# VLP Generation

## *Sf21* Transfection

For the composition of Grace's medium, refer to: https://www.thermofisher.com/nl/en/home/technical-resources/media-formulation.71.html (retrieved: 29-09-2017)

1. Seed the Sf21 cells in a 6-wells plate. Use around $8*10^5$ cells/well in 2mL Grace's medium (no antibiotics).
2. Give the cells 2 to 3 hours to attach to the well, or incubate overnight.
3. Combine 10µL Bacmid DNA and 90µL Grace's medium and set aside. Avoid pipetting up and down!
4. Prepare the transfection mixture by combining 5µL ExpresS2 TR and 95µL Grace's medium. Add this to the 100µL DNA mixture and incubate for 20 minutes at room temperature.
5. Add the full Transfection:DNA mix to the medium of the cells, in a dropwise manner.
6. After 4 hours, replace the culture medium with 2mL fresh Grace's medium+antibiotics. Incubate at [...]ºC.
7. In 2-3 days, monitor the cells for presence of baculovirus signs.

## Bacmid DNA Isolation

1. Pellet the cells in a table top centrifuge at 2000x g for 15 min, at 4°C.
2. Resuspend the cell pellet in 200µl chilled GTE and transfer it to an Eppendorf tube.
3. Add 400µl NaOH solution and mix by inverting the tube a few times. Incubate for 5 minutes at RT afterwards.
4. Add 300µl KAc solution, invert the tube several times, avoid vortexing! Incubate on ice for 5 minutes.
5. Centrifuge 10 min at max speed in the Eppendorf centrifuge at 4°C.
6. Transfer 1ml supernatant to a new Eppendorf tube and add 0.6x volume (600µl) ice-cold 100% isopropanol and mix by inverting the tube.
7. Centrifuge for 15 min at max speed, 4°C. Remove the supernatant afterwards.
8. Wash the pellet with 500µl 70% ethanol and centrifuge again for 5 min, max speed at 4°C.
9. Remove the supernatant. If there is still a lot of liquid left, spin an additional 5 min to remove all liquid.
10. Air dry the pellet for 15-30 minutes.
11. Add 40µl $dH_2O$ to the pellet and leave at RT for 15-30 minutes.
12. Dissolve the pellet by flicking the tube (!do not pipette up and down!) and store the bacmid DNA at 4°C.

## RNA Isolation

**Sample Lysis**

1. Pellet the cells by centrifugation and discard the supernatant.
2. Add TRIzol™ Reagent to the sample in a final 3:1 ratio (TRIzol:sample). Homogenize the mixture by pipetting up and down.
3. Incubate for 5 minutes at room temperature and add 0.2mL chloroform per mL of TRIzol™ Reagent added previously.
4. Incubate for 3 more minutes, then centrifuge the sample for 15 minutes at 12000x g, 4°C.
5. Transfer the upper aqueous phase to a new tube.

**RNA Isolation**

6. Add 0.5mL isopropanol per mL of TRIzol™ Reagent used for sample lysis and incubate for 10 minutes.
7. Centrifuge for 10 minutes at 12000x g at 4°C and discard the supernatant.
8. Resuspend the pellet in 1mL of 75% ethanol per mL of TRIzol™ Reagent used for lysis.
9. Vortex briefly, then centrifuge for 5 minutes at 7500x g at 4°C. Discard the supernatant.
10. Air dry the pellet for 10 minutes.
11. Resuspend the pellet in 20-50µl MQ. Homogenize by pipetting up and down.
12. Use RNA for further applications or freeze in -20°C.

## Semi-Dry Blotting

1. Soak per gel 2x 3 Whatmann filter papers in 1x Semi-dry blotting buffer.
2. Place the Immobulon-P membrane in a 96% ethanol solution.
3. Assemble the following construct onto the blotting machine, using this order:

    3x Whatmann paper>Immobulon-P membrane>SDS-gel>3 Whatmann filter papers

4. Attach the lids of the machine and blot with a constant 0.05A/gel for 1 hour.
5. Remove the blot from the machine afterwards and place it into a 50mL tube, with 10mL 1% ELK added.
6. Incubate at room temperature for 1 hour, or overnight at 4°C.
7. Discard the ELK and add 5mL of primary antibody in 1% ELK using the correct dilution.
8. Incubate at room temperature for 1 hour, or overnight at 4°C.
9. Discard the solution and wash 3x with 10mL PBS-T for 5 minutes.
10. Add 5mL of secondary antibody, diluted in PBS-T.
11. Incubate at room temperature for 1 hour, or overnight at 4°C.
12. Discard this solution as well and wash with 3x 10mL of PBS-T for 5 minutes.
13. Add 10mL AP-buffer and incubate for 10 minutes at room temperature.
14. Add 5mL NBT/BCIP solution (made by adding 75µl to 5mL AP-buffer).
15. Develop the membrane until bands appear, then wash with 3x water to stop the reaction.

## Baculovirus Titration by End Point Dilution Assay (EPDA)

1. Prepare a dilution series of the baculovirus suspension from $10^{-1}$ to $10^{-9}$ in culture medium.
2. The $10^{th}$ sample will be a negative control, only containing 90µl *Sf*9-ET medium.
3. Dilute the Sf cells to a concentration of $1.5 \cdot 10^6$ cells/mL.
4. Add 90µl *Sf*9-ET cell suspension to each tube containing virus dilution and the negative control. Mix well.
5. Add 10µl of each tube to a 6 wells of a 60-wells microtiter plate. Incubate 4-7 days at 27ºC in a humid box.
6. Count the number of infected wells and calculate the $TCID_{50}$/mL (Tissue Culture Infected $Dose_{50}$) using the following formula:

$$TCID_{50} = 200 \cdot 10^{(a+b)}$$

In which:
a = the highest dilution of which the percentage of AIW is higher than 50%
b = (c-50%)/(c-d)
c = percentage of AIW of dilution a.
d = percentage of AIW of a ten times dilution of dilution a.

## Transmission Electron Microscopy (TEM) sample preparation

1. Prepare tungsten TEM grids in a vacuum evaporator.
2. Apply 5µL of sample and incubate for 1 minute. Remove excess liquid with blotting paper.
3. Apply 5µL of phosphotungstic acid staining and incubate for 30 seconds RT. Remove excess liquid here as well.
4. Use sample for microscopy.