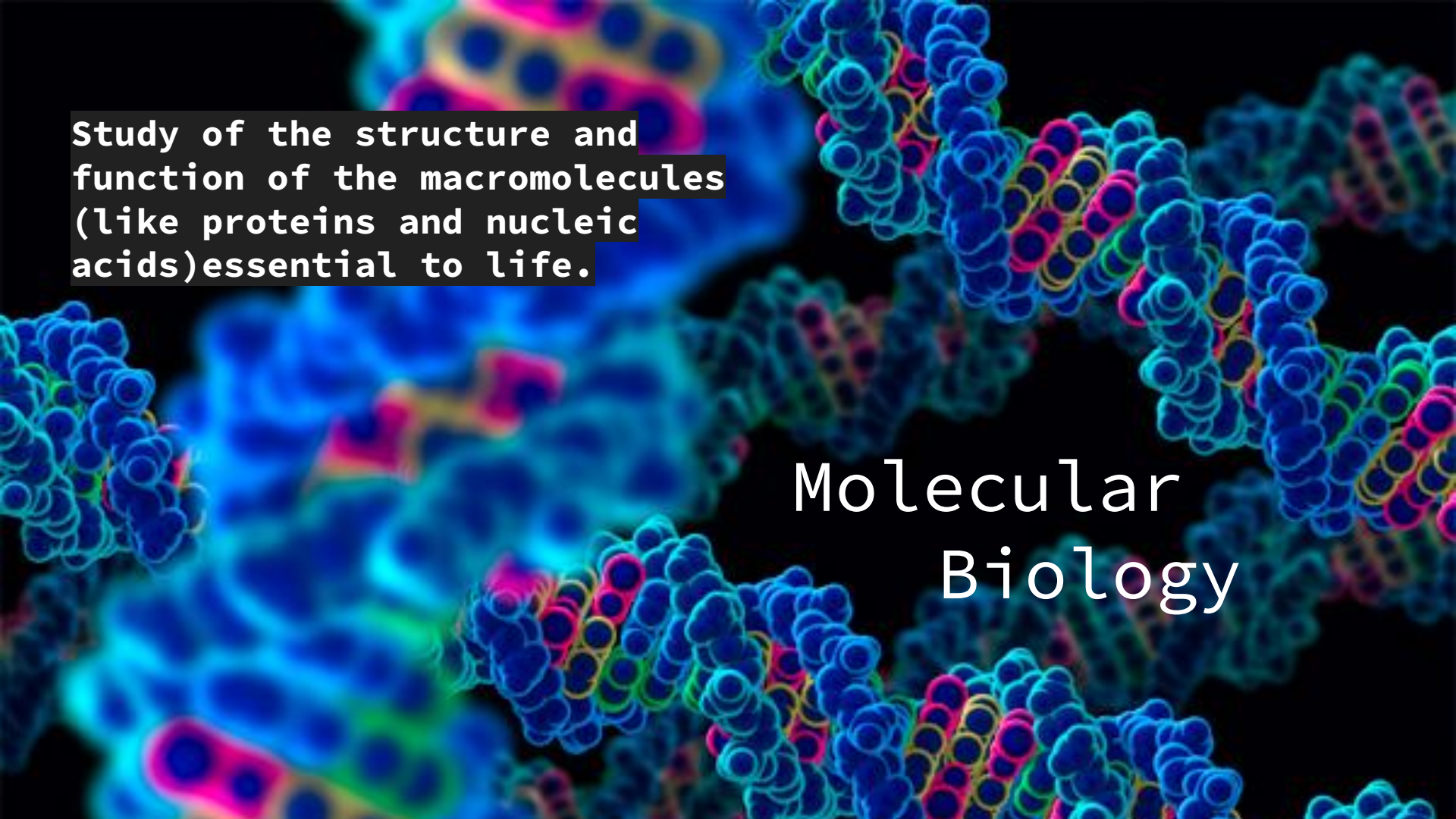
Summer@TutorsForChange2022 – Biology and Design Camp – Day 2

Tutors For Change

Molecular Techniques

SAPTA SENSE
UNIVERSITY OF ROCHESTER

Presented by University of Rochester iGEM Team 2022



Study of the structure and function of the macromolecules (like proteins and nucleic acids)essential to life.

