TRONOLAB 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 50µl 2.5M CaCl₂ and shake briefly, keep in RT for 20-25min, add dropwise 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
  
  *Bring pH to 7.05*

- 2.5M CaCl₂
- bi-distilled water