

Genetic Engineering

Using Biology to Solve Real-World Problems

INTRODUCTION :: Real-World Problem Example

PROBLEM: Plastic Waste Build-Up

- Plastic takes hundreds of years to break-down naturally in the environment
- Plastic pollution builds up rapidly in global ecosystems
- No easy, quick way to get rid of plastic waste



INTRODUCTION :: Biodegradation

How does organic waste breakdown in nature?

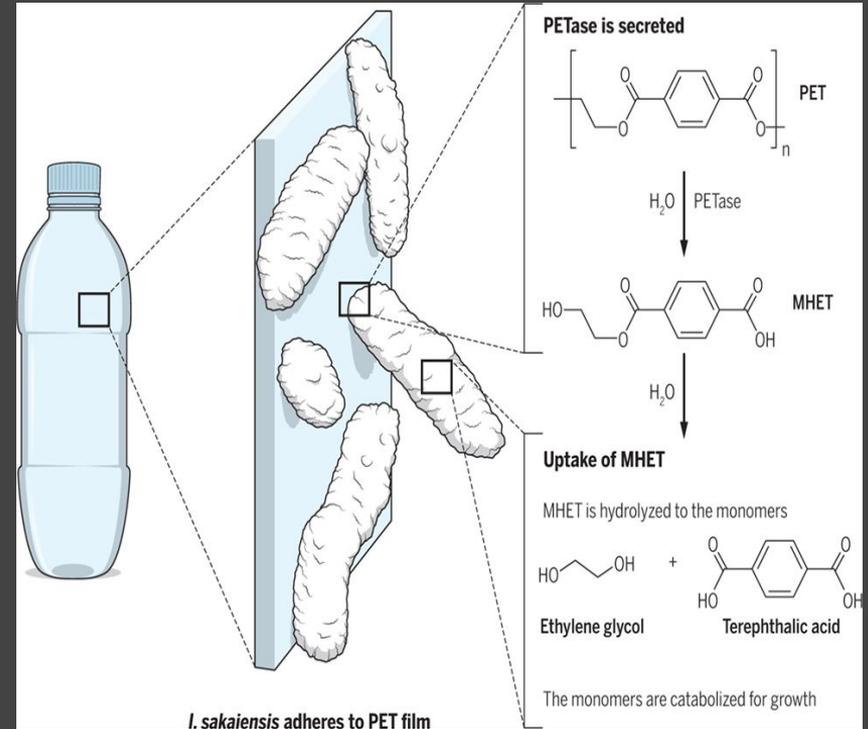
- Microbes like bacteria and fungi do most of the work
- Over time, microbes slowly disintegrate organic material via a biochemical process called **BIODEGRADATION**



INTRODUCTION :: Plastic Degradation

Can plastic be biodegraded?

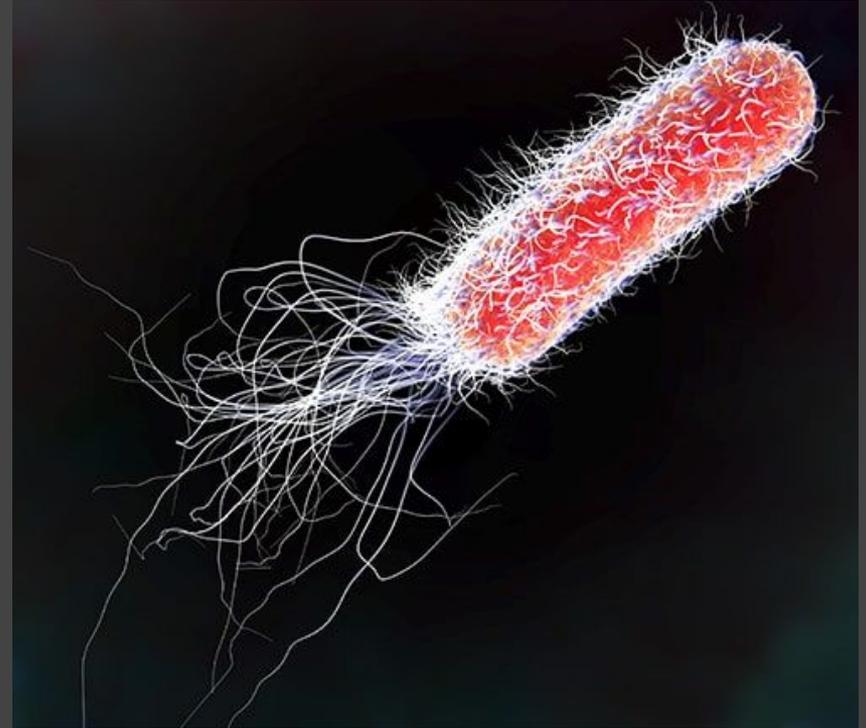
- Most microbes have not evolved ability to biodegrade plastic
- In 2016, a new species of bacteria was discovered to produce enzymes needed to degrade **PET plastics** (type of plastic used in water bottles)
- **PROBLEM:**
 - Species is not well-studied and degrades PET slowly



