Wax Embedding Protocol*
(Danforth Plant Science Center, IMF)

1. Fix tissue:
--small pieces, to facilitate wax infiltration
--glutaraldehyde gives optimum cross-linking but also makes the tissue fluorescent
--formaldehyde (~4%, 1-2 hours) is commonly used for specimens to be used for fluorescence
--consult the literature for specialized applications

2. Dehydrate in a graded water/ethanol series: 10%, 25%, 50%, 75%, 95% for 1 h each with constant agitation, changing solutions after 30 min.

3. Infiltrate fixed and dehydrated tissues in a graded series of Histoclear in ethanol: 50%, 75%, 95% Histoclear for 2 h each, then in Histoclear overnight.

4. Infiltrate in 1:1 Histoclear/wax mixture for 8 h at 56 degrees C.

5. Infiltrate in wax for 72-96 h refreshing the wax three times per day.

6. Orient tissues in embedding mold with fresh wax and cool to room temp overnight.

7. Remove from mold, trim block, section at 5-15 µm.