TRONOLAB PROTOCOLS, EPFL 2007

Production of lentivectors by Calcium phosphate in 293T cells.

This protocol was adapted for 15 cm dishes but it can be adapted to smaller or bigger plates. The timing has also been optimized for our needs but it supports some flexibility.

Friday: 293T plating

293T are maintained in 0.45um filtrated DMEM 10% serum and 1% PS.
Typically five 15cm dishes are used for one production.
Plate $2.5.10^6$ cells / 15cm dish.

Monday: Transfection

Ideally you have around 11.10^6 cells / 15cm dish (around 70% confluence)
Two hours before transfection, replace the medium with 22.5 ml of fresh medium preheated at 37C.

For 5 dishes (15 cm dishes), prepare the following transfection mix in 50 ml falcon tubes:

- 112,5µg vector plasmid (with your gene of interest)
- 39,5µg envelop plasmid (pMD2G codes for the broad range VSV-G envelope)
- 73µg packaging plasmid psPAX2 (suitable for most studies)

Then add in this order:

- 3,3ml of TE 0,1X
- 1,75ml water
- 565µl CaCl2 2,5M

Briefly mix, then add 5.7 ml of HBS2X, dropwise under agitation by vortexing.
Wait at least 5 min (but no more than 30min) at RT
Add dropwise 2.25 ml/dish of the precipitate, and mix gently by rotating the plate.

Tuesday: Medium replacement and supernatant collecting

Remove medium around 14-16h post-transfection (typically early in the morning) and put 14ml/dish of fresh pre-heated medium.

Collect supernatant for the first time in the evening, if possible not before 5-6 pm.

Wednesday: **Supernatant collecting**

Supernatant can be harvested 2 or 3 times, every 12 hours. Keep it at 4°C over the collecting period.
Pool the collected supernatants, centrifuge 5 min at 1500 rpm to remove cell debris and filtrate on 0.22µm.
The cleared supernatants can be kept at 4°C for 4-5 days.
Supernatants can be used directly, stored at -80°C in aliquots, or concentrated if needed.

Thursday: **Vector concentration**

If needed, vector can be concentrated by ultracentrifugation at 47.000g (RCF average) for 2 hours at 16°C in a swinging rotor. We turn at 19.500 rpm in a Beckman SW32 Ti rotor.
Discard supernatant and resuspend pellet in 100-200µl PBS1X total if possible (try to make a 100 or 1000 fold concentration). The cleaner the virus is, the clearer the pellet.
The concentrated vector can be used directly or aliquoted and stored at-80°C for future use.

Reagents

Commercially kits are available but home made reagents can be prepared and maintained sterile as well:

HBS 2X: NaCl 280mM
Hepes 100mM
Na2HPO4 1.5mM, 7.11≤pH≤7.13

TE 0.1X: Tris 1mM
EDTA 0.1mM pH8.8

CaCl2: 2.5M in bi-distilled water