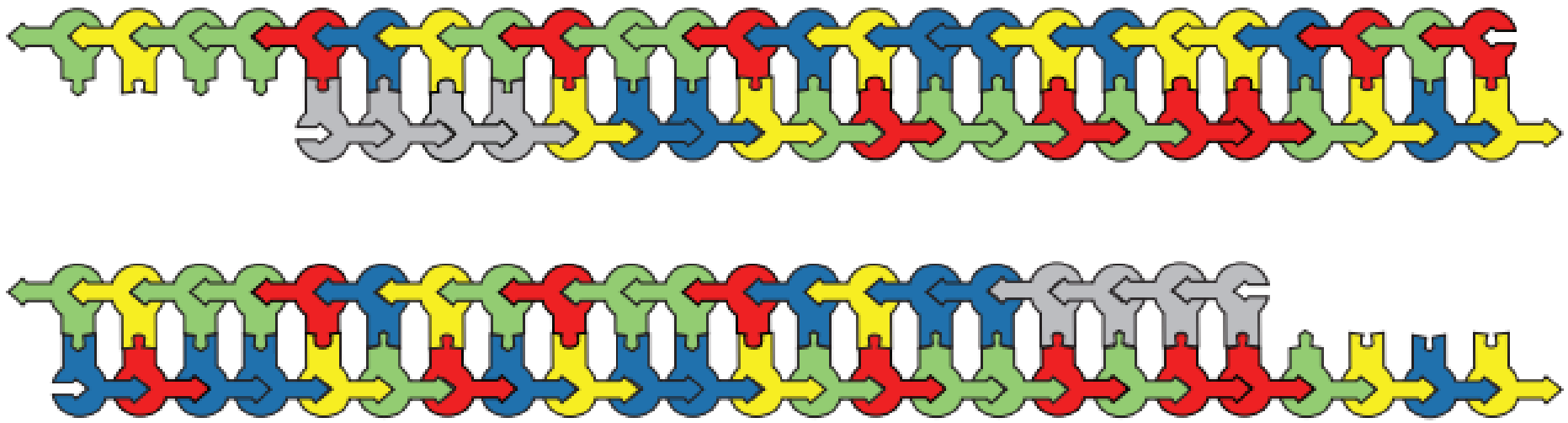


Anneal



Primers will bind according to Watson and Crick base pairing rules

Extend



Starting at the 3’ end of your primer, copy the template strand. Remember that *Taq* Polymerase can only add nucleotides in one direction (5’ -> 3’)

*Note the unpaired ends here- The targeted sequence is dependent upon the primers

