**DAS and SCIENCE EXPRESS EXPERIMENT: DETECTING BIOTECH CORN**

**EXPERIMENTAL PROCEDURE**

**Purpose:** To detect a traited (GMO) corn plant using biotechniques including PCR and Gel Electrophoresis.

**PCR Protocol: Day 1**

**Reagents and Supplies**

**Reagents:**

* PCR master mix I (green)
* PCR master mix II (purple)
* GMO Positive DNA control (red)
* GMO Negative DNA control (blue)
* Genomic Corn DNA 1µg/µl (yellow)
* Unknown Corn DNA- 1 (yellow)
* Unknown Corn DNA- 2 (yellow)

**Supplies:**

* Thermocycler
* PCR tubes – strip tubes
* P200 Micropipette and tips

**PCR Set-up:**

**Step 1:** Remove master mixes and DNA tubes from freezer and allow to thaw on ice, once thawed, spin briefly in a micro centrifuge

**Step 2:** Label the PCR strip tubes as appropriate and micropipette the corresponding amounts

 into the correctly labeled tubes. Microfuge the tubes for 1min; or Shake down gently

 **Tube # Master Mix DNA**

 **1 30 µL MMI 20 µL Unknown DNA 1**

 **2 30 µL MMI 20 µL Unknown DNA 2**

 **3 30 µL MMI 20 µL GMO + DNA**

 **4 30 µL MMI 20 µL GMO - DNA**

 **5 30 µL MMI 20 µL water**

**Step 2b:**  If desired, set up another set of tubes using MMII

**PCR Set-up: continued**

**Step 3:** Program the thermocycler with the following conditions

|  |  |  |
| --- | --- | --- |
| **PCR Parameters:**  |  |  |
| **CYCLE** | **TEMP (0C)** | **TIME** |
| **Initial Denaturation**  | **96** | **10 minutes** |
| **Denaturation**  | **95** | **30 sec** |
| **Annealing** | **58** | **30 sec****40 cycles** |
| **Elongation** | **72** | **1 minute** |
| **Final Elongation** | **72** | **10 minutes** |
| **Cool down** | **4** | **forever** |

* Add strip tubes to thermocycler, tighten lid, and start program
* After PCR program is completed, spin down and store at 40C until use

**Instruction:**

* Explain basic principles of PCR and the components in the master mix and the purpose of each component
	+ Primers specific for WT- corn gene called Invertase
	+ Primers specific for inserted gene, called CamV35S Promoter
	+ PCR Buffer including MgCl2, the importance of pH
	+ Taq polymerase enzyme
* Explain Thermocycling conditions and what is happening during cycles
	+ Denaturation
	+ Annealing – How is this temperature derived
	+ Elongation
	+ Log Phase of Amplification
* Explain a Biplex PCR and the expected results.
	+ Internal control gene is a gene in which every corn plant has in their genome. The positive, negative control DNA and Unknown samples should have a band at 98bp.
	+ If traited (GMO) corn, a second band should be detected. This band represents the gene which was inserted into the corn genome.
		- In MMI: the band size is ~150bp
		- In MMII: the band size is ~450bp

**Gel Electrophoresis: Day 2**

**Reagents and Supplies**

 **Reagents**

* Molecular Weight Ladder
	+ (1 Kb Plus or Promega 2-Log DNA ladder, 0.1ml, cat# N3200S)
* 5x Loading dye
* Agarose
* 1xTAE Buffer, dilute 10x TAE buffer (mol bio grade, 1L, cat# V4271)
* SYBR Safe Dye (cat# 533102)

or

* Fast Blast DNA Stain (500x) (Bio-Rad cat# 166-0420EDU)
* PCR Amplified Product

**Supplies**

* Electrophoresis rig with casting tray
* Power supply
* Balance
* Glassware – bottle or flask (heating Agarose)
* Graduated Cylinder
* Microwave – heating Agarose
* Staining tray – Fast Blast Stain
* Gel Imaging system (optional if using Fast Blast Dye)
* Micropipette – P10 and P200

**Gel Electrophoresis Set-up Protocol**

**Making Agarose Gel:** For a 1.2% Agarose gel:

**Step 1:** Weigh out .9 grams of Agarose and place into a glass container (bottle/flask).

**Step 2:** Using a graduate cylinder, measure 75mls of 1xTAE buffer and pour into glass container. (Dilute 10xTAE buffer with water to a 1xTAE solution)

