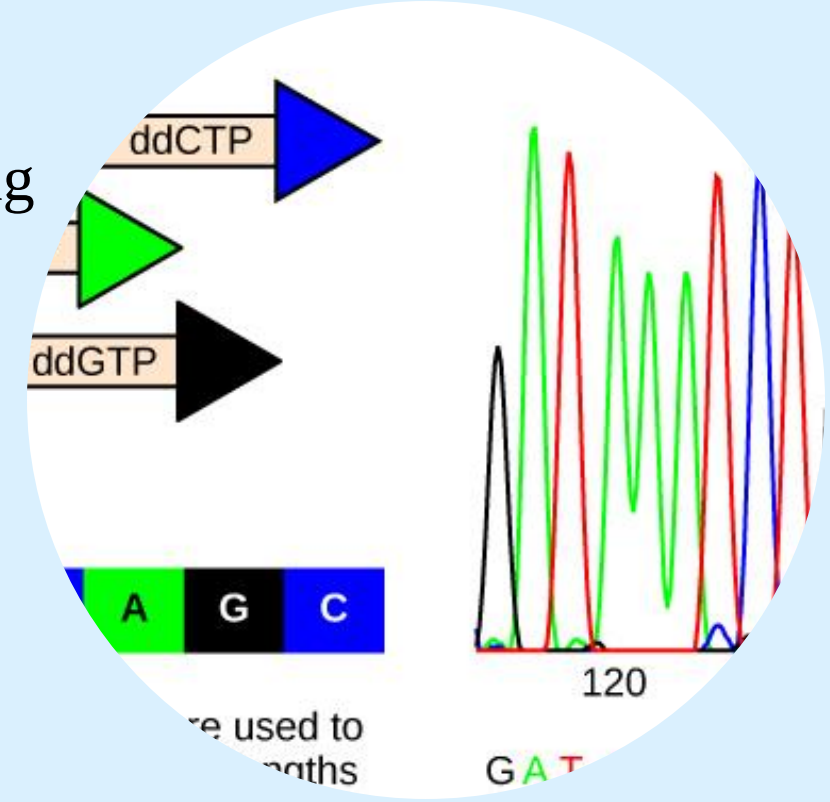
3d molecular designs
Transforming Science Learning

Getting the letters out

Modeling Sanger sequencing

Mark Arnholt
Science Educator
3D Molecular Designs



Mark Arnholt

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3D Molecular Designs

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

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

Workshop Learning Goals

- 1) Understand how DNA replication is completed in vitro
- 2) Model the use of ddNTPs in genetic sequencing
- 3) Show how a sequence can be obtained
- 4) Understand that science is a process
- 5) Have fun!

Intended Grade Level(s):
This activity has been written at the high school level. However, this activity can be tailored to your student population and differentiated as needed.

Prior Student Knowledge Required:
It is expected that students understand the following concepts prior to working through this model:

