Calcium Phosphate Transfection Protocol For Primary Hippocampal Cultures

This protocol is optimized for transfection of primary hippocampal neurons:

- Cultures are prepared from P0 rat pups, as described in Yu and Malenka, Nature Neuroscience, 2003.
- Neurons are plated on 12 mm round glass coverslips in 24-well plates, in 1 ml Neurobasal medium, scale up all reagents accordingly for large coverslips.
- Transfection efficiency is highest for days *in vitro* (DIV) 5-9, but also works at a lower efficiency for DIV 1-14. For transfection of neurons less than 4 DIV, plate on astrocyte monolayer (Yu and Malenka, Neuropharmacology, 2004)
- The protocol works best for neurons cultured in media not containing serum or containing less than 2% FBS.
- The DNA used for transfection is prepared using Qiagen maxiprep kit (in distilled water, at concentrations of 1μg/μl or higher, and not lower than 0.5μg/μl) and stored at –20C.

Stock buffers:

- 2xHBS buffer （溶于水，全部用 Sigma 公司的试剂）

Concentration	Amount for 100ml
274 mM NaCl	1.6 g
10 mM KCl	75 mg
1.4 mM $Na_2HPO_4.7H_2O$	38 mg
15 mM glucose	270 mg
42 mM HEPES	1.0 g

  pH to 7.05 with NaOH. Filter, aliquot, store at –20C. (The pH is really important, so it is best to make several batches, at pH 7.01, 7.05, 7.09 and test them all to find the batch with the best transfection efficiency).
- 2M $CaCl_2$, filtered, aliquot, store at –20C

Protocol:

1. Day before transfection, feed the cultures 50% with new medium and save the 0.5 ml conditioned medium from the cultures in the fridge.
2. In a 1.5ml Eppendorf tube, mix DNA and $CaCl_2$. For each well:
   1.9μl sterile 2M $CaCl_2$
   2-4 μg DNA in water
   sterile water to 15 μl
3. **slowly**, add 15 μl pH 7.05 2x HBS per transfection, while **flickering** the tube containing DNA.
4. Let sit, shielded away from light for 30 minutes at room temperature (in hood).
5. Before adding the transfection mixture onto neurons, remove 0.5 ml of conditioned media from each well (0.5ml remaining) and pool with conditioned medium from the day before, equilibrate in the incubator. Add 30 μl of the transfection mixture to each plate, dripping slowly to cover the cells. The more medium in the well, the longer one waits for precipitates.
6. Return cells to incubator for 40 –90 minutes, and look at appearance of cells at regular intervals. Wait until the calcium phosphate precipitates cover the cells in a fine sandy layer. Longer incubations increase the efficiency and extent of transfection, but prolonged incubation can eventually kill the cells.
7. Wash neurons twice with 90% exchange with 1 ml serum-free pre-equilibrated media.
8. If there is still a lot of precipitate, leave the neurons in the conditioned medium for an hour before proceeding to the next step.
9. Add back the 1 ml conditioned media to each well.
10. Return cells to incubator and look for green cells the next day.

注：磷酸钙转染的优点之一就是共转率很高，通常都能共转上。

* All the medium labeled with “NB + 1% B27” is addition with Glutamax which is a relative of glutamine and less excitotoxic than glutamine in our lab.