Transfections into JSL1 cells for Stable Cell Lines

Day 1

1. Split stock of JSL1 cells to about 0.5 million per ml in a volume of at least 10 mls per sample.

Day 2

1. For each construct to be transfected spin down 10-20 million JSL1 cells (at density of 0.5-1.2 million/ml)

2. Resuspend in 400 μl serum-free RPMI

3. Add cells to a new cuvette and add 10 μg DNA in a volume of not more than 10 μl H2O.

4. Flick cuvette gently to mix and let stand up to 5 minutes

5. Set electroporator for 250mV, Capacitance = 960 and switch dial to read time constant.

6. Place each cuvette in holder and depress both buttons until you hear beep (then let go!)
   (note: time constant should be around 24-26)

7. Flick vigorously to mix pH gradient which has been formed and let stand for ~ 5 minutes.

8. Remove cells from cuvette with transfer pipet and add to a well of a 6 well plate to which 6-8 mls of RPMI+10% FCS has been added (no drugs at this point!)

Day 4-5

1. Resuspend cells from 6 well plate and count.

2. Make 3 dilutions of cells: $1x10^5$ cells/ml, $3x10^4$ cells/ml, $1x10^4$ cells/ml with each dilution totaling at least 20 mls. (in RPMI+10%FCS+**drug**)
   (this is most easily done by making serial dilution into sterile basins).

3. Using multichannel pipet fill a 96 well flat-bottom plate with each dilution (200 μl/well)

4. Place plates in incubator and allow to grow for 10-20 days. Then expand single colonies.