STO-134

Genetic Engineering: Way to Grow

Teacher Information

science take·out
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Summary

How can genetic engineering be used to help a child who is not growing normally? Students conduct simulated tests to determine what is causing the child's slow growth. They model how genetic engineering could be used to create bacteria that produce human growth hormone.

Core Concepts

- Lack of human growth hormone results in abnormally slow growth.
- Hormone replacement therapy may be used to treat human growth hormone deficiency.
- Genetic engineering can be used to create bacteria that produce human growth hormone.

Time Required

Two 40-minute class periods

Kit contains

- Tube of Jose's blood plasma
- Hormone Test Kit containing hormone test strip and instructions
- Paper plasmid DNA strip
- Paper human DNA strip
- BAM enzyme "card" transparency
- "Bacteria" plastic bags
- "Protein" paperclips

Teacher Provides

- Safety goggles
- Clear adhesive tape (not glue)
- Scissors

Optional

- Page iii of the teacher information provides an optional Part 4 that can be used as an extension for the kit activities. Internet access is needed.
- Page iv of the teacher information provides an optional reading on how growth hormone affect the body.

Warning: Choking Hazard

This Science Take-Out kit contains small parts. Do not allow children under the age of seven to have access to any kit components.

Reusing *Genetic Engineering* kits

Kits may be refilled and reused. Allow approximately 15-30 minutes for refilling 10 student kits. Teachers will need to instruct students on how to handle clean-up and return of the re-usable kit materials. For example, teachers might provide the following information for students:

Discard	Return to kit
• Used hormone test strip • Used Plasmid and Human DNA strips • Used Hormone Fast-Test Kit Instructions	• Tube of Jose's blood plasma • BAM enzyme card (transparency) • "Bacteria" plastic bags • "Protein" paperclips

Refills for *Genetic Engineering* kits are available at www.sciencetakeout.com. The **10 Kit Refill Pack** includes the following materials:

- Instructions and Quick Guide for refilling kits
- 15 ml Jose's Blood Plasma
- 10 hormone test strips
- 10 Human DNA strips
- 10 Bacterial Plasmid DNA strips
- 10 Hormone Fast-Test Kit Instructions
- 10 BAM enzyme card transparencies
- 1 graduated transfer pipet (for refilling the microtubes)

Optional Extension

Part 4: Explore More

Select one of the following exploration activities. Complete the activity and be prepared to briefly explain one important thing that you learned and one interesting thing that you learned from your exploration.

1. Using the Internet, search for the phrase "genetic engineering products" and make a chart like the one below that briefly describes 15 products of genetic engineering and their potential uses.

Product	Description of what the product does

2. The Internet has many websites that offer human growth hormone (HGH) for sale. These websites claim that HGH can be used to:
   - Help short children grow taller to improve their social standing or athletic ability.
   - Reverse the effects of aging.
   - Increase muscle size and muscle performance for athletes and body builders.

   Do you think that people should be allowed to use HGH for these purposes? Explain why or why not. Include scientific evidence for the effectiveness of HGH and possible side effects of HGH in your explanation. This website makes a good starting point for your research:http://www.webmd.com/fitness-exercise/guide/human-growth-hormone-hgh.

3. Scientists have used genetic engineering to produce "genetically modified" plants and animals that are used for human food.
   - What are examples of genetically modified foods?
   - What are the pros (benefits) and cons (risks) of genetically engineered foods?

4. In the future, scientists hope that they can use "gene therapy" to insert normal genes into the cells of people who have inherited defective genes that do not make essential proteins.
   - How is gene therapy different from enzyme replacement therapy?
   - What are some examples of diseases that may, in the future, be cured by gene therapy?
   - Describe the pros (benefits) and the cons (risks) of gene therapy?

Optional Reading

How Growth Hormone Affects the Body

Growth hormone is a protein of about 190 amino acids that is synthesized and secreted by the pituitary gland. Growth hormone has two types of effects—direct effects and indirect effects.

- **Direct effects** are the result of growth hormone binding to its receptor on target cells. For example, fat cells have growth hormone receptors. Growth hormone stimulates fat cells (adipocytes) to break down lipids (fats) and it suppresses the ability of fat cells to take up and accumulate circulating lipids.