Cycle 1:
Complete

Begin Cycle 2
Denature

Anneal

Extension

*Note: Unpaired bases are still present

Cycle 2
Complete

Begin Cycle 3
Denature

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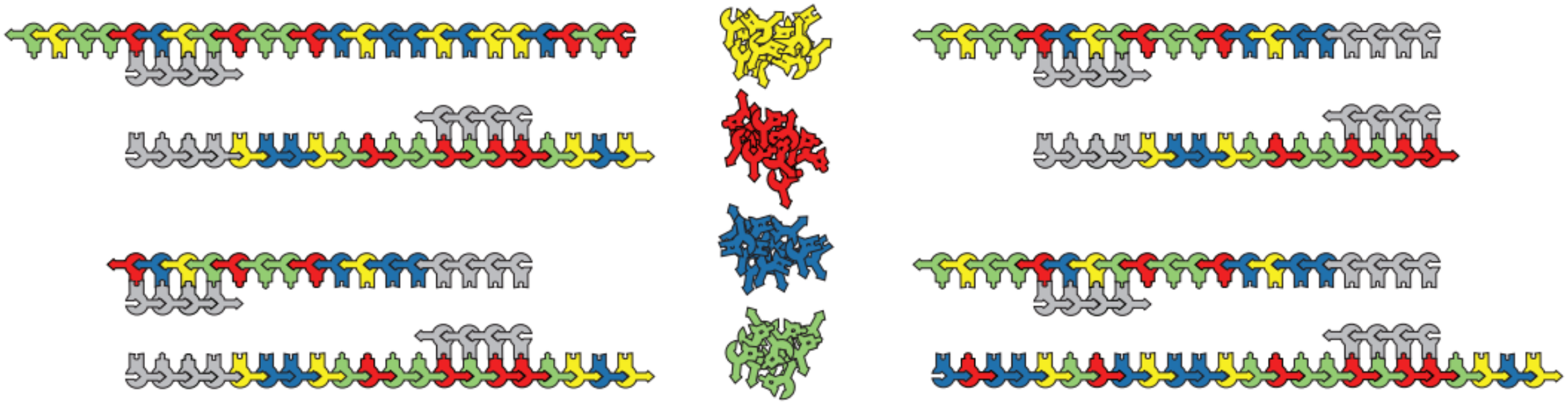
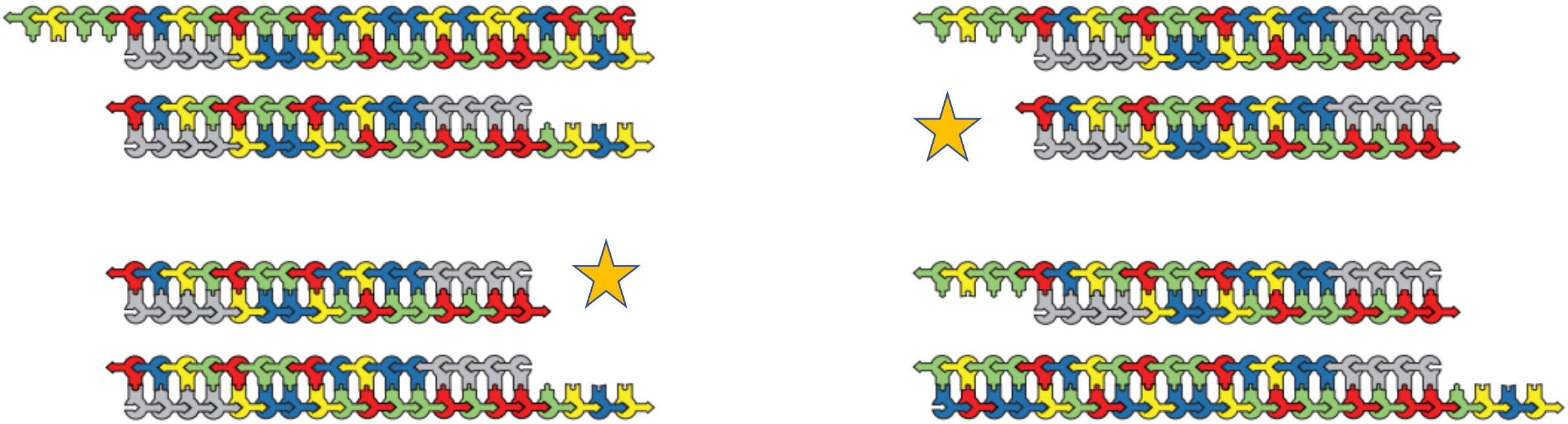
Anneal



Extend



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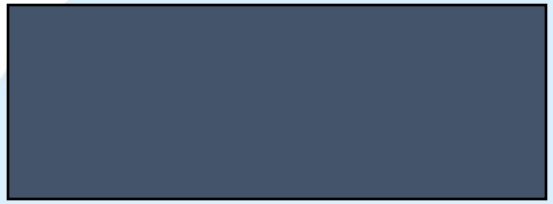
Lather, rinse, repeat…

The goal? Make as many copies of a specific sequence of DNA as possible.

How many copies of the sequence will we make if we run 30 cycles?



