

Activity 1: Coming Attractions

Based on video content

15 minutes (10 minutes before and 5 minutes after the video)

Setup

Before watching the video, answer a few quick questions about genetically modified organisms. Don't think too long about the questions—just write down your first response. You might already know the answers to some of them; otherwise, just take an educated guess. During the video, listen for the topics addressed by the questions. After the video, review the questions and the answers as a group.

Materials

- Transparency of the Coming Attractions Questions (master copy provided)
- Transparency of the Coming Attractions Answers (master copy provided)

Coming Attractions Questions

1. What two U.S. crops are the most heavily genetically modified?
2. What fraction of U.S. corn is genetically engineered?
3. What percent of U.S. processed food contains some genetically modified corn or soybean product?
4. All known food allergies are caused by one type of molecule. Is it proteins, sugars, or fats?
5. What is the substance called "bt" used for?
 - a. Bonus: What does "bt" stand for?
 - b. Bonus: When was "bt" first registered with the USDA?
6. What does "totipotent" mean?
7. What nutrient is produced by "golden rice"?
8. According to the World Health Organization, how many children go blind each year from vitamin A deficiency?
9. What is the normal function of "anti-thrombin"?
10. For what was "Dolly the sheep" famous?
11. What's the difference between a "transgenic" animal and a "cloned" animal?
12. Yes or no: Are all "transgenic" animals also "cloned"?
13. Which are easier to clone: animals or plants?
14. What do "restriction enzymes" do?
15. What is the function of "microinjection" in genetic engineering?

Coming Attractions Answers

1. What two U.S. crops are the most heavily genetically modified?
Corn and soybeans
2. What fraction of U.S. corn is genetically engineered?
Almost one-third
3. What percent of U.S. processed food contains some genetically modified corn or soybean product?
70%
4. All known food allergies are caused by one type of molecule. Is it proteins, sugars, or fats?
Proteins
5. What is the substance called "bt" used for?
It's an insecticide.
 - a. Bonus: What does "bt" stand for?
Bacillus thuringiensis
 - b. Bonus: When was "bt" first registered with the USDA?
1961
6. What does "totipotent" mean?
A cell or cells with the ability to develop into the whole organism
7. What nutrient is produced by "golden rice"?
Beta-carotene, a vitamin A precursor
8. According to the World Health Organization, how many children go blind each year from vitamin A deficiency?
Up to half a million
9. What is the normal function of "anti-thrombin"?
It's a blood protein, involved in anti-coagulation/clotting and anti-inflammation.
10. For what was "Dolly the sheep" famous?
She was the first cloned mammal.
11. What's the difference between a "transgenic" animal and a "cloned" animal?
A transgenic contains a foreign gene; a cloned animal contains an entire nucleus of genetic material from another individual.
12. Yes or no: Are all "transgenic" animals also "cloned"?
No
13. Which are easier to clone: animals or plants?
Plants. Many plants can be cloned with cuttings or by grafting. Animals require nuclear transfer into an egg, followed by development in a surrogate mother.
14. What do "restriction enzymes" do?
Cut DNA at specific sequences into small pieces
15. What is the function of "microinjection" in genetic engineering?
It introduces DNA into cells like eggs or embryonic cells.

Activity 2: What's the Difference?

Based on video and online text content

50 minutes

Setup

Many of the terms used in genetic engineering have multiple, alternate definitions. Many sound similar to other terms that have a very different meaning. This exercise asks "What's the difference?" between two or more closely related genetic engineering terms. Work in pairs, with each pair taking at least one of the questions to work on. Use the Genetically Modified Organisms online text chapter as a reference. After five minutes to come up with a concise explanation for the difference between the terms, go over the questions as a group. First, spend a few seconds letting each person think of how he or she might answer the question. Then the pair who was working on this question will give the answer they came up with. Others can ask for clarification or add to the answer. Explanations of similarities are welcome, too!