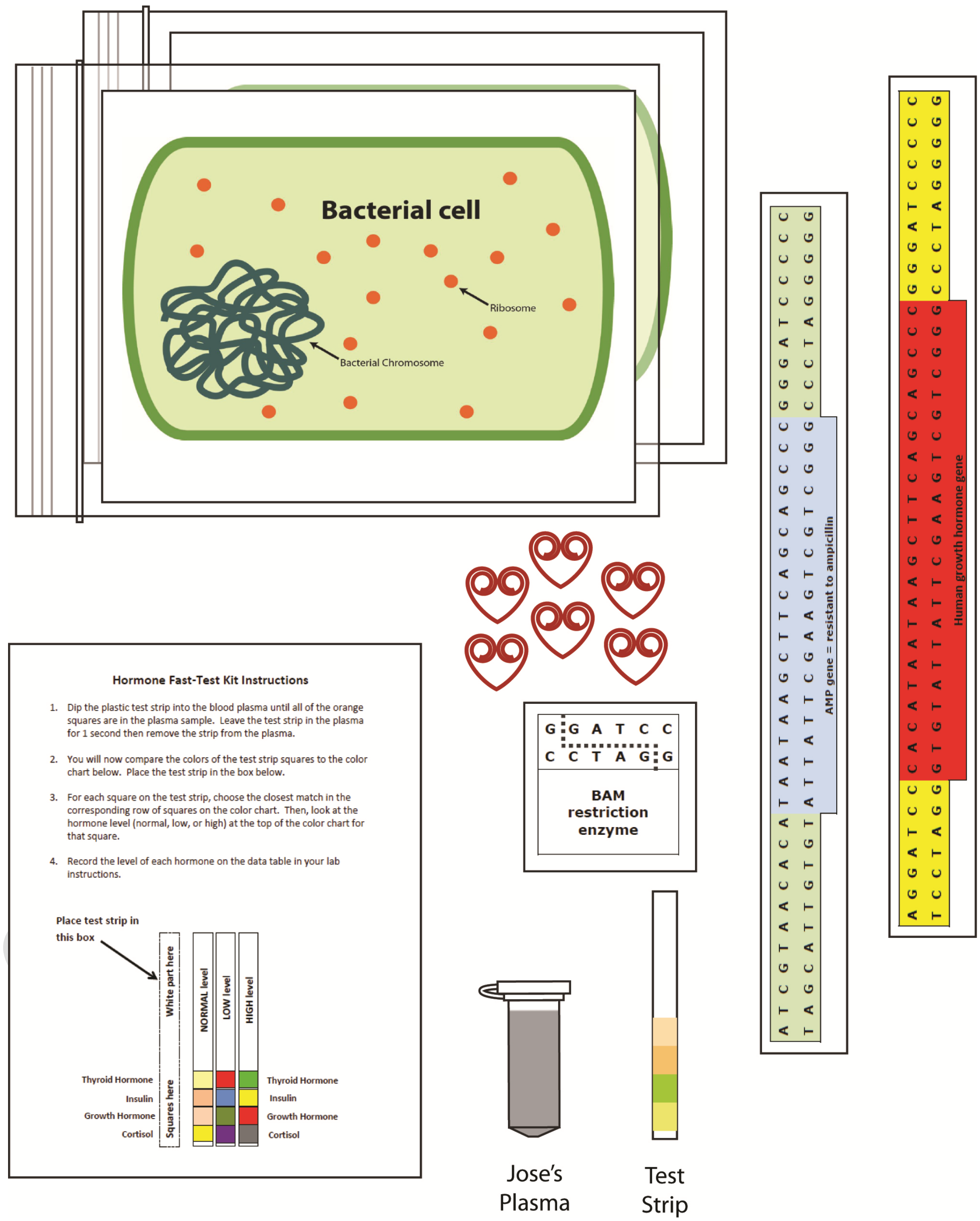
- **Indirect effects** are due to insulin-like growth factor (IGF), a hormone that is secreted from the liver and other tissues in response to growth hormone. IGF stimulates production of cartilage cells (chondrocytes), resulting in bone growth. IGF also stimulates the production of myoblasts, the cells that form muscles.

Growth hormone produced using recombinant DNA technology has led to many different applications. Growth hormone is used as a drug for both humans and animals.

Human growth hormone is commonly used to treat children of significantly short height. There is concern that this practice will be extended to treatment of essentially "normal" children, to enhance their height. Similarly, growth hormone has been used by some people to enhance their athletic performance. Several companies sell anti-aging cosmetics with human growth hormone. However, there are concerns that use of growth hormone may have unpredictable side effects and health risks, particularly for people whose bodies are making normal amounts of growth hormone.

Bovine (cow) growth hormone is currently approved and marketed for increasing milk production in dairy cattle. There is no evidence that drinking milk from cattle treated with bovine growth hormone poses a risk to human health. However, many people are wary of drinking milk from cows treated with growth hormone.

Kit Contents Quick Guide



Read these instructions before using Science Take-Out kits

Parental or Adult Supervision Required

This kit should be used only under the supervision of an adult who is committed to ensuring that the safety precautions below, and in the specific laboratory activity, are followed.

Safety Goggles and Gloves Strongly Recommended

We encourage students to adopt safe lab practices, and wear safety goggles and gloves when performing laboratory activities involving chemicals. Safety goggles and gloves are not provided in Science Take-Out kits. They may be purchased from a local hardware store or pharmacy.

Warning: Choking and Chemical Hazard

Science Take-Out kits contain small parts that could pose a choking hazard and chemicals that could be hazardous if ingested. Do not allow children under the age of seven to have access to any kit components. Material Safety Data Sheets (MSDS) provide specific safety information regarding the chemical contents of the kits. MSDS information for each kit is provided in the accompanying teacher instructions.

Chemicals Used in Science Take-Out Kits

Every effort has been made to reduce the use of hazardous chemicals in Science Take-Out kits. Most kits contain common household chemicals or chemicals that pose little or no risk.

