Gene Editing: How can we accelerate artificial selection?

Introduction (~10 minutes)

If you've ever eaten blueberries, you may have noticed that each one has a slightly different taste. (You may even have kept eating berries until you found that one with the perfect flavor!) In contrast, every banana with the same ripeness tastes exactly the same.

Every banana (Musa acuminata) at your grocery store is probably a Cavendish banana known as Grand Nain. Every Grand Nain banana is the same genetically, so they are basically clones! This is true of many seedless fruits, though some fruits have more varieties. For example, there are many types of seedless grapes.

You might think that's a good thing. You know exactly what to expect when you buy a banana! Sadly, the fact that they are all the same puts them in danger. Cavendish bananas are threatened by banana wilt. Banana wilt has already destroyed many banana plantations (farms) in Asia and Australia. In fact, it nearly completely destroyed the last cultivar of banana.

You may have also wondered why banana candies taste different than banana fruits. Banana flavoring tastes more like Gros Michel bananas. Between the 1950s and 1960s, these bananas were wiped out by the same disease.

The fungus that causes banana wilt has spores that can survive in soil for decades. No known Cavendish bananas have resistance to this fungus.

What can we do? Scientists have found other bananas with resistance to the fungus, but those bananas are not edible. If we can add this resistance to the Grand Nain banana, perhaps our bananas can stay disease free.

Researchers modified the DNA of Grand Nain bananas to make 5 transgenic banana lines. They added genes from a wild Indonesian banana that was resistant to banana wilt. These new banana lines were planted alongside unmodified plants and the results are below.

The bananas in the control group had unmodified genes. The bananas in the transgenic group had new genes from a wild banana.

Banana Line	**Gene Type**	**Percent of Plants Infected or Killed**
GCTCV 218	Control Group	68.8
Williams	Control Group	66.7
DPM25	Control Group	100
Grand Nain	Control Group	87.5
RGA2-2	Transgenic	20

Banana Line	Gene Type	Percent of Plants Infected or Killed
RGA2-3	Transgenic	0
RGA2-4	Transgenic	20
RGA2-5	Transgenic	14.3
RGA2-6	Transgenic	66.7
RGA2-7	Transgenic	60

Were bananas in the control group or the transgenic group most susceptible to banana wilt? (Remember, 100% means every plant was infected or killed.)

Which specific line of bananas was least impacted by banana wilt? (Remember, the lower the percent, the less disease.)

Why do you think some lines of bananas were more likely to be infected than others? (Make sure you think about genetic factors, the environment, and more.)

Explore (~20 minutes)

Explore Materials List

- About 100 beads or similar objects, with around 25 each of 4 tactually different shapes.

In Natural & Artificial Selection, we learned how farmers improve crops to have desired features. This process has been used for a long time. It allows us to have more unique crops. For example, there are more than 7,500 types of apples around the world! Farmers' efforts have also led to seedless bananas and grapes.

The process of artificial selection is not an easy one. As farmers change features within a crop, undesired traits may appear. What if there was a way to choose favorable alleles without the undesired traits?

In 2012, Drs. Jennifer Doudna and Emmanuel Charpentier came up with just such a method. They knew that bacteria were able to pick up DNA from attacking viruses. The bacteria keep a copy of this DNA to fight later infections.

If the bacteria recognize an invading virus’ DNA, they make an enzyme. This enzyme's job is to cut up the viral DNA. This saves the bacteria.

Drs. Doudna and Charpentier adapted this method to cut the DNA of interest in an organism. They received the 2020 Nobel Prize in Chemistry for their work. We can insert a CRISPR-Cas9 protein into an organism, and it will cut the DNA exactly where we need it to.

Let's model this. Gather beads or similar small objects. You will need about 100 pieces of 4 different shapes.

Keep track of which shapes match which letter.

Shape	**Letter**
	A
	T
	C
	G

These shapes represent four different bases: adenine, thymine, cytosine, and guanine. These are often shortened as A, T, C, and G. These are the four nucleic acids found in the double-stranded DNA that stores all your genes (and the genes in bananas too!).

If you've ever heard that we share about 50% of our genes with bananas, that's true. Both humans and bananas have many of the same proteins, and we do some important things in the same way. For example, we both break down sugars for energy. However, our total DNA is only about 1%

similar. (DNA doesn’t just encode genes. It also has instructions for how to use those genes and some instructions we don’t fully understand.)

Line up 40 shapes side-by-side on a grid. You can use tactile graph paper or another tool to help keep your shapes in line. Don’t place the shapes down in any specific order or pattern. Make their order random.

Now use the key you made earlier to add another line of 40 shapes. Place each shape below a corresponding shape in your original line to fill a 40×2 grid. Where you have an A, add a T (and vice versa). Where you have a C, add a G (and vice versa).

Nice job making a paired, double-strand of DNA! Our shape model misses a lot of details, but it does a good job representing the nucleic acids inside DNA. In the real world we also need to worry about transcribing DNA to RNA. We will keep things simple and focus on what happens to DNA.

Pick out 2 more shapes: two Gs. This is the first sequence we will be looking for. Search from one end of your DNA to the other. Can you find those shapes in that sequence? How many times?

