

Jul 13, 2022

Small scale Lentivirus Production and Infection

DOI

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

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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>

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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





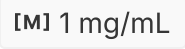
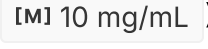
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21KB



Materials

Materials:

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

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





- 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. 




Day 0

- 3 Prepare DNA mix for transfection:

- 3.1 Add the following to  100 μ L Optimem per well for transfection: 

	A	B
	1 μ g	PsPAX2 1 μ g helper plasmid (Addgene ##12260)
	0.5 μ g	VSV-G / pMD2.g (Addgene #12259)
	1 μ g	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 μ g PEI for  2.5 μ g DNA mix. 

- 3.3 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**






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

Day 2

- 5  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.

2d

Day 3




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

3d

5m

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 ,  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  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 ( 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  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 .