CMBL – CRJ/JJR
6/10/08 preliminary

Protocol: Subcloning Cells

Materials

- See Protocol for subculturing
- 24-well Tissue Culture plates

Purpose: To obtain a uniform culture of cells derived from a single cell of a heterogeneous population. After transfection and selection with antibiotics, stably transfected cells are highly heterogeneous (e.g. some cells will have higher expression levels of the transfected gene, some may not express the desired gene – only antibiotic resistance, some will divide faster and take over the culture over successive passages, etc.).

Procedure

1. Follow steps 1-14 of the subculturing protocol
   - use a 50 mL centrifuge tube on step 11
2. After counting cells make a serial dilution[1] of cells to obtain 40 mL at ~1.4 cells/mL
   - if cells are stably transfected you do not need to add the selection antibiotic to subcloned cells
3. Place 0.5 mL of dilute cells into each well of three 24-well plates (72 wells total @ ~0.7 cells/well)
4. Plate remaining cells from step 2 as during a standard subculturing

After 7-10 days

5. Check wells for single cell-derived colonies; mark wells (should have at least 10 marked wells)
6. Change media on marked wells
7. Allow cells to continue growing until each well/colony is large enough to trypsonize and transfer to a 100 mm tissue culture dish (may take an additional 5-7 days)

1.) Example serial dilution for $1x10^6$ cells in 5mL of medium
- Add 20 mL of medium and mix (pipet up and down) cell suspension to obtain 40,000 cells/mL
- Remove 250 uL and place in a new 50 mL tube with 24.75 mL medium; thoroughly mix to obtain 400 cells/mL
- Remove 2.5 mL and place in a new 50 mL tube with 24.75 mL medium; thoroughly mix to obtain 40 cells/mL
- Remove 1.4 mL and place in a new 50 mL tube with 38.6 mL medium; thoroughly mix to obtain 1.4 cells/mL