

# *Transgenic or Volunteer?*

*Adapted from Damon Lisch procedure: All Natural (no organics) Genomic Mini-prep*

## **Background:**

A *b* locus encodes a transcription factor that regulates the expression of genes that produce purple anthocyanin pigment. The genotypes of maize plants are *B'B'* (streaked), *BI/BI* (purple), or *b/b* (green). The introduction of the trans gene into a *b/b* maize plant causes the expression of anthocyanin turning the plant purple. A stray green maize plant was found amongst a family of transgenic purple maize plants. It is your job to determine if this plant is a silenced transgenic mutant or a volunteer.

## **Materials:**

- Extraction Buffer (for 50 ml)
- 5 ml 1 M Tris, pH 8 (final conc. 100 mM)
- 5 ml 0.5M EDTA (final conc. 50 mM)
- 5 ml 5 M NaCl (final conc. 500 mM)
- 35 ml sterile water
- TE
- 10mM Tris
- 1 mM EDTA
- 10% SDS
- 5M Potassium Acetate (KOAc)
- 100% Isopropanol
- 70% Ethanol
- TE or Water (TE is better when you don't trust the quality of the prep.)
- Ice Bucket
- 2 eppendorf (1.5 ml) tubes per prep
- Miracloth (cheese cloth can be substituted)

## **Procedures:**

### **DNA Extraction**

1. Measure (¼ teaspoon) \*\*\*grams of leaf tissue. Add 1000 µl of Extraction Buffer, and grind some more in the buffer.
2. Pour the slurry into a 1.5 ml eppendorf tube.
3. Add 120 µl of 10% SDS. Keep samples on ice until all are done.
4. Put at 65°C for 20 minutes.
5. Add 300 µl 5M KOAc. Mix well by inverting several times (**important!**), then place on ice 5-30 minutes (5 is fine).
6. Spin for 5 minutes at top speed in microfuge. Squirt about 700 µl of the supernatant through miracloth (make small funnel, place tip directly onto the miracloth at the tip of the funnel and squirt through – do not allow the whole funnel to get soaked).
7. Add 600 µl of isopropanol. Mix the contents thoroughly by inverting. DNA precipitate may or may not be visible at this point; don't worry if you don't see much. A really good prep (excellent grinding of tissue) should result in visible DNA at this stage, however. Can put in the freezer for a while at this point, or proceed immediately to the next step
8. Spin for 1 minute at top speed.
9. Pour off supernatant, add 500 µl of 70% ethanol and flick until the pellet comes off the bottom (for best washing results).
10. Spin briefly, then pour off the ethanol. Suck off the rest of the ethanol with a pipette.
11. Let air dry in hood for around 10 minutes.
12. Re-suspend the DNA in 100 µl water or TE.
13. Let sit at room temperature for 30 minutes, then mix by pipetting. Best to then let sit for another 30 minutes and pipette mix a final time.
14. (Optional) Divide your DNA samples into 5 tubes of 20 µl each. Label them well. Freeze 4 of the tubes for future use.
15. (Optional) Dilute DNA 10 fold for PCR. Put 10 µl of DNA prep into 90 µl of water.

### PCR Reactions' Setup

For PCR you will need to set up on reaction for each plant plus the negative control.

Use these steps to set up PCR:

1. Label PCR tubes (0.2 ml) and one 1.5 ml tube for the master mix.
2. Fill in chart below:

Reagent	1 reaction (µl)	___ reactions
Water	16.0	
2X Master Mix	25.0	
Primer <i>MCG700A</i>	2.0	
Primer <i>MCG700B</i>	2.0	
DNA	5.0	-----
<i>Total</i>	50.0	

*2X Master Mix includes Taq, dNTPs, and buffer. It should always be on ice.*

*MCG700A (35S/adh1 reverse) GAT CCG TCG ACC TGC AGG TCT GTC CTC TCC*  
*MCG700B (35S/adh1 forward) CAA AGG GTA ATA TGG GGA AAC CTC CTC GG*

3. KEEP ON ICE!
4. Prepare the master mix (Can buy premade).
5. Add 45 µl to each PCR tube.
6. Add 5 µl plant DNA to the correct tube.
7. Add 5 µl water to the negative control.

Checklist to use when preparing samples:

		DNA						PCR Position
		Master Mix	B/B	b/b	b/b trans	unknown	Water	
1	B/B	45 µl	5 µl					
2	b/b (non-transgenic)	45 µl		5 µl				
3	b/b (transgenic)	45 µl			5 µl			
4	unknown	45 µl				5 µl		
5	water	45 µl					5 µl	

Cycling Conditions	Temp. (°C)	Time (min./sec.)
Denature	95 °C	5 min. 30 sec.
Anneal	63 °C	30 sec.
Extension	68 °C	~11 min.
Hold	4 °C	Indefinitely
Number Cycles: 30		

## Creating, Loading, and Analyzing Gel

1. Pour a 1% Gel while PCR is running.
2. Load DNA markers in 1<sup>st</sup> and last wells.
3. Load Samples 1-5 in middle wells.
4. Let gels run till dye reaches the middle of the gel.
5. Stain gels.
6. Photograph gel and analyze in the following way:
  - a. Draw a line along the 'bottom' of the wells.
  - b. Label Semi-log paper
    - i. x-axis: distance traveled (cm or mm)
    - ii. y-axis: DNA length (bp or kb depending on ladder)
  - c. Measure distance from well to the 'bottom' or 'leading' edge of the band.
  - d. Plot distances. Students can plot all bands but should only use ones similar in size to the unknown band.
  - e. Draw a line through the dots if they approximate a straight line. If not draw a fit line by eye. If all bands were plotted, separate lines may need to be drawn. This demonstrates how electrophoresis estimates are only valid on similar sized standards.
  - f. Measure distance to leading edge of the experimental bands.
  - g. Plot those and that gives approximate size. This can be done in a spreadsheet. A regression equation can be generated to a fit. The problem is that this is too exact for gel electrophoresis. This could be a good demonstration of matching appropriate exactness with the limitation of the measurement.