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We use this protocol and it's working

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Virus production

In 2 collections

michela.deleidi[1]

[1]German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany

Federico Bertoli

ABSTRACT

Lentiviral production protocol

Virus production

1 For lentiviral production, HEK cells were transfected with the plasmids expressing the shRNAs together with the lentiviral packaging plasmid psPAX2(RRID:Addgene_12260) and the envelope

plasmid pMD2.G (RRID:Addgene_12259) using TransIT-X2 (Mirus).

2 HEK 293 T cells were seeded in a 10-cm dish to reach 80% confluency the day after transfection.The next day, the medium was changed.

3 On days 4 and 5 after transfection, the medium was collected and filtered through a0.45-µm PVDF membrane.

4 To concentrate the virus, the filtered supernatant was centrifuged in a Vivaspin column (Sartorius Stedim Biotech) at 3000 RCF at 4 °C until the volume reached 500 –1000µl.

5 The virus-containing supernatant was collected, and the concentration of p24 particles was determined with Lenti-X GoStix Plus (Takara).

6 Cells were infected with equal concentrations of p24 particles for the scramble and respective shRNA.