

BASIC CELL CULTURE:

Overview: Recover cells from liquid nitrogen,
Pass Cells with trypsin

Count Cells to measure viability

Transfect cells

X-gal staining

NOTE: All tissue culture (TC) plates are located in the cell incubator.

2 mls of lacZ solution (yellow liquid)

50 µl of X-gal solution (frozen at -20)

2 ml of 0.05% glutaraldehyde

Students:

Extra experiment for 1st TC class:

- remove medium from small TC plate, labeled "hsp".
- add 2 ml of PBS, swirl to rinse, and remove the PBS with the same pipette that you used to add the PBS.
- add 2 ml of 0.05% glutaraldehyde.
- leave for 3 minutes at room temp.
- While waiting, mix 40 µl of Xgal solution with 2 ml of lacZ solution.
- Remove glutaraldehyde.
- add 2 ml of PBS to the plate, swirl to rinse, and remove the PBS with the same pipette that you used to add the PBS.
- add the Xgal+lacZ solution to the plate.
- incubate in the oven at 37 for at least 1 hour.
- look with the microscope.

Protocol for recovery of cells from liquid nitrogen

- 1) Take vial from liquid nitrogen container
- 2) Dip in a water bath at 37C until contents melt
- 3) Pipette out contents with a yellow 1 ml pipette
- 4) Add complete medium (5mL) drop by drop (slowly)
- 5) Spin tube for 5 mins at 1500 rpm in large centrifuge
- 6) Remove all medium without dislodging the cell pellet
- 7) Add medium (5mL) to pellet
- 8) Pipette up and down gently until cells are resuspended
- 9) Add contents into a 6 cm tissue culture and disperse evenly by swirling

Important: The medium that prevents the cells from forming crystals while frozen is toxic to the cells once they have thawed. It is important to pipette all the medium and wash thoroughly with the new medium. Spin should be maintained at 1500 rpm max or else the cells will just break apart. Pipetting should be done very gently as not to destroy the cell integrity.

Protocol for Passing Cells

- 1) Take 1.5 cm plate from incubator
- 2) Remove medium by aspirating with a Pasteur pipette
- 3) Add 1 ml of PBS onto plate and swirl
- 4) Remove all of the PBS by aspiration
- 5) Add 400 µl of trypsin to plate
- 6) Put cells back in incubator at 37C for 5 min
- 7) Get plate back add 2 ml of medium
- 8) Pipette cells up and down to lift off plate
- 9) Put cells in a 15 ml conical tube
- 10) Spin 1500 rpm for min in large centrifuge

- 11) remove supernatant
- 12) add 3 ml of medium pipette up and down to resuspended pellet
- 13) count cells

Important: If the culture is to be maintained, the cells must be subcultured (also called splitting or passing cells) by placing some of the cells in a new dish at low density. This is accomplished by gently digesting the cells with trypsin. After several minutes, the trypsinized cells round up, they are removed from the monolayer and they float in the medium. The released cells are resuspended in fresh medium and about 10% are transferred to a new dish. The cells settle down, attach, and start to grow again as a secondary culture.

Protocol for Counting Cells:

- 1) take 50 μ l of cells from the conical tube
- 2) mix with 5 μ l of trypan blue solution
- 3) get 10 μ l from the mixture and add the drop to both wedges on the slide
- 4) put chamber in stage of microscope and locate the central 25 squares
- 5) count the number of cells in the square
- 6) number of cell per ml is equal to the number counted multiplied by 10,000

Important: trypan solution is readily absorbed by dead cells that have no more control of their membrane, so do not count the blue cells, only the clear cells.

Protocol for Transfection of Cells

- 1) In an eppendorf tube, mix the following components (for each plasmid)
 - a. 5 μ l of DNA
 - b. 31 μ l of 2M CaCl₂
 - c. 204 μ l of water
- 2) in another tube prepare the 250 μ l of 2X HBSS

- 3) take the tubes to the TC hood
- 4) get a small 35 mm dish from the incubator
- 5) mix the contents from the tube A into tube B pipette vigorously to mix
- 6) add the mix A+B onto the liquid of the tissue culture plate
- 7) return plate to incubator
- 8) 10 min later take a look at the plate to assess quality of transfection