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Virus production



In 1 collection

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Protocol status: Working

We use this protocol and it's working



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Abstract

Lentiviral production protocol

Troubleshooting

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 a 0.45- μ m PVDF membrane.
- 4 To concentrate the virus, the filtered supernatant was centrifuged in a Vivaspinn column (Sartorius Stedim Biotech) at 3000 RCF at 4 °C until the volume reached 500–1000 μ l.
- 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.