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
  o 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/CaCl₂/dH₂O mix:
  o Envelope plasmid: pMD-G 3.5 µg
  o Packaging construct: pCMV-ΔR8.2 6.5 µg
  o Rev-encoding plasmid: pRSV-Rev 2.5 µg
  o pL-transfer construct: 10.0 µg
  o 2.5M CaCl₂: 50.0 µl
  o dH₂O: Adjust to 500 µl
• Add 500 µl 2xHBS (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% CO₂
• Replace medium with 6ml fresh medium
• Incubate the cells o/n, 37°C, 5% CO₂
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 Na₂HPO₄·H₂O (MW = 177.99)
  - 50 mM HEPES (MW = 238.3)
  - Dissolve in dH₂O
  - 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.!