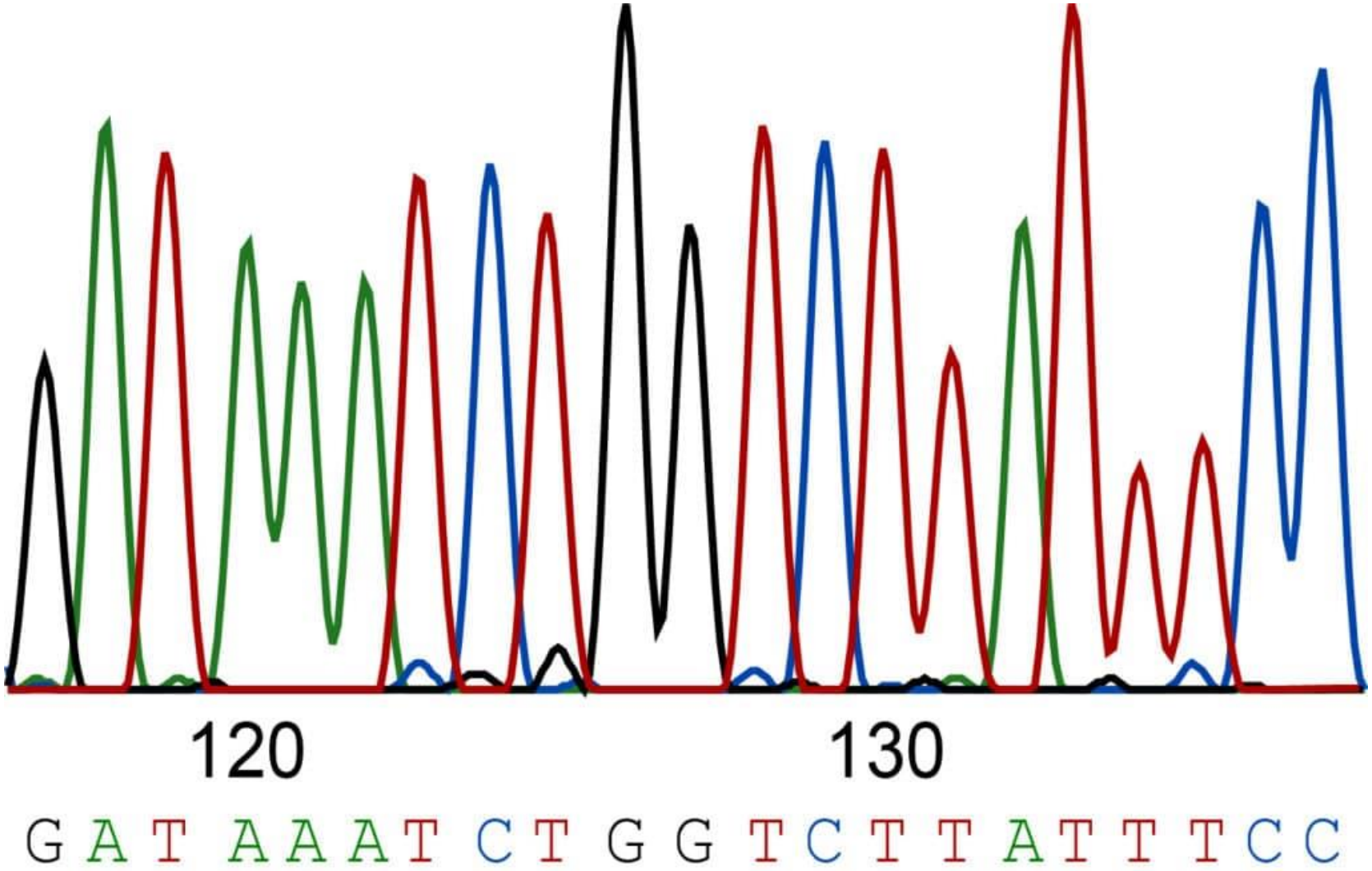
- Hydrogen and covalent bonding
- DNA structure
- Deoxyribose structure compared to ribose structure
- Chromosomes, genes, and alleles
- Base pairing rules
- DNA replication
- PCR Reactions

Key Words:

- Sanger Sequencing
- DNA
- Gene
- Nucleotides (dNTP)
- Base pairing
- Complementarity
- DNA polymerase
- Hydrogen bonds
- Covalent bonds
- Taq polymerase
- Buffer
- Thermal cycling
- Primers
- Denaturation
- Annealing
- Extension
- Electrophoresis
- Next Generation Sequencing