60 will have unmatched base pairs
All of the rest will be the targeted DNA sequence

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Lather, rinse, repeat…

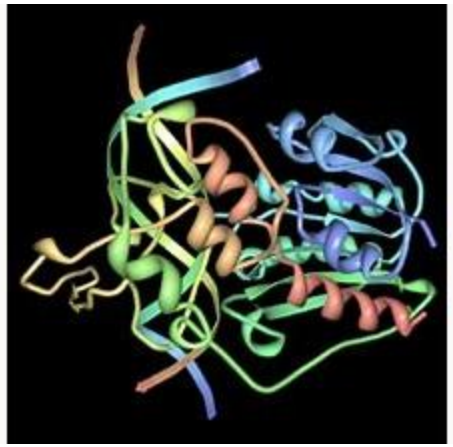
The goal? Make as many copies of a specific sequence of DNA as possible.

How many copies of the sequence will we make if we run 30 cycles?

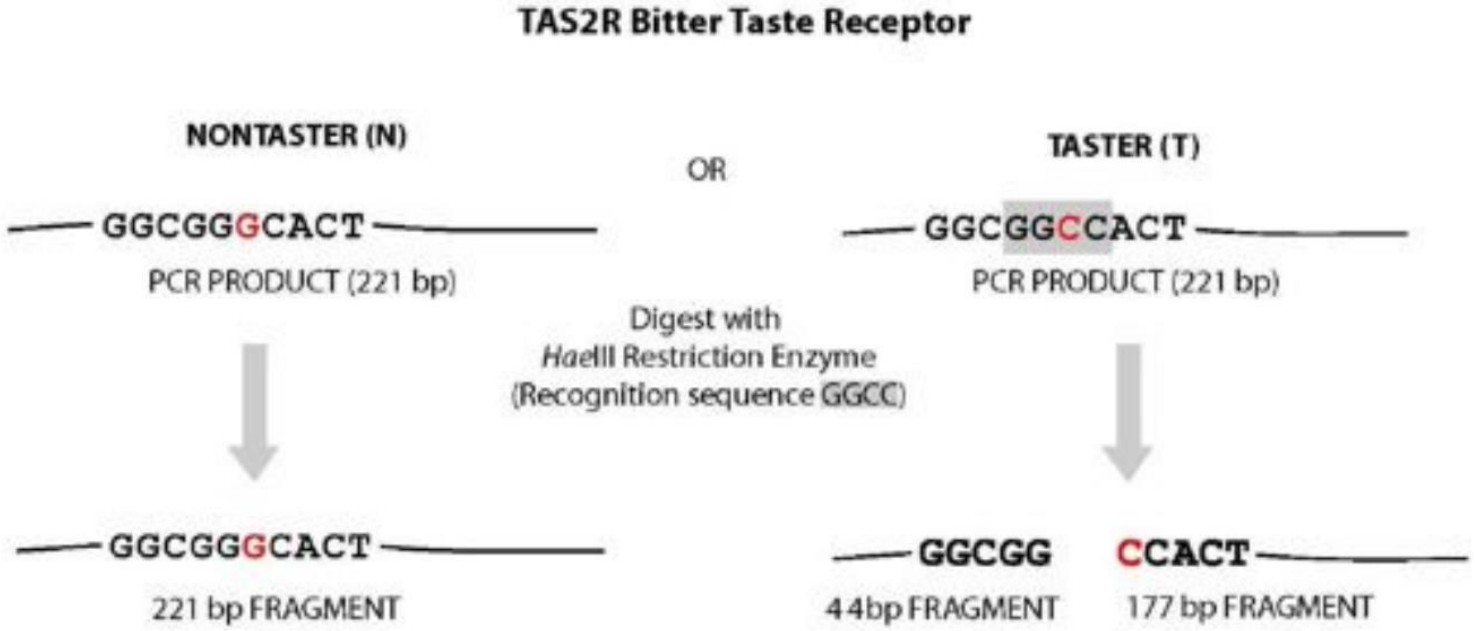
$2^{30} = 1.02 \times 10^{9}$

60 will have unmatched base pairs
All of the rest will be the targeted DNA sequence

How can we differentiate between the 2 different DNA sequences?

