

## **Titration of virus**

### **Virus transduction**

1. When aliquoting the virus, prepare 1 vial with 6 ul for titration. This vial must go through one cycle of freeze /thaw before titration and if you want to titer in conjunction with aliquoting you can put the vials on dry-ice for a bit.
2. Seed cells in 6 well plates seed 100,000 cells – in 2-3 ml media. For each virus - 3x wells are required. Plate 6x additional wells for control and reference batch.
3. Dilute the virus 1:10 before adding it to the cells (54 ul PBS + 6 ul virus).
4. Then add 3 ul, 10ul and 30 ul of the diluted virus to the cells (corresponding to 0.3 ul, 1 ul and 3 ul undiluted virus).
5. Leave for 72 hours before DNA is isolated.

### **Isolation of DNA**

1. Look at the cells under the microscope and compare with control
2. Remove media and wash 1x with 1 ml PBS (Use 1000 ul Pipette and don't add the PBS directly on the cells to avoid flushing them off)
3. Add 500 ul Trypsin and leave for a few minutes in the incubator.
4. After incubation with Trypsin, add 500 ul PBS or Media and transfer cells to 1.5 ml tubes.
5. Spin down for 5 min at 300 x g.
6. Remove supernatant with a pipette to not disturb the pellet
7. Make a master mix of 200 ul PBS + 20 ul Protein K per sample and resuspend the pellet in 220 ul mastermix
8. Add 200 ul Buffer AL (w/o ethanol) and mix thoroughly by vortexing.
9. Bring samples to DNA isolation room and follow the instructions from the Qiagen DNeasy Protocol: Purification of Total DNA from Animal Blood or Cells (Spin-Column Protocol). Start with incubation at 56°C for 10 min and then move directly to step 3.

### **qPCR (Taqman).**

Standard qPCR protocols were then performed using the following primers:

- a. Woodchuck hepatitis virus post-regulatory element (WPRE):  
WPRE FP- GGCACGACAATTCCGTGGT,  
WPRE RP-AGGGACGTAGCAGAAGGACG  
WPRE probe- 5'Fam-ACGTCCTTTCCATGGCTGCTCGC -Tamra-3'.
- b. Human albumin was used as a standard:  
ALB FP-5'-TGAAACATACGTTCCCAAAGAGTTT-3'  
ALB RP 5'-CTCTCCTTCTCAGAAAGTGTGCATAT-3'  
ALB probe- 5'Fam-TGCTGAAACATTACCTTCCATGCAGA-Tamra-3'.
- c. LV 2 for LV not using WPRE:  
LV2 FP; 5'-ACTTGAAAGCGAAAGGGAAAC-3'  
LV2RP; 5'-CACCCATCTCTCTCCTTCTAGCC-3'  
LV2 PROBE ; 5'.FAM AGCTCTCTCGACGCAGGACTCGGC-TAMRA-3'

Relative quantification of WPRE/LV2 and albumin content compared to reference batch (virus of known titre) was performed by the  $\Delta\Delta CT$  method.