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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. Prepare & Run CaMV 35S PCR Amplification

1. Label five 0.5ml PCR tubes with the food sample (below) and/or your group number. 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	***Total Master Mix Volume*** *(for six reactions)* **COMBINE THE FOLLOWING COMPONENTS IN ORDER:**	***Each Reaction***
Nuclease-Free Distilled Water	**126.5µL**	21.1µL
5X PCR Buffer	**60µL**	10µL
$MgCl_2$	**30µL**	5µL
35S Forward Primer	**8µL**	1.3µL
35S Reverse Primer	**8µL**	1.3µL
dNTPs	**6µL**	1µL
Taq DNA Polymerase	**1.5µL**	0.3µL
TOTAL	**240µL**	**40µL**

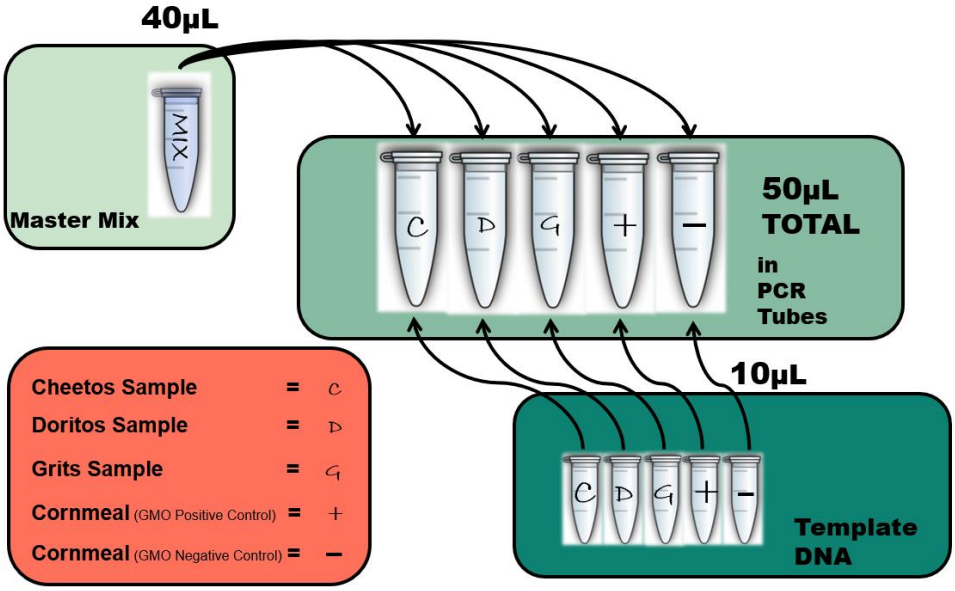
Table 1. PCR Reaction

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

BioPharmaceutical Technology Center
I N S T I T U T E

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

4. **ON ICE!!!** Use a pipette to transfer 40µL Master Mix into the five labeled 0.5ml Thin Walled PCR 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. Carefully add one drop of mineral oil to the top of each reaction and seal the tube.
7. Spin the PCR tubes in a minicentrifuge for 10 seconds.
8. 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: **PCRGMO2**

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 2 hours.
Analyze results on a 2% agarose gel. **The PCR product for the CAMV 35s Promoter is ~195 bp**

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I N S T I T U T E

II. 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 at room temperature for **10 minutes**. (22-25^{O}C).

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.

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.

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I N S T I T U T E

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

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.

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^{O}C for 5 minutes**.

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

20. Transfer **10ul** of your sample to a new 1.5ml centrifuge tube.

21. Add **2ul** Loading Dye to the new tube.

22. Load **10ul** of your dyed sample into a 1% agarose gel.

23. Record the gel and well locations of your sample in the following Loading Diagram:

Loading Diagram
Gel Number:_____

1	2	3	4	5	6	7	8	9	10	11	12

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III. Gel Electrophoresis of PCR

Note: Agarose Gel Preparation [*steps 1 through 6*] is often done for you.
If this is the case, then please proceed to *step 7*.

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 2+ 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 by raising the buffer dams and securing them in place, and insert a 6-well comb into the upper groove on the casting tray.
6. Once the molten gel has cooled to around 65°C Pour approximately 35ml of molten agarose into the casting tray and let cool.
7. When gel has cooled, place it into the electrophoresis cell and remove the comb.
8. Add about 325-350mls of 1X sodium borate buffer to the electrophoresis chamber, buffer should just cover the gel.
9. Label five 0.5ml tubes, one for each of your PCR amplifications.
10. Transfer **10µl** from each of your PCR reactions to the appropriate tubes that you just labeled. **BE CAREFUL** to put your pipette tip beneath the level of the mineral oil so that you draw PCR sample (and not oil) with your pipette.
11. Add **2µl** EZ-Vision loading dye to each of your sample-containing tubes.
12. Load **10-12µl** 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 **8µl** marker).

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**

13. Place the lid on the gel box; connect the electrodes to the power supply. Make sure that the black wire goes into the black plug and the red into red.
14. Turn on the power supply and set it at **250 V**. Bubbles at the electrodes indicate that the current is running. The gel will run for 20 minutes.
15. After the gel has run, remove it from the gel box.

III. Gel Electrophoresis of PCR Reactions (continued)

16. Place the gel on the UV light box to visualize the DNA and to photograph it.

Lane	Sample
1	Marker 100bp DNA Ladder
2	Positive Control GM(+) Corn Meal
3	Cheetos
4	Doritos
5	Grits
6	Negative Control GM(-) Corn Meal