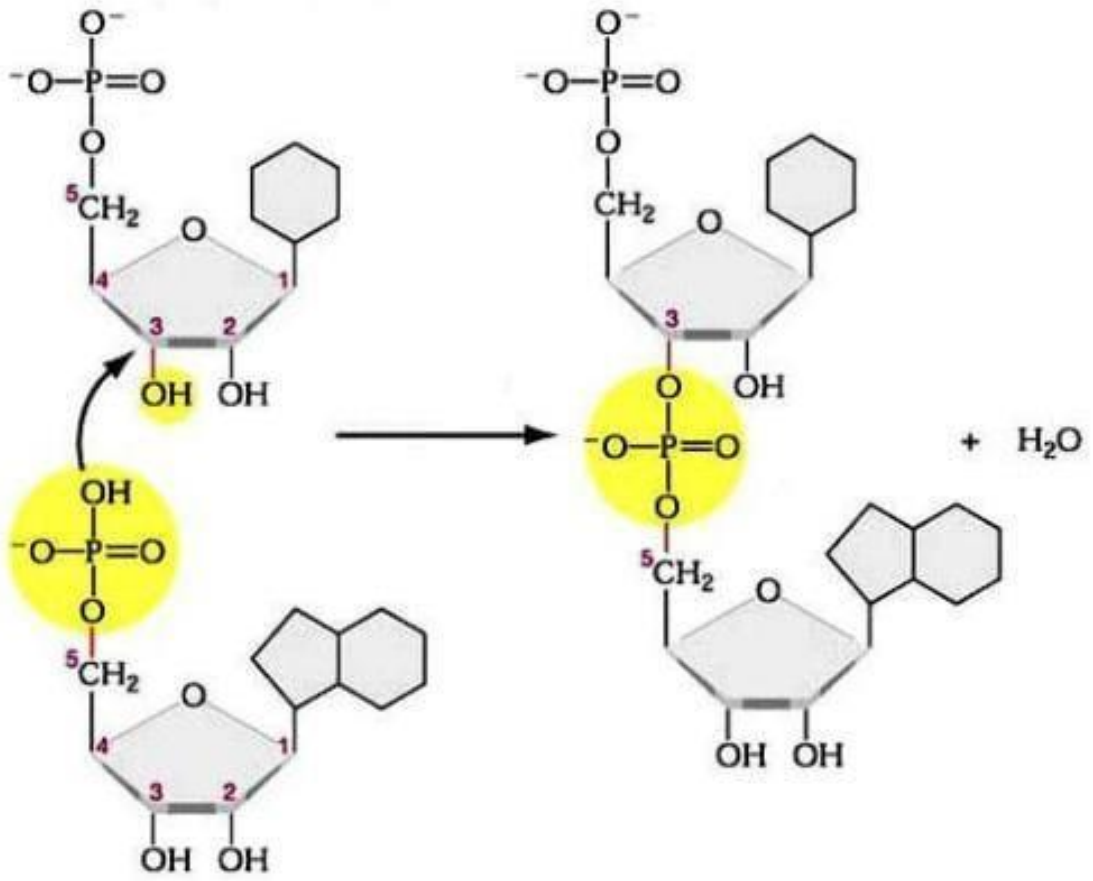
Biotechnology Kit

How do we get this data?

