# Better Bananas: Gene Editing Team

As the **gene editing** team, you are tasked with **changing an existing gene** within the banana plant. Your team will mimic the process of using *Agrobacterium,* a common soil bacterium, to insert a *CRISPR/Cas 9* molecule that allows scientists to edit existing *genes* within the plant’s cells. This changes the *traits* the plant expresses.

**Banana Background:**

Bananas are an important agricultural product in the U.S.. In 2017, the U.S. consumed so many bananas that it is estimated each American ate an approximately 29 pounds of bananas over the course of the year. Bananas continue ripening after they are picked. Because most of the bananas that Americans consume are grown in South America or Asia, the time it takes to transport bananas from these countries to the U.S. can make it difficult to ensure the bananas in grocery stores are ripe. Ripening allows bananas to become sweeter, softer, and juicier. However, overripe bananas are usually soft or have brown spots on the peel. These characteristics discourage consumers from purchasing or consuming overripe bananas and contribute to substantial food waste. In fact, bananas are one of the most wasted produce products in grocery stores, which has environmental and social impacts for farmers and consumers. Subsequently, scientists are looking for ways to delay the ripening process for bananas.

**Step 1:** As the **gene editing** team, you are tasked with **editing an existing gene** within the banana plant to delay it’s ripening process. There are many genes that contribute to how a plant ripens. Review the list of genes associated with ripening below. As a team, choose a gene from the list that you would like to try to edit to delay banana ripening. **Put an X on the line of the gene you’ve chosen**.

**Genes**

\_\_\_\_***REDUCE ETHYLENE PRODUCTION****:* ethylene is a natural hormone associated with plant growth, development, and ripening. One the plant is fully mature, the plant’s production of ethylene triggers a series of biological pathways that change the fruit’s color, soften the fruit, and develop distinct tastes and aromas associated with overripe fruit. Scientists may be able to delay ripening by editing the gene that is responsible for initiating this pathway to disrupt it’s process.

**DNA Sequence:** GCT AAC CGA TTC ACG TAC GGC TGC CTC

\_\_\_\_***REDUCE PECTIN PRODUCTION****:* pectin is a plant substance that maintains the integrity of cell walls. During the ripening process, plants produce an enzyme that breaks down pectin. This softens the fruit because there is not as much pectin available to maintain the stability of the cell walls. Scientists may be able to delay banana ripening by editing the gene that is responsible for producing the enzyme that breaks down pectin, allowing cell walls to maintain stability for a longer period of time.

**DNA Sequence:** TAG GCT ATA AGG TAC CGT TCG GAG CAT

\_\_\_\_***ESTABLISH RESISTANCE TO FUSARIUM WILT****:* Fusarium wilt is a deadly disease caused by a fungus that lives in the soil. The fungus enters the plant through the roots. It can cause discoloration of the leaves and fruit, wilting, and plant death. This can contribute to plants exhibiting signs of ripening. Scientists may be able to establish resistance to fusarium wilt by editing the gene that makes encourages the fungus to infect the banana.

**DNA Sequence:** CAT GCT ATA AGG CGA TTC ACG TAC GGC

**Why did your team choose to edit this gene within the banana instead of the others?**

**Step 2**: Your team of scientists needs to search the gene pool to find the genetic material that controls the ripening trait you want to edit.

* Search the gene pool for the strip of paper that contains the DNA sequence of the trait that you identified in Step 1 above.
* Once you’ve found this crucial genetic material, submit it to your teacher.
	+ There are 10 copies of your genetic material in the gene pool. For each DNA strand your team would like to try to insert into banana cells, you must find and submit a copy of the genetic material to your teacher to randomly receive a numbered ball.

**Step 2a:** Identify a team member to act as the guide RNA within the CRISPR/Cas 9 molecule. This person will come in handy later and ideally has the best aim.

**Name of guide RNA student:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Step 3**: Scientists now need to get the genetic material into the cell. One method to do this uses Agrobacterium, which enters the cell and can insert DNA into the nucleus. The other uses a gene gun to insert tiny little pellets covered in DNA into the cell. Both of these processes have a lot of uncertainty and can be very random. Scientists do not know if the DNA will get into the cells at all or if they will get into the nucleus, the portion of the cell where the DNA is housed. For this activity, your team will act as Agrobacteria that are inserting a CRISPR/Cas 9 molecule with the genetic material associated with the trait you are editing.

* Take turns throwing the DNA at the plant cell side of the felt board until at least one sticks to the nucleus portion of the cell (light blue felt).
* Because scientists often use multiple agrobacteria to try to successfully insert new DNA in a petri dish of plant cells, you can throw as many balls as you would like at the board at each time.
* IMPORTANT: Keep track of the total number of balls you throw at the board throughout the entire activity so you can calculate your success rate at the very end.
* Once you successfully insert the DNA into the nucleus of the cell, have your teacher make a note of which numbered ball(s) successfully made it. **Only successful balls can be used in the next steps.**

**Step 4**: Flip over the board to the side with two chromosomes.

**Step 5**: Translate the DNA to RNA. The guide RNA tells the Cas 9 molecule where to cut the banana DNA to insert the new DNA sequence you found in the gene pool.

* Guide RNA student (identified in step 2a):
	+ locate the DNA strand from the “gene pool” you found in step 2 and write the sequence down below.
	+ Use the DNA to RNA table to translate your DNA sequence to RNA.

|  |  |
| --- | --- |
| **DNA Nucleotides** | **RNA Pair** |
| A - Adenosine | U – Uracil (replace T in RNA) |
| C - Cytidine | G – Guanine |
| T - Thymidine | A – Adenosine |
| G - Guanine | C - Cytidine |

* + Take your new RNA sequence to your teacher who will give you a corresponding 3-color sequence.

**DNA Sequence (copy from above): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Translated RNA Sequence: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**RNA Color Sequence: \_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Step 6:** Determine the 3-color DNA sequence that pairs with your 3-color RNA sequence (above) using the table below.

|  |  |
| --- | --- |
| **RNA COLOR** | **DNA PAIR COLOR** |
| Blue | Red |
| Red | Blue |
| Yellow | Green |
| Green | Yellow |

**DNA Color sequence: \_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Step 7:** Get your DNA strand into the proper location within the chromosome.

* Locate the DNA color sequence from step 6 above on the DNA helix on the chromosome side on the board.
* Aim your DNA at that portion of the DNA helix. Throw until you successfully land in the correct area.

**Step 8**: Sometimes DNA can be inserted into a chromosome and not be taken up into the chromosome and replicated during the DNA replication process. You need to make sure that even though your new genetic material landed in the chromosome, it is being replicated into future copies of the cell.

* Call your teacher over to determine whether the ball(s) that landed on the chromosome portion of the felt board were successful.

 **Step 9**: Calculate your success rate.

* How many balls did you throw at the felt board until you were successful (the DNA was successfully replicated)? \_\_\_\_\_\_\_\_\_
* Divide 1 by this number (above) to determine your success rate. \_\_\_\_\_\_\_\_\_\_\_