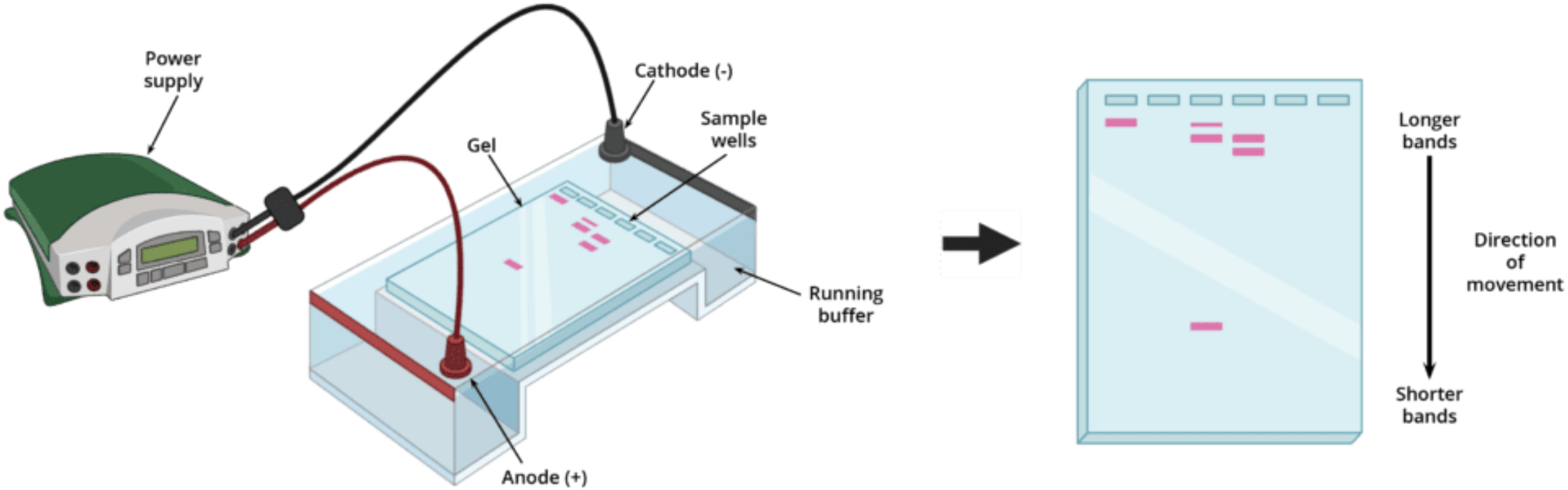


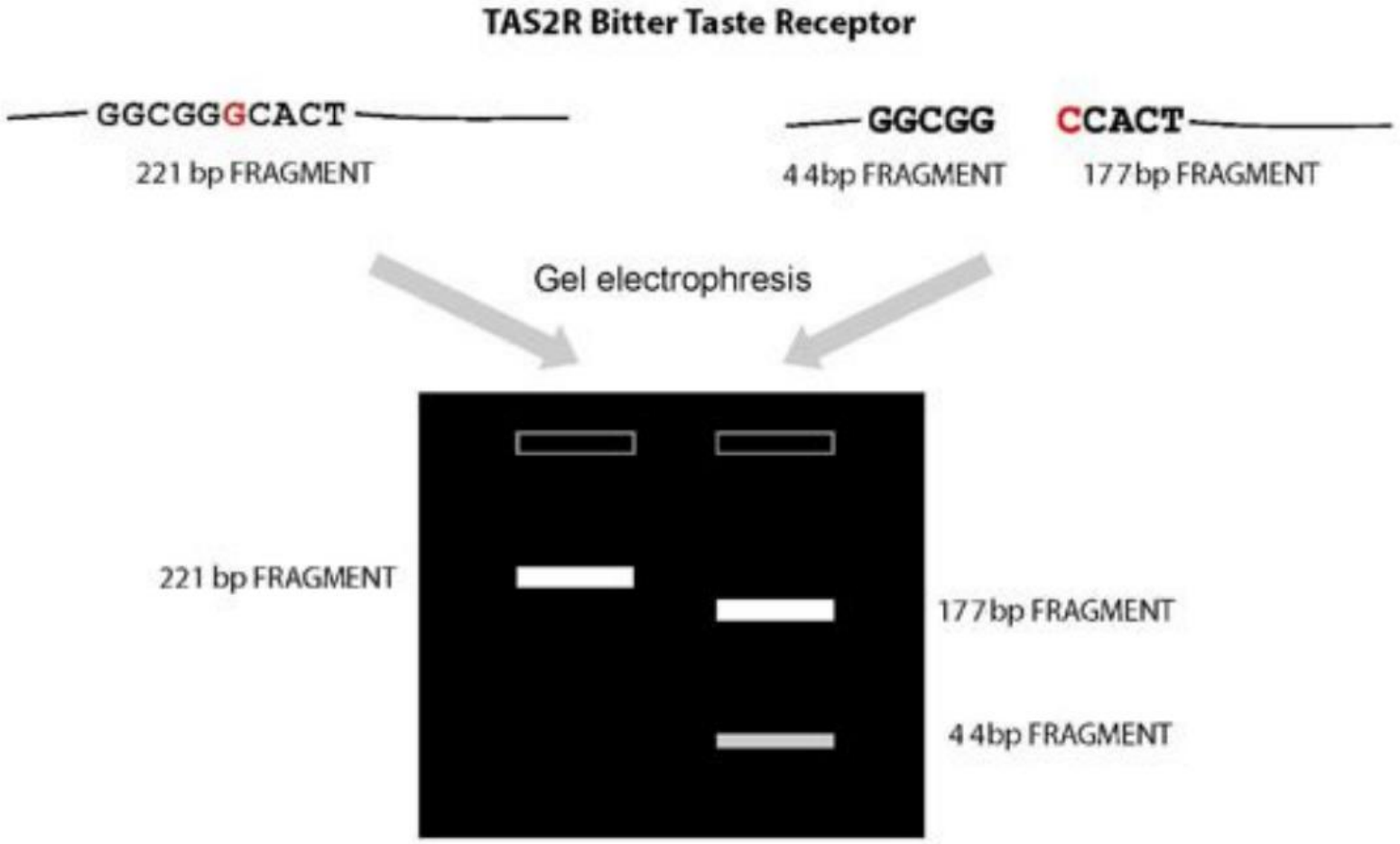
*Hae*III



The amplified PCR products of non-tasters and tasters are treated with the restriction enzyme, HaeIII, which contains the restriction sequence - GGCC -. This sequence is not present in the amplified PCR product of non-tasters. Thus, the PCR product remains uncut with a length of 221 bp. By contrast, the PCR product of tasters contains the recognition sequence and is cleaved into two fragments: of 44 bp and 177 bp.

Gel Electrophoresis allows us to separate DNA based upon size.