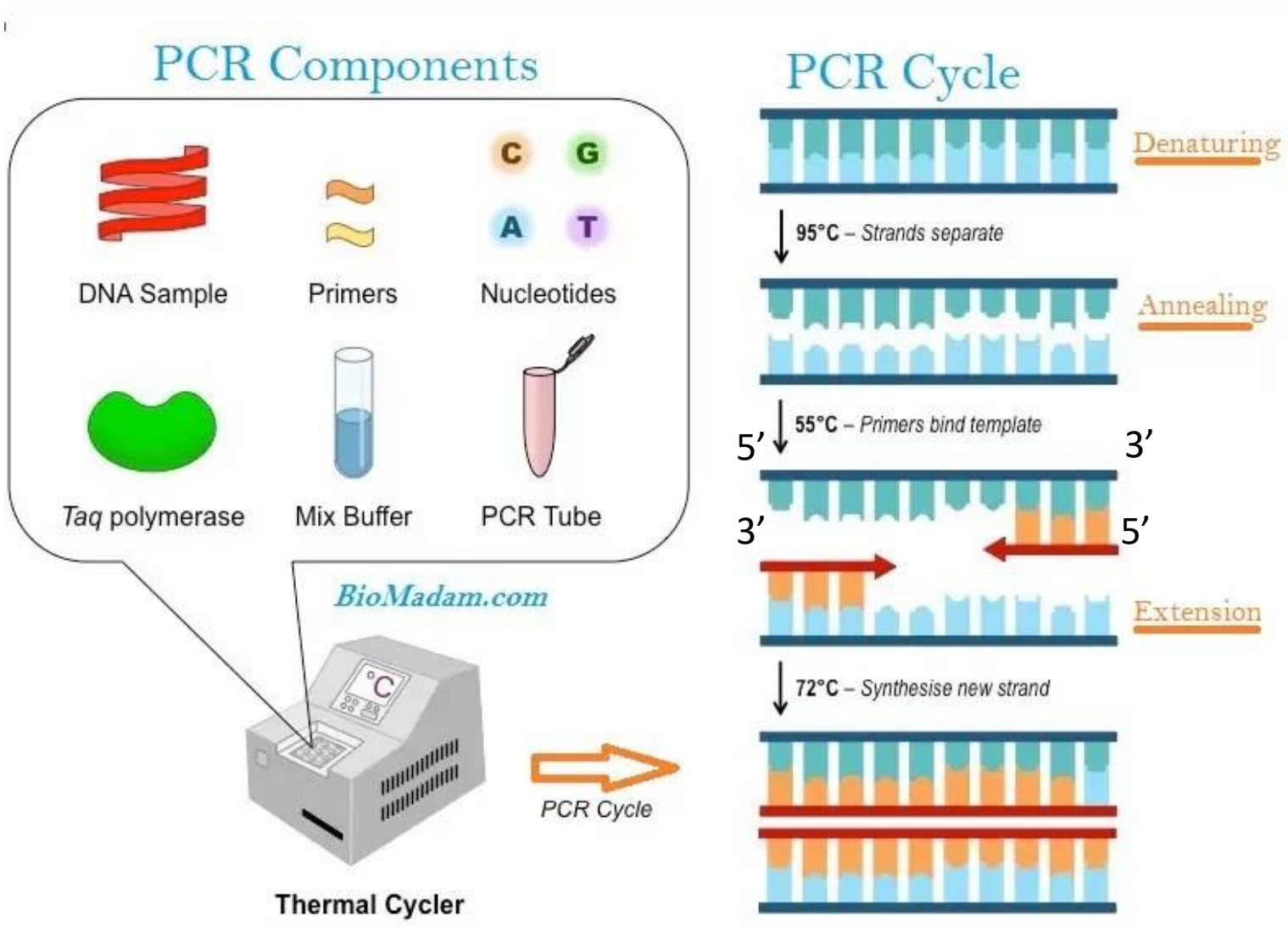


Review of DNA

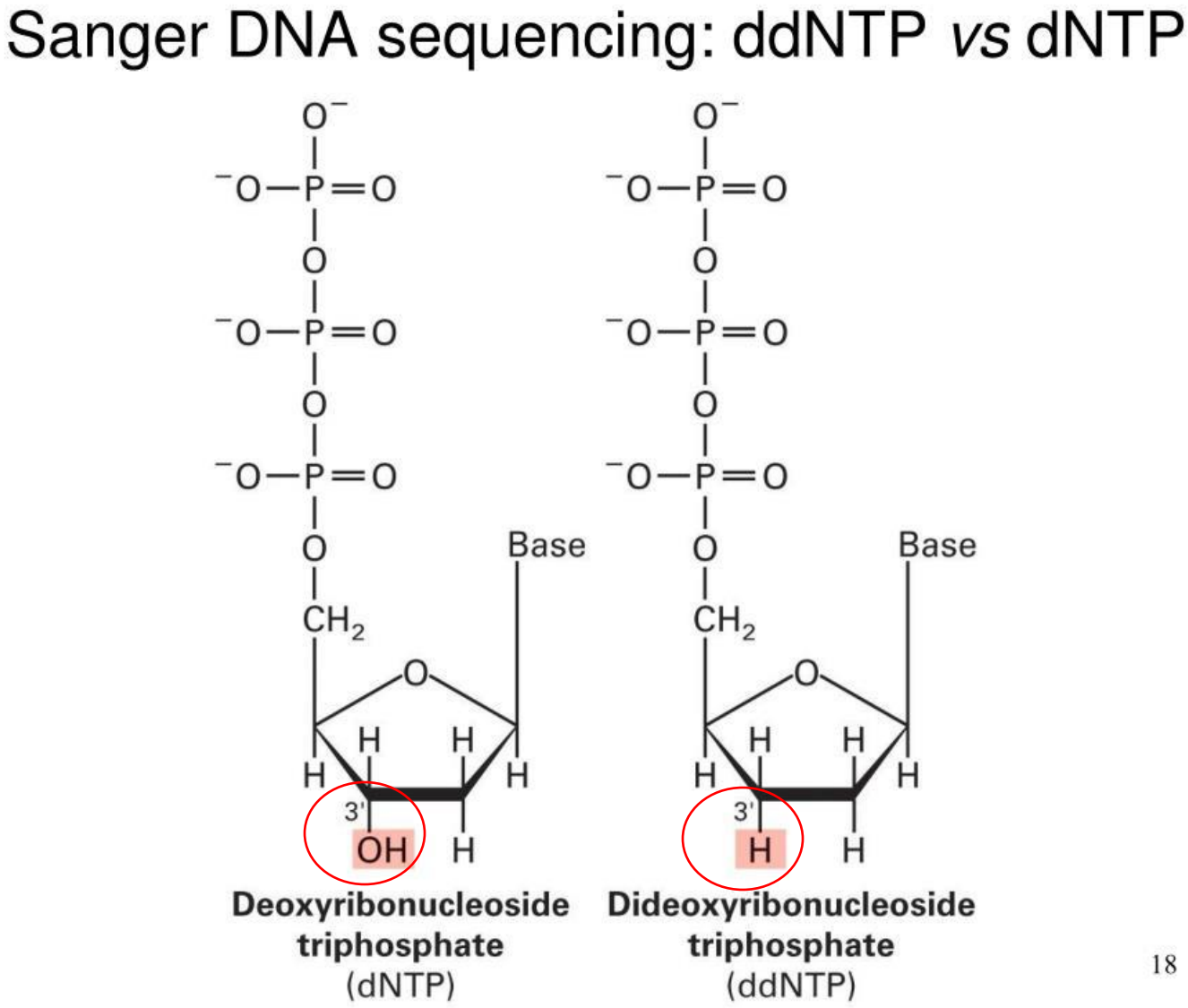
- DNA replication is dependent upon an OH- on the 3’Carbon of Deoxyribose.