Materials

- One copy of What's the Difference? Questions per person (master copy provided)
- Transparency of What's the Difference? Questions (master copy provided)
- Tips and Suggested Answers
- One copy of the Genetically Modified Organisms online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)

What's the Difference? Questions

1. What's the difference between a "cloned" gene, a "cloned" bacterium, and a "cloned" sheep?
2. What's the difference between traditional, selectively bred animals and transgenic animals?
3. What's the difference between "cloning" a plant and "cloning" an animal?
4. What's the difference between the term "vector" (when applied to genetic engineering) and the term "plasmid"?
5. What's the difference between an intron and an exon?
6. What's the difference between DNA, cDNA, and T-DNA?
7. What's the difference between Dolly (a cloned sheep) and Polly (a transgenic sheep)?
8. What's the difference between microinjection and a gene gun?
9. What's the difference between a cell that contains a transferred nucleus (from somatic cell nuclear transfer) and a cell that contains recombinant DNA?
10. What's the difference in technique, objective, and outcome in reproductive human cloning and human cloning for therapeutic or research purposes?
11. What's the difference between cloned human cells and human embryonic stem cells? (What's the connection between these cell types?)
12. What's the difference between the laws regarding human cloning in the U.S. and in other countries?

Note: For possible ways to answer these questions, see the Tips and Suggested Answers that follow.

What's the Difference? Suggested Answers

1. What's the difference between a "cloned" gene, a "cloned" bacterium, and a "cloned" sheep?
"Cloned" in this case means genetically identical. When a gene is cloned, it means it has been genetically engineered into a bacterial cloning vector, usually a plasmid, so many identical copies of it can be generated. Bacteria that grow asexually naturally make genetically identical, cloned populations. A cloned animal has been generated by transferring the nucleus of an adult animal into an enucleated egg. If the implanted egg develops, the result is a genetically identical copy of the adult who donated the nucleus to the enucleated egg.
2. What's the difference between traditional, selectively bred animals and transgenic animals?
Traditional selective breeding relies on sexual reproduction to mix genes of organisms. Then humans select individuals with the desired traits. Over many generations, populations with suitable traits are obtained. Transgenic animals contain introduced genes that may be from another organism. These genes introduce desired traits directly, without relying on sexual reproduction or mutation to generate these traits randomly.
3. What's the difference between "cloning" a plant and "cloning" an animal?
Cloning a plant can often be done by grafting, or taking a cutting and getting the stem of the cutting to develop roots. Individual cells can be cultured into fully developed plants by plant tissue culture methods. Plants are commonly totipotent, so their cells maintain the ability to develop into almost any specialized plant cell.

Cloning an animal requires replacing the nucleus of an enucleated egg with the nucleus of the individual to be cloned and stimulating division into a few embryonic cells. At that point, the embryo must be implanted into a surrogate mother for development into the cloned animal.
4. What's the difference between the term "vector" (when applied to genetic engineering) and the term "plasmid"?
A vector is a more general term that means any piece of DNA that can be used to introduce recombinant DNA into a cell. Some are engineered viral chromosomes and some are engineered plasmids. A plasmid is a small, extrachromosomal piece of naturally occurring DNA, commonly found in bacteria, and easily isolated from and re-introduced into bacterial cells. They are used to clone genes and introduce them into cells.
5. What's the difference between an intron and an exon?
Both are parts of genes, but the DNA of exons contains "coding information" or instructions for amino acid sequences of the protein encoded by the gene. Introns are intervening sequences that are transcribed into RNA, but spliced out before translation.
6. What's the difference between DNA, cDNA, and T-DNA?
All are "DNA" (deoxyribonucleic acid). cDNA has been made as a double-stranded DNA copy of a messenger RNA (mRNA), using the retroviral enzyme reverse transcriptase. T-DNA is a plasmid carried by the plant pathogen *Agrobacterium tumefaciens*. It is used to introduce genes into genetically modified plants.

