Detecting GMOS via PCR & Gel Electrophoresis

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11th Grade

Introduction

- Genetic Engineering
  - Encode beneficial traits into plants and animals
- 448 million acres of GM crops globally in 2014
  - 82% of Soy
  - 68% of Cotton
  - 30% of Corn
  - 25% of Canola

Background Info

- Genetically Modified Organism
  - “Unnatural” changes
- Transgenes – genes from another organism
- 2 Important Example
  - Bacillus thuringiensis – Toxic to Corn Borers
  - Glyphosate-resistance gene – protects from common herbicides
    - Higher yield, lower environmental impact, etc.

Objective

- To determine if certain “Non-GMO verified” claims are valid (35S Promotor and 225 NOS)
  - Triscuit Thin Crisps
  - Soybeans (Generic Brand)
  - Banza Spagetti
  - Popcorn Cakes
  - Peanuts (Generic Brand)
  - Post Great Grains Cereal

Foods


SKINNY
POP
POPCORN
CAKES
40
- SEA SALT -
NON GMO
GLUTEN FREE


Post
great grains
CEREAL
BANANA NUT CRUNCH
BANANA CLUSTERS
ALMONDS & WALNUTS
FLAKES
7g
43g
NON GMO


NON GMO
Triscuit
ThinCrisps
starts with
3 Simple
Ingredients
ORIGINAL
MADE WITH
SEA SALT


Banza
LINGUINE MADE
FROM CHICKPEAS
NET WT 8 OZ (227G)
POWERED BY
CHICKPEAS

Hypothesis

- I believe that the non-GMO verified claims on the foods that have them are true.
  - Foods w/ “Non-GMO”
    - Cereal, Triscuit, Spagetti, Corn
  - Foods w/o Labels
    - Peanuts & Soybeans

Procedure

- Isolation
  - Extract samples of DNA from food products
- Amplification
  - Use PCR to replicate the DNA for larger pool
- Gel Electrophoresis
  - Look for the specific DNA bands to determine if GMO

Materials

- Mortar & Pestle
- Non-GMO Grain Sample (The Control)
- Pipettes & Distilled Water
- Water Bath & Assorted Tubes
- Insta-Gene Matrix (Microscopic Beads)
- Scale
- Centrifuge

Isolation

1. Pulverize the food sample in a sterile mortar & pestle into a fine powder
    A. Weigh out ~1 gram and mix with 5 ml of water
2. Pipet 50 µl of slurry into a tube with 500 µl of Insta-Gene
3. Repeat previous steps for each food sample & control
4. Water Bath @ 95° C for 5 min, then centrifuge

Materials

- PCR Thermal Cycler
- Plant PSII (green) & GMO (red) primers
- Master Mix
- GMO-positive sample (Another Control)
- Pipettes & Distilled Water
- Ice-bath & Assorted Tubes

Amplification

1. Pulse-spin all tubes & template (50 µl); Avoid Supernatant
2. Dilute primers with the Master Mix
    A. Ratio of 11 µl primer : 550 µl master mix; Store on Ice
3. Create the following mixtures via pipette in PCR tubes:
    A. 20 µl plant + 20 µl food sample
    B. 20 µl GMO + 20 µl food sample
4. Repeat for all test foods and controls; Made 2 of each

Amplification (pt. 2)

5. Place the PCR tubes in the Thermal Cycler

A. 94° C @ 2 min

B. Begin 40 Cycles:
   A. 94° C @ 1 min
   B. 59° C @ 1 min
   C. 72° C @ 2 min

C. 72° C for 10; Hold @ 4° C

Materials

- Agarose Gel (Made from Powder)
- 1X TAE buffer (Tris-acetate-EDTA) & Methylene Blue Dye
- Orange Loading Dye & Molecular Weight Ruler
- Pipettes & Distilled Water & Assorted Tubes/Flasks
- Transilluminator (LightBox)
- Gel Electrophoresis Chamber & Equipment
- Weighting Dishes/Ziplock Bags