It’s likely you found the sequence, and it may have occurred more than once. It was a short sequence!

In the CRISPR process, this short sequence is a first step in the search for a specific gene. It is easier to find two matching shapes side by side than it is to find a more complex pattern.

Every time your 2-shape sequence is found, we are going to do another check. We will look for a 20-shape sequence coming right before it. This longer sequence will be all or part of the gene we want to modify. In a

banana, we might be looking for the place to add a gene to help the banana plant resist banana wilt.

Look before each GG. Do you see the following exact sequence? If so, CRISPR would unzip the DNA strands and cut them within the 20-shape sequence.

ACGC TGAC ACGC CTAC GTAG

You likely did not find that exact 20-base sequence. Why not?

Think about how long it took you to complete your search. How much longer would it take to search through 500 million shapes instead of 40? (There are over 500 million bases in the banana genome!) The CRISPR system can do something that would be very difficult for us. It can very quickly search all the DNA in a cell!

Cutting the DNA is just the start of the process. What do you think your cell would do if it found two cut ends of a strand of DNA?

a. re-attach the two strands
b. add a few bases, and then re-attach the two strands
c. remove a few bases, and then re-attach the two strands
d. all of the above

Explain (~10 minutes)

When a cell finds a broken length of DNA, it will repair it. Most of the time, it will re-attach the two strands with no changes. Sometimes it will add or remove a few bases. If there are any changes at all, it is often enough to do what scientists need CRISPR to do. Adding nearby bases results in a mutation.

Often, this will break the gene. If scientists want to turn off a gene, breaking it might be enough. These kinds of mutations happen naturally, but by targeting all the cells in a seed, a mutation caused by CRISPR is more likely to take hold.

A scientist may want to add specific DNA instead. For example, they may want to add a protein that would make a banana resistant to banana wilt. They just need to include copies of the DNA to make that protein. Scientists insert "donor DNA" into the cell at the same time as the CRISPR enzyme. When the cell goes to repair the cuts in the DNA, the donor DNA will be handy and is likely to be included.

Imagine you are a molecular biologist. Which method would you want to use in each of the situations on the next page? Make your choice, and explain why you think it is the better option. The first two have been completed for you as an example.

Situation	Method (choose one)	Reasoning
You want to add a gene to make your crop more resistant to drought.	Include donor DNA	Adding a gene requires donor DNA. Anytime a new or specific gene is needed, it must be included.
You want to reduce the amount of erucic acid in a crop since it may affect human heart health.	Mutation only	Reducing the impact of a gene just requires mutation. A mutation will “break” the normal gene.
You want to reduce the odor of a crop to make it more palatable as food.	Mutation only or include donor DNA	
You want to decrease the amount of fiber in a plant’s seed to increase germination.	Mutation only or include donor DNA	
You want to increase the amount of oil in a plant’s seed to make it more valuable.	Mutation only or include donor DNA	

Extend (~20 minutes)

Extend Materials List

- Domesticated pennycress (yellow) seeds and undomesticated pennycress (black) seeds. Any set of seeds from related plants can work, including kale and cauliflower.

- A jeweler’s loupe or microscope

Nearly every crop is being carefully gene edited. Scientists use CRISPR and other gene editing methods to explore the effects of small changes to DNA. These studies take place in greenhouses and labs.

Many studies look at mutations. Mutations are a natural part of a plant’s growth, but gene editing allows humans to make changes in years instead of decades. Gene editing is much faster than artificial selection!

One helpful mutation is one that changes black-seeded (wild) pennycress into yellow-seeded (domesticated) pennycress. Use your jeweler’s loupe to examine your seeds. Draw and describe the differences you see between the seeds.

Seed 1 Name, Drawing, & Description:

Seed 2 Name, Drawing, & Description:

If you looked at pennycress seeds, you likely noticed the yellow seed was smaller, smoother, and (of course!) a different color. All three of these changes were the result of one mutation. The mutation reduced the amount of fiber in the seed coat. With less fiber, the seed also has more oil in less space.

If you looked at kale and cauliflower, you likely noticed the cauliflower seeds were smaller, less spherical, and lighter in color. These are likely the result of many genetic changes as ancient wild cabbage was turned into kale and cauliflower by artificial selection.

All of these changes were the result of mutations. Domesticating pennycress was much faster than kale and cauliflower, though.

Why does domestication take less time now?

Think back to the activities you completed in Natural and Artificial Selection. Which of the changes you noticed in your seeds were beneficial changes for humans?

Which of the changes you noticed in your seeds would offer an advantage to the wild plant?

Reflect (~10 minutes)

Gene editing can be beneficial because:

Gene editing can be potentially risky because:

Think about what you had for lunch. What one change would you like to make to a food you ate? Do you think that is a change that could be made with DNA? Why or why not?

Molecular biologists work with things that are very small. What is an example of another career where humans work at a very small scale?

Career Connection: Biochemist

Biochemists study the chemistry of life. They may look at the chemicals that plants produce, or they may monitor the effects of chemicals on plants. In either case, they seek to understand how chemistry helps organisms grow and develop.

Biochemists need 4-year degrees, even to work in a lab. Leading a lab usually requires a master's or doctoral degree.

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