Culturing & Splitting Mammalian Cells

Introduction

Culturing HEK-293 (also applies to CHO and other adherent cells.)

Materials

- 70% Ethanol (in a spray bottle)
- Paper towels
- Trypsin/EDTA (stored at -20°C)
- PBS or Versene (stored at room temperature in the iGEM drawer)
- P1000 pipette tips (only use the ones from the orange boxes)
- Aspirator adapter
- 10cm tissue culture dish
- 15mL conical tube, 50 ml conical tube
- Complete tissue culture media (stored at 4°C)

Procedure

Setup

1. 45 minutes or an hour before splitting cells, move the trypsin to the dry bath set to 37°C.

2. 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: 20 ml for each source dish, plus 10 ml for each destination dish. (Ie, if you're splitting one plate into three plates, you'll need 50 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

3. If cell culture is in the incubator, remove and check under the microscope for confluence: under 20X magnification, should cover the bottom of the plate

4. 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

5. Move the trypsin and 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....)

6. Put a aspirator 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.

7. Attach a P1000 tip to the glass pasteur pipette. Holding the dish with one hand such that the lid is ajar, tip the dish to one side so the media pools against the wall of the dish. Aspirate the media from that side of the dish.

8. Immediately add 10ml of PBS or Versene, pipetting it *gently* down the side of dish.

9. Slosh the PBS or Versene back and forth over the cells once or twice, then aspirate it off in the same manner as above.

10. Quickly and gently add 3mL of trypsin to the dish. Gently tip the dish around to spread out the trypsin, then let sit 3-4 min.

    HEK and CHO cells will detach at room temperature in the hood. More adherent cells may require returning the dish to the 37°C incubator for 5 minutes or so.

11. Tip the plate back and forth and make sure that the cells have detached and are running freely down the plate.

12. Add 7mL of complete media to quench the trypsin. Pipette gently up and down twice or three times to completely dissociate the cells, then transfer into a 15 ml conical tube.

13. Centrifuge at 125 xg for 5 min at room temperature.

    Make sure the rotor is balanced!

14. Aspirate the media off the top, being careful not to disturb the pelleted cells at the bottom.

15. Add 10 ml of complete culture media and pipette gently up and down several times to resuspend the cells.

16. Optional: count the cells on the hemacytometer. This is particularly important if you are seeding a plate for transfection. In a plate seeded for transfection, you want 5x10*4 cells TOTAL per well.

    - Get the hemacytometer (in its blue plastic case) out of the common supplies drawer.
    - Make sure that the hemacytometer and its cover glass are clean. If not, spray with 70% EtOH, wipe with a Kimwipe, and dry thoroughly.
    - Place the hemacytometer glass on top of the hemacytometer. **You must use a hemacytometer glass; you can't use a standard cover slip.**
    - 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.

17. Make the split: there should be 10mL of liquid in the culture dish at the end. How much is fresh media and how much is cells depends on how hard you want to split the cells.

    for 1:10 split: put 9 mL of media into culture dish, slowly add 1 mL of cell culture
    for 1:5 split: 8mL of media, 2 mL of cell culture
    for 1:2 split: 5mL of media, 5mL cell culture

18. Tilt the plates back and forth a few times to evenly distribute the cells.

19. 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).

20. 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.