Gel Electrophoresis

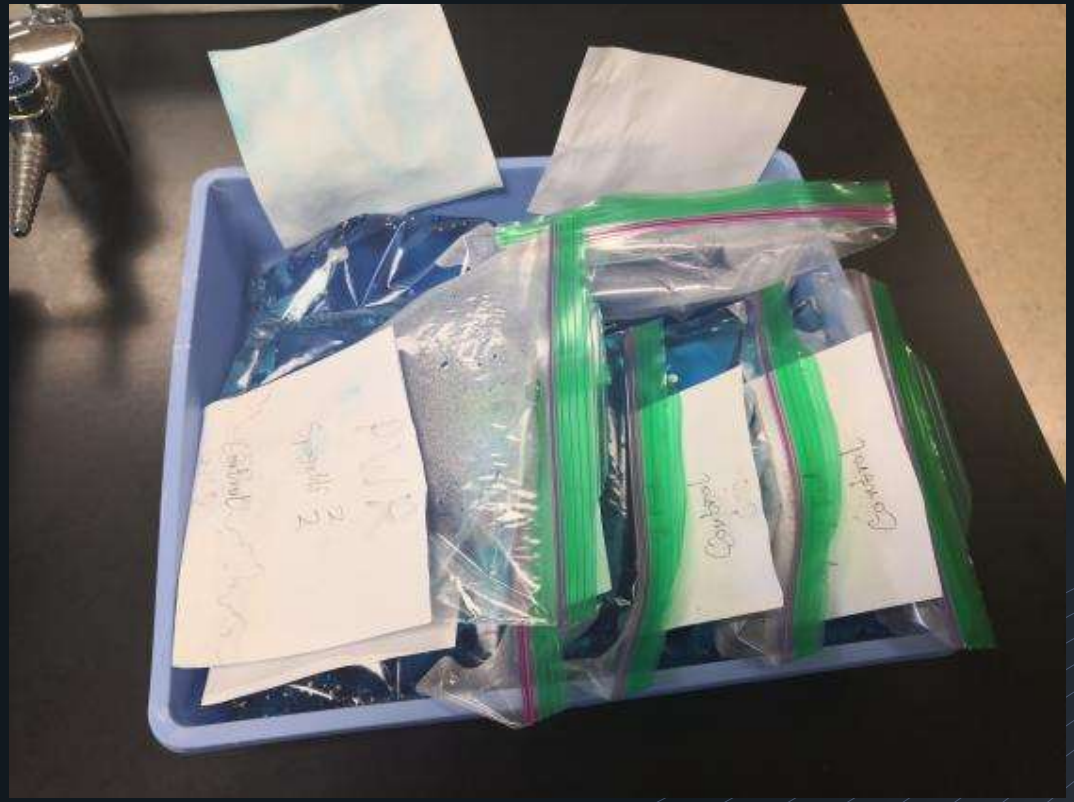
1. Prepare 3% agarose gels with 6 wells each & place in station
    A. Set up gel electrophoresis station & pour TAE buffer in
2. Remove PCR tubes and place in a tray
3. Pipette 10 µl of orange loading dye into each tube
    A. Add 40 µl to 1000 µl of MWR & mix well
4. Load 20 µl of each sample into individual lanes
    A. Requires 1 MWR in each gel

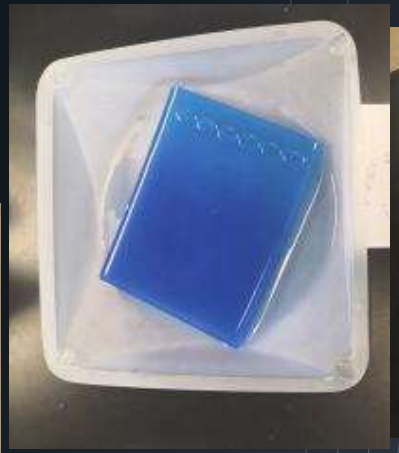
Pictures

Gel Electrophoresis (pt. 2)

5. Run the gels until ~80% orange front (150 V @ 45 min)
6. Stain with Methylene blue dye overnight in weighing boats
    A. 1X concentration (Diluted from 500X)/Ziplock bags
7. Wash off gel and soak in distilled water
    A. Be careful of dehydration
8. Repeat until bands are visible under light box
9. Record the data and analyze

