Aug 16, 2024

Titration of Lentivirus

Forked from Titration of Lentivirus

DOI
dx.doi.org/10.17504/protocols.io.14egn6yj6l5d/v1

Titration of lentivirus

Anita Adami[1]

[1]Lund University

ASAP Collaborative Rese…

Laura Castilla-Vallmanya
Lund University

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.14egn6yj6l5d/v1

Protocol Citation: Anita Adami 2024. Titration of Lentivirus. **protocols.io https://dx.doi.org/10.17504/protocols.io.14egn6yj6l5d/v1**

Protocol status: Working
We use this protocol and it's working

Created: August 16, 2024

Last Modified: August 16, 2024

Protocol Integer ID: 105751

Keywords: Titration, lentivirus, DNA isolation, qPCR, titer, ASAPCRN

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- GGCACTGACAATTCCGTGGT,
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-TGCTGAAACATTCACCTTCCATGCAGA-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 ΔΔCT method.

Safety warnings

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

Virus transduction

3d

1 When aliqouting the virus, prepare 1 vial with 6 µl for titration. This vial must go through one cycle of freeze / thaw before titration and if you want to titer in conjunction with aliqouting 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 µL PBS + 6 µL virus].

4 Then add 3 µl, 10 µl and 30 µl of the diluted virus to the cells [corresponding to 0.3 µl, 1 µl and 3 µl 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 ul pipette and don´t add the PBS directly on the cells to avoid flushing them off].

8 Add 500 µL trypsin and leave for a few minutes in the incubator.

9 After incubation with trypsin, add 500 µL 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 µL PBS + 20 µL Protein K per sample and resuspend the pellet in 220 µL mastermix .

13 Add 200 µL 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. 10m

15 Assess titre using qPCR.