General Safety Precautions

1. Work in a clean, uncluttered area. Cover the work area to protect the work surface.
2. Read and follow all instructions carefully.
3. Pay particular attention to following the specific safety precautions included in the kit activity instructions.
4. Goggles and gloves should be worn while performing experiments using chemicals.
5. Do not use the contents of this kit for any other purpose beyond those described in the kit instructions.
6. Do not leave experiment parts or kits where they could be used inappropriately by others.
7. Never taste or ingest any chemicals provided in the kit – they may be toxic.
8. Do not eat, drink, apply make-up or contact lenses while performing experiments.
9. Wash your hands before and after performing experiments.
10. Chemicals used in Science Take-Out experiments may stain or damage skin, clothing or work surfaces. If spills occur, wash the area immediately and thoroughly.
11. At the end of the experiment, return ALL kit components to the kit plastic bag. Dispose of the plastic bag and contents in your regular household trash.

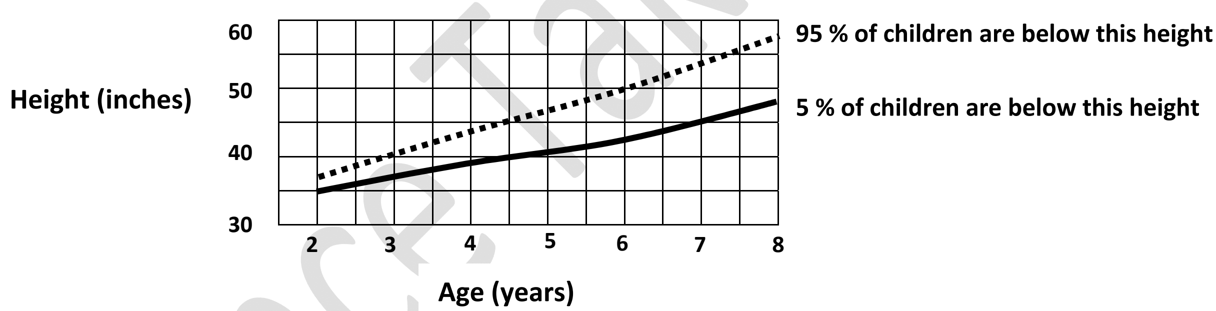
No blood or body fluids from humans or animals are used in Science Take-Out kits. Chemical mixtures are substituted as simulations of these substances.

Genetic Engineering: Way to Grow - *Teacher Answer Key*

Part 1: Jose's Story

Jose is a healthy and active six-year old. The doctor at the health clinic determined that Jose is 35 inches tall. She showed Jose's parents a growth chart with expected heights for boys at different ages.

1. Plot a point surrounded by a circle (◉) on the growth chart to represent Jose's height at age 6.



2. Based on the growth chart above, what can you conclude about Jose's growth?

Jose’s doctor suggested a blood test to measure the concentrations of several hormones that could be affecting Jose’s growth. Thyroid hormone, insulin, growth hormone, and cortisol are four hormones that may affect growth.

Your lab kit contains a sample of Jose’s blood plasma (the liquid part of his blood). Use the materials and instructions in the “Hormone Fast-Test Kit” to test the hormone levels in Jose’s blood plasma.

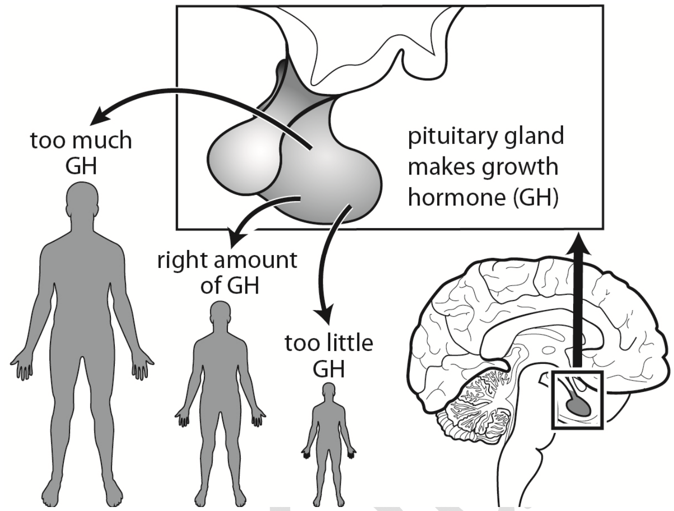
3. Record the results of the hormone tests in the chart below.

Hormone	Hormone Level (low, normal, or high)
Thyroid hormone	
Insulin	
Growth Hormone	
Cortisol	

4. Based on Jose’s blood tests, how would you explain why Jose is growing slowly?

The doctor explains that Jose has a medical condition called growth hormone deficiency. Children with this condition grow unusually slowly because their pituitary gland does not make enough growth hormone.

Luckily, Jose can be treated with hormone replacement therapy. He can receive injections of growth hormone to help him grow normally.



Before the 1980's, growth hormone for injections was isolated from human pituitary glands collected during autopsies. Supplies of this "cadaver" growth hormone were so limited and so expensive that only a few children could be treated. Some of the "cadaver" growth hormone was contaminated with pathogens that caused brain damage.

In the 1980's, scientists began using genetic engineering to create bacteria that contained the genes for human growth hormone. These engineered bacteria can be used to safely make large amounts of human growth hormone. Most children with growth hormone deficiency now can be offered affordable hormone replacement therapy that allows them to grow normally.

