Protocol: Vascular labelling with *Lycopersicon esculentum* lectin
(Masahiro Inoue and Mallika Singh 9/99, modified by Mikala Egeblad 1/04)

I. Perfusion

- Anesthetize the mouse with Avertin.
- Inject 0.1 ml of 1 mg/ml lectin (in PBS, made up the same day) through a tail vein, taking 20-30s (faster will kill the mouse).
- Wait 5 min.
- Open chest, insert an 18-guage butterfly needle attached to the perfusion apparatus. Cannulate right atrium and perfuse as follows:
- 4% paraformaldehyde in PBS, 120-140 mm Hg, 5 min.
- Hold the needle in the heart steady with a forceps while maintaining the pressure. The liver should clear and the tail will often twitch when perfusion is adequate. The mouse becomes rigid after fixative is perfused through.

II. Dissection and dehydration

- Remove tissues of interest and fix in 4% paraformaldehyde overnight.
- Dehydrate through increasing sucrose concentrations for 1-12 hours each, 12%, 15%, 18%, 25% in PBS (very flexible)
- Mount tissues in OCT and freeze on dry ice. Store at -80C until cutting.
- Cut thick sections (60-80 μm), and use a confocal microscope to visualize stained vessels.

III. Reagents and perfusion apparatus

- Lectin: *Lycopersicon esculentum* (tomato) lectin, FITC conjugated. FL-1171 from Vector labs (1 mg). Refrigerate and make up in PBS just before use.
- Fixative: 4% PFA
- A standard sphymanometer to monitor pressure hooked up by tubing to corks on a 500 ml bottle with the PFA. The 500 ml glass bottle has outlets at the bottom and is connected to tubing that ends in the 18-guage needle for perfusion. The flow of liquid is controlled by pinch corks.