Molecular Biology

So... What do Molecular Biologists even do?

Well, a majority of what they do revolves around working in the lab!

This includes:

- Coding
- Writing procedures for experiments
- Compiling research and results
- And take a wild guess…

A glimpse into where we do our molecular biology work:

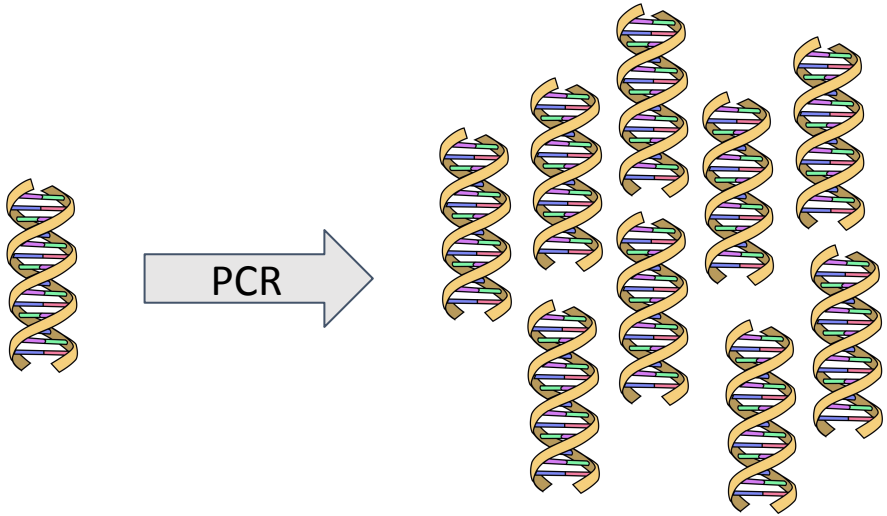
What are some common techniques and instruments molecular biologists use in the lab?

Polymerase Chain Reaction

(PCR)

What is PCR and why do we need it?

PCR is used to **amplify** DNA.



Using a special machine called a **thermal cycler**, we can make millions of copies of a single DNA molecule!

https://www.thermofisher.com/order/catalog/product/A24811

How does the thermocycler make copies of DNA?

