Excision of an Active TE in Arabidopsis

In this experiment you will determine whether Osmar5 is active in Arabidopsis. To do this you will observe plant leaves or evidence of OSmar excision. You will also confirm excision at the DNA level using a PCR based strategy.

Over the next few days you will:

1. Observe the plants for GFP fluorescence.
2. Isolate DNA from the plants.
3. Use PCR to amplify the TE insertion site.
4. Analyze the results using gel electrophoresis.

The plants you will are:

1. Wild Type (un-transformed).
2. GFP.
3. OSmar TE, no transposase.
4. OSmar TE with transposase.

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Damon's All Natural (no organics) Genomic Miniprep
Damon Lisch

1) Harvest 5-6 plants. There is no need to put the plants on dry ice; the DNA is stable for several hours.

2) Grind tissue in liquid nitrogen to a fine powder in a small mortar. Add 1000 µl of Extraction Buffer, and grind some more in the buffer. Pour the slurry into a 1.5 ml eppendorf tube. Don't use too much liquid nitrogen or your tissue will freeze into an unworkable frozen mass.

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. Pour off supernatant, add 500 ul of 70% ethanol and flick until the pellet comes off the bottom (for best washing results). Spin briefly, then pour off the ethanol. Suck off the rest of the ethanol with a pipet. Let air dry in hood for around 10 minutes.

9) Re-suspend the DNA in 100 µl water or TE. Let sit at RT for 30 minutes, then mix by pipetting. Best to then let sit for another 30 minutes and pipetting a final time.

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10) (Optional) Divide your DNA samples into 5 tubes of 20 µl each. Label them well. Freeze 4 of the tubes for future use.

11) (Optional) Dilute DNA 10 fold for PCR. Put 10 µl of DNA prep into 90 µl of water.

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Reagent and Supply List

<u>Extraction Buffer (for 50 ml)</u>

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

<u>TE</u>

10mM Tris
1 mM EDTA

10% SDS
5*M* 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)

If you use liquid Nitrogen use a metal ladle to dispense it. (Don't use plastic it may break.)

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Analyzing Gel

Photograph gel and analyze in the following way:

1. Draw a line along the 'bottom' of the wells.
2. Label Semi-log paper
   x-axis: distance traveled (cm or mm)
   y-axis: DNA length (bp or kb depending on ladder)
3. Measure distance from well to the 'bottom' or 'leading' edge of the band.
4. Plot distances. Students can plot all bands but should only use ones similar in size to the unknown band.
5. 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.
6. Measure distance to leading edge of the experimental bands. Plot those and that gives approximate size.
7. 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.

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Set up the PCR Reactions. 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 out the sheet attached.
3. Prepare the master mix.
4. Add ___ to each PCR tube.
5. Add plant DNA to the correct tube.
6. Add water to the negative control.

Pour a 1% Gel. Details to follow.

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PCR Protocol using NEB 2X Master Mix

Purpose:

KEEP ON ICE!

Reagent	1 reaction (µl)	_____ reactions
Water	16.0	
2X Master Mix	25.0	
Primer ______	2.0	
Primer ______	2.0	
DNA	5.0	--------
Total	50.0	

Aliquot 45 µl into each tube. Add 5 µl of DNA.

Tube Labels:

Cycling conditions:

Denature ____°C

Anneal ____°C

Extension ____°C

Number of Cycles ____

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

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