INTRODUCTION :: Genetically Engineering a Solution

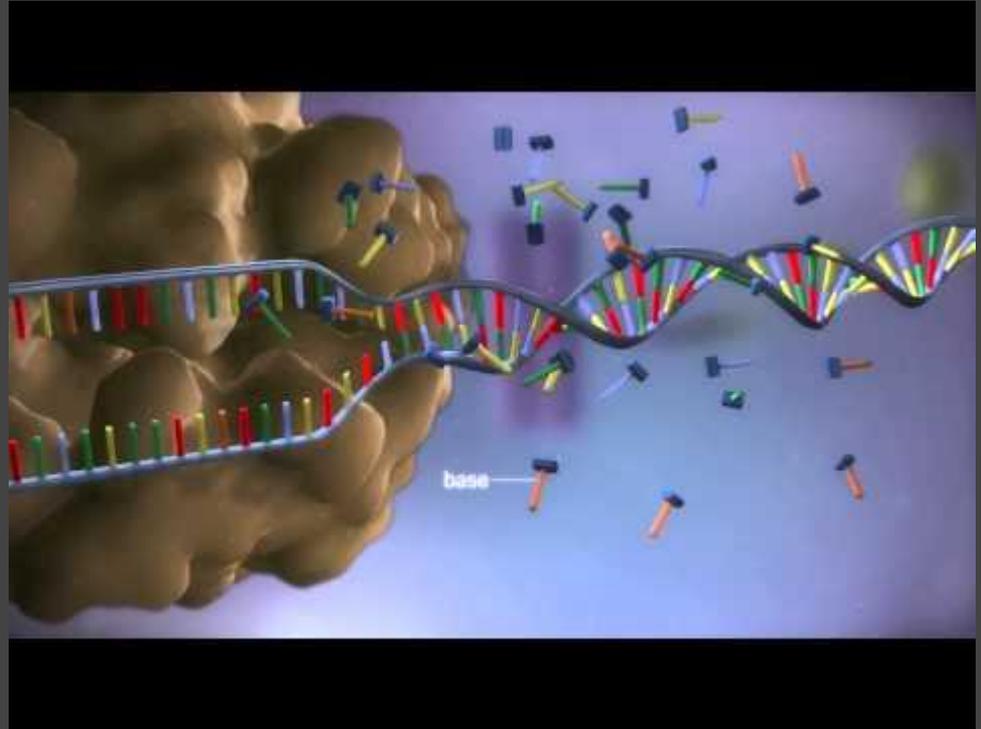
How could we genetically engineer a solution?