PCR basics


PCR Components
DNA Sample
Primers
C
G
A
T
Nucleotides
Taq polymerase
Mix Buffer
PCR Tube
BioMadam.com
°C
PCR Cycle
Thermal Cycler
PCR Cycle
Denaturing
95°C – Strands separate
Annealing
55°C – Primers bind template
5’
3’
3’
5’
Extension
72°C – Synthesise new strand

ddNTP's


Sanger DNA sequencing: ddNTP *vs* dNTP
Base
3'
OH
Deoxyribonucleoside triphosphate (dNTP)
Base
3'
H
Dideoxyribonucleoside triphosphate (ddNTP)
18

Let's Model!

1) Grab needed materials
   - Nucleotide bags
   - DNA template
   - Primer Sequences; placed to the side in a stack

2) Using the Unknown Sequence as a guide and following nucleotide base pairing rules:
   - First add the primer to the 3' end of the unknown sequence.
   - Grab one color ddNTP and add it to your nucleotide mix
   - Then, add free nucleotides to the 5' end of the primer.

3) When you pull a dideoxynucleotide, ddNTP, where N stands for any one of the nucleotide bases, then that strand is complete. When you arrive at your ddNTP, look away from your pile and grab one at random. When your sequence ends with a ddNTP, separate that single strand from the Unknown Sequence.

4) Repeat this process, starting first with adding a primer to the 3' end of the Unknown Sequence and then add nucleotides as you did in step three