5. What is meant by the term "hormone deficiency"?

6. What is "hormone replacement therapy"?

7. How are genetically engineered bacteria different from normal bacteria?

8. List <u>three</u> ways that using growth hormone produced by genetic engineering is better than using "cadaver" growth hormone?

Part 2: Modeling Genetic Engineering

The growth hormone used to treat Jose's hormone deficiency is made by genetically engineered bacteria. In Part 2, you will model the genetic engineering process that scientists use to create bacteria that produce human growth hormone.

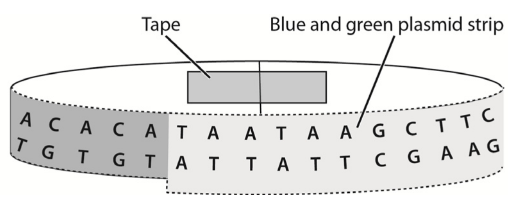
1. Bacteria contain two types of DNA rings. A large coiled ring of chromosomal DNA and many smaller rings of DNA called **plasmids.** For genetic engineering, scientists use the plasmids from bacteria.

Chromosomal DNA
a large ring of bacterial DNA that has most of the genes needed for bacteria.

Plasmids
Smaller rings of bacterial DNA that carry a few genes for antibiotic resistance.

2. What is a plasmid?

3. Take out the blue and green strip of paper from your kit. This blue and green strip of paper simulates plasmid DNA. Follow the instructions below to make a model of a ring-shaped bacterial plasmid that contains the **AMP resistance gene**:



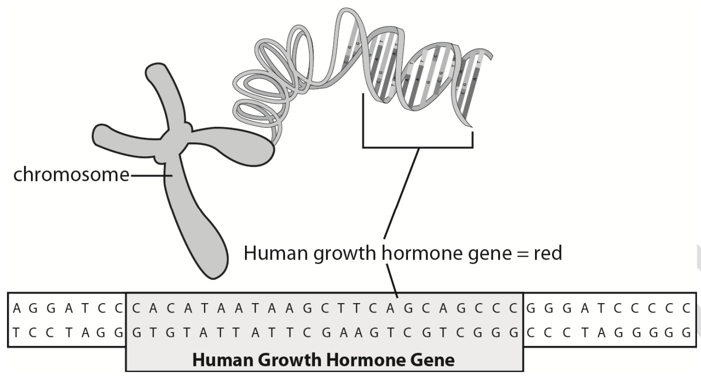
Plasmid with AMP resistance gene

   a. Cut the blue and green strip of paper along the dotted lines.

   b. Tape the green ends of the strip together to make a ring. Make sure there is no overlap between the letters on the ends of the green strip. You have now made a model of a ring-shaped bacterial plasmid.

   c. The blue area of your plasmid is the **AMP resistance gene.** Bacteria containing plasmids with the AMP resistance gene are resistant to ampicillin (an antibiotic). These bacteria will <u>not</u> be killed by ampicillin. Bacteria that do not have the AMP resistance gene are killed by the ampicillin antibiotic.

4. Now, take out the red and yellow strip of paper from your kit. This red and yellow strip of paper simulates human DNA. Follow the instructions below to make a model of a part of a human chromosome that contains the **gene for making human growth hormone.**



Part of a human chromosome with the gene for human growth hormone

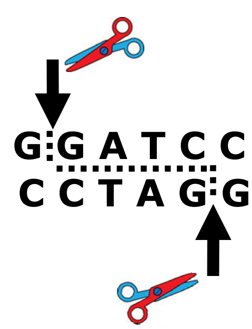
a. Cut along the red and yellow strip of paper along the dotted lines. (**Note**: Human DNA is linear, not circular, so <u>do not</u> tape this strip into a ring.) You have now made a model of part of a human chromosome.

b. The **red area** of the chromosome represents the **gene for human growth hormone.**

You will use your model of a ring-shaped bacterial plasmid and your model of part of a human chromosome to simulate the genetic engineering process.

The first step in the genetic engineering process is to cut the plasmid DNA and the human chromosome DNA at very specific locations. Genetic engineers use special proteins called **restriction enzymes** to cut DNA base sequences at specific locations. Restriction enzymes recognize and bind to specific DNA base sequences and act like scissors to cut the DNA in a very specific way.

For example, a restriction enzyme called BAM binds to and cuts DNA whenever it encounters the DNA base sequence GGATCC. The diagram on the right shows where the BAM restriction enzyme cuts the GGATCC base sequence. The cut site is shown with a dotted line (▪▪▪▪▪▪). Note that the DNA is double-stranded. The top strand of the DNA has the sequence GGATCC, while the bottom strand of the DNA has the sequence CCTAGG.