- Isolate the plastic-degradation genes from new bacteria species
- Insert genes into a well-studied and faster growing species of bacteria (i.e. *E. coli*)
- Newly-inserted genes should give *E. coli* the ability to also degrade PET plastics



THEORY :: The Central Dogma of Biology

- The big theme of biology
- Explains how cells work, and how we can manipulate them
- Describes the flow of information in a cell
- DNA --> RNA --> proteins



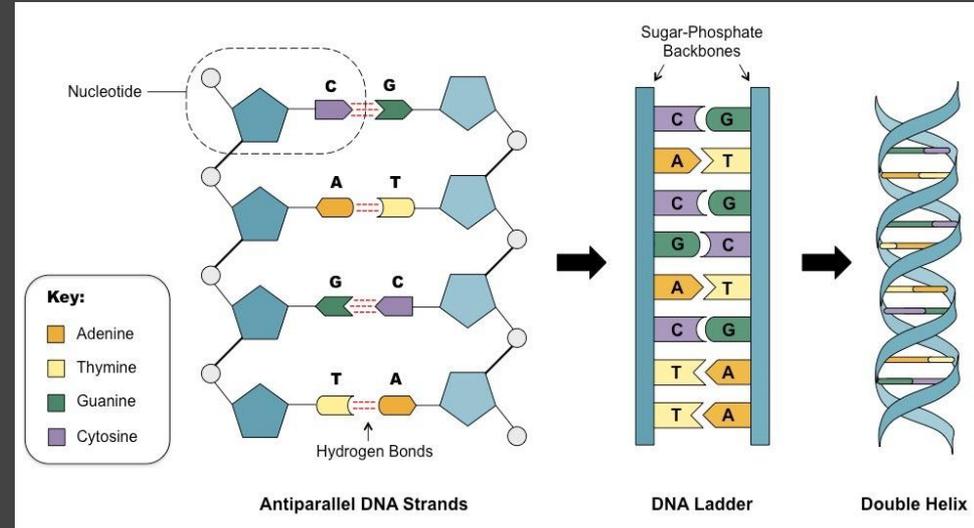
THEORY :: What is DNA?

- DNA stands for DeoxyriboNucleic Acid
- All living things have DNA
- DNA is a double stranded helical structure
- The building blocks of DNA encode instructions for the cell



THEORY :: The building blocks of DNA

- Consists of 4 bases that we call nucleotides
 - Adenine (A)
 - Guanine (G)
 - Cytosine (C)
 - Thymine (T)
- Nucleotides bind to each other in a specific manner
- Instructions for a cell come from the specific order of nucleotides in DNA
 - Similar to how the alphabet can “code” for english words and sentences



THEORY :: Complementary Strands - how DNA works

- DNA strands bind to each other in a **complementary** manner
- This means that the nucleotides on one strand bind to their partner on the other strand
- From one strand of DNA, a cell can construct the complementary strand of DNA
- This process is used for more than just DNA!

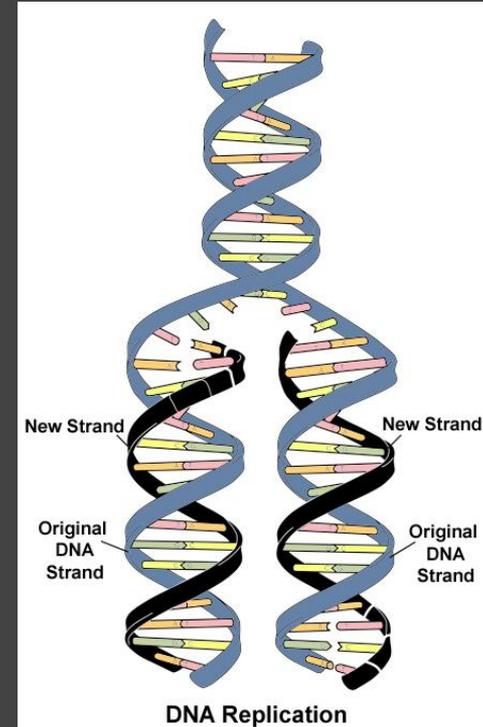
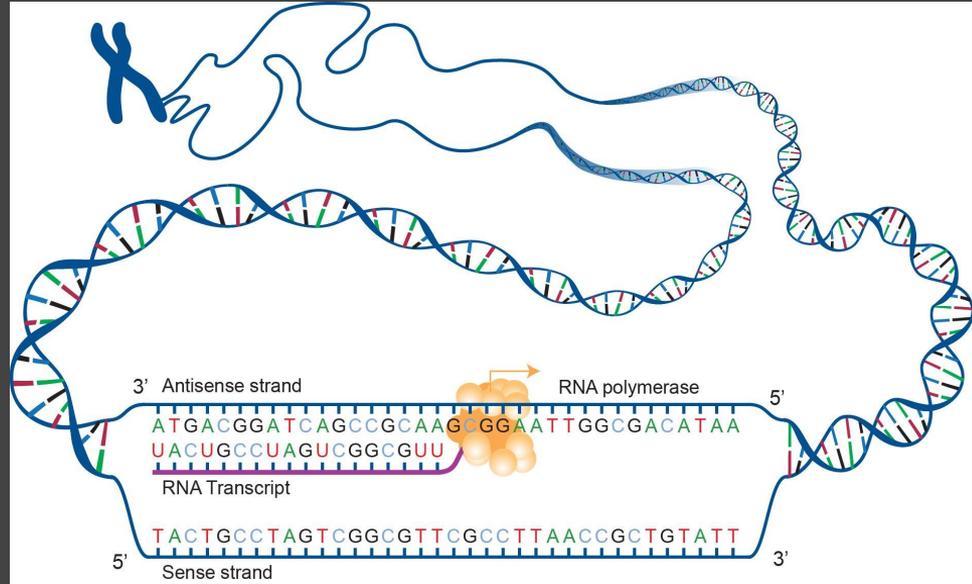