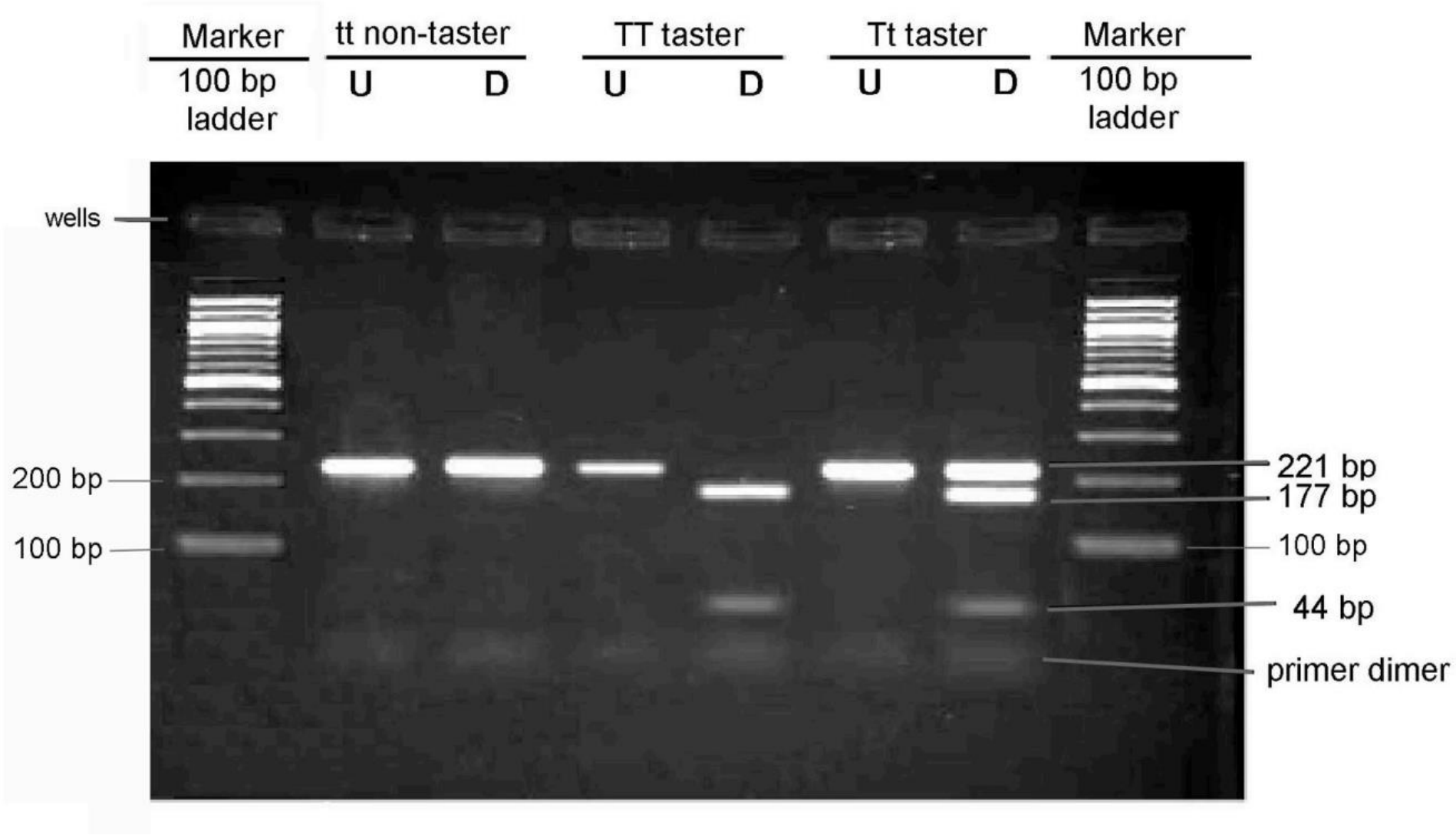
The amplified PCR product of a taster contains the sequence - GGCC -, which is recognized and cut by the restriction enzyme, *Hae*III. When run on an agarose gel the digested PCR product gives two DNA fragments: 177 bp and 44 bp. By contrast, the amplified PCR product of a non-taster lacks the recognition sequence and is not cut by *Hae*III. Gel electrophoresis gives one fragment of 221 bp, the same as the undigested PCR product.

Challenge Question!

What about a heterozygote for this trait?

