Laser Microdissection

[Reagent preparation]

1. Xylene

2. 95% ethanol

3. RLT buffer from Qiagen RNeasy kit 

4. OCT (optimal cutting temperature compound)

5. Slides

6. Agilent RNA 6000 Pico Assay chips 

[Procedure]

1. Debark the sections from internodes between 15 and 20 of 6-month-old Populus trichocarpa, and cut into 0.5-mm segments.

[note] The thickness of the stem segments should not be too thin. It would be hard for make cross sections (see below) with thin stems.

2. Collect the 0.5-mm segments in a 50-ml tube and freeze in liquid nitrogen immediately.

[note] Based on our experience, freshly frozen stem can provide the best RNA quality

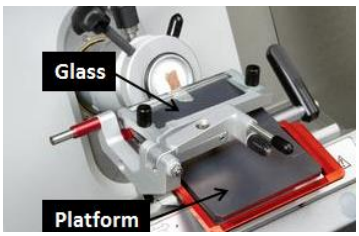
3. Attach the stem segments to a specimen chuck using optimal cutting temperature compound at -20°C for 10 min

[note] This step make the liquid nitrogen frozen stems equilibrate at -20^{o}C

Specimen chuck  , Optimal cutting temperature compound 

4. Cut 10-µm-thick cross sections cut using a cryostat at -20°C.

[note] We prefer to cut stem cross section through the platform and the glass (see picture)



5. Attach 6~8 cross sections to a slide, and keep at -20^{o}C. Prepare 3~5 slides for laser microdissection.

6. Dip the slides with stem sections in 95% ethanol for 2 min, transferred into 100% xylene for 2 min, and air-dry for 10~15 min.

7. Use LMD7000 to cut off different cell types, and the collect the tissue in RLT buffer for RNA extraction.

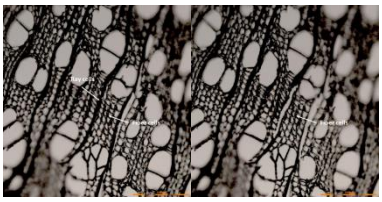
(**[Fiber cell isolation]** Use laser to burn away ray cells then avoid vessel cells)

8. Use the RNeasy Plant RNA isolation kit (Qiagen) to isolate total RNA

9. Check the RNA quality using an Agilent 2100 bioanalyzer and Agilent RNA 6000 Pico Assay chips.

Example: (Fiber cell isolation)

Step1: burn away the ray cells



Step2: choose fiber cells and avoid vessel cells