Microwave Enbedding Procedure for Arabidopsis
(NAIST, June 2006)

Reagents

Sorensen's buffer (10 mM sodium phosphate buffer) pH 7.2

1M NaH_2PO_4	6.8 mL
1M $Na_2 HPO_4$	3.2 mL
measure up to 1 L (use DEPC treated water)	

<Fixation>

1. Dissect leaves into 5 mm strip in 10 mM Sorensen's buffer (pH 7.2) with a sharp razor blade and keep on ice
2. Place samples in 20 mL glass vials
3. Turn on the microwave and set the power at 150W (35%).
4. Place the vials in a water bath. Insert the probe in the vial, make sure the probe is immersed with solution.
5. Microwave fix the sample three times in fresh 10 mM Sorensen's buffer at 37 °C for 15 min. Change the water of water bath with fresh tap water each time. Cool samples in an ice bath while changing the 10 mM Sorensen's buffer.

<Dehydration>

1. Dehydrate the sample at 67 °C for 1 min. 15 sec (1.3 m) each step using 30%, 50%, 70%, 80%, 95%, 100%, 100% with Safranin-O (0.1% Safranin-O in absolute ethanol). Change the water in the water bath each time.
2. Replace ethanol with 50% ethanol (use absolute ethanol)/50% isopropanol, then 100% isopropanol. Microwave for 1 min.30 sec. at 77 °C each step. Do not change the water in the water bath at the last isopropanol change.

<Infiltration>

1. Insert the probe in the water bath for the rest of microwave procedure. Do not have to change the water in the water bath.
2. Pour off some isopropanol, add melted paraffin wax (Fisher, Paraplast Plus) to 50%.

Microwave samples at 77 °C for 10 min.

3. Replace solution with melted paraffin wax. Microwave the sample at 67 °C for 10 min.

4. Replace the wax and microwave the sample at 67 °C for 2.5 hrs, replacing the wax every 30 min.

5. One hour before the cycle is complete, turn on the embedding hot plate. Print out the label.

6. Using forceps, remove samples from wax and place into fresh melted paraffin for orientation. Let wax harden slowly to reduce the possibility of bubbles.