double-stranded DNA

STEP 1:
Denature

STEP 2:
Anneal

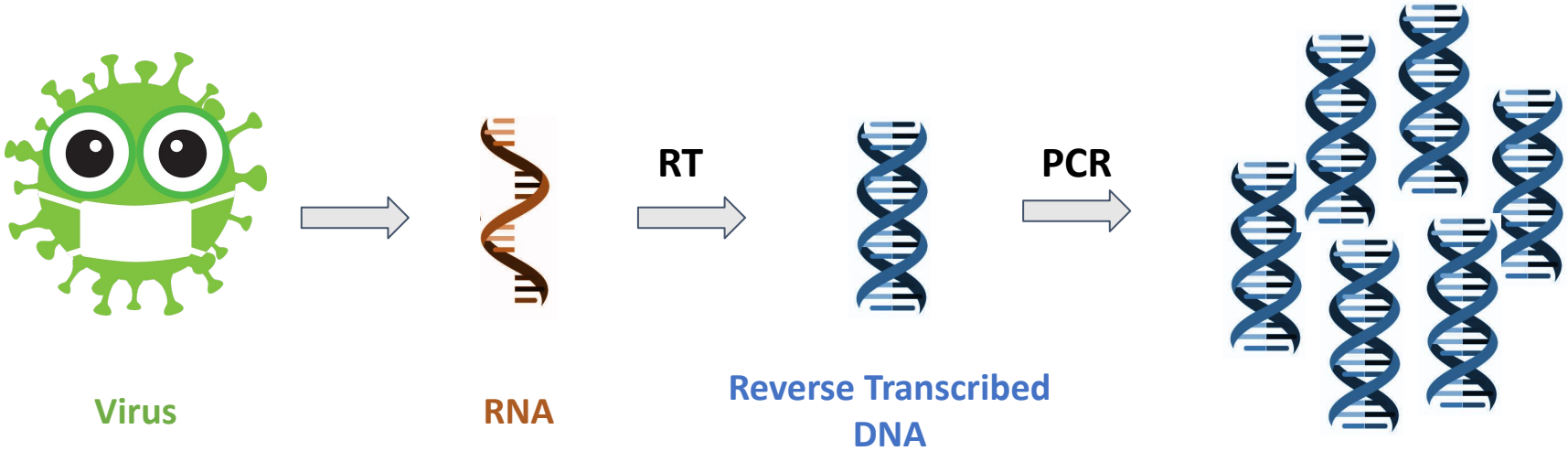
STEP 3:
Extend

source:https://www.yourgenome.org/facts/what-is-pcr-polymerase-chain-reaction

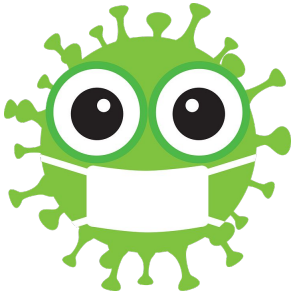
Does this work the same way as a COVID PCR Test?

There are many different types of PCR! Most COVID-19 PCR tests rely on a special type known as **Reverse Transcription (RT) PCR.**

RT-PCR is similar to regular PCR except that the starting material is **RNA** instead of **DNA**.



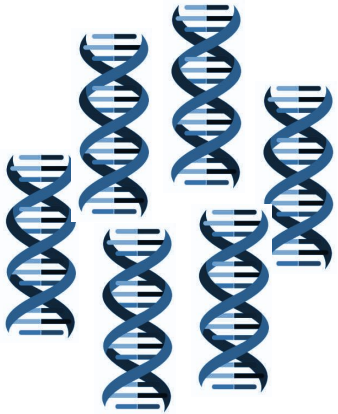
Sample Collection (Nasal Swab)