7. What's the difference between Dolly (a cloned sheep) and Polly (a transgenic sheep)?

A cloned sheep like Dolly is a genetically identical copy of an existing adult sheep. The sheep from which Dolly was cloned donated a nucleus to an egg, which was taken from a different female sheep and "enucleated" so the chromosomes were removed. Dolly contained no non-sheep genes. A transgenic sheep like Polly contains an introduced gene, which may be from another organism (for example, the gene for production of human anti-thrombin III). A transgenic animal is not necessarily a clone or an identical copy of another, although it is possible to make a clone of a transgenic animal.
8. What's the difference between microinjection and a gene gun?

Microinjection uses a microscopic needle to introduce DNA directly into the nucleus of a cell or egg. A gene gun shoots pellets coated with DNA into cells—especially cells with an otherwise impenetrable cell wall, like those of plants. Some of the DNA may get shot into the nucleus and might be incorporated into the chromosomes.
9. What's the difference between a cell that contains a transferred nucleus (from somatic cell nuclear transfer) and a cell that contains recombinant DNA?

Like the difference between a cloned animal and a transgenic animal, the transferred nucleus contains ALL the genetic information of a cell. If the transferred nucleus is put into an enucleated egg, and the egg is successfully implanted and develops, a cloned individual will result. A cell that contains recombinant DNA contains only one or a few introduced genes.
10. What's the difference in technique, objective, and outcome in reproductive human cloning and human cloning for therapeutic or research purposes?

Both reproductive and therapeutic cloning starts with an egg. The nuclear DNA is removed, usually using microinjection technology. The "cloning" part is the introduction of a nucleus from a different cell, with subsequent cell division. The next step is the difference in technique and objective between reproductive and therapeutic cloning. In reproductive cloning, the embryonic group of cells would be implanted into a "surrogate mother" for the purpose of generating an entire new individual, a clone of the nucleus donor. Therapeutic cloning does not implant the embryonic cells, but uses them for research or for generating cells for transplants. Cloned cells that are genetically identical to a transplant recipient are less likely to be rejected.
11. What is difference between cloned human cells and human embryonic stem cells? (What's the connection between these cell types?)

These are two different types of cells but they are often discussed in the same context.

Cloned human cells would be generated by the technique described in the answer for "What's the difference in technique, objective, and outcome in reproductive human cloning...?" They are genetically identical to the nucleus donor.

Embryonic stem cells are the pluripotent cells from an embryo, which have the potential to become any cell type. Embryonic stem cells can come from an embryo generated by fusing a sperm and an egg. In that case, they are genetically unique. Embryonic stem cells could, in theory, come from an embryo generated by therapeutic cloning techniques. In that case, they would be clonal, or genetically identical, to the nucleus donor. Differentiated cells generated from them could be used for a relatively rejection-free transplant.
12. What's the difference between the laws regarding human cloning in the U.S. and in other countries?

The answer will depend on changing referenda and laws.

Activity 3: Troubleshooter

Based on video and online text content

50 minutes

Setup

Amazing techniques have been developed for making genetically engineered or cloned organisms, but the process is not always easy. Here are several cases of a failed attempt at a genetic engineering experiment. You will be the troubleshooter trying to figure out what went wrong.

Working in teams of two or three, read about one of the failed experiments. Each case comes with several possible explanations. Evaluate each possible explanation as the source of the failure, and think of an experiment that would test it, with possible results and conclusions. (The Genetically Modified Organisms online text might help in understanding the different techniques.) After about five or ten minutes of consideration and discussion, give the handout explaining the case to a neighboring team and receive a new one from an adjacent team.

After all teams have had a chance to work on all the cases, go over them quickly as a group and see if there is discussion about the possible causes. What kinds of experiments did the teams come up with and which would the group most like to try?

