Jul 12, 2022

Small scale Lentivirus Production and Infection

Herschel Dhekne[1], Suzanne R R R Pfeffer[1]

[1]Stanford University Department of Biochemistry

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

Suzanne R R Pfeffer

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

465-974.docx

DOI

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

PROTOCOL CITATION

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

KEYWORDS

Small scale Lentivirus Production , Small scale Lentivirus Infection

CREATED

Jun 16, 2022

LAST MODIFIED

Jul 12, 2022

OWNERSHIP HISTORY

Jun 16, 2022 maria.s

Jun 28, 2022 Suzanne R R Pfeffer

PROTOCOL INTEGER ID

64696

MATERIALS TEXT

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

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

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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 **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

20m

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

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

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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** .

> 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

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

12 

Add **1 µL** , **10 mg/mL** Polybrene.

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 **4 µg/ml** .

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

Concentrating the virus

15

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**).

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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.

The concentrations of PEG-8000 and NaCl in the stock solution are 40% (w/v) and **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  4h 0m 20s

Mix by gentle shaking for ~ **00:00:20** then incubate with constant rocking at least **04:00:00** at **4 °C** .

Overnight rotation or rocking will enhance recovery.

24  1h

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Spin down at **1600 x g** for **01:00:00** at **4 °C** .

25 Carefully remove supernatant without disturbing the pellet.

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

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  5m

Spin down at **250 x g** for **00:05:00** at **Room temperature** .

30   1h 30m

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

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.

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

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