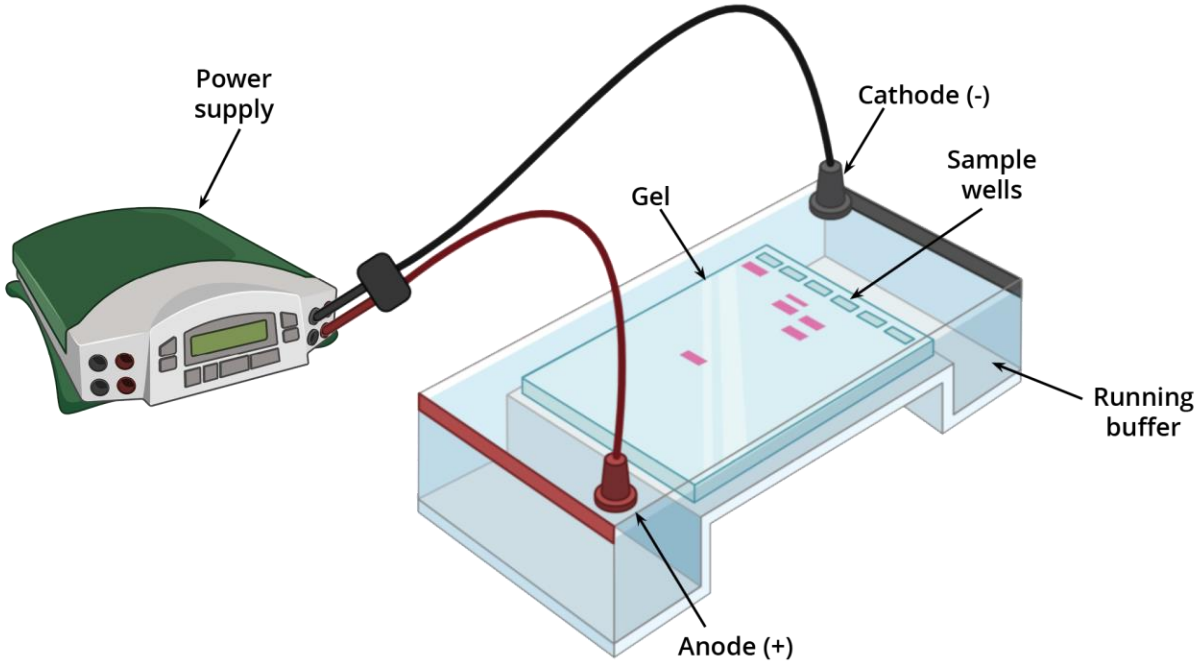
Each group is assigned a single ddNTP

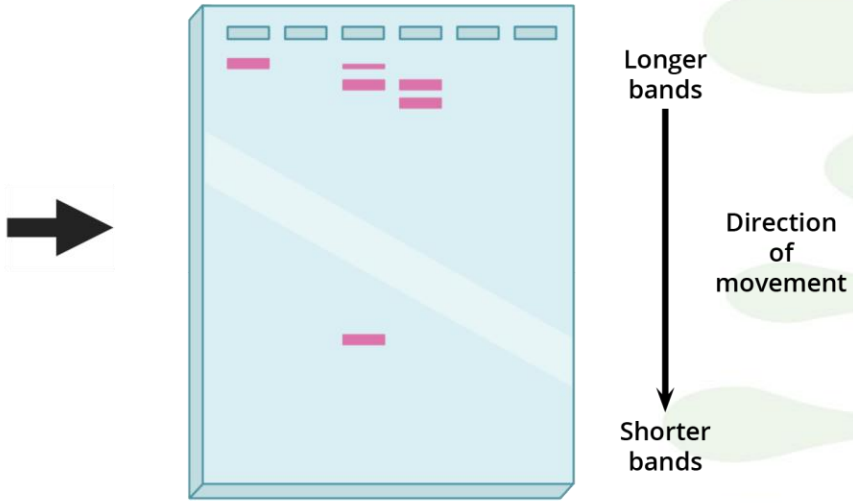
The initial protocols for Sanger sequencing required 4 different PCR reactions. All 4 PCR products were run in a gel and the data was pieced together.

We will simulate a gel by taking your individual products and spreading them out on a table. Longest strands at the top, shortest strands at the bottom.