SARS-CoV-2 Virus

SARS-CoV-2 RNA

RT-PCR Test



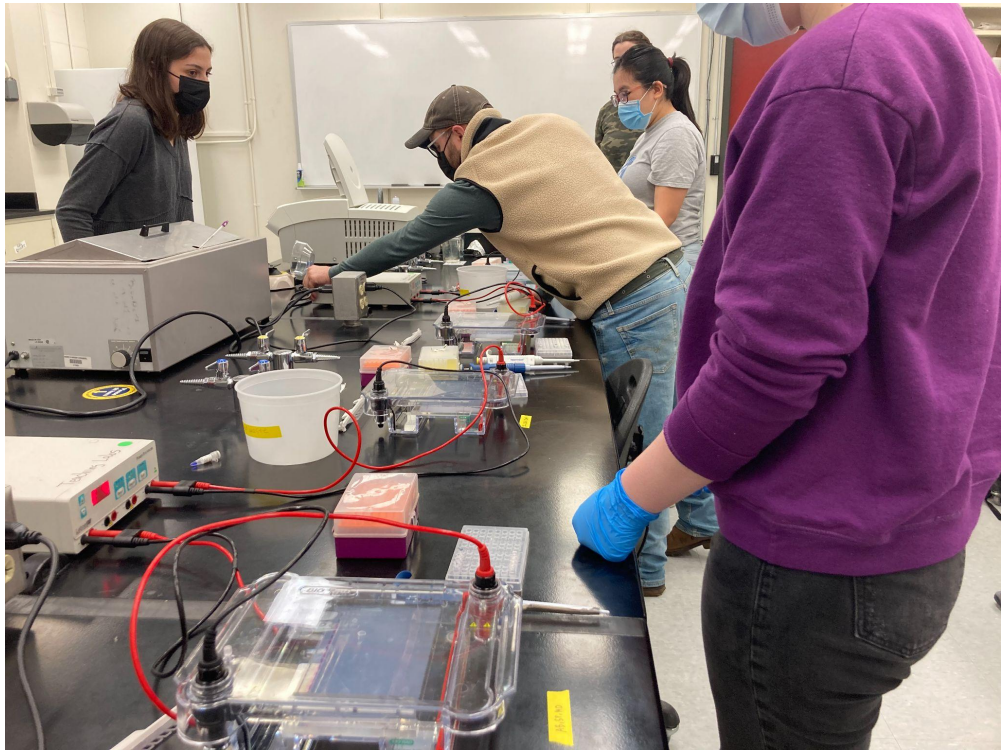
SARS-CoV-2 DNA

POSITIVE FOR COVID-19!!!

COVID PCR tests provide a reliable way to determine if someone has COVID-19 or not.

This is just one example of how scientists have used molecular biology to solve problems in our world!

Gel Electrophoresis

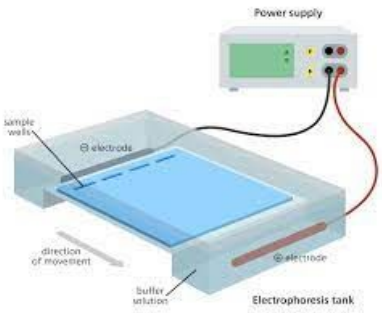


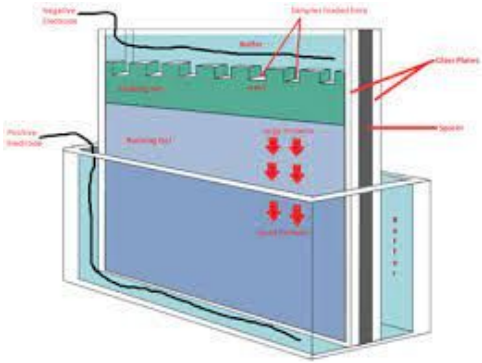
What is gel electrophoresis?

Gel Electrophoresis is used to separate DNA, RNA, or proteins based on their size!

Components…

- Gel
  - Made of materials like agarose or polyacrylamide
  - Has wells to place your sample
  - Has pores
- Tank
  - Holds gel and buffer
  - Has ports for connecting to electrodes
- Buffer
  - Maintains pH
  - Carries electrical current
- Power Supply
  - Has negative (anode) at top, next to samples
  - Has positive (cathode) electrodes at the bottom





Why do we need it? What is used for?

So… why do these biological molecules just travel down the gel? **Take a guess!**

Hints:

*Electric current flows between the positive and negative electrodes

*DNA, RNA, and proteins (when treated with detergent) are **negatively** charged

*Like charges repel each other and opposite charges attract each other

*Samples are loaded on the same side as the **ANODE**

Why do we need it? What is used for?

Different ways to use electrophoresis…

To confirm the size of your desired DNA fragment from PCR

To determine the size of an unknown DNA/RNA fragment

To separate and identify proteins

To validate the sequence of a known or unknown fragment

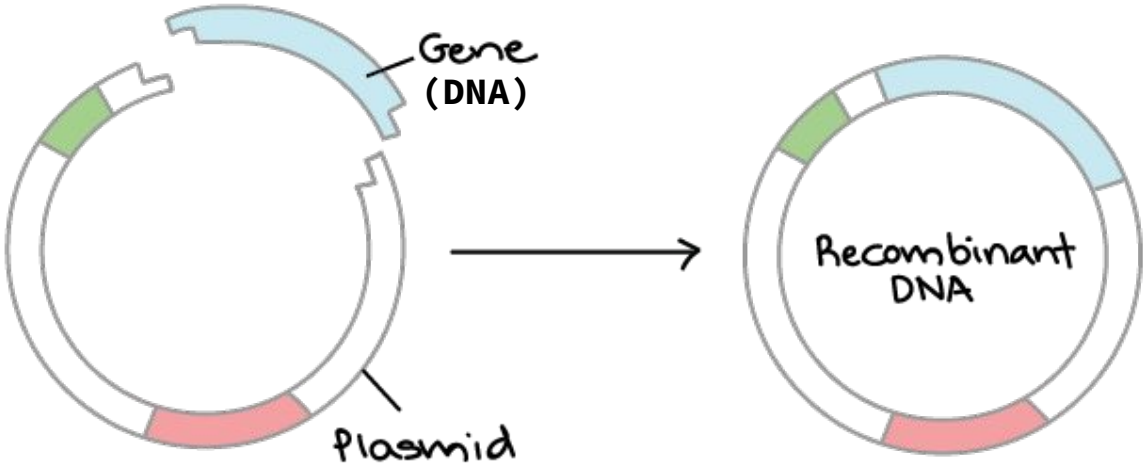
To observe the effects of gene expression over time

