CALCIUM PHOSPHATE MEDIATED TRANSFECTION

Materials:

1) 2 M $CaCl_2.2H_20$ — 29.4 g/100mls
   Filter
2) 100X PO_4
   - 70 mM Na_2HPO_4 — 0.994 g/100mls
   - 70 mM NaH_2PO_4 — 0.966 g/100mls
   - Filter
3) 2X HBS
   - 10 g/liter Hepes
   - 16 g/liter NaCl
   - make 100 mls, pH to 7.15, Filter.

Procedure: (Amounts refer to a transfection of one p100 of cells)

1) Precipitate 10-20 µg of DNA. DNA must be extremely pure. 2 CsCl gradients are highly recommended.
2) In hood, remove ethanol.
3) Without disturbing pellet, fill tube with cold 100% ethanol to sterilize. Remove 100% ethanol carefully. Rinse cap.
4) Invert to dry. Drying occurs rapidly in hood. Do not overdry.
5) Resuspend pellet in 440 µl H_20.
6) Add 60 µl 2 M $CaCl_2$.
7) Incubate at 37°C >4 hrs to ensure that the DNA is fully dissolved. This DNA/calcium solution is called solution B.
8) Make solution A (in hood).
   - 500 µl 2X HBS
   - 10 µl 100X PO_4.
9) Very slowly, dropwise, add solution B to solution A.
10) A very fine, cloudy precipitate should form. Ideally no particles should be visible, only a grayish haze. If extensive clumping occurs, start over. Clumping can be due to doing step 9 too fast or by failing to completely resuspend the DNA in step 7. Let precipitate sit in hood for 15-30 minutes to allow precipitate formation to go to completion.
11) Pipette precipitate vigorously to break up any minor clumping that might have occurred.

Steps 12 -- differ for 293 and Hela Cells.

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293 cells

12) Wash cells (~85% confluent) 2X with warm DME. (Do this while ppt is sitting)
13) Add 7mls DME to plate.
14) Dropwise, add ppt to plate. Tilt plate to mix.

15) Let incubate O/N, 37°C, 5% CO_2.

16) Remove media.
Replace with 8mls normal growth media (containing serum).

17) For transients harvest 24-48 hours later.
For stable lines, split 1:5 to 1:10 24 hours later. Incubate 24 hours and then select. (400 µg/ml G418).

Hela cells

12) Wash cells (~85% confluent) 2X with warm DME. (Do this while ppt is sitting)
13) Add 1 ml ppt directly to cells. (No DME)
14) Incubate 30', 37°C, 5% CO_2.
15) Add 7 ml DME + 2% Fetal Calf Serum.
16) Incubate 6-8 hours, 37°C, 5% CO_2.
17) Remove media.
Shock with 2ml DME + 25% glycerol.
Shock for 2-3 minutes.
18) Wash with warm DME 2X.
19) Add 8ml normal growth media (containing serum).
20) For transients, harvest 24-48 hours later. For stable lines, split 1:2 to 1:5 24 hours later. Incubate 24 hours, then add selective media (400 µg/ml G418).

Comments:

Pooling of 293 cells can be done after 2-3 weeks. Helas, >1 month. While selecting change media when dead cells become the majority or if the media becomes orange.