5. Follow the instructions below to simulate using a BAM restriction enzyme to cut your plasmid DNA. Your kit contains a clear plastic strip – this simulates the BAM restriction enzyme. The top portion of the clear plastic strip has the DNA base sequence that is recognized by the BAM restriction enzyme (GGATCC on the top strand and CCTAGG on the bottom strand).

    a. Slide the clear plastic BAM restriction enzyme along your paper model of the <u>bacterial plasmid</u> ring to find the DNA base sequences that BAM would cut on the plasmid. (Hint: Look for the DNA base sequence GGATCC. There should only be one location on your plasmid with GGATCC. Draw **dotted lines** (like the ones shown in the previous diagram) on your plasmid to show where it would be cut by the BAM restriction enzyme.

    b. Use scissors to cut the plasmid along the **<u>one</u>** cut site (the dotted line that you drew) for the BAM restriction enzyme. Be careful to leave the overhangs, called sticky ends, attached to the plasmid DNA. *Do <u>not</u> cut the clear plastic BAM restriction enzyme piece.*

6. Now, follow the instructions below to simulate using a BAM restriction enzyme to cut human chromosome DNA.

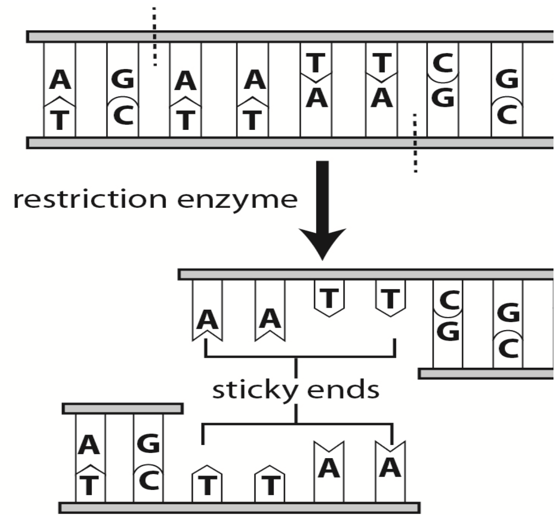
    a. Slide the clear plastic BAM restriction enzyme along your paper model of the <u>human chromosome</u> to find the DNA base sequences that BAM would cut on the human chromosome. (Hint: Look for the DNA base sequence GGATCC. There should be **<u>two</u>** locations on the human chromosome DNA with GGATCC). Draw **dotted lines** (like the ones shown in the previous diagram) on the human chromosome to show where it would be cut by the BAM restriction enzyme.

    b. Use scissors to cut the human DNA along the **<u>two</u>** cut sites (dotted lines) for the restriction enzyme. Be careful to leave the overhangs, called sticky ends, attached to the human growth hormone gene. *Do <u>not</u> cut the clear plastic BAM restriction enzyme piece.* Note that you have used the BAM restriction enzyme to cut out the Human Growth Hormone gene from the human chromosome.

7. What do restriction enzymes do?

Notice that the paper models of the bacterial plasmid DNA and the human growth hormone DNA have little overhangs from where you "cut" them with the BAM restriction enzyme. These overhangs are called "sticky ends." The term "**sticky ends**" is science slang for the complementary DNA overhangs left after a DNA molecule is cut by a restriction enzyme.

These overhangs are called sticky ends because they have a tendency to form weak hydrogen bonds with other sticky ends that have complementary (opposite) DNA bases.

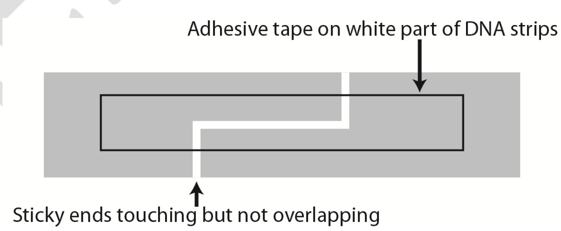


Sticky ends are important for genetic engineering because they allow the DNA from the human growth hormone gene to stick to the plasmid DNA.

8. What is meant by the term "sticky ends"?

9. You will now use your cut plasmid DNA (with sticky ends) and the human growth hormone gene (with sticky ends) to make a **recombinant DNA plasmid** ring that includes the plasmid and the human growth hormone gene.

   a. Match the sticky ends from the plasmid with the complementary sticky ends on the human growth hormone gene. *Remember: A pairs with T and C pairs with G.*



   b. To hold the sticky ends together firmly, genetic engineers use an enzyme called **ligase.** Use clear adhesive tape to simulate ligase and attach the human growth hormone gene to the plasmid DNA as shown in the diagram on the right.

