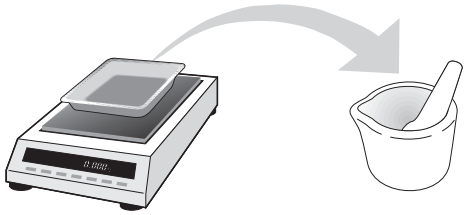
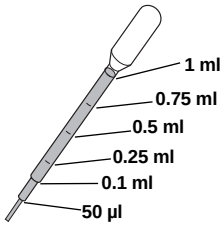
Quick Guide

Day One: Extraction of DNA From Food Samples

1. Find your screwcap tubes and label one “non-GMO” and one “test”.
2. Weigh out 0.5–2 g of certified non-GMO food and put it into the mortar.



3. Add 5 ml of distilled water for every gram of food. To calculate the volumes of water you need, multiply the mass in grams of the food weighed out by 5 and add that many milliliters.

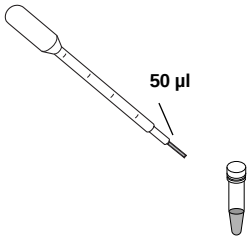


Mass of food = ____ g x 5 = _____ ml

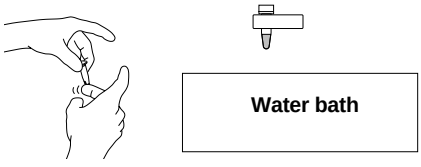
4. Grind with pestle for at least 2 min to form a slurry.

5. Add 5 volumes of water again and mix or grind further with pestle until smooth enough to pipet.

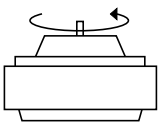
6. Pipet 50 µl of ground slurry to the screwcap tube containing 500 µl of InstaGene labeled “non-GMO” using the 50 µl mark on a graduated pipet. Recap tube.



7. Repeat steps 2–5 to prepare the test food sample.
8. Pipet 50 µl of ground test food slurry to the screwcap tube labeled “test”. Recap tube.
9. Shake or flick the non-GMO food and test food InstaGene tubes and place tubes in 95°C water bath for 5 min.



10. Place tubes in a centrifuge in a balanced conformation and centrifuge for 5 min at max speed.



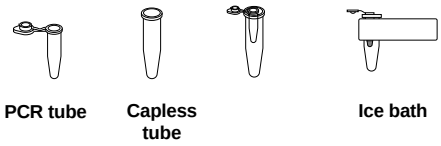
11. Store tubes in a refrigerator until next lesson.

Day 2: Set Up PCR Reactions

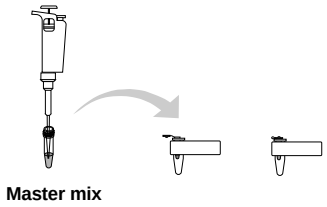
1. Number PCR tubes 1–6 and initial them. The numbers should correspond to the following tube contents:

Tube number	Master Mix	DNA
1	20 µl Plant MM (green)	20 µl Non-GMO food control DNA
2	20 µl GMO MM (red)	20 µl Non-GMO food control DNA
3	20 µl Plant MM (green)	20 µl Test food DNA
4	20 µl GMO MM (red)	20 µl Test food DNA
5	20 µl Plant MM (green)	20 µl GMO positive control DNA
6	20 µl GMO MM (red)	20 µl GMO positive control DNA

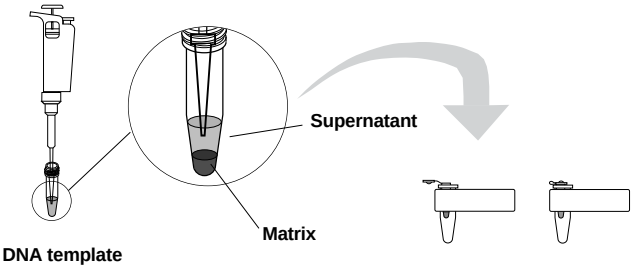
2. Place each tube in a capless microtube adaptor and place in the foam float on ice.



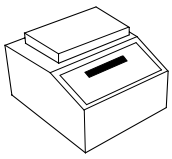
3. Referring to the table and using a fresh tip for each addition, add 20 µl of the indicated master mix to each PCR tube, cap tubes.



4. Referring to the table and using a fresh tip for each tube, add 20 µl of the indicated DNA to each PCR tube, being sure to avoid the InstaGene pellet at the bottom of the tubes. Mix by pipetting gently up and down; recap tubes.



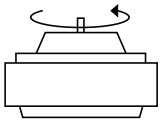
5. When instructed, place PCR tubes in thermal cycler.



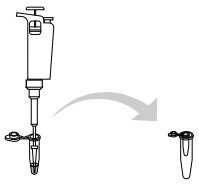
Day 3: Electrophoresis of PCR products

1. Set up your gel electrophoresis apparatus as instructed.

2. Obtain your PCR tube from the thermal cycler and place in the capless microtube adaptor. Pulse-spin the tube for ~3 seconds.

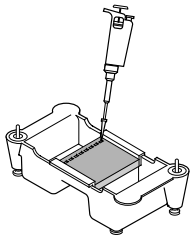


3. Using a fresh tip each time, add 10 µl of Orange G loading dye (LD) to each sample and mix well



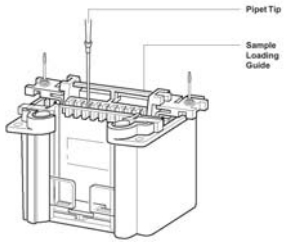
4. Load 20 µl of the molecular weight ruler and 20 µl each sample into your gel in the order indicated below:

Lane	Sample	Load volume
1	Sample 1: Non-GMO food control with plant primers	20 µl
2	Sample 2: Non-GMO food control with GMO primers	20 µl
3	Sample 3: Test food with plant primers	20 µl
4	Sample 4: Test food with GMO primers	20 µl
5	Sample 5: GMO positive DNA with plant primers	20 µl
6	Sample 6: GMO positive DNA with GMO primers	20 µl
7	PCR molecular weight ruler	20 µl
8	Leave empty	



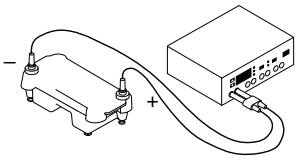
Agarose Gel

or



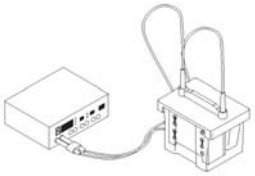
Polyacrylamide Gel

5. The run time and voltage will depend on the type of gel you are running. Run an agarose gel for 30 min at 100 V and run a polyacrylamide gel at 200 V for 20 min.



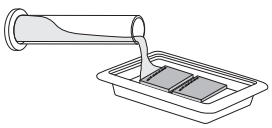
Agarose Gel Electrophoresis

or



Polyacrylamide Gel Electrophoresis

6. Stain in Fast Blast DNA stain. Refer to specific instructions depending on gel type.



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