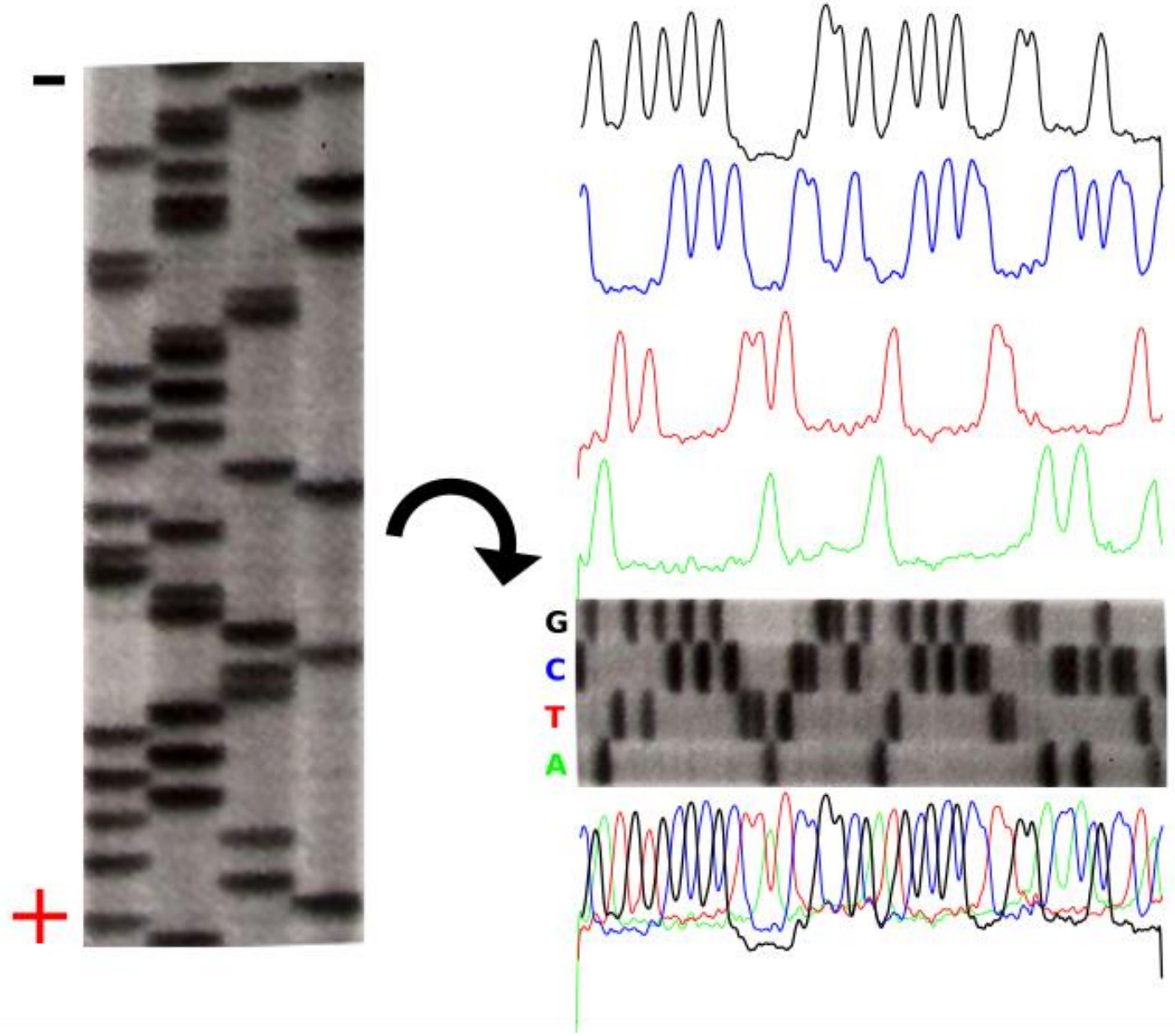
Together, we can determine the genetic sequence for the gene!

