Jul 13, 2022

Small scale Lentivirus Production and Infection

DOI
dx.doi.org/10.17504/protocols.io.bp2l61z2zvqe/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.bp2l61z2zvqe/v1

Protocol Citation: Herschel Dhekne, Suzanne R Pfeffer 2022. Small scale Lentivirus Production and Infection. **protocols.io https://dx.doi.org/10.17504/protocols.io.bp2l61z2zvqe/v1**

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

Created: June 16, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 64696

Keywords: Small scale Lentivirus Production, Small scale Lentivirus Infection, ASAPCRN, small scale lentivirus production, lentivirus plasmid, shrna, infection this protocol

Abstract

This protocol can be used for production and transduction of lentiviral sgRNA, shRNA and protein overexpression in conjunction with generation 2 and generation 3 lentivirus plasmids.

Attachments

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21KB

Materials

Materials:

- BSL-2+ facility cell culture lab
- Addgene plasmids - psPAX2 **addgene Catalog #**12260 , pMD2.G **addgene Catalog #**12259), lentiviral vector
- Polyethyleneimine (PEI, Polysciences) [M] 1 mg/mL stock
- HEK 293T cells
- Polyethylene glycol (PEG) 8000
- Polybrene ([M] 10 mg/mL)

- **4X lentivirus concentrator solution**6. Store at 4 °C .

- Ultracentrifuge and compatible tubes

Troubleshooting

Make lentivirus

6h

1 Plate 293T cells at 40% confluency in a 6 well tissue plate submerged under 2 mL medium per well.

2 After 06:00:00, most cells will have attached. 6h

Day 0

3 Prepare DNA mix for transfection:

3.1 Add the following to 100 µL Optimem per well for transfection:

	A	B
	1 µg	PsPAX2 1µg helper plasmid (Addgene ##12260)
	0.5 µg	VSV-G / pMD2.g (Addgene #12259)
	1 µg	Lentivirus vector (see below)

3.2 Add PEI (from a [M] 1 mg/mL stock) to this mixture solution at ratio 5:1 w/w (PEI:DNA). Example, 12.5 µg PEI for 2.5 µg DNA mix.

3.3 Mix DNA mix gently and incubate for 00:20:00 at Room temperature. 20m

3.4 Add the mix to the cells dropwise.

Day 1 (16 hours later)

4 Check for cell viability; at this time, >70% of the cells should be transfected and virus is already being produced and is being released into the supernatant.

Note

NOTE: Removal of residual PEI at this stage by medium change is not essential but will be present in the supernatant.

Day 2

5 2d

48:00:00 after transfection, collect the culture supernatant in a BSL-2+ facility; centrifuge in an enclosed rotor and remove supernatant with care. This is “Day-2 virus”.

6 Carefully add an additional 2 mL complete DMEM medium into each well without splashing or disturbing the monolayer.

7 Bleach all tips and pipettes used to collect the virus.

Day 3

8 3d

72:00:00 after transfection, collect the culture supernatant in BSL-2+ facility as before. This is “Day-3 virus". Day-2 and Day-3 virus are then pooled; Day-2 titre is lower than Day-3.

9 5m

The pooled virus (~ 4 mL) is transferred into a 15ml tube and centrifuged at 250 x g for 00:05:00 .

Note

The pellet represents cell debris as well as 293T cells that can contaminate the target cell line to be infected with the virus; care should be taken when aspirating the virus supernatant. Filtration can decrease viral titre and is not required.

10 Prepare 0.5 mL aliquots of the lentivirus and freeze at -80 °C .

Lentivirus Infection

11 Thaw a 0.5 mL virus aliquot in a 37 °C water bath, flicking tube gently to facilitate gentle thaw.

12 Add 1 µL , [M] 10 mg/mL Polybrene.

Note

NOTE: Polybrene enhances infectivity but is not essential. Use at 2-8µg/ml depending on the cell type; polybrene can be toxic to cells so take care. HeLa, MEF, 3T3 and A549 cells tolerate up to 8 µg/ml.

13 Transfer virus mixture to the medium covering 1 well of a 6 well plate containing the target cell line. Polybrene will become diluted in the cell medium to a final concentration of [M] 4 µg/ml .

14 48:00:00 post infection, cells are ready for analysis or selection. 2d

Concentrating the virus

15

Note

Rationale: To achieve 100% infection and/or if you have low titers or do not care about precise multiplicity of infection, it is beneficial to concentrate the lentivirus.

4×Lentivirus Concentrator Solution

16 Dissolve 80 g PEG-8000 and 14.0 g NaCl in 80 mL MilliQ water.

17 Add 20 mL , 10X PBS (pH 7.4).

18 Mix with gentle stirring, heating gently only if necessary, until the solids are dissolved then adjust pH to 7.0~7.2.

19 Adjust the final volume to 200 mL.

20 Sterilize by passage through a 0.2 µm filter.

Note

The concentrations of PEG-8000 and NaCl in the stock solution are 40% (w/v) and [M] 1.2 Molarity (M), respectively.

Virus concentration protocol

21 Carefully transfer the virus supernatant into a new 50 ml tube.

22 Add 1 volume of concentrator solution to 3 volumes of virus supernatant (eg. 1 mL concentrator solution for 3 mL virus).

23 Mix by gentle shaking for ~ 00:00:20 then incubate with constant rocking at least 04:00:00 at 4 °C. 4h 0m 20s

Note

Overnight rotation or rocking will enhance recovery.

24 Spin down at 1600 x g for 01:00:00 at 4 °C. 1h

25 Carefully remove supernatant without disturbing the pellet.

Note

Pellet size does not necessarily correlate with virus yield.

26 Thoroughly resuspend the viral pellet in PBS or desired medium using 1/10~1/20 of the original volume by gentle pipetting using a 1ml Pipetman.

27 Aliquot and store at -80 °C until use.

Alternative Centrifugation- based Virus concentration method

3d 1h 35m

28 3d

Note

In case of low transduction efficiency, consider ultracentrifugation as follows:

72:00:00 after transfection, collect the virus-containing supernatant in a BSL-2+ facility (take only Day 3 supernatant).

29 Spin down at 250 x g for 00:05:00 at Room temperature. 5m

30 Transfer the precleared supernatant to ultracentrifuge tubes and pellet at 90000 x g for 01:30:00 at 4 °C. 1h 30m

31 Remove the supernatant and leave a little less than 1 mL in the tube. Use a 1 mL pipette to recover the remaining pellet which may be difficult to see.

32 Make aliquots of 0.2 mL concentrated virus and freeze at -80 °C.