SOP 72 - Kleinfeld Laboratory - 14 June 2017

Protocol for vascular fills of adult mice

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Perfusion (performed *without* fixative):

- Anesthetize mouse with Fatal Plus
- Clip ventricle, insert cannula, and then clip atria

Perfuse with 50 ml of warm (~ 36C), 0.9 % (w/v) saline, pH 7.2, with 20 units/ml Na-heparin (Sigma H3393) at 10 to 100 ml/min.

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- Inject 20 ml of Gel perfusate, maintained at 42C
- Immerse head in ice-bath to solidify Gel
- Decapitated animal
- Post fix the entire head in 4 % (w/v) PFA in PBS on a shaker at 4C
  (a small incision to the skull can facilitate fixation)
- Extract brain
- Soak brain overnight in 4 % (w/v) PFA at 4C

Composition of Gel perfusate:

- 10 % (w/v)* gelatin (Sigma G1890)
- 0.4 % (w/v)** (Blinder et al. 2013) to 2.0 % (w/v) (Blinder Lab 2017) FITC-albumin (Sigma A9771)
- 0.1 % (w/v) Na-azide (Sigma S2002)
- All in PBS

To make 100 mL of Gel perfusate:

- Add 65 mg azide to 100 ml of PBS
- Bring to boil
- Stir in 10 g of gelatin
- Cool to just below 50C
- Filter with pre-wetted Whatman paper;
- Stir in 400 mg of FITC-albumin for 0.4 % fluorescein solution (note: Albumin denatures above 63C)
- Store in 42C oven

* Tests show that 10 % (w/v) gelatin is the "sweet spot"; it provides sufficient structural stability for large vessels, so that they maintain they roundish shape and do not collapse, while allowing a complete fill of all small capillaries.

** FITC albumin is labeled at a substitution rate of 7 to 12 Moles FITC per Mole albumin (MW = 66 kDa). For simplicity, we assume labeling at 10 Moles FITC per Mole albumin, i.e., 6.6 g = 1 mMole FITC. Therefore, 0.4 % (w/v) requires 400 mg of material equivalent to ~ 600 µM FITC. Cost = $160 (@ $400/g) per 100 ml, enough for 5 animals.