**CHICKEN EMBRYO FIBROBLASTS**
makes 50 plates from 12 eggs

**Solutions:**
- 500 ml Trypsin (1x in PBS)
- 500 ml PBS
- 2 x 500 ml Ham's F-10 + 10% FCS + 1% Chicken Serum
- 70% EtOH
- 100 ml Freezing media (25% FCS, 8% DMSO in Ham's F-10)

**Equipment:**
- 2-200 ml beakers autoclaved with stirbars
- 2-150mm Plates
- 500 ml beaker
- stir plate
dissection tools (2 tweezers, 1 pair scissors)
- 50 plates P-100
- 2 corning 50 ml centrifuge tubes
- 500 ml beaker of Tupperware container of same size
- sterile slide and razor blade.

**Procedure:**
1) On day 11-12 candle 1 dozen fertile white leghorn eggs. Mark abnormalities on egg.
   On day 12, warm Trypsin, 1 bottle media, and 2 bowls of water.
   Place in hood (w/ UV on) beakers, 150 mm plates, dissection tools in EtOH.
2) In hood, half fill one of the plates w/PBS. Place 50ml beaker in the other half, place 50 ml of Trypsin in both 200 ml beakers. Sterilize eggs with 70% EtOH.
   Place egg in beaker pointed end down.
3) Crack open wide end. Remove shell.
4) Pull out embryo. Rinse by pouring PBS over embryo over 500 ml beaker.
5) Put embryo into dry plate. Remove head close to body, limbs, and internal organs (heart, lungs, liver, kidney).
   Place embryo into plate with PBS to rinse.
6) Mince on slide with sterile razor blade. Slide tissue into beaker of trypsin.
   Collect 5-6 embryos in 1 beaker of trypsin.
7) Stir at medium speed in 37°C bowl of water about 5 min.
8) Spin down cell debris at #2 for 10 min.
9) Pour supernatant (containing cells) into a new corning tube. Spin at #5 for 5 min.
10) Resuspend cell debris in about 40 ml trypsin and return to beaker with stirbar. Continue stirring as before for 5 min. Repeat spins pooling cells each time.
11) When all the cells are pelleted, resuspend in 10ml PBS and break up clumps.
    Spin @ #5 for 5 min.
12) Bring pellet up in 50 ml Media. Place 1 ml of cells into 50 plates each with 7 ml media.
    Place in incubator.
Next day: Rinse cells with PBS to remove floaters and add fresh media.

Following day: Trypsinize plates, pool cells in 2 corning tubes.  
Add 10 ml Media to inactivate trypsin.  
Spin cells down #5 for 5 min.  
Resuspend each tube in 50 ml Freezing media.  
Aliquot out 1 ml into each freezing tube.  
Freeze in -80°C.  
If cells are to be maintained, passage with no more dilution than 3 or 5 (i.e., 1 plate split into 3-5 new plates.)