LEVITT/SQ LAB PROTOCOLS

Protocol for Production of Lentiviral Vectors in 293T cells

Day 1 Plating (9-10am)

Plate 2-2.5x10^6 of 293T cells per 10cm plate

Day 2 Transfection (9-10am)

Prepare calcium-phosphate precipitate (1ml/10cm plate)
• Transfer vector - 20µg
• Packaging plasmid - 15µg (**3rd generation:** pMDL g/p RRE - 10µg + pRSV-Rev - 5µg)
• Envelope plasmid - 6µg

Add water to 0.5ml, add 0.5ml 2xHBS and mix well. Add 50µgl 2.5M $CaCl_2$ and shake briefly, keep in RT for 20-25min, add drop wise on a plate and mix gently with a medium.
Change medium (6-8hrs later); remove medium with precipitate and add 6ml/plate of fresh medium.

Day 4 Collection (9-10am)
• Collect medium
• Spin 3000rpm/5min/RT
• Filter through 0.45 µm
At this point virus can be used for transduction, frozen at -70°C for future use, or concentrated

Concentration
Transfer 30ml of virus to 33ml Beckman conical tubes spin at 26.000rpm/2hrs/4°C in Beckman SW28 swingle bucket rotor. After spin discard supernatant and resuspend the virus in a desired volume of serum-free medium (e.g. Cellgro or Episerf) or PBS/ 1% BSA, aliquot and store at -70°C. For transduction of very delicate cells the virus can be concentrated on sucrose cushion, just put 4ml of 20% sucrose on the bottom of the tube and overlay with 26ml of viral supernatant.

Reagents:
- 2 x HBS (for 500ml)
• NaCl - 8g
• KCl - 0.38g
• Na2HPO4 - 0.1g
• Hepes - 5g
• Glucose - 1g