Image adapted from: National Human Genome Research Institute.

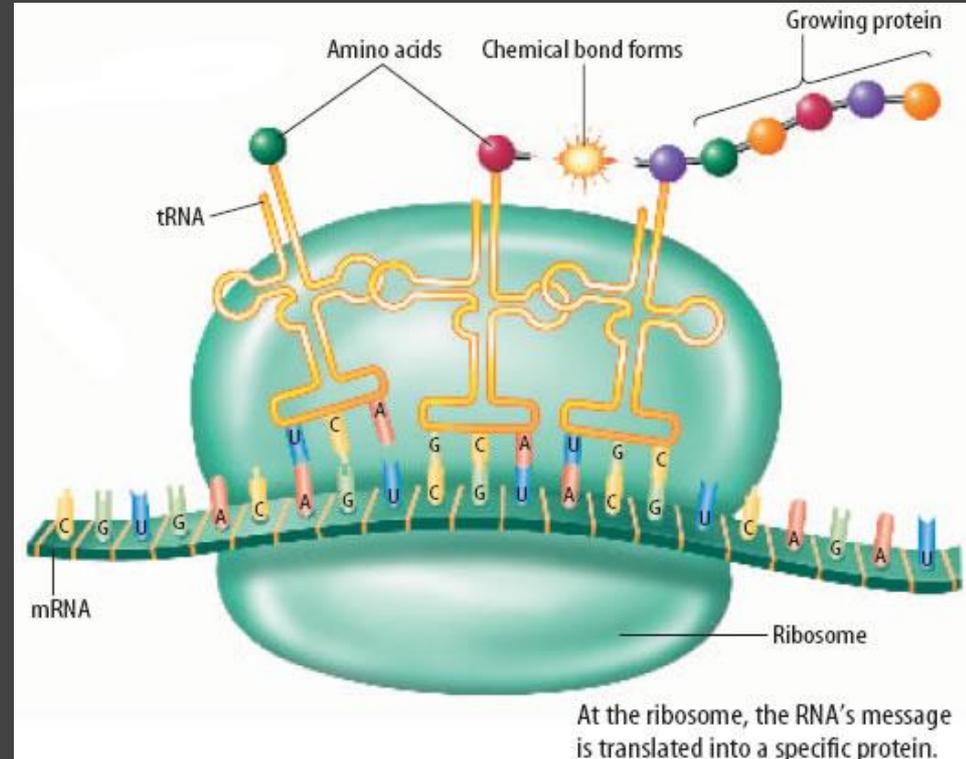
THEORY :: What is RNA?

