Transfection of 293T cells

- Calcium Phosphate transfection reagents (self-made or kits, e.g. Promega ProFection or Sigma #CAPHOS):
  - 2x Precipitation-buffer (2x HBS)
  - H_2O
  - 2.0M $CaCl_2$ (maybe 2.5M in some kits)

- Medium: DMEM with Glutamine and high Glucose (4.5g/l)
  + 10% FBS
  + 1mM Sodium Pyruvate
  + 20mM HEPES (or 25mM)
  + Penicillin/Streptomycin

- 25mM Chloroquine in PBS (= 1000x stock solution. Available e.g. from Sigma)

- Plasmids
  - Vector-Plasmid: 10 - 20 µg/dish (depends on specific construct)
  - Gag/Pol-Plasmid: 10 µg/dish pMDLg/pRRE
  - Rev-Plasmid: 5 µg/dish pRSV-Rev
  - Envelope-Plasmid: (only one of them) 2 µg/dish phCMV-VSV-G; 8 µg/dish phCMV-RD114/TR; 4 µg/dish Eco-Env (#522, K73); 4 µg/dish phCMV-GALV-C_{4070A}

Day 1:
- In the afternoon seed 5 x 10^6 293T cells per 10cm dish (one dish per vector construct)

Day 2:
Early:
- Thaw reagents and plasmids, they should be at room temperature. Warm up the medium.
- Dilute plasmids in water to 437.5µl then add 62.5µl 2,0M-$CaCl_2$-solution (or 50µl if 2.5M).
- Fill 500µl of 2x HBS into 15ml conical-bottom tube.
- Add DNA/$CaCl_2$-solution to 2x HBS drop wise, while blowing air through HBS with pasteur pipette.
- Incubate the mixture for 10 to 20min at room temperature.
- Remove old medium from the cells.
- Add 10ml medium including 25µM Chloroquine (the Chloroquine is used in this step only).
- Add DNA-mixture drop wise to the cells, swirl gently.
- Incubate the cells for 6-12h in incubator.

Late:
- Change medium to 8ml.
- From now on harvest the medium every ~12 hours, it contains the viral particles.

Day 3:
Early:
- Harvest the supernatant (supernatant 1) with a syringe and filter it through 0.45µm or 0.22µm syringe-filter into 2ml tubes. For the titration an additional tube with only ~300µl supernatant is needed.
- Add 8ml medium to the cells for the second supernatant.
- Quickly freeze the virus-containing supernatant at -80°C.

Late:
- Optional: Use fluorescence microscope to check transfection, cells should be already very bright.
- Harvest supernatant 2 in the same way (don't forget the small aliquot for the titration).
- Add 8ml medium over night.

Day 4:
- Early: harvest supernatant 3
- Late: harvest supernatant 4 and discard the cells.

Alternatively harvest only two supernatants every 24 hours, this is only recommended when using VSV-G. Use 10ml medium per supernatant in that case.