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PCR Detection of Genetically Modified (GM) Foods

Protocol

Purpose

Isolate DNA from corn-based food so that the Polymerase Chain Reaction can be used to determine whether the selected foods have been genetically modified.

Protocol

I. Isolation of Genomic DNA from Food Material

1. You will receive 200mg crushed food material in a 2ml microcentrifuge tube. **Label** your 2ml microcentrifuge tube with your initials.

2. **Tilt the tube to the side** so that the food material is covering the side of the tube. Add **500ul Lysis Buffer A** to the food material. Cap the tube and vortex for **30 seconds**.

3. Add **5ul RNase A** to the tube. Cap the tube and **vortex** for **30 seconds**.

4. Add **250ul Lysis Buffer B** to tube. Cap the tube and **vortex** vigorously for **10-15 seconds**. Place the tube on its side.

5. **Incubate** the tube on its side at room temperature (22-25°C) for **10 minutes**.

6. Add **750ul Blue Precipitation Solution**. Cap the tube and **vortex** for **30 seconds**.
   - The sample should be evenly suspended. If not, vortex or mix with a **yellow** pipette tip by hand.

7. **Spin for 10 minutes** in a microcentrifuge at maximum speed. (13,000 x g)

8. **Transfer the supernatant** (200 to 800 µl) to a fresh 2ml microcentrifuge tube. There will be some non-digested food material in the bottom of the tube. Dispose of the pellet-containing tube once the supernatant has been removed to a new tube.
   - If there is floating material on top of the liquid phase, carefully pipette under it.

9. Mix the bottle of MagneSil® Paramagnetic Particles (PMPs) by vortexing for 15-30 seconds to make sure that the brown PMPs are thoroughly resuspended. Add **50ul of resuspended particles** to the lysate now in the clean 2ml microcentrifuge tube.

10. Add **1ml Isopropanol** to the tube containing the lysate and PMPs. Cap the tube and invert the tube in your hand 10-15 times to mix.

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I. Isolation of Genomic DNA from Food Material (continued)

11. **Incubate** the tube at room temperature for **5 minutes**, mixing the tube by inversion by hand continuously to prevent resin clumping.

12. Insert the tube into the **magnetic separation stand** and leave in place for **1 minute.**
    - You will see the PMPs move to the side of the tube closest to the magnetic stand.

13. **Leave the tube in the magnetic stand.** Once all the PMPs have collected on the side of the tube, remove the cap and remove the liquid phase by **gently** flicking liquid out of tube (and into the sink) while it is **still in the stand.**

14. **Remove the tube from the stand.** Add **1ml 70% ethanol** wash solution to the particles. Cap the tube and mix by inversion by hand 10-15 times to mix.

15. Place the tube back into the magnetic stand.

16. **Leave the tube in the magnetic stand**. Leave the cap off the tube. **Gently** flick ethanol supernatant into the sink. Let the tube sit at room temperature for **5 minutes** with the lid open to allow the alcohol to evaporate. While you wait, set a pipette for 50µl and use it to remove (from the bottoms of the tube) any residual ethanol that may weep off of the resin. Squirt removed ethanol into the sink, or eject the liquid-filled tip into the waste container.

17. **Remove the tube from the stand**. Add **100µl of Nuclease-free water and vortex to resuspend the particles in the water.**

18. **Incubate at 65°C for 5 minutes**.

19. **Place the tube back into the magnetic stand**.

20. Transfer **50ul** of your sample to a new 0.5ml centrifuge tube. Label the top of the tube with your initials and food sample. Your purified DNA will be quantified.

    Food sample: _____

    DNA concentration: _____

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II. Prepare & Run CaMV 35S PCR Amplification

1. Locate five 0.2ml thin-walled PCR tubes. Label the top of each tube with the name of a food sample, as listed below:

D – Doritos
C – Cheetos
G – Grits
(+) – Cornmeal GMO Positive Control
(-) – Cornmeal GMO Negative Control

2. Assemble a PCR master mix **(ON ICE!!!)** by combining the following required PCR reagents in the 0.5mL blue tube. Make enough for your group's 5 reactions plus 1, for a total of 6.

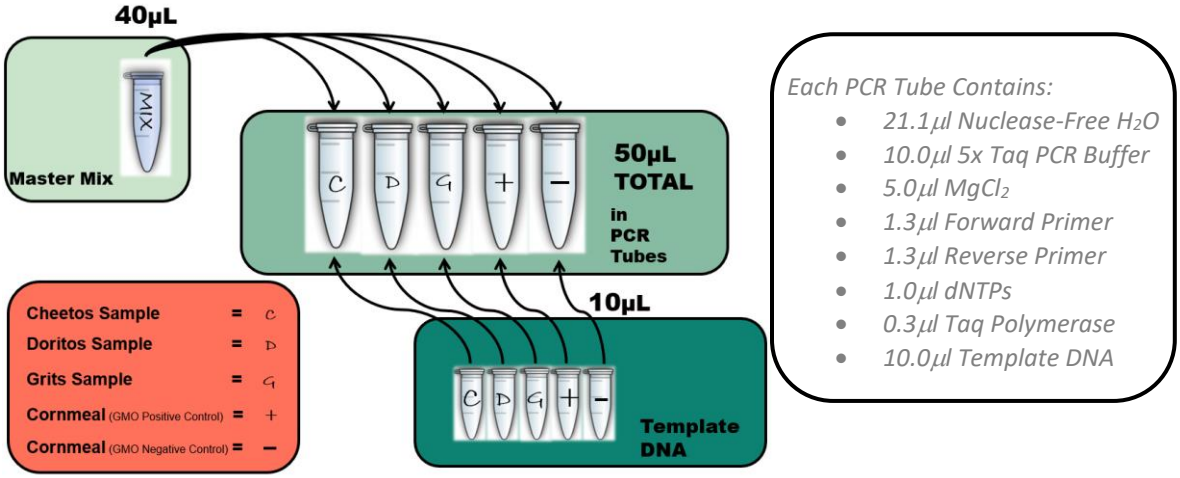
Important: **SPIN ALL TUBES BEFORE ASSEMBLING MASTER MIX!!!**