Gel Electrophoresis

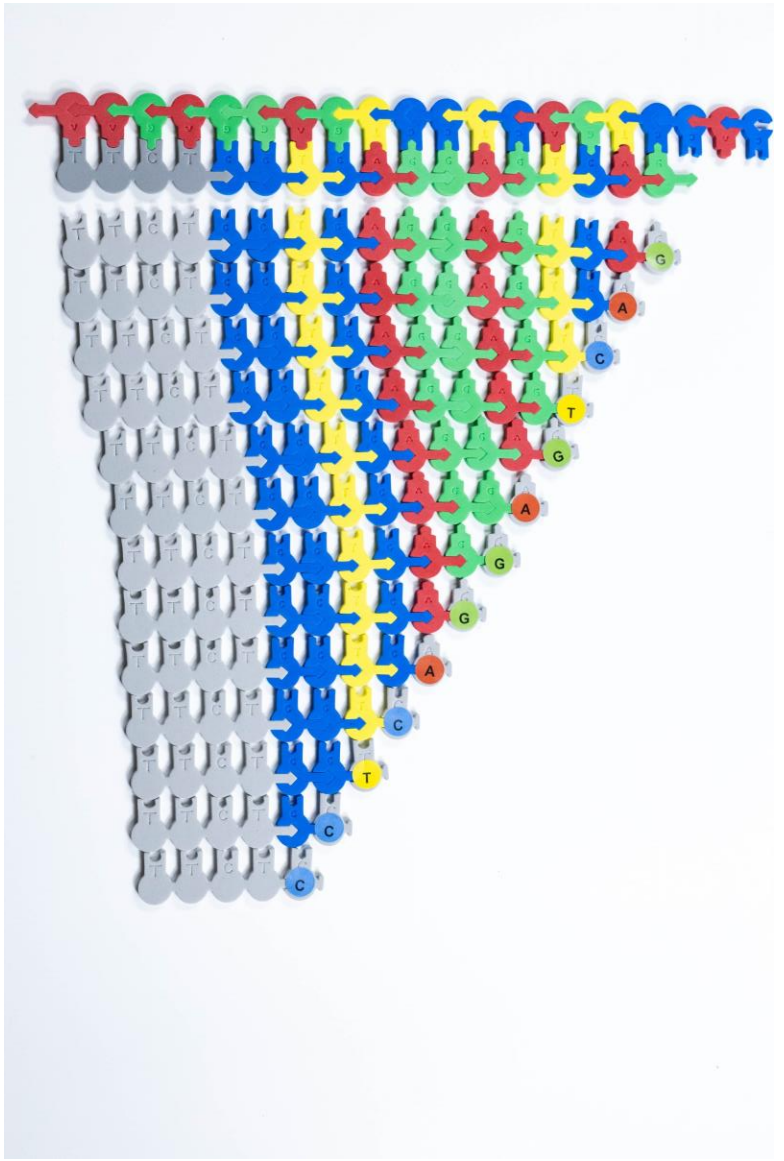




Dyes added to ddNTP's allow us to visualize



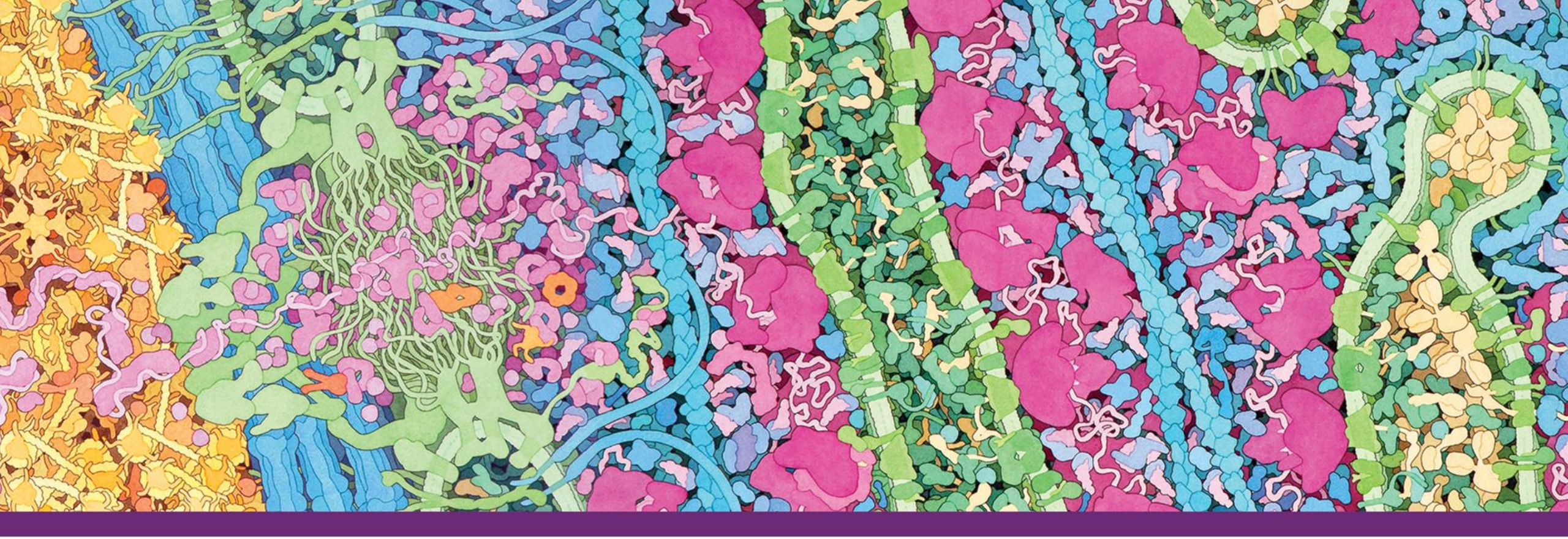
Our end goal...



Workshop NGSS Connections

SEPs	CCCs	DCIs
Asking Questions	**Patterns**	**HS-LS1-1**
Developing and Using Models	**Cause and Effect**	**HS-LS1-2**
Constructing Explanations	**Scale, Proportion, and Quantity**	**LS-1A**
	Stability and Change	**LS-1C**
		LS-1D

Scan me!



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