To analyze modifications of proteins

AND MORE!!

Recombinant DNA/Cloning

Recombinant DNA technologies can be used to manipulate and isolate DNA and provide scientists with many useful tools.



https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/overview-dna-cloning

For example, DNA can be inserted into a **plasmid** using special proteins called restriction enzymes. Scientists can then insert the plasmid back into bacteria to study the function of the gene!

Question…

Genetically Modified Organisms (GMOs)

GMOs are any type of organism that has undergone **genetic engineering**

Genetic engineering is defined as the manipulation of an organism's genome via 3 steps:

1. **Identify** a trait of interest from organism #1

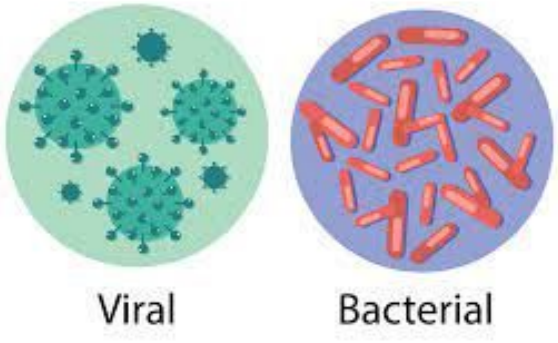
2. **Isolate** the genetic trait (aka find the gene) that codes for this trait from organism #1

3. **Insert** the desired trait into a new organism!

How are GMOs made?

In order to transfer genetic information from organism to organism, we need to apply **recombinant DNA technologies** to make a plasmid!

Once the plasmid with the recombinant DNA is made, it is inserted by means of a vector depending on the organism being modified and the method of modification

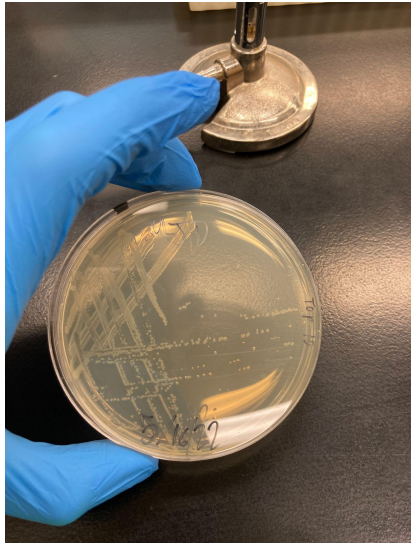


A vector is a particle used as a vehicle to carry recombinant DNA. This includes:

- Plasmids -Viral vectors -artificial chromosomes

How are GMOs made?