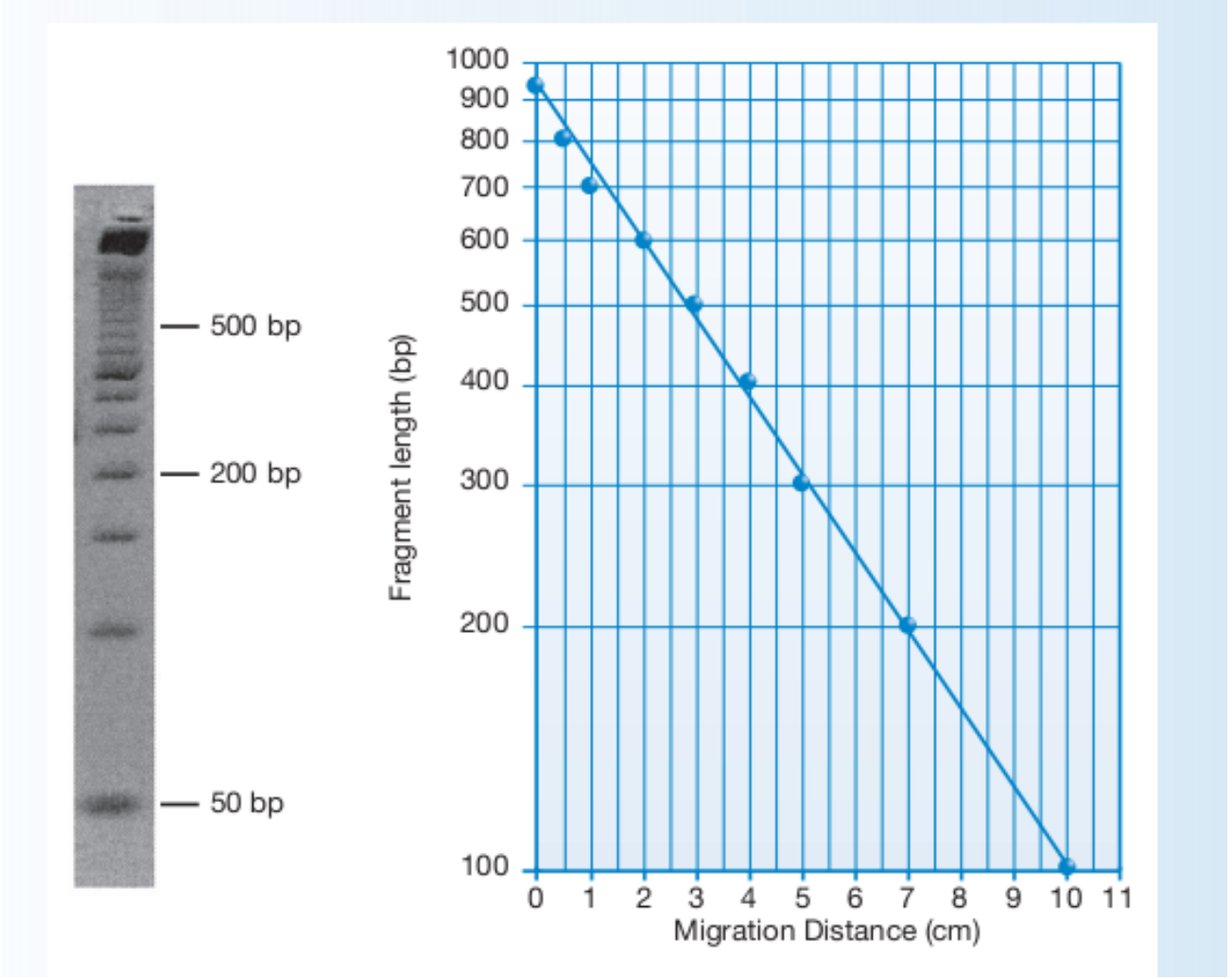
Predict what their lane would look like.

Electrophoresis results



Semi-log graphing:

Why do we make a semi-log graph?



Application of the technology

Diagnosis of Genetic disorders

- Sickle Cell Anemia (Single Nucleotide Polymorphism)
- BRCA mutations (Short Tandem Repeats)

Bacterial Contaminants in Food or Water Samples

Amplification of crime scene DNA – suspect identification

Genotyping

DNA Sequencing

Synthetic Biology – Recombinant DNA

Cloning

Assessment Ideas

- Research a Single Nucleotide Polymorphism (SNP) Disease and determine if an enzyme exists to differentiate between them
- Explain how Short Tandem Repeats (STR) could be used to identify BRCA mutations
- Demonstrate how a plasmid could be constructed with a gene of interest inserted (GFP Lab)
- Demonstrate how a Multiplex PCR would allow identification of biological contaminants
- Create a video explaining PCR – Live action or stop motion

Citations

- Additional reading / resources:
- PCR Overview | GoldBio
- Wet Lab resources - Carolina
- Wet Lab resources - Edvotek
- HHMI – Genetics of Bitter Taste