Pictures



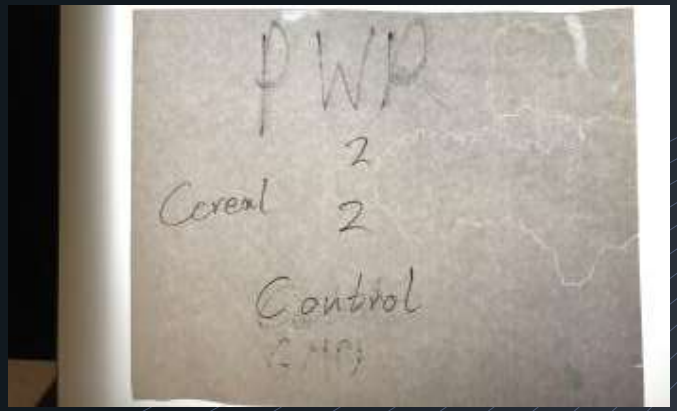


Diagram

Note: Control Refers to POSITIVE GMO

	Gel #1	Gel #2	Gel #3	Gel #4	Gel #5	Gel #6	Gel #7
Lane #1	MWR	MWR	MWR	MWR	MWR	MWR	MWR
Lane #2	Cereal GMO	Spaghetti GMO	Corn GMO	Triscuit GMO	Peanuts GMO	Soy GMO	Grain GMO
Lane #3	Cereal GMO	Spaghetti GMO	Corn GMO	Triscuit GMO	Peanuts GMO	Soy GMO	Grain GMO
Lane #4	Cereal Plant	Spaghetti Plant	Corn Plant	Triscuit Plant	Peanuts Plant	Soy Plant	Grain Plant
Lane #5	Cereal Plant	Spaghetti Plant	Corn Plant	Triscuit Plant	Peanuts Plant	Soy Plant	Grain Plant
Lane #6	Control GMO	Control GMO	Control GMO	Control GMO	Control Plant	Control Plant	Control Plant

Pictures



True/False Chart

	Gel #1	Gel #2	Gel #3	Gel #4	Gel #5	Gel #6	Gel #7
Lane #1	MWR	MWR	MWR	MWR	MWR	MWR	MWR
Lane #2	Cereal GMO	Spaghetti GMO	Corn GMO	Triscuit GMO	Peanuts GMO	Soy GMO	Grain GMO
Lane #3	Cereal GMO	Spaghetti GMO	Corn GMO	Triscuit GMO	Peanuts GMO	Soy GMO	Grain GMO
Lane #4	Cereal Plant	Spaghetti Plant	Corn Plant	Triscuit Plant	Peanuts Plant	Soy Plant	Grain Plant
Lane #5	Cereal Plant	Spaghetti Plant	Corn Plant	Triscuit Plant	Peanuts Plant	Soy Plant	Grain Plant
Lane #6	Control GMO	Control GMO	Control GMO	Control GMO	Control Plant	Control Plant	Control Plant

Conclusion

- My hypothesis was partially correct.
- Determined to be non-GMO
  - Cereal, Spaghetti, Corn, Triscuit, Peanuts
- Soy discarded due to Error, etc.
- The controls worked as expected for the majority
- Plant Primer?

Limitations/Errors

- Voltage control
- Sensitivity of agarose gels during formation
- Staining issues with band variation
- Lack of time/resources to process more trials
- Potential Contamination in Mortar/Pestle
- Inconsistency between separate gel runs

Further Inquiry

- More trials and larger sample sizes
- More types of food
- What is the threshold for non-GMO certified?
- Calculate Retardation factor to normalize results
- Perform horizontal gene transfer in plant growth
- CRISPR research

Works Cited

"Genetically Modified Organism." *Wikipedia*, Wikimedia Foundation, 23 Feb. 2019, en.wikipedia.org/wiki/Genetically_modified_organism.

"Horizontal Gene Transfer." *Welcome -Antimicrobial Resistance Learning Site For Veterinary Students*, amrls.cvm.msu.edu/microbiology/molecular-basis-for-antimicrobial-resistance/acquired-resistance/acquisition-of-antimicrobial-resistance-via-horizontal-gene-transfer.

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Questions

Extra