Production and purification of HHV-6A

Materials:
- HHV-6A GS
- HSB2 cells, mycoplasma free
- RPMI medium + 10% FCS, tissue cultures flasks and tubes, centrifuges

Protocol:

Day 1
- **Preparation cells**
  - HSB2 cells from 2x 225cm² (300 – 500 ml culture)
  
  Spin cells down in Falcon tubes

- **Infection**
  
  Add virus to the pellet (MOI 0.01-0.5 per cell)
  
  Re-suspension cells with less volume as possible of RPMI 0% (500 µL)
  
  37°C, for 2-3h (5% CO₂)

  Re-suspension in RPMI+10% FCS (around 10⁶ cells/mL not need to be precise but it is necessary to keep a high concentration of cells, as better for virus spreading)
  
  37°C 3 days (5% CO₂)

Day 2/3/4 (or longer is started with small MOI)

- Check cells several times per day; look at the size of cells (10x the usual size)
- Check cell viability by trypan blue staining
- As soon as there is lot of cell debris, freeze the cells (Ideally 80-90% of cell are enlarged at that stage)

Day 4 or 5

- **Freezing process**
  
  Put the flask in -80°C or transfer cells into 50 ml falcon tubes and freeze;

- **Purification**
  
  Thaw rapidly cells in waterbath 37°C and vortex vigorously (30sec)
  
  Freeze again at -80°C
  
  Thaw rapidly in waterbath 37°C and vortex vigorously (30sec)
  
  Centrifuge at 1500 rpm during 10 min at 4°C
=> supernatant (SN1) => Centrifuge 1500 rpm 10 min 4°C  
=> supernatant (SN2) keep on ice  
=> Cell pellet 2 => resuspend in 1mL cold RPMI medium  
=> Cell pellet 1 => Resuspend in 1mL cold complete RPMI medium and keep on ice

Add cell pellet 2 to cell pellet 1 => Lyse by two freeze-thaw cycles (10min on dry ice, about 5min in incubator at 37°C => centrifuge (1500 rpm, 10 min, 4°C)  
  ✴ Supernatant (SN3)  
  ✴ Cell debris => discard

Total supernatant SN2 + SN3 => centrifugation 20min 4560 rpm at 4°C  
  ✴ Supernatant 4 (SN4) => 2h ultracentrifugation at 4°C on 20% sucrose cushion (4mL) at 100,000g (rotor SW32: 28500rpm)  
    ✴ Supernatant discard  
    ✴ Virus pellet: resuspended in 0.5-1ml (depending on starting volume) of RPMI+ 10% FCS and aliquot per 20-50µL/tube, freeze at -80°C.