Transfection of 293T Cells (Modified CaPO4 protocol) SS 10/99 (Malim lab)

Reagents

10x NTE (100ml)
NaCl 8.77g
1M Tris-HCl pH7.4 10ml
0.25 M EDTA pH8.0 4ml
pH to 7.4 w HCl
H_2O to 100ml

Store at -20°C in aliquots. Can freeze/thaw multiple times

0.5M HEPES - pH 7.1 +/- 0.05 w NaOH pH is critical
2M NaCl
200mM Na_2HPO_4 (pH 7.0 w phosphoric acid)
2M $CaCl_2$ Make fresh weekly store at -20°C

One day prior to transfection:

1. Gelatinize plates: Dilute 2% soln of gelatin (SIGMA) 1:20 in H_2O
Add diluted gelatin to plates and leave 15 mins
Rinse 1x w media
(This is necessary as 293Ts are not very adherent and w/out gelatin may dislodge upon media changes later following transfection)

2. Seed 293T cells so 70% confluency the next day. 0.8x10E6 cells in 6 well
5x10E6 in 10cm dish

Transfection
1. Make up fresh 2x Transfection buffer (good for ~1wk at RT?)

0.5M HEPES	0.5ml
H_2O	4ml
2MNaCl	0.45ml
200mM Na_2HPO_4	50ul

	5mls total

2. Make up DNA cocktail and mix well. The amts below are for 6 well plate w 2mls of media. Multiply everything by 5 for a 10cm dish w 10mls media.

DNA	6 ug (2ug of each) [*pBMN-whatever, VSV-Env, gag-pol*]
10xNTE	10 ul

2M $CaCl_2$ 12.5 ul
H_2O to 100 ul

3. Add the DNA Cocktail dropwise to an equal vol of 2x Transfection buffer. Using a pasteur gently blow a stream of bubbles through the mixture to promote formation of precipitate. (This part is all Voodo - do whatever works best for you - some people add dropwise whilst vortexing at low speed. The aim is to get a fine precipitate but not clear what is the best way to achieve this)

4. Add the DNA Cocktail/Transfection buffer mix dropwise to the cells (some protocols recommend waiting 15-30mins for precipitate to form but I get better transfection when I don't wait)

5. Incubate 6-8 hrs at 37°C

6. Rinse cells 1x w PBS, add fresh media and return to 37°C
(Make sure media is warm else cells will detach)

7. Harvest at ~24 hrs post transfection for virus or ~ 48hrs for protein expression

Infection

1. Harvest supe from transfected 293Ts. Virus has settled to bottom so pipette vigorously. Spin down to get rid of cells. then filter through .45 uM (Note: .2uM is too small want whole virus particles)

2. Infect 2x10E6 cells in 10cm dish. Add 1ml of viral supe to 1ml of cells or to 1 well of a 6-well plate. Also add 8ug/ml polybrene (helps cells take up virus). For suspension cells use 4ug/ml polybrene.

3. If have plate attachments for a table top swinging bucket centrifuge, spinning the plates 45 min RT 1800 rpm may improve infection.

4. 6hrs post infection: wash cells w media. Change media.
(Polybrene is toxic to some cells. Half life of virus is 4-6hrs so by this time most of the virus is dead)

For TIPS on getting beter infection see Nolan lab home page at Stanford.