10. Why are “sticky ends” important in genetic engineering?

11. What does the enzyme ligase do?

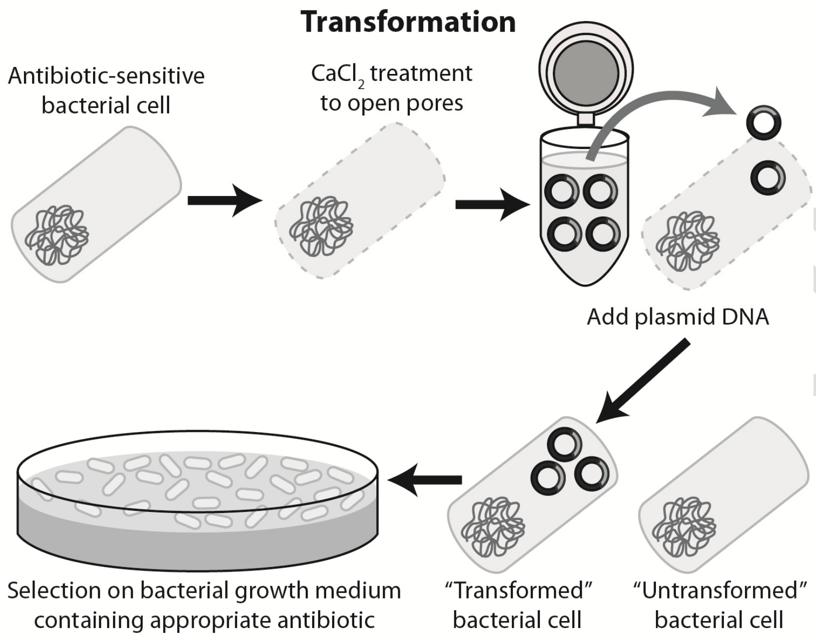
Congratulations! You have successfully engineered a recombinant DNA plasmid. A recombinant DNA plasmid is a ring of DNA that combines the genetic material of one organism with the genetic material of another organism.

12. What is meant by the term “recombinant DNA”?

However, now you need to put the recombinant DNA plasmid into a bacteria cell. This is important because the recombinant DNA plasmid cannot make human growth hormone unless it is inside a bacteria cell. The bacteria cell provides the enzymes and materials necessary to make human growth hormone. Also, the bacteria with the plasmid can reproduce asexually to produce millions of bacteria that contain the genes for human growth hormone.

13. Explain <u>two</u> reasons why recombinant plasmids must be put into bacteria cells before human growth hormone can be produced.

To make bacteria that can produce human growth hormone, you need to get the plasmid into a bacterial cell. The process used to get a plasmid into bacteria is called **transformation**. The diagram below and steps 14 through 17 describe the processes involved in transformation.



14. Bacteria are first treated with calcium chloride ($CaCl_2$) to open pores that allow the bacteria to "take up" DNA from their environment. Your kit contains two bags labeled "Bacteria." Simulate treating bacteria with calcium chloride, by opening the tops of <u>both</u> bags labeled "Bacteria."

15. The bacteria are then mixed with recombinant plasmids. Some, but not all, of the bacteria "take up" the recombinant plasmids and are "transformed." Simulate this by putting the recombinant plasmid you made into only <u>**one**</u> of the bags to represent a transformed bacterial cell.

16. Describe the steps in the "transformation" process used to produce transformed bacteria that contain plasmids with the recombinant DNA plasmid.

Now that you have transformed bacteria with the recombinant plasmid DNA, you need to allow these transformed bacteria to grow and multiply.

17. The bacteria are grown on a culture medium (jelly-like material) that contains ampicillin (an antibiotic). The ampicillin in the culture medium is used to **select** bacteria that have been transformed.

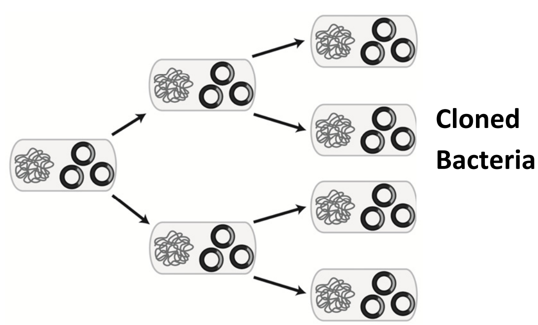
    a. **Untransformed bacteria** that did not take up plasmids with the AMP gene are killed by the ampicillin in the culture medium. Simulate the death of the untransformed bacteria. Take the bag representing and untransformed bacterial cell that would not survive on a culture medium, and place this bag back into your kit.

    b. **Transformed bacteria** that contain plasmids with the AMP gene are resistant to ampicillin. These bacteria will survive and multiply in a culture medium that contains ampicillin. Simulate the survival of the transformed bacteria in the culture medium by keeping the bag that represents a transformed bacterial cell.

18. How is the "transformed bacterial cell" different from the "untransformed bacterial cell"?

19. Why was it important to use a plasmid that contained the AMP gene that makes the bacteria resistant to the antibiotic ampicillin?

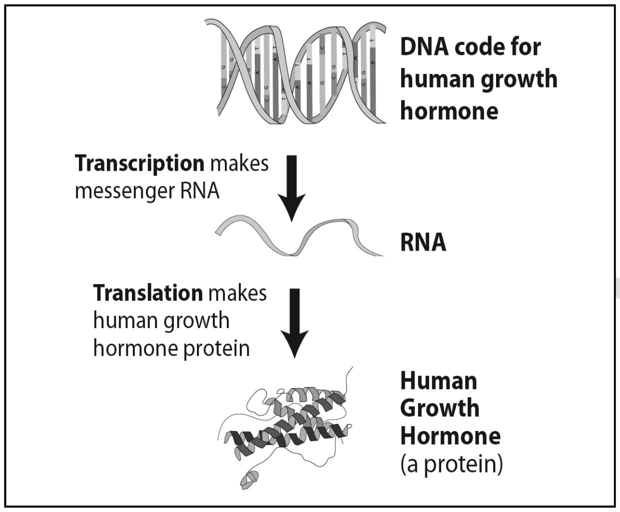
20. The transformed bacteria can reproduce asexually to make thousands of genetically identical bacteria (**clones**) that all contain the recombinant plasmids and can make human growth hormone.



