## Labeling leaf disks with [14C]-Sucrose

Required Items:

Suitable plant material

24-well microtitre plate(s)

20 mM MES [2(N-morpholino)ethane-sulfonic acid] plus 2 mM CaCl2, pH 5.5, with KOH

MES/CaCl2 buffer as above with 1 mM Suc, 30 kBq (0.81 µCi) mL-1; 0.5 – 1.0 mL per well

3MM or filter paper for blotting

Aluminum foil envelopes, 1 per well, labeled

Powdered dry ice or liquid nitrogen

Autoradiography film, scintillation vials and fluid

* + 1. Grow plants of interest under suitable conditions: For Arabidopsis, larger shade leaves are desirable (12 / 12 light / dark or less; ~100 µmol photons m-2 sec-1); choose the desired leaves (e.g., leaves 8, 9, 10, 11, 12 source leaves, or sink leaves, etc.)
    2. Cut the petiole near the stem, transfer to a Petri dish lined with filter paper and MES buffer to a depth of a few mm. Under MES buffer, remove a leaf disk with a cork bore (~8 mm diameter); transfer the leaf disk abaxial side down to the well of a 24-well microtitre plate containing 1 mL MES buffer to keep they hydrated while processing all the samples; pool 3-4 disks in each well.
    3. Once all the disks and wells are prepared, transfer the leaf disk to a fresh plate with the abaxial side UP; do not stack them, but distribute evenly across the bottom of the well; make sure they are the bottom and not riding up the side of the well. Process all the disks as efficiently as practical.
    4. Gently add 3 mm glass beads to cover the disks and weigh them down so they will not float.
    5. Add 0.5 mL MES/CaCl2/[14C]-Suc solution to each well to cover the disks and vacuum infiltrate the MES/CaCl2/[14C]-Suc solution into the leaf disks: In a bell jar, pull a vacuum on the microtitre plate until the disks stop bubbling (removes the air from the air spaces) and then release the vacuum (solution fills the voided air spaces). It is important to keep the disks submerged for effective infiltration: repeat as necessary until the disks are solution saturated. Disks imbibed with solution look “saturated” compared to disks that still have air in them. Air trapped under the disks will get pulled back into the disk, and disks protruding from the surface of the solution will also suck in air rather than solution. This is why care must be taken in steps 3 and 4.
    6. Incubate the disks for 20 min with gentle shaking; carefully remove the MES/CaCl2/[14C]-Suc solution to a labeled Falcon tube and freeze. This solution can be reused several times since only a small portion of the [14C]-Suc is absorbed (it is a good idea to filter sterilize it before freezing it, but invertases released from the disks may still degrade Suc). Process wells as quickly as safely practical to minimize deviations in incubation times.
    7. Wash the disks three times with 1 mL MES/CaCl2 buffer, 10 minutes per wash; treat all wash solution as radio-active liquid waste.
    8. Blot the leaf disks on absorbent paper and place them in a small, perforated aluminum foil envelope (prepared and labeled in advance and perforated with a tack). Place the envelopes immediately in LN2 or powdered dry ice, and keep them frozen until all samples are processed.
    9. Lyophilize the disks/envelopes in a mesh rack placed amongst the cooling coils of the lyophilizer for 48 hours
    10. While the envelopes are still cold from the cooling coils, transfer to a bell jar with Drierite desiccant: it is important that condensation or other moisture does not contact the dried samples to prevent diffusion of the label.
    11. Use steel plates and a vice to press thin the disks and arrange on high-resolution auto-radiography film (Kodak BioMax MR Film), or a phosphor-imaging screen to obtain an image of the labeled tissues, and/or proceed to scintillation counting.
    12. For scintillation counting, place the leaf disks in a scintillation vial, add 300 µL 80% ethanol with gentle shaking for 20 minutes to extract the tissue, add 300 µL bleach with gentle shaking for 20 minutes to clear the extract solution and tissue (adjust volumes and incubations as necessary), add 5 mL scintillation fluid and mix thoroughly, count cpm on [14C] channel. Use a negative counting control that also contains 80% ethanol and bleach.

Required Solutions and Notes:

* Instruction here on making the MES/CaCl2/[14C]-Suc solution:
* (yet to come)