Reagents	Volume in 6x Master Mix
Nuclease-Free H_2O	126.5µl
5x Taq PCR Buffer	60.0µl
$MgCl_2$	30.0µl
35S Forward Primer	8.0µl
35S Reverse Primer	8.0µl
dNTPs	6.0µl
Taq Polymerase	1.5µl
Total:	**240.0µl**

3. Spin the completed master mix briefly in a minicentrifuge, then blend the master mix by pipetting up and down gently 5-10 times.

4. **ON ICE!!!** Use a pipette to transfer 40µL Master Mix into the five labeled 0.2ml tubes. Add Master Mix to the bottom of the PCR tubes.

5. Add **10µL** DNA template (labeled **C**, **D**, **G**, **+** or **-**) to each corresponding PCR tube. Add DNA to the bottom of the tube and pipette up and down to mix.



6. Spin the PCR tubes in a minicentrifuge with the appropriate 0.2ml tube adaptor for 10 seconds.

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II. Prepare & Run CaMV 35S PCR Amplification (continued)

7. Place tubes in Thermal Cycler and begin cycling using the following conditions:

PCR Amplification of Food DNA

Thirty cycles of PCR will be used to amplify the 35S promoter sequence. Your instructor will program the thermal cycler to carry out the following temperature changes: PCR Program name: **PCR GMO**

Temperature	PCR Step	Time	Number of Cycles	Purpose
94°C	Initial Denaturation	3 minutes	N/A	Ensures that all the template DNA molecules are denatured.
94° C 58° C 72° C	Denaturation Primer Annealing Extension	15 seconds 25 seconds 25 seconds	29	Amplification of target DNA sequence (CaMV35S promoter)
94° C 58° C 72° C	Denaturation Primer Annealing Final Extension	15 seconds 25 seconds 3 minutes	1	Gives DNA polymerase extra time to complete all of the PCR products.

A total of thirty PCR cycles will be completed by the thermal cycler and will take ~1.25 hours.
Analyze results on a 2% agarose gel. **The PCR product for the CAMV 35s Promoter is ~195 bp**

III. Gel Electrophoresis of PCR

1. Obtain a 2% gel from an instructor and set up your MiniOne electrophoresis system.
2. Place the gel tray with the gel in the clear buffer tank.
3. Add ~135ml of 1x sodium borate buffer to the buffer tank. There is a marking in the buffer tank to help guide you. The buffer should just cover the gel. Do not overflow!
4. Label five 0.5ml tubes, one for each of your PCR amplifications.
5. Add **2µl** 6x loading dye to each of your labeled tubes.
6. Transfer **10µl** from each of your PCR reactions to the appropriate tubes.
7. Load **10µl** of each dyed sample into the appropriate wells of the gel - look at the diagram below. You will be provided with 100bp ladder for Lane 1 (Load **6µl** marker).

Lane 1	2	3	4	5	6
100bp DNA Ladder Marker 6µl	GMO Positive Control + 10µl	Cheetos C 10µl	Doritos D 10µl	Grits G 10µl	GMO Negative Control -- 10µl

8. Place the orange bonnet on top of the MiniOne gel box and push the power button. To see the DNA bands in the gel, push the light bulb button along the right side of the MiniOne unit.
9. When the gel has separated far enough to see your PCR bands relative to the DNA Ladder, push the power button to stop the electrophoresis. Use a camera to take a picture of your gel through the window at the top of the orange MiniOne bonnet.

IV. Appendix

1. Weigh out 2g agarose and put in a 250ml Erlenmeyer flask.
2. Measure 100ml of 1X Sodium Borate Buffer in a graduated cylinder.
3. Pour the 100ml of 1X Sodium Borate Buffer in to the Erlenmeyer flask containing the agarose and gently swirl. This will make enough agarose for 4+ gels.
4. Heat the agarose in the microwave until the solution is clear, and no more agarose is visible.
5. Assemble your gel-casting tray and insert a 6-well comb into the groove on the casting tray.
6. Once the molten gel has cooled to around 65°C, add 10µl GelGreen™ DNA stain to the molten gel and swirl gently for all the orange color to diffuse.
7. Pour approximately 15ml of molten agarose into each casting tray and let cool.
8. When the gel has cooled, remove the comb.