Materials

- One copy of each of The Genetic Engineering Experiment Cases (master copy provided)
- Tips and Suggested Answers
- One copy of the Genetically Modified Organisms online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)

Troubleshooter: The Genetic Engineering Experiment Cases

Case 1

Background

Someone in your lab tried to clone a gene from *Salmonella* bacterium into a plasmid in *E. coli* bacterium. He cut the gene out of the *Salmonella* chromosome with a restriction enzyme, cut the plasmid with the same restriction enzyme, and used ligase to combine the *Salmonella* gene into the plasmid. He used electroporation to introduce the plasmid into *E. coli*, and then plated onto plates containing ampicillin. The next day, instead of a few colonies of cells that had acquired the plasmid, the plate was covered with a "lawn" of cells, as though every single cell had grown.

What could have gone wrong? Which of the reasons below is a possible explanation for the problem? Think of an experiment that would test if this is the explanation. What results would be expected?

Possible Explanations

1. Electroporation cannot be used on bacterial cells; it works only on plant cells.
2. He left out the selecting antibiotic when making the medium, so all the cells grew whether they had the plasmid or not.
3. The *E. coli* he used were already resistant to the antibiotic.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter: The Genetic Engineering Experiment Cases

Case 2

Background

Someone in your lab tried to clone a gene from *Salmonella* bacterium into a plasmid in *E. coli* bacterium. He cut the gene out of the *Salmonella* chromosome with a restriction enzyme, cut the plasmid with the same restriction enzyme, and used ligase to combine the *Salmonella* gene into the plasmid. He used calcium chloride to introduce the plasmid into *E. coli*, and then plated onto plates containing ampicillin. The next day, instead of a few colonies of cells that had acquired the plasmid, there were no colonies.

What could have gone wrong? Check off those that are a possible explanation for the problem. Then, think of an experiment that would test if this is the explanation. What results are expected?

Possible Explanations

1. The restriction enzyme cut the plasmid in the middle of the gene for ampicillin resistance, disrupting the gene and making antibiotic resistance impossible.
2. He neglected to add antibiotic to the medium.
3. The treatment to make them calcium-competent killed the cells.
4. The mixture of cut gene and cut plasmid requires an enzyme (ligase) to covalently connect them and he forgot to add this enzyme. Neither the cut *Salmonella* gene alone nor the cut plasmid can confer antibiotic resistance.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter: The Genetic Engineering Experiment Cases

Case 3

Background

Your lab has two sibling mice, one male and one female, that seem to be living longer than other mice. So far, they have lived 25% longer than the normal mouse lifespan. Thinking they might be good models for human aging, you would like to perpetuate them but are not sure if they will be able to reproduce.

You direct your technician to try cloning the mice. She obtains 10 eggs from the long-lived female and 10 eggs from another, unrelated female. After removing the nucleus from all 20 eggs, she introduces a nucleus into each one. Ten eggs receive a nucleus from the long-lived male and ten receive a nucleus from the long-lived female. All are implanted into different surrogate mothers. Two mice are born. When they are tested, one is genetically identical to the long-lived female in every way. The other is nearly genetically identical to the male but has a few differences. Your technician is disappointed in the results.

What could have gone wrong? Check off those that are a possible explanation for the problem. Then think of an experiment that would test if this is the explanation. What results are expected?

Possible Explanations

1. Nothing went wrong; these are actually good numbers. Animal cloning has a very low success rate.
2. The small genetic differences between the long-lived male mouse and his clone are because of mitochondrial DNA, present in the egg and not removed by the microinjection technique.
3. The small genetic differences between the long-lived male mouse and his clone are because not all the chromosomal DNA of the egg was removed by the microinjection technique.
4. It isn't possible to get an exact clone of the male because the surrogate mother mouse will always contribute some DNA.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter: The Genetic Engineering Experiment Cases

Case 4

Background

Your lab is trying to produce a human protein that acts as a fat-regulating hormone for therapeutic purposes. Normally the protein is secreted by adipose tissue. The most efficient way to produce a large amount of protein seems to be to clone the human gene into a plasmid that can be introduced into bacteria, grow the bacteria in culture, and have the bacteria produce large amounts of the protein.

