Isolation and culture of rat peritoneal macrophages

1. **In vivo induction of macrophages**
   There are two techniques for inducing macrophages in vivo.

   a. Thioglycollate
   This is **NOT** the preferred technique but can be necessary in some situations (rarely).
   Prepare a 1% solution of thioglycollate in sterile dH2O and let sit overnight at room temperature.
   Inject 12 ml intraperitoneally per rat.
   A good induction will result in necrosis of the tip of the tail 2-3 days after injection.

   b. Concanavalin A
   This is a much better technique, a lot less stressful on the rats.
   Prepare a 0.2 ml/ml solution of concanavalin A in PBS.
   Inject 1 ml intraperitoneally per rat.
   The best time for macrophage collection is 3 days after injection. There will be no visible signs of the injection (no tail necrosis, no change in rat behavior).

2. **Preparation of media and buffers**

   a. PBS-PS
   Add 5 ml of penicillin-streptomycin stock to a 500 ml bottle of sterile PBS.
   Store in the fridge.
   Warm to 37°C before rat anesthesia.

   b. Medium
   DMEM 500 ml
   Penicillin - Streptomycin - L-Glutamin 1 bottle or aliquot
   Sodium pyruvate 5 ml
   Non essential amino acids 5 ml
   RPMI vitamins 5 ml
   β-mercaptoethanol 175 ul stock in the fridge
   Store in the fridge for no more than 10 days.
   Warm to 37°C before rat anesthesia.

   c. FBS
   Make sure you have some thawed and warmed to 37°C at time of plating macrophages.

   d. TCGF (T cell growth factor)
   Home-made (see attached recipe). We always have a stock at -20°C.
   Make sure you have some thawed and warmed to 37°C at time of plating macrophages.

3. **Collection of macrophages**
   Deeply anesthetize the rat with isoflurane and place on the back, using the nose cone for continuous delivery of isoflurane.
   Do a cardiac puncture to remove blood as much as possible. Do not cardiac perfuse as macrophages will start adhering to tissue really fast.
   Open the abdominal skin using sterile conditions and instruments.
   Inject 10 ml of **warm** PBS-PS intraperitoneally.

Updated 10/15/2014
Gently massage the abdomen. Make a small incision in the abdominal wall and collect the fluid into a sterile 50 ml conical tube using a sterile transfer pipet.
Rinse the abdominal cavity twice with warm PBS-PS and collect the fluid.
Ensure animal is dead by direct inspection of the heart and, if necessary, cutting the heart. Collect fluid from different rats in different tubes in case there is a contamination by rupture of the intestines in one rat.

4. Plating of macrophages
Centrifuge the tube containing the abdominal fluid for 8 min at 250g. Resuspend into 10 ml of warm medium (no FBS, no TCGF) and count the large cells only. You should get 30-80 million macrophages (large cells) per rat. Make sure there are no bacteria in your cells.
Dilute to 0.2 million large cells/ml medium (no FBS, no TCGF) by mixing cells from all rats and plate:
   a. 0.1 ml per well in a flat-bottom Corning 96-well plate.
   b. 10 ml per dish in Corning ø 10 cm culture dishes.
Incubate at 37°C (5% CO₂) for 2-3 hours.

5. Stimulation of macrophages
Use warm medium (no FBS, no TCGF) to gently wash off non-adherent cells. Add complete medium containing 5% FBS and 10% TCGF. Incubate for 24 hours at 37°C (5% CO₂).