Transfection of neurons in culture

A.Ghosh, H. Dudek, M.E. Greenberg (1995)

Transfection Procedure:
1. Replace media with warm DMEM (save culture media to return to plates after transfection).
2. Return plates to incubator for 1 hour.
3. While the plates are in the incubator prepare DNA precipitates (ppt).
4. After the cells have been in DMEM for 1 hour, add DNA ppt dropwise over the surface of the media, rock back and forth to distribute evenly. A fine sandy ppt should be visible under the microscope.
5. Return plates to incubator for 10-30 minutes (determine the best time for your cells empirically).
6. Aspirate DMEM media w/ ppt.
7. Wash cells 2X w/ DMEM.
8. Replace original media (saved in step 1) and return cells to incubator.
9. Detect transfected gene by procedure of choice between 1 and 5 days after transfection.

Making CaCl2/DNA ppts:
For 24-well plates: 500 µl media per well; 3 µg DNA in 30 µl ppt per well.

Recipe to make 300 µl ppt (10 wells):
1. Make 150 µl DNA mix: 2.0 M CaCl2 15µl (bring CaCl2 to 37 C in water bath), plasmid DNA 30 µg (in TE. We typically use double banded CsCl maxiprep DNA), & sterile dH2O to 150 µl total
2. Add DNA mix to 150 µl 2X HeBS (brought up to 37C on heat block, then placed in TC hood), dropwise, while swirling.

3. Place in dark for 10-25 minutes (room temp). At the end of this period the ppt should look a little cloudy compared to straight HeBS. This is now ready to be added to the cells.

HeBS (for 2 liters)
NaCl 32 g
KCl 1.42 g
$Na_2HPO_4 \cdot 7H_2O$ 0.76 g
D-glucose 5.4 g
Hepes (free acid) 20 g
Add components to 1.8 liters dH_2O. pH w/ 10N NaOH to 7.05. Bring vol. to 2 liters. Recheck pH (7.05-7.07). Filter sterilize. Aliquot and freeze (-20). Thaw individual aliquots to use.
[It is useful to make 20 mls of HeBS at a range of pH's from 7.05 to 7.09, and test each of them for transfection efficiency]

Notes:
1. For newborn cultures one should add Kynurenic acid (1mM final) to block glutamate receptors and reduce excitotoxic cell death during the transfection.
2. Check a few batches of HeBS with slightly different pH's to find the most effective one (try a range of pH's between 7.05 and 7.09).
3. You may need to play with the duration for which the ppt is left on the cells to get the best results.
4. We typically optimize the transfection conditions using a b-gal expression plasmid, and we use the Promega anti-b-gal antibody (monoclonal) to detect the product 2 days post-transfection. One should routinely get around 5% of the cells transfected. Plasmids using the CMV and RSV promoters give high levels of expression.
5. If ppt is too clumpy, try a lower concentration of $CaCl_2$. If it doesn't form fast enough, try a higher concentration of $CaCl_2$.