- A cell can't understand the code in DNA
- In order to use instructions, a cell converts a **template** DNA sequence into a complementary RNA sequence
 - This process is called **transcription**
- RNA stands for **R**ibo**N**ucleic **A**cid
- RNA uses the same nucleotides as DNA with one change; Thymine is replaced with Uracil
- Where DNA acts as the code for the cell, RNA acts as the message or signal for a cell to make something



THEORY :: What are proteins?

- Proteins are the tools that carry out the functions of a cell
- Proteins are made from 20 amino acids
- Once a cell has RNA, the cell can start to make proteins using the message in the RNA
 - This process is called **translation**
- Translation is done by the ribosome
- Using the RNA message, the ribosome translates the **genetic code** into amino acids, which make up proteins



ACTIVITY :: Decode the Secret Message

Decode the Secret Message from the Template DNA Sequence using what you have learned about the Central Dogma of Biology!

Template DNA Sequence:

TCG ACG TAA CTT TTG ACG CTT TAA TCG CCG GCG CTT CGC TGG

		Second letter					
		U	C	A	G		
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	Third letter	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }		U C A G
	A	AUU } AUC } Ile AUA } AUG Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }		U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }		U C A G

ACTIVITY :: Decode the Secret Message

Decode the Secret Message from the Template DNA Sequence using what you have learned about the Central Dogma of Biology!

Template DNA Sequence:

TCG ACG TAA CTT TTG ACG CTT TAA TCG CCG GCG CTT CGC TGG

mRNA Sequence:

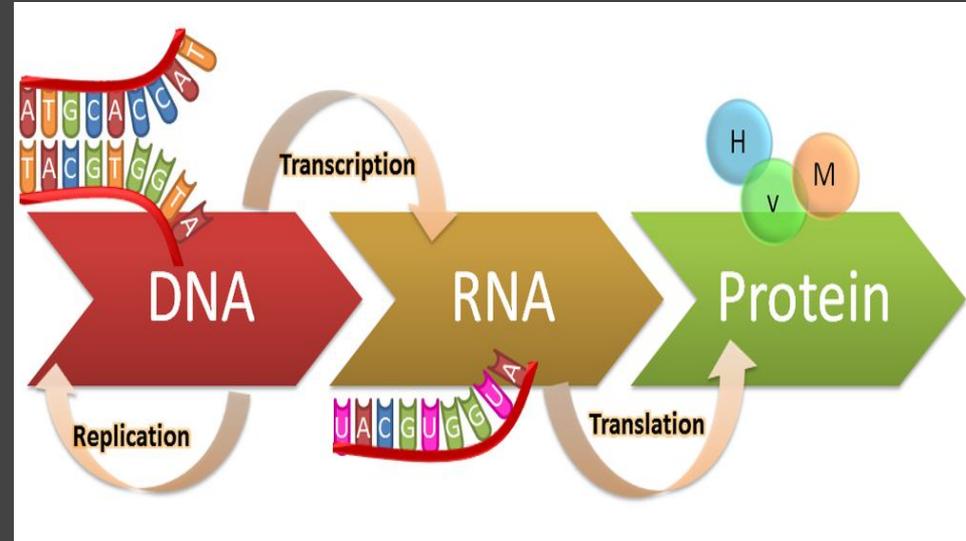
AGC UGC AUU GAA AAC UGC GAA AUU AGC GGC CGC GAA GCG ACC

Amino Acid Sequence:

S C I E N C E I S G R E A T

THEORY :: From proteins back to DNA

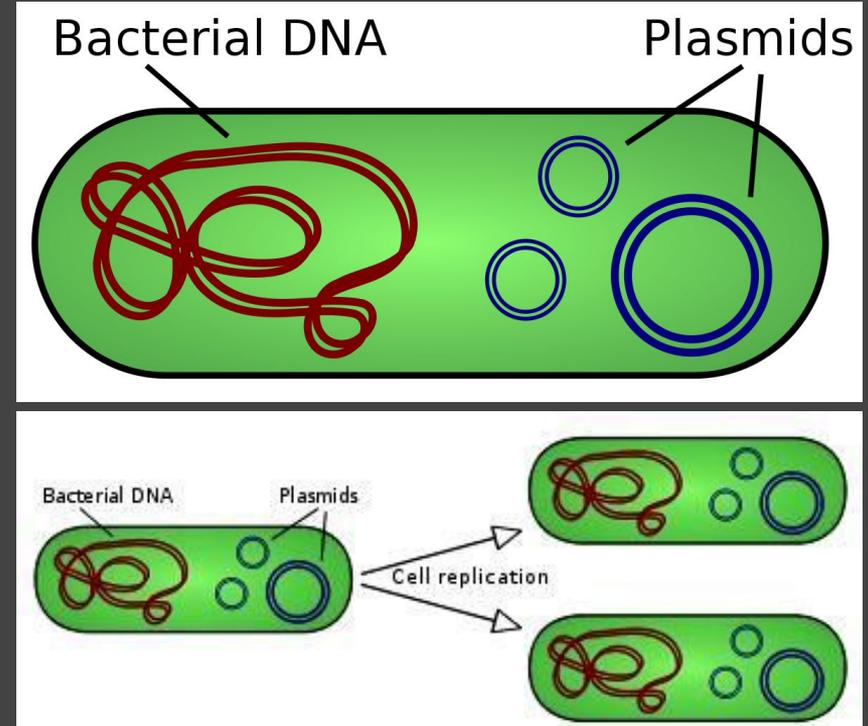
- Now that we understand the central dogma of biology, we can use it
- See a problem that requires a specific protein to fix
 - A cell digesting a new food source
- In order to make the new protein, we need the right message of RNA
- In order to have the right message of RNA, the cell needs the right code in DNA
- We can insert DNA into a cell in order to give it new proteins and new functions



APPLICATION :: Plasmids

What are plasmids?

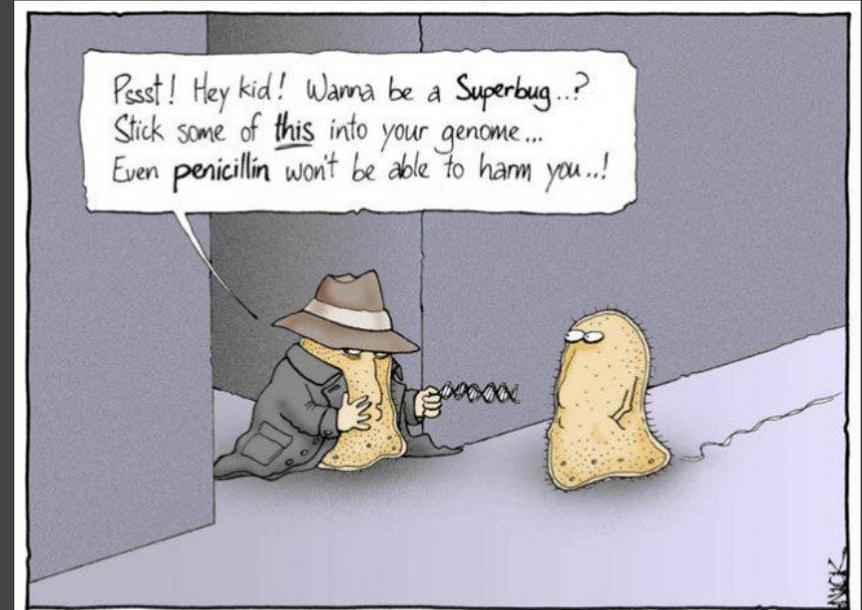
- Small, circular pieces of DNA
- Separated from the rest of a cell's DNA
- Replicates independently
- Super important for genetic engineering
- Can act as a “flash drive” for genetic information



APPLICATION :: Using Plasmids

Why are plasmids useful?

- Plasmids carrying new foreign genes can be transferred into bacteria cells
- Newly transferred plasmid can give cells new abilities and functions (depending on what genes are on the plasmid)
- Allows biologists to introduce new genes into various cells



It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

APPLICATION :: Constructing Plasmids

How to insert genes into plasmids?