You clone the gene into a bacterial plasmid and successfully introduce the plasmid into lab strains of *E. coli*. When you harvest some of the bacteria after they have grown in culture, they still have the correct plasmid but have produced no protein.

What could have gone wrong? Which of the reasons below is a possible explanation for the problem? Think of an experiment that would test if this is the explanation. What results would be expected?

Possible Explanations

1. Bacterial cells and human cells are too far apart, evolutionarily, to produce each other's proteins. For example, bacteria and humans do not use the same codons to encode the amino acids methionine, tryptophan, and histidine.
2. The human gene contains introns, and the bacterial cell cannot splice them out of the mRNA.
3. The human protein might require modifications that the bacterial cell cannot perform, such as glycosylation (addition of sugars) or post-translational processing. Without these modifications, the protein might be unstable so it can't be detected.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter: The Genetic Engineering Experiment Cases

Case 5

Background

Your lab is attempting to introduce the gene for a special enzyme into corn plants. This enzyme will change the color of the corn endosperm, making it pink. A large food processing company is interested in this plant because they think pink corn chips will be a big marketing hit.

DNA containing the gene for the enzyme is coated onto gene gun pellets and shot into totipotent plant cells. From these cells, transformed plants are cultivated. Two plants are obtained that make the special enzyme but they do not express it in the endosperm.

What could have gone wrong? Which of the reasons below is a possible explanation for the problem? Think of an experiment that would test if this is the explanation. What results would be expected?

Possible Explanations

1. The cloned gene did not contain the proper regulatory sequences required to produce the protein in the endosperm.
2. When the DNA was shot into the cells, the only cells that acquired it and integrated it into the chromosomes were non-endosome cells like the cells of roots or leaves.
3. The gene gun technique works on only certain kinds of plants. The introduction of DNA should have been done with *Agrobacterium tumefaciens*, which introduces DNA into any kind of plant.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter Answers

Case 1 Answers

1. Electroporation cannot be used on bacterial cells; it works only on plant cells.
This is not a possible explanation.
2. He left out the selecting antibiotic when making the medium, so all the cells grew whether they had the plasmid or not.
This is a possible explanation that could be tested by putting antibiotic-sensitive bacteria on the plate. If they grow, the plates do not contain antibiotics.
3. The *E. coli* he used were already resistant to the antibiotic.
This is a possible explanation that could be tested by putting the non-recombinant *E. coli* onto a plate with antibiotics without electroporation to add a plasmid. If they grow, they were already antibiotic resistant.

Case 2 Answers

1. The restriction enzyme cut the plasmid in the middle of the gene for ampicillin resistance, disrupting the gene and making antibiotic resistance impossible.
This is a possible explanation, which could be tested by transforming with the plasmid, without cutting with the restriction enzyme; or checking the “map” or the sequence of the plasmid to see if the restriction enzyme has a recognition site within the amp resistance gene.
2. He neglected to add antibiotic to the medium.
This is not a possible explanation. In that case, everything would grow.
3. The treatment to make them calcium-competent killed the cells.
Probably not, but it could be tested by putting the calcium-treated cells directly onto medium with no antibiotic. They should all grow.
4. The mixture of cut gene and cut plasmid requires an enzyme (ligase) to covalently connect them and he forgot to add this enzyme. Neither the cut *Salmonella* gene alone nor the cut plasmid can confer antibiotic resistance.
This is a possible explanation that could be tested by starting over from the step combining the gene and the cut plasmid, this time adding ligase.

