Culturing and Splitting Suspension Cells (NCI-H82, etc)

Introduction

Suspension cells are easier to split than adherent cells: there's no rinsing or trypsin, you just split by dilution. HOWEVER, they are more sensitive to seeding density, so you MUST count them before you split them.

Materials

- 70% Ethanol (in a spray bottle)
- Paper towels
- P-200 pipette tips (only use the ones from the orange boxes)
- Aspirator adapter
- Hemacytometer and cover glass
  - Hemacytometer glasses are thicker than regular microscope cover glasses. If you can't find the hemacytometer glass, DON'T just use a regular microscope cover glass.
- 10cm tissue culture dishes
- 50 ml conical tube
- Complete tissue culture media (stored at 4°C)
  - RPMI-1640 base media
  - 10% FBS
  - Supplemented with non-essential amino acids

Procedure

Setup

1. 45 minutes or an hour before splitting cells, aliquot out the amount of media you'll need, so as to avoid warming up the entire bottle.

   - Compute the amount of complete media you'll need: 10 ml for each destination dish. (Ie, if you're splitting one plate into three plates, you'll need 30 ml of media.)
   - Wipe down a hood with ethanol sprayed on all interior surfaces (including the glass face shield!)
   - Get the bottle of complete media from the 4°C refrigerator. Spray off with ethanol and put in the hood.
   - Aliquot out the amount of media you'll need into 1 or more 50ml conical tubes.
   - Label the conical tubes.
   - Put the conical tubes in the 37°C dry bath and the media bottle back in the refrigerator.

Splitting

2. Prepare the hood: fill in the sign-in sheet, turn on the regular light, wipe down with ethanol *including the glass face shield.*

   From this point forward, spray off everything that goes into the hood with 70% ethanol

3. Move the complete media from the 37°C dry bath to the hood.

   SPRAY THEM ALL DOWN WITH ETHANOL BEFORE PUTTING THEM IN THE TC HOOD. (who knows what's growing in that dry bath....)

4. Put a aspirator adaptor on the vacuum tube. To turn on the vacuum, there is a yellow knob on the left. "On" means the knob is facing the hose.

5. Move the hemacytometer into the hood. If it's dirty, clean it with a liberal amount of 70% EtOH. Dry the hemacytometer and cover glass and place the glass on the hemacytometer.

6. If cell culture is in the incubator, remove and check the color of the indicator in the media. If it is pale yellow, check on the microscope for infection.

7. To dissociate the cell aggregates, pipette the culture up and down *twice* with a 10ml pipette.

   Don't pipette up and down more than necessary; excessive shear stress can damage the cells.

8. **Count the cells on the hemacytometer**.

   - Pipette 20 ul of the cell suspension into the hemacytometer, introducing it to one side through the notch on the edge.
   - Take the hemacytometer to the microscope and bring the grid into focus.
   - The hemacytometer has a number of levels. The largest grid is 3x3. The corner squares are subdivided further into a 4x4 grid; the middle square has very small subdivisions.
   - Count the number of cells IN ONE OF THE LARGE CORNER SQUARES (the one of the ones divided 4x4.) The volume in this square is 0.1 ul.
   - Multiply your count by 10,000 to get the number of cells per ml.

9. Transfer enough of the cells into the fresh media so that the final density is **50,000 cells/ml**. Mix thoroughly, then transfer 10 ml of the new culture to each new TC dish.

10. Label plates with the cell type, initials, date, passage number, and the date of the next splitting (if 1:10, add 3 days, if 1:5, add 2 days, if 1:2, add 1 day).

11. Clean up.

    - Suck up any media spilled, or any leftovers in the containers. - Glass tips go in the sharps, other tips and stripettes go in the biowaste bag.
    - NO MATTER HOW LITTLE MEDIA IS THERE, disassemble the aspirator and add some bleach. Swirl around until the media turns white, then empty into the bleach bucket in the sink.
    - NO MATTER HOW LITTLE PLASTIC WASTE THERE IS, move the biowaste to the burn box. DO NOT TIE THE GARBAGE BAG, that makes it hard to compress. Put a fresh bag in the waste bin.
    - Close the hood, turn the UV on, sign out.