21. What is a clone?

The transformed bacteria (containing the recombinant plasmid) will use the coded information in the human growth hormone gene to direct the synthesis of human growth hormone.

- The human growth hormone gene is copied to make messenger RNA. This process is called **transcription.**
- Ribosomes use the code in messenger RNA to direct the synthesis of human growth hormone (a protein). This process is called **translation.**



Human growth hormone protein image from http://www.rcsb.org/pdb/explore.do?structureId=1H

22. When bacteria copy the human growth hormone DNA to make messenger RNA, the process is called __________________. When ribosomes use the information in messenger RNA to make protein, the process is called _______________________.

23. Simulate the production of human growth hormone by the transformed bacteria by placing human growth hormone molecules (paper clips) into the transformed bacteria (the bag containing the plasmid with the gene for human growth hormone).

24. Lastly, scientists **isolate** the human growth hormone from the transformed bacteria. This human growth hormone is used to make the injection that is given to the patient. Simulate the isolation of the human growth hormone by removing the paper clips from the bag and placing them on the vials in the diagram on the right.

Note: Do <u>not</u> tape or glue the clips to the vials. You will return the paper clips to the kit.

25. Organize the steps below by writing numbers to indicate the correct order in the genetic engineering process. *Note: The first and last steps have already been numbered. Write the correct order for the other steps.*

___**1**___ Use a restriction enzyme to cut bacterial plasmids and create sticky ends

_______ Use ligase to attach the sticky ends together to make recombinant plasmids

_______ Use a restriction enzyme to cut out the growth hormone gene and create sticky ends

_______ Mix the plasmids with growth hormone genes

_______ Mix recombinant plasmids with treated bacteria cells

_______ Treat bacteria to open pores so bacteria can take up DNA

_______ Select transformed bacteria by growing on a culture medium that contains an antibiotic

___**8**___ Isolate growth hormone produced by bacteria

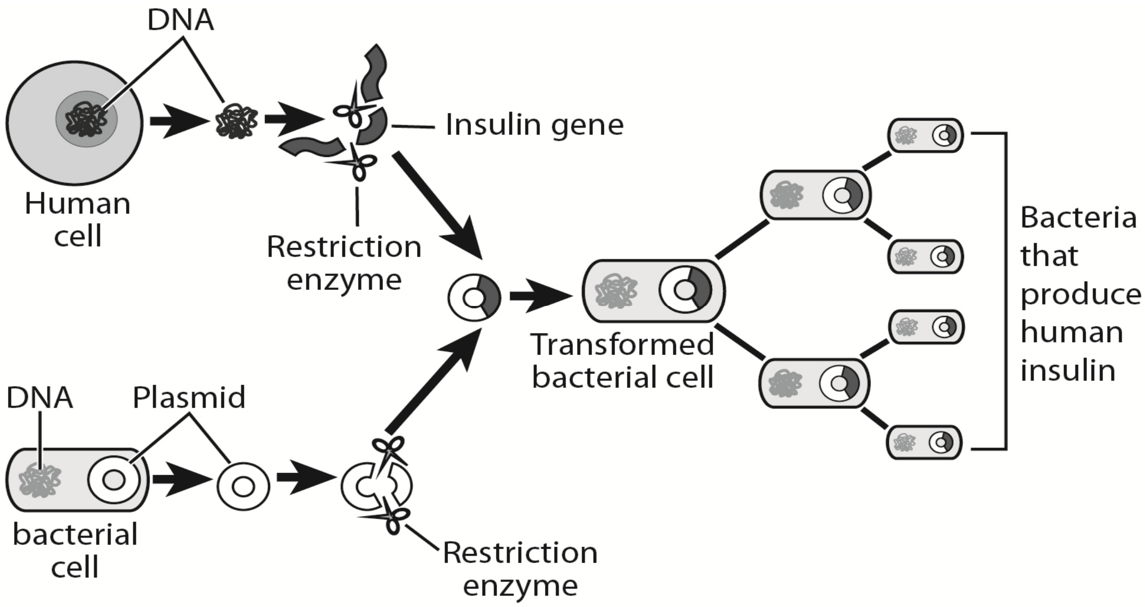
Jose's Story Continued

Jose has received daily growth hormone injections for three years. He has grown 11 inches and has gained 19 pounds since beginning treatment. Jose's doctor assures his parents that with continued growth hormone therapy, Jose will become a normal height adult.

Jose's parents are hoping that he will be able to continue growth hormone therapy until he finishes puberty. However, growth hormone treatments are very expensive – $20,000 a year on average. Jose's parents are having increasing difficulties getting his hormone therapy approved by their insurance company.

Part 3: Genetic Engineering to Make Insulin

Genetic engineering has also been used to create genetically engineered bacteria that produce **human insulin** that can be used to treat diabetes. The diagram below summarizes the steps in using genetic engineering to produce **human insulin.**



On a separate sheet of paper, write a brief essay that describes the genetic engineering process used to produce bacteria that make **human insulin.** Include the following words in your paragraph and underline them.

