Making and testing LIF

Making:

For a large batch of LIF, use 15 flasks (75 ml) of COS 7 cells grown to confluency. This makes 500 ml of LIF. It requires about 1.200 µg of pC10 DNA (no need for linearization; prepare by NucleoBond, Qiagen, or any other high purity column purification method). Grow COS 7 cells in ES cell medium without LIF/ mercapto-ethanol.

1. Trypsinize flasks in batches of 3. No need to count cells. Pool the cells from 3 flasks, spin down (3 min 1.000 rpm) and resuspend in 1.6 ml of HBS buffer.

2. Electroporate 0.8 ml of cells with ~120 µg of pC10 DNA; 200 V, 500 µFd.

3. Leave cells for 10 min., then transfer each 0.8 ml of electroporated cells into a separate 50 ml blue cap tube with 10 ml of medium and plate into a 140 mm dish. Add a further 20 ml of medium without antibiotics to each dish. This yields 10 dishes in total.

4. The next day, the dishes should be nearly confluent. Aspirate medium and add 25 ml of fresh medium. Leave 3 days.

5. Collect the medium and add another 25 ml. Leave for further 2 days and collect again.

6. As a precautionary measure, collect the medium into 3 separate bottles. Know which bottle is being used to collect medium from a particular dish. After collection is completed, and if there is no evidence of any culture contamination, pool the medium and filter. Use pre-filter, 0.45 µm and 0.1 µm filters. Pipette 5 ml aliquots into tubes and store at -20°C. Set up a sterility test on the first and last aliquots.

In our experience, a preparation can be used for up to 5 years without a drop in quality.

Testing:

1. Trypsinize a healthy culture of HM-1 cells in the morning. Plate 1 000 cells/well into a gelatinized 24-well microtitre plate. Also, plate 250 cells/well into a separate plate.

2. After ~4-5 hrs, aspirate medium and replace with 1 ml/well of complete medium containing the new batch of LIF at dilutions of 1.000-, 2.000-, 5.000- and 10.000-fold. Also, set up a row of 4 wells with no LIF, and with the current batch of LIF at the appropriate dilution.

3. Fix and stain after 7 days. Compare plating efficiency, colony size and, most importantly, level of differentiation in the dilutions of the new batch of LIF against the standard, which should show only a low level (no more than 5-10 %) of differentiation.

10 x Hepes-Buffered-Saline (HBS) for electroporation

16	g NaCl
0.74	g KCl
0.252	g Na_2HPO_4 dihydrate
2	g D-Glucose (Dextrose)
10	g HEPES

Dissolve all in 180 ml cell culture grade water, adjust pH to 7.2, bring up to 200 ml and filter through 0.1 µm pore filter. Store at -20° C. To prepare 1 x HBS, dilute with sterile water which brings pH to 7.05.