Some methods of inserting these vectors include…



Plants

- “Gene guns” that shoot metal nanoparticles coated with the vector into seeds

Bacteria/Plants

- Expose vector to organism by means of “infection”
    - Bacteria often need to be electrically/heat shocked in order to allow for **transformation**

Mammals

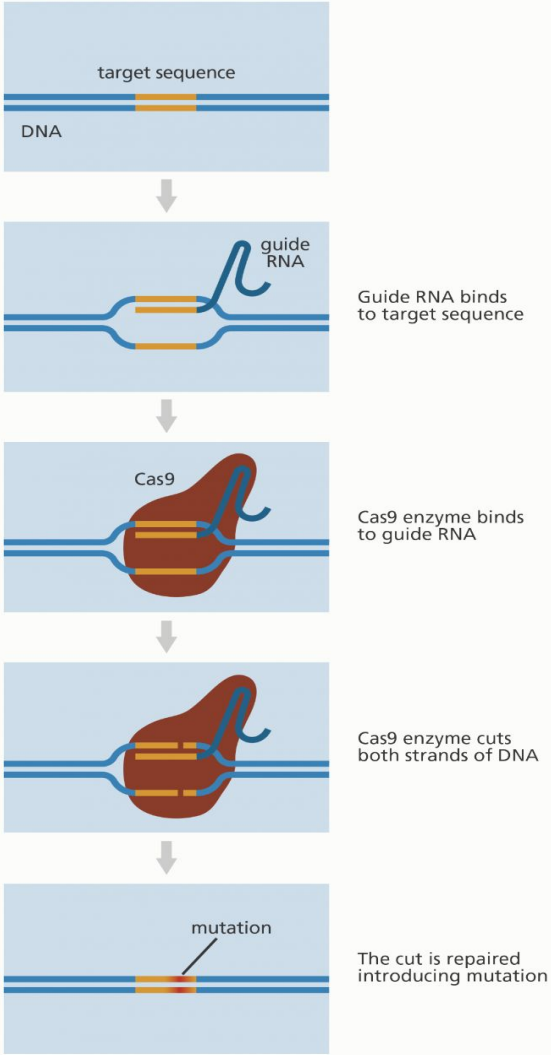
- Injection or IV exposure to vector
- Exposure of vector to cell sample that is then placed back into patient

Gene editing advances: CRISPR

CRISPR-Cas9 is a gene editing tool developed from bacterial immune systems.

CRISPR-Cas9 is an enzyme that acts like the “scissors” of the DNA, and is led to it via a guide RNA. Today other enzymes have been developed, like Cas13, to refine this mechanism. It is one of the leading research focuses of cancer treatment!

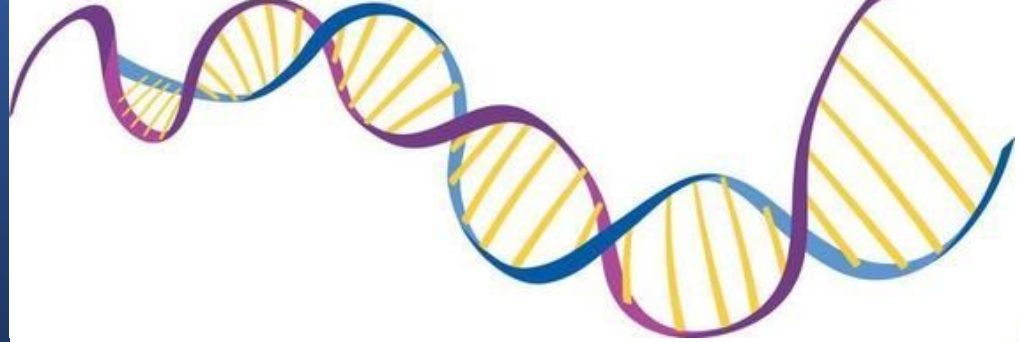
Fun Fact, the CRISPR part of the name is only there to specify the enzyme, and does not mean we need this type of genetic code in order to cut out genes from other organisms!



Questions?

At home DNA Extraction Activity!

At home DNA Extraction Activity!



Materials:

- ½ cup of Isopropyl (rubbing) alcohol
- ½ cup of water
- 2 teaspoons of dish detergent
- 2 plastic cups
- 1 ziploc baggie
- 1 coffee filter
- 1 coffee stirrer or chopstick

Link to instructions:
https://www.genome.gov/Pages/Education/Modules/StrawberryExtractionInstructions.pdf

THANK YOU!!

Tutors For Change

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If you enjoyed today's session, be sure to head back on Friday for more biology and fun interactive activities!!