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Titration of Lentivirus

Forked from a private protocol

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lentivirus

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol is about titrating lentivirus.

Attachments



[Lentivirus titration...](#)

71KB

Materials

qPCR [Taqman]

Standard qPCR protocols were then performed using the following primers:

- a. Woodchuck hepatitis virus post-regulatory element [WPRE]:
WPRE FP- GGGACTGACAATTCCGTGGT,
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'CACCCATCTCTCCTTCTAGCC-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.

Safety warnings

 For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).



Virus transduction

3d

- 1 When aliquoting the virus, prepare 1 vial with 6 μ L 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 mL -  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 μ L PBS +  6 μ L virus].
- 4 Then add 3 μ L, 10 μ L and 30 μ L of the diluted virus to the cells [corresponding to 0.3 μ L, 1 μ L and 3 μ L undiluted virus].
- 5 Leave for  72:00:00 before DNA is isolated.

3d

Isolation of DNA

10m

- 6 Look at the cells under the microscope and compare with control.
- 7 Remove media and wash 1x with  1 mL PBS [Use 1000 μ L pipette and don't add the PBS directly on the cells to avoid flushing them off]. 
- 8 Add  500 μ L trypsin and leave for a few minutes in the incubator. 
- 9 After incubation with trypsin, add  500 μ L PBS or Media and transfer cells to 1.5 ml tubes.
- 10 Spin down at  300 x g, 00:05:00 . 
- 11 Remove supernatant with a pipette to not disturb the pellet.



- 12 Make a master mix of  200 μ L PBS +  20 μ L Protein K per sample and resuspend the pellet in  220 μ L mastermix .
- 13 Add  200 μ L Buffer AL [w/o ethanol] and mix thoroughly by vortexing.
- 14 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  00:10:00 and then move directly to step 3.
- 15 Assess titre using qPCR.

10m