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

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. Food options may include Cheetos (C), Doritos (D), Grits (G), or cornmeal (+ or -). **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 **500µl Lysis Buffer A** to the food material. Cap the tube and vortex for **30 seconds**.

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

4. Add **250µl 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 **750µl Blue Precipitation Solution**. Cap the tube and **vortex** for **30 seconds**.
   - The sample should be evenly suspended. If not, vortex or mix with a small 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 **50µl 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 **50µl** 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: _____