SCHNABLE LAB
Plant Genomics

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LCM FIXATION AND CRYOPROTECTION

(Last Revised: April, 2007)

Protocol used by the Schnable Laboratory (Iowa State University). Please contact Dr. Patrick Schnable (schnable@iastate.edu) regarding questions or corrections.

1. Immerse tissue (5 mm sections) in vials containing fixative (3:1 Ethanol:Acetic acid) and vacuum infiltrate (400 mm Hg) for 20 minutes on ice.
2. Swirl vials and rotate at 4°C for 1 hour. Decant fixative or remove with RNase-freePasteur pipette.
3. Repeat steps 1 and 2 one time. Infiltrate overnight at 4°C.
4. Decant fixative or remove with RNase-freePasteur pipette.
5. Add 10% sucrose (in 1X PBS) and vacuum infiltrate (400 mm Hg) for 15 minutes on ice.
6. Swirl vials and rotate at 4°C for 1 hour. Decant fixative or remove with Pasteur pipette.
7. Add 15% sucrose (in 1X PBS) and vacuum infiltrate (400 mm Hg) for 15 minutes on ice and allow to stand for a minimum of 1 hour (or overnight). Decant fixative or remove with Pasteur pipette.
8. Fill a plastic sample holder nearly full with TissueTek OCT medium and orient sample as desired. After all air bubbles are removed.
9. Freeze the sample carrier in liquid nitrogen by placing it in a floating plastic lid (to keep the sample out of direct contact with the liquid nitrogen).
10. Store the samples at -80°C.