**Step 3:** Heat in microwave to boiling point, but do not boil over. Swirl container and ensure all Agarose has gone into solution

**Step 4:**  Allow to cool ~ 5-10 minutes

* + For SYBR Safe Stain: Add 7.5µL of SYBR stain into the Agarose and swirl
	+ For Fast Blast Stain: Add 1µL of 500x Fast Blast DNA Stain into the Agarose and swirl

**Gel Electrophoresis: Day 2 continued**

**Gel Rig Set-up:**

**Step 1:** Ensure the casting tray is sealed and add the appropriate size comb, at least 8 wells

**Step 2:** Once Agarose is cooled to touch, pour slowly into the casting tray. Avoid bubbles.

**Step 3:** Allow Agarose to fully solidify (~ 10-15 min)

**Step 4:** Place Agarose gel with tray into the gel rig, remove the comb.

**Step 5:** Pour 1xTAE buffer over the gel and into the Electrophoresis chamber until the gel is fully submerged and the wells are covered. The gel is ready to load.

**Note:**  If gel will be saved for next day loading, remove comb and slide off casting tray.

Wrap gel in Saran wrap and store in refrigerator. Begin next day with **Step 4**

**Sample Preparation for Gel Loading:** (while Agarose Gel is solidifying)

**Step 1:** Remove PCR products from refrigerator or thermocycler, quick spin in a micro centrifuge and ensure all contents are at the bottom of the tube

**Step 2:** Pipette 2µL of 5x Loading dye into each PCR sample tube, use a new pipette tip for each sample. Gently pipette up and down to mix the sample and dye. Quick spin samples.

**Step 3:** Prepare the Molecular Weight Ladder into a microfuge tube and label as Tube 6

* For 1Kb plus ladder: 28µL ladder + 2µL 5x Loading dye

**Step 4:** Prepare Genomic Corn DNA sample (optional), and label as Tube 7

* Pipette 25µL of 1µg of Corn DNA (tube 0) + 5 µL 5x Loading dye

**Step 5: Load 30µL of each Sample onto the gel in the following order**

 **Lane# Sample Load Volume**

1 PCR Tube 1, Unknown DNA 1, MMII 30µL

2 PCR Tube 2, Unknown DNA 2, MMII 30µL

3 PCR Tube 3, GMO + Control, MMII 30µL

4 PCR Tube 4, GMO - Control, MMII 30µL

5 PCR Tube 5, water blank 30µL

6 Tube 6, Molecular weight ladder 30µL

7 Tube 7, Genomic Corn DNA 30µL

8 Blank or 5µL 5xLoading Dye 5µL

**NOTE:** Samples maybe prepped then stored in the refrigerator to load the next day

**Gel Electrophoresis: Day 2 continued**

**Step 6:** Plug Electrophoresis into power supply, set Voltage for 110 Volts for at least 30 min.

 This is a guideline, time and voltage may vary depending on type and size of gel.

 **NOTE: Always remember “Run to Red”, (Negative to Positive)**

**Step 7:** If used a **SYBR Safe** gel stain, (No Post Staining is Required)

* View gel with a UV-Illuminator with the following settings examples

(dependent upon type of imagining system)

* + 530 nm Emission filter; Excitation, broadband UV (VersaDoc Bio-Rad)
	+ 530 nm Emission filter; Excitation 402nm (Gel Doc XR+, Bio-Rad)
	+ 530 nm Emission filter; Excitation Blue Light, 480nm (Vernier)

**Gel Staining Protocol** (for Fast Blast DNA Stain) **Day 3**

**Step 1:** Remove gel from gel tray and gently slide into staining tray

**Step 2:** Make **.1x dilution** of 500x Fast Blast DNA stain

* For 200 mls: Micropipette 40µL of 500x Stain into 200mls DI water

**Step 3:** Pour Fast Blast dilution into staining tray and ensure entire gel is covered.

 Place on rocker with low speed (40-60 rpm) overnight.

 Bands will appear visual to eye

**Explanation of Results**

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**Discussion Points:**

* Difference between genomic DNA and PCR Amplified DNA
* Fail results between a negative result
* Difference between positive band sizes