LIPOFECTION PROCEDURE- CHO (PF)

Complete medium: 5-10% FCS, P/S, glutamine

Selection medium: 10% dFCS (dialyzed), P/S, no glutamine, 0-100 μM MSX

Cells: Use CHO cells that have been in culture less than one month and have been maintained subconfluent for several passages in advance. (A 10 cm plate of CHO cells which have just reached confluence has about 10^7 cells on it. Densities of 2-5 X 10^7 cells per 10 cm plate can be achieved by maintaining a confluent monolayer in complete medium with 10% FBS.)

Lipofection using DNAs for secreted proteins:

Day 1: Using stock cultures that are 80% confluent or less, plate 10^6 cells per 10 cm dish (or split one just subconfluent 10 cm plate onto 4-5 10 cm dishes). At this point, be prepared to apply DNA within 18-24 hours.

Day 2: 18-24 hours after seeding cells should be 20% confluent. About 4 hr before adding DNA, change medium on cells.

Prepare DNA:

Combine plasmids to be transfected, using 30 μg total DNA. Take volume to 85 with water

Filter DNA by centrifuging through .2 μ filter (mostly this removes spores that can germinate in your transfected cultures). Alternatively, you can combine DNA's and EtOH precipitate them together, then take them up in 85 μl. The filtration is preferred, because of spores.

Combine DNA with 80 lipofectin reagent (1 mg/ml) in polystyrene snap cap tubes, from stockroom Falcon tubes #2054 (polyethylene adsorbs the reagent so regular μfuge tubes should not be used). Wait 15 minutes.

Prepare cells:

While DNA is incubating with lipofectin reagent, wash cells with 1xPBS 2 times.

Add 10 ml 1% FCS medium (with glutamine).

Add DNA to cells:

Add lipofectin/DNA mixture drop by drop around the plate. The solution with DNA should be milky without obvious precipitate. Let grow over night.

Day 3-4: When cultures are confluent (or nearly so), trypsinize them, dilute all cells into 50 ml complete medium, and distribute them into five 96 well plates, 100 per well.

Day 4-5: When cells are 20% confluent in the wells, replace the medium with 200 per well selection medium containing 25 μM MSX using multi-channel pipetter (be careful to avoid contamination!!!).

Day 9: Check the cells and change the medium (again with 25 μM MSX).

Day 13: Check cells; change the medium using 100 μM MSX to start amplification of transfected DNA.

Day 17: Check cells, change medium (100 μM MSX).

Day 20: Score trays for colonies (I keep track of the numbers). Take supernatants from wells with cells for ELISA. Change the medium.

After screening by ELISA, trypsinize and transfer cultures you want to keep to 12 well trays (2 ml medium per well). When confluent, transfer cells to two 6 or 10 cm dishes. Freeze down a good supply as soon as you can. Expand and harvest the remainder.

If an ELISA is not available for the expressed protein, colonies can be transferred to 12 or 24 well trays to be screened by immunoprecipitation. I divide the cells between two trays, one for passage, one for metabolic labelling. (One well of a 12 well tray holds $3X10^6$ CHO cells when densely confluent, enough to give readily detectable protein on metabolic labelling in experience).

Clones should appear after 7-14 days of selection (25 μM MSX); amplification will take an additional 7-14 days in 100 μM MSX. You should get 30-50 "clones" i.e., wells with cells growing in them) in a good lipofection. For truly clonal expressing cell lines cultures should be subcloned by limiting dilution after amplification (I haven't bothered to so far).

MSX is not stable in solution (I don't know what the half life is). For best selection/amplification, use freshly prepared medium.

Lipofection using DNAs for cell surface proteins:

(Similar to above EXCEPT cells are not split into 96 well trays. Expressers are identified and separated out by FACS.)

Day 1: Prepare cells as above

Day 2: Apply DNA as above

Day 3: Change medium to selection medium with MSX

Day 4: Change medium to selection medium with 25 μM MSX

Day 8: Change medium (25 μM MSX).

Day 12-14 Colonies should be readily visible through the bottom of the dish

There are two options (at least) at this point:

1. Split each 10 cm dish onto 3 new 10 cm dishes using complete medium. Grow these "populations" to confluence in selection medium with 25 μM MXS. Use two plates for FACS sort, one plate to freeze. Grow up sorted cells, apply 100 μM MSX for 10-14 days and resort to get high expressors. Do limiting dilution cloning with FACS sort if clonal lines are wanted. Grow up high expressers and freeze numerous vials as soon as possible.

2. Trypsinize and replate cells in complete medium to get even distribution. Apply selection medium containing 100 μM MSX next day. Grow another 10-14 days in 100 μM MSX to amplify transfected DNA. Split to 3-5 10 cm dishes and grow up "populations". Use 2 plates for FACS sort, one plate to freeze down. Grow up high expressers from sort, cloning by limiting dilution if desired. Freeze numerous vials as soon as possible.