- recombinant DNA
- sticky ends
- human insulin gene
- ligase
- restriction enzymes
- plasmid
- bacteria
- transformation
- insulin
- isolation of insulin

Be certain to attach your essay to this lab report.

MATERIAL SAFETY DATA SHEET

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name (as printed on the label): "Jose's Plasma"

Product identity: Buffer pH 7

Distributor:SchoIAR Chemistry; 5100 W. Henrietta Rd, Rochester, NY 14586; (866) 260-0501; www.Scholarchemistry.com

Telephone number for information: (718)338-3618 Medical emergency phone number (Chemtrec): (800) 424-9300

Date of this MSDS: 12/1/12

2. COMPOSITION/INFORMATION ON INGREDIENTS

Ingredients	CAS Numbers	% Weight/Volume (balance is water)
Potassium phosphate monobasic	7778-77-0	< 1%
Sodium hydroxide	1310-73-2	<1%

For all the ingredients
OSHA PEL: TWA – none estab. STEL – none estab.
ACGIH TLV: TWA – none estab. STEL – none estab.
NIOSH REL: TWA – none estab. STEL – none estab.
NIOSH ILDH: none estab.

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW
Do not ingest. Avoid skin and eye contact. Avoid exposure to vapor or mists.

Potential Health Effects
This material is not considered hazardous.
Target organs: None known.

4. FIRST AID MEASURES

EYES - Flush with water for at least 15 minutes, raising and lowering eyelids occasionally. Get medical attention if irritation persists.

SKIN - Thoroughly wash exposed area for at least 15 minutes. Remove contaminated clothing. Launder contaminated clothing before reuse. Get medical attention if irritation persists.

INGESTION - Do not induce vomiting. If swallowed, if conscious, give plenty of water immediately and call a physician or poison control center. Never give anything by mouth to an unconscious person.

5. FIRE FIGHTING MEASURES

NFPA Rating: Health: 0 Fire: 0 Reactivity: 0
Extinguisher Media: Any means suitable for extinguishing surrounding fire
Special Firefighting Procedures: Firefighters should wear full protective equipment and NIOSH approved self-contained breathing apparatus.
Unusual Fire and Explosion Hazards: No data available

6. SPILL OR LEAK PROCEDURES

Ventilate area of spill. Clean-up personnel should wear proper protective equipment and clothing. Absorb material with suitable absorbent and containerize for disposal.

7. HANDLING AND STORAGE

Store in a cool dry place. This Material is not considered hazardous. Handle using safe laboratory practices.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Respiratory Protection: n/a

Ventilation: Local Exhaust: Preferred Mechanical(General): Acceptable Special: No Other: No

Protective Gloves: Natural rubber, Neoprene, PVC or equivalent.
Eye Protection: Splash proof chemical safety goggles should be worn.
Other Protective Clothing or Equipment: Lab coat, apron, eye wash, safety shower.

9. PHYSICAL AND CHEMICAL PROPERTIES

Melting Point: ~0°C
Vapor Pressure: information not available
Specific Gravity (H_2O=1): ~1
Evaporation Rate: information not available
Appearance and Odor: Clear yellow liquid

Boiling Point: ~100°C
Vapor Density: information not available
Percent Volatile by Volume: >99
Solubility in Water: soluble

10. STABILITY AND REACTIVITY

Stability: Stable
Hazardous Decomposition Products: none known

Materials to Avoid: strong acids and bases
Hazardous Polymerization: will not occur

11. TOXICOLOGICAL INFORMATION

Sodium Hydroxide: LD50 [oral, rabbit]; N/A; LC50 [rat]; N/A; LD50 Dermal [rabbit]; N/A

Material has not been found to be a carcinogen nor produce genetic, reproductive, or developmental effects.

Effects of Overexposure:

Acute: Essentially non-hazardous.
Chronic: None known.
Conditions aggravated/Target organs: none known
Primary Route(s) of Entry: Ingestion or skin contact.

12. ECOLOGICAL INFORMATION

No ecological data available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods: Dispose in accordance with all applicable Federal, State and Local regulations. Always contact a permitted waste disposer (TSD) to assure compliance.

14. TRANSPORTATION INFORMATION

D.O.T. SHIPPING NAME: Not regulated

15. REGULATORY INFORMATION

EPA regulations: RCRA Hazardous waste number (40 CFR 261.33) – not listed
RCRS Hazardous waste classification (40 CFR 261) – not classified
SARA Toxic Chemical (40 CFR 372.65) – not listed
SARA EHS (Extremely Hazardous Substance (40 CFR 355) – not listed
OSHA regulations: Air Contaminant (29 CFR 1910.1000) – not listed

16. ADDITIONAL INFORMATION

The information provided in this Material Safety Data Sheet represents data from the manufacturer and/or vendor and is accurate to the best of our knowledge. By providing this information, Science Take-Out LLC makes no guarantee or warranty, expressed or implied, concerning the safe use, storage, handling, precautions, and/or disposal of the products covered or the accuracy of the information contained in this fact sheet. It is the responsibility of the user to comply with local, state, and federal laws and regulations concerning the safe use, storage, handling, precautions, and/or disposal of products covered in this fact sheet.