Production of lentiviruses

Version LG-23/08/2005

Adapted protocol from Dirac and Bernards (2003) J Biol Chem 278:11731-11734

Please check http://www.nki.nl/nkidep/vansteensel for updated versions of this protocol.

Cell culture

- Grow 293T cells in 10 cm tissue culture dishes
    - 293T cells are cultured in DMEM + 10% FCS + P/S
- Transfect cells at 80-90% confluency

Transfection (per 10 cm tissue culture dish)

- Cells are transfected by calcium-phosphate co-precipitation
- Prepare the DNA/$CaCl_2$/dH_2O mix:
    - Envelope plasmid: pMD-G 3.5 µg
    - Packaging construct: pCMV-ΔR8.2 6.5 µg
    - Rev-encoding plasmid: pRSV-Rev 2.5 µg
    - pL-transfer construct: 10.0 µg
    - 2.5M $CaCl_2$: 50.0 µl
    - dH_2O: Adjust to 500 µl
- Add 500 µl 2×HBS (pH 7.00), dropwise, while vortexing
- Incubate the precipitate 5 min. at RT
- Add 1 ml precipitate to the tissue culture dish, dropwise
- Gently shake the plate to mix the precipitate with the medium
- Transfer the transfected 293T cells to the lentivirus lab
- Incubate the cells o/n, 37°C, 5% CO_2
- Replace medium with 6ml fresh medium
- Incubate the cells o/n, 37°C, 5% CO_2

Harvest virus

- Harvest virus on 3 consecutive days:
    - Take off medium
    - Add 6 ml fresh medium to the dish
    - Filter through 0.45 µm filter
    - Store filtered medium at 4°C
- After the final harvest:
    - Combine filtered media
    - Aliquot in 1 ml fractions
    - Store at -70°C

Notes

- All plasmids are ampicillin-resistant
- 2×HBS (pH 7.00):
    - 140 mM NaCl (MW = 58.44)
    - 1.5 mM $Na_2HPO_4 \cdot H_2O$ (MW = 177.99)
    - 50 mM HEPES (MW = 238.3)
    - Dissolve in dH_2O
    - Set pH with 0.5 M NaOH
    - Filter-sterilise (0.22 µm filter)
    - Aliquot (5 ml fractions)
- Transfection by calcium-phosphate co-precipitation is very pH-sensitive. Make various batches of 2×HBS with different pHs, ranging from 6.80 to 7.20. Do a test-transfection with these 2×HBS batches with a GFP-expressing plasmid.
- 293T cells easily detach from the culture dish. Be very careful with pipetting medium etc.!