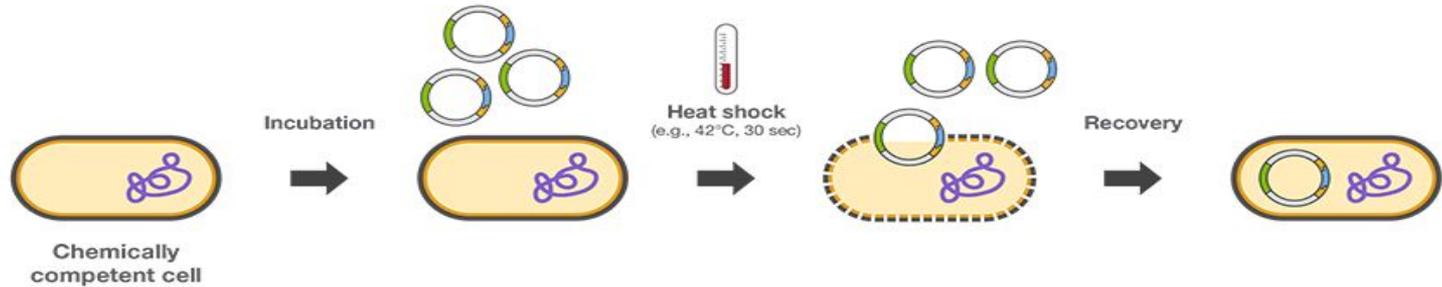
- 1) Isolate the DNA sequence for the **gene** of interest
- 2) Start with an empty plasmid template called a **vector**
- 3) Use **restriction enzymes** to cut complementary **sticky ends** on the gene and vector
- 4) Use an enzyme called **ligase** to fully bond the gene to the vector

The diagram, titled "Steps in Cloning a Gene", shows a circular plasmid vector and a linear DNA fragment. The plasmid has a single-stranded sequence of nucleotides (ACGG and TCGA) on its ends. The DNA fragment has complementary single-stranded sequences (GATCTGGC and ACGTCTAG) on its ends. The text "Cohesive 'sticky' ends" is written above the diagram. Below the diagram is a control bar with "Play" and "Pause" buttons, a progress indicator, and "Audio" and "Text" buttons. A text box at the bottom explains: "Each fragment has a single-stranded sequence of nucleotides on its ends that is capable of hybridizing with DNA that has been fragmented with the same restriction enzyme." The copyright notice "Copyright © The McGraw-Hill Companies, Inc." is at the bottom right.

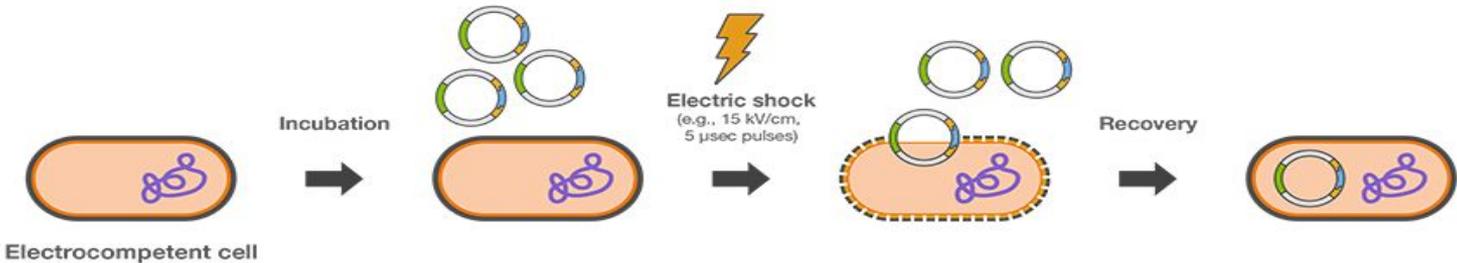
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APPLICATION :: Getting Plasmids into Cells

Chemical transformation



Electroporation



ACTIVITY :: Making Glowstick Plasmids

