Maize DNA Isolation in Microcentrifuge Tubes

- Turn the water bath on to 65 degrees C and put the KOAc and isopropanol on ice or in the freezer.

1. Place narrow pieces of maize tissue (1.5 cm by 2.5 cm, cut into narrow strips), a single 3 mm tungsten carbide bead, and 500 ul of the Candela extraction buffer in each tube.

Use 1.7 ml or 2 ml microcentrifuge tubes or 1.5 ml 96 well plates

2. Macerate tissue using Mixer Mill (ask to be trained!). Mill for 1 min at 25 Hz. Un-assemble the plate and adaptor set and switch the orientation of the plate. Mill again for 1 min at 25 Hz. If the tissue is not completely disrupted, mill again.

Alternatively, use blue pestles.

3. Add 70 ul of 10% SDS to each tube. Invert several times and incubate at 65 degrees C for 10 minutes.

Longer incubation times (1-2 hrs) ok.

4. Add 130 ul of ice-cold 5M KOAc and mix by inverting the tubes several times. Incubate on ice for 5 mins.

This step precipitates SDS-bound proteins and excess SDS.

5. Spin at max speed in microcentrifuge for 10 min.

If using 96 well plates, spin at 4000 RPM in JS-4.3 rotor for 15 min. Rpt if necessary.

6. Transfer supernatant (~400 ul) to fresh tubes. Add equal volume of isopropanol and 1/10th volume of 3M NaAc to each tube and mix by inversion. Incubate for 30 min (room temperature, ice, -20 all ok).

This step precipitates DNA. Longer incubations ok, can put tubes in -20 overnight.

7. Spin at max speed in microcentrifuge for 10 min.

If using 96 well plates, spin at 4000 RPM in JS-4.3 rotor for 15 min. Rpt if necessary.

8. Dump out or aspirate off the supernatant and wash with 500 ul of 70% EtOH. Spin for 5 min at max speed in microcentrifuge.

If using 96 well plates, spin at 4000 RPM in JS-4.3 rotor for 10 min.

9. Remove the supernatant and allow the pellet to air dry (overnight OK).

Alternatively, dry in speed vac.

10. Re-suspend pellets in 100 ul of water or TE with 10ng/ul RNAse. After the pellet is re-suspended, incubate at 37 C for 30 mins prior to use

RNase treatment critical for PCR!

Candela Extraction buffer (final concentrations):

100mM Tris-Cl pH 8.0
50 mM EDTA
100mM NaCl
10 mM β-mercaptoenthanol (add fresh – 14 ul per 20 ml)