

## **Vascular labelling with tomato lectin (or Vascular leakage with FITC-dextran)**

### **Perfusion**

1. Anesthetize the mouse.
2. Inject 0.1 ml of 1 mg/ml lectin through a tail vein, taking 20-30s (faster will kill the mouse).
3. Wait 5 min.
4. Open chest, insert an 18-gauge butterfly needle attached to the perfusion apparatus. Cannulate right atrium and perfuse as follows:
  - a. 4% paraformaldehyde in PBS, 120-140 mm Hg, 5 min.
  - b. 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.

### **Dissection and dehydration**

5. Remove tissues of interest and fix in 4% paraformaldehyde overnight.
6. Dehydrate through increasing sucrose concentrations for 1-12 hours each, 12%, 15%, 18%, 25% in PBS (very flexible).
7. Mount tissues in OCT and freeze on dry ice. Store at -80C until cutting.
8. Cut thick sections (50  $\mu$ m), and use a confocal microscope to visualize stained vessels.