- Images:
- Denatured protein
- File:Polymerase chain reaction Wikimedia Commons
- Coffee face
- Taste bud / tongue
- PTC image

Workshop NGSS Connections

SEPs	CCCs	DCIs
Asking Questions and Defining Problems (HS-LS3-1)	Cause and Effect (HS-LS3-2)	Structure and Function (HS-LS1-1)
Developing and Using Models (HS-LS1-4)	Scale Proportion, and Quantity (HS-LS3-3)	Inheritance of traits (HS-LS3-1)
Analyzing and Interpreting Data (HS-LS3-3)	Systems and System Models (HS-LS3-3)	Variation of Traits (HS-LS3-2)
	Science is a Human Endeavor (HS-LS3-3)	

We put teachers first!

Your opinions are important to us.

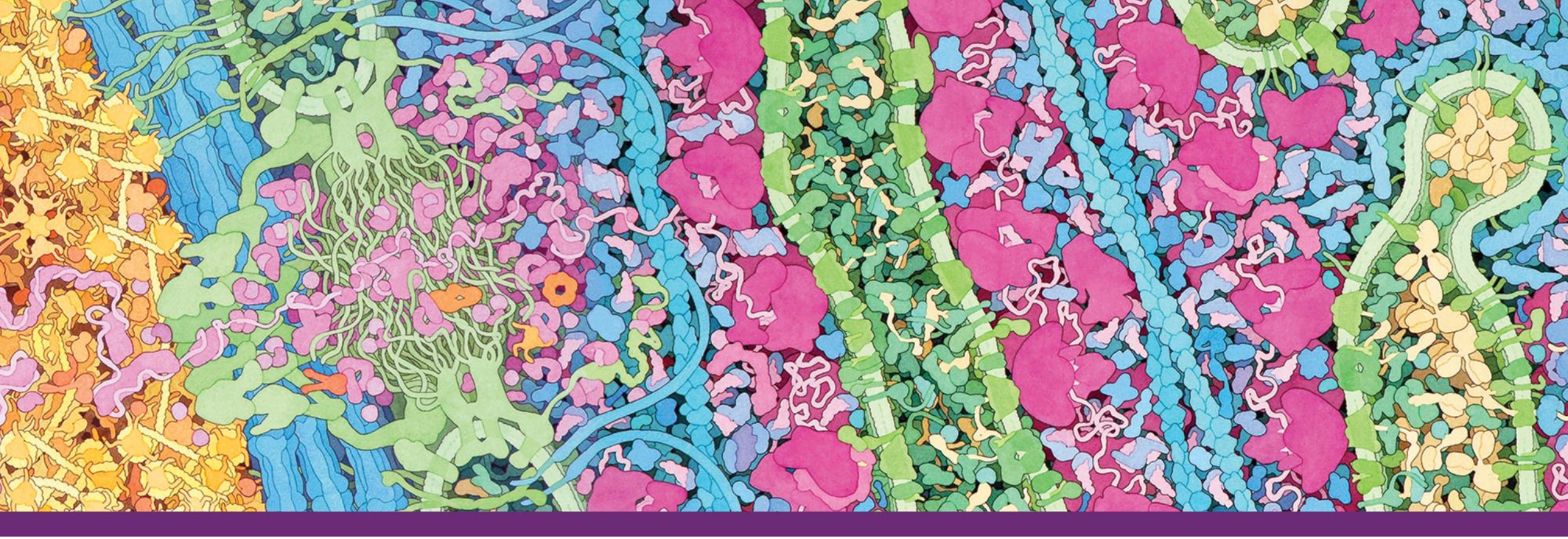
We will email you
the digital resources associated
with this session.

THANK YOU FOR ATTENDING!

- Purchase kits at **3dmoleculardesigns.com**.
- Use discount code **STATE22** to receive 20% off most items in our catalog. *Student Modeling Packs are not eligible for discount.*

Kits and models used in this session:

Biotechnology Kit



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