Calcium phosphate precipitation for MDCK cells

from Karl, rewritten by EY 7-95

References: Wigler et al. (1979) PNAS 76,1373-1376.

Prep of Cells:

Split a confluent plate 1:20 the day before the transfection.

Transfection:

1. Mix fresh 2x HEPES buffer (280 mM NaCl , 50 mM HEPES, 1.5 mM $NaPO_4$, pH 7.1)
2. Mix fresh 0.25 M $CaCl_2$ from 1M stock and filter sterilize
3. Test precipitate formation by adding 0.25 ml $CaCl_2$ dropwise to 0.25 ml 2x HEPES on vortex. Use clear plastic tube. Note cloudyness that forms after a few minutes.
4. Add 20 to 50 µg DNA to 1 ml 2x HEPES buffer.
5. Add 1 ml 0.25 M $CaCl_2$ dropwise shaking vigorously after each drop (keep tube on vortex all the time).
6. Allow precipitate to form 30 minutes at room temperature.
7. Vortex and add to 10 cm cell culture plates (~ 30 - 50% confluent). Incubate 20 minutes at 37°C. Mix once during this time.
8. Add 7ml of warm media and incubate overnight.
9. Remove media. Glycerol shock with 12.5 % sterile glycerol in medium. Incubate 1 to 2 min at room temperature
10. Remove glycerol solution and rinse cells two times with PBS.
11. Add medium with serum (DMEM + 10%FCS).
12. 24 hours later: change medium to selection medium -- with 0.6mg / ml G418.
13. 24 hours after selection: split cells into 2 big dishes (15cm).
    Resuspend in 5 ml and spilt 1 / 10th (0.5 ml) and 9 / 10ths (4.5ml) in 35ml medium w / G418.
14. A few days later, change to fresh media.
15. It will take a couple of days for the cells to die off and you should be able to pick colonies about 2 weeks after selection.

Picking Clones:

1. Mark the colonies that you want to pick by circling them on the bottom of the plate.
2. Add trypsin with a pipettman tip, till the outer cells of the colonies start to round up. Aspirate the trypsin solution off. Put cells back into incubator till they come off.
3. Using a 1ml plastic pipette, fill with media and touch tip to a colony. Several drops of media will come out allowing you to suck up the cells into the plastic pipette.
4. Transfer the media with cells to a 24 well plate. Pick about 24 colonies. Do not let the cells dry out.
5. Also trypsinize the rest of the plate and grow as a crude fraction in a 10cm plate. Freeze some.

Screening:

1. Screen first by IF (with C7 for LDL-R).
2. Grow positive clones in a 6 well plate and then in 10cm dishes. Freeze some now. You should have 3-4 different clones.
3. Plate them on filters and do polarity assays.

Reagents:

2x HEPES BUFFER		
0.5 M HEPES pH 7.1	5 ml	667 µl
3.0 M NaCl	4.65 ml	623 µl
1.0 M $NaPO_4$ pH 7.1	75 µl	10 µl
dH_2O to volume	50 ml	6.67 ml

Bring to 90 % total volume and pH to 7.1. Bring volume to 100 %. Filter sterilize. Make fresh for each use.

HEPES buffer must be made fresh and the pH is of unmost importance (7.1 ± 0.05).