Case 3 Answers

1. Nothing went wrong; these are actually good numbers. Animal cloning has a very low success rate.
This is a possible explanation. Hundreds of eggs were enucleated and received nuclei, and 13 were implanted in order to get one cloned sheep, Dolly.
2. The small genetic differences between the long-lived male mouse and his clone are because of mitochondrial DNA, present in the egg and not removed by the microinjection technique.
This is a possible explanation. Most “cloned” animals are not exactly genetically identical to the nucleus-donating cell because the cloned animals cells contain the mitochondrial DNA from the egg donor. To test this, a Southern blot or other “DNA fingerprinting” type test could be performed, probing specifically for mitochondrial DNA, and comparing the egg-donating mouse’s mitochondrial DNA with the mitochondrial DNA from the long-lived male and the clone. If the difference is in mitochondrial DNA only, the clone will have the mitochondrial DNA of the egg donor, but all other DNA fingerprint markers will match the long-lived male who donated the nucleus.

3. The small genetic differences between the long-lived male mouse and his clone are because not all the chromosomal DNA of the egg was removed by the microinjection technique.
This is a possible, but unlikely, explanation. It could be tested with a Southern blot or other "DNA fingerprinting" test. A test like this might be able to detect egg DNA that remained and incorporated into the chromosomes of the developing embryo.
4. It isn't possible to get an exact clone of the male, because the surrogate mother mouse will always contribute some DNA.
This is not a possible explanation. The surrogate mother will not contribute DNA to the embryo.

Case 4 Answers

1. Bacterial cells and human cells are too far apart, evolutionarily, to produce each other's proteins. For example, bacteria and humans do not use the same codons to encode the amino acids methionine, tryptophan, and histidine.
This is not a possible explanation. Although there are a few examples of non-standard codon usage, the codon table is otherwise universal. *E. coli* cells and human cells use the same codons to encode the same amino acids.
2. The human gene contains introns, and the bacterial cell cannot splice them out of the mRNA.
This is a possible explanation. There are many ways to test this, including determining the base sequence of the entire gene. In fact, this would almost certainly be done before the cloning step. One way to cleanly remove the intron so the gene could be expressed in bacteria would be to generate a cDNA copy from mRNA of the gene extracted from eukaryotic cells. The mRNA would have the intron "spliced out," so the cDNA copy made from it would not contain the intron and bacteria would be able to produce the protein properly.
3. The human protein might require modifications that the bacterial cell cannot perform, such as glycosylation addition of sugars or post-translational processing. Without these modifications, the protein might be unstable so it can't be detected.
This is a possible explanation that is frequently a difficulty in producing eukaryotic proteins in bacteria. There are many possible tests for this, but one possibility is to have a eukaryotic cell, like yeast, produce the protein and see if it is modified properly.

Case 5 Answers

1. The cloned gene did not contain the proper regulatory sequences required to produce the protein in the endosperm.
This is a possible explanation, although difficult to test precisely. A version of the gene with known endosperm-expressing regulatory sequences should be made and introduced into the plants.
2. When the DNA was shot into the cells, the only cells that acquired it and integrated it into the chromosomes were non-endosome cells like the cells of roots or leaves.
This is not a possible explanation. When DNA is introduced into totipotent cells, they are not yet differentiated into roots and leaves. They have the ability to develop into any kind of cell. If a totipotent cell that acquires recombinant DNA is grown into an entire plant, all its cells will contain the introduced DNA although they may not express it into proteins.
3. The gene gun technique works on only certain kinds of plants. The introduction of DNA should have been done with *Agrobacterium tumefaciens*, which introduces DNA into any kind of plant.
This is not a possible explanation. The gene gun technique is actually more universal than introduction of DNA by infection with an *Agrobacterium*.

Activity 4: Two Thumbs Up?

Based on video content

5 minutes

Setup

Now that you've seen a video addressing some of the issues surrounding genetically modified organisms, discuss the following questions in teams of three or four.

Materials

- One copy of the Discussion Questions per person (master copy provided)

Discussion Questions

1. What information in the video or subsequent activities surprised you the most?
2. Was there anything that you hadn't heard of before? If so, do you think this would be completely new information for high school students as well?
3. Compared to the average adult, do you think your high school students would be more or less accepting of the use of genetically modified organisms in food and medical production? Why?

Notes
