Aug 19, 2024

# Lentivirus Production

Forked from a private protocol

DOI
**dx.doi.org/10.17504/protocols.io.8epv5ro85g1b/v1**

Lentivirus production

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**DOI:** dx.doi.org/10.17504/protocols.io.8epv5ro85g1b/v1

**Protocol Citation:** Anita Adami 2024. Lentivirus Production. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5ro85g1b/v1



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

**Created:** August 19, 2024

**Last Modified:** August 19, 2024

**Protocol Integer ID:** 105905

**Keywords:** lentivirus, production, LV, ASAPCRN

**Funders Acknowledgement:**
**Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research**
**Grant ID: ASAP-000520**

## Abstract

This protocol is about the production of lentivirus.

## Attachments

PDF

**Lentivirus Productio...**

178KB

## Materials

Materials:

- PEI stock solution [**Steps**]
- 15 ml and 50 ml culture Flasks
- $T_{175}$ Cell culture Flasks
- Complete DMEM [DMEM with Glutamax + 10% FBS + Peniclillin [100U/ml] + Streptomycin [100ug/ml]]
- DNeasy blood and tissue kit [Qiagen AB, Sollentuna, Sweden]
- Plasmids
    1. transfer plasmid,
    2. pMD2G [envelope]
    3. pRSV [REV]
    4. pMDL [GAG/POL]

| PLASMID | AMOUNT NEEDED PER BATCH |
|---|---|
| pMD2G [envelope] | 5,5 ug |
| pRSV [pack. 1, REV] | 3,9 ug |
| pMDL/RRE [pack. 2, GAG/POL] | 7,5 ug |

| TRANSFER PLASMID SIZE | AMOUNT OF PLASMID | PEI |
|---|---|---|
| 8 KB | 17 ug | 102 ul |
| 9 KB | 19 ug | 108 ul |
| 10 KB | 21 ug | 114 ul |
| 11 KB | 23 ug | 120 ul |
| 12 KB | 25 ug | 126 ul |
| 13 KB | 27 ug | 132 ul |
| 14 KB | 29 ug | 138 ul |
| 15 KB | 31 ug | 144 ul |

## Safety warnings

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

## PEI stock solution preperation

1 Dissolve Polyethyleneimine [Polysciences PN 23966] by swirling using de-ionized $H_2O$ heated to 80 °C and using ~90% of volume required to reach a final concentration of [M] 1 Mass Percent [1 µg/µl].

2 Cool to Room temperature.

3 Set to pH 7.0 by adding HCl.

4 Add de-ionized $H_2O$ to reach final concentration of [M] 1 µg/µL.

5 Filter using a 0.22 µm membrane and divide into aliquots. Store in -20 °C freezer.

6 Thaw in 37 °C water bath until precipitates have fully dissolved.

7 Store thawed aliquot in fridge [ 4 °C ].

8 Re-dissolve precipitates by incubating at 37 °C before use if needed.

## Lentivirus production [Day -4 or -3]

5m

9 Make media, warm in water bath.

10 Seed cells on Thursday [0.9 x $10^6$] or Friday [1.8 x $10^6$] before [25 ml media]. This will yield approx. 20 million cells per T175. Seed 8-9 flasks for a 6 virus batch.

11 Wash with 10 mL PBS [on the roof, then submerge carefully].

12 2 mL trypsin , 00:05:00 in incubator, hit on the side. 5m

13 10 mL media , spin 400 x g, 00:05:00 .

14 Resuspend in 5 mL , count, [80 ul PBS + 10 ul cell susp, i.e. 1:10 dil].

15 Add the right number of cells to each flask [see above], plus 10 mL - 20 mL media .

## Lentivirus production [Day 0: Seeding of cells] 5m

16

> **Note**
>
> Perform this section approximately 24 hours before transfection.

17 Warm media in water bath.

18 Wash with 10 mL PBS [on the roof, then submerge carefully].

19 2 mL trypsin , 00:05:00 in incubator, hit on the side. 5m

20 Add 5 mL - 7 mL medium to each flask to neutralize trypsin.

21 Rinse flasks and transfer cells to a [or two] 50 ml tube.

22 Spin down at 400 x g, 20°C, 00:05:00 .

Note

Pressing start saves settings.

23 Resuspend cells in appropriate volumne [about 20 mL ].

24 Count.

25 Add 20 mL media to each flask [DMEM Glutamax + 10% FBS + Penicillin 100 U/ml + Streptomycin 100 U/ml].

26 Plate 12.5 undetermined cells per T175 flask. Need 12 for 6 virus batch.

27 Add the calculated cell suspension to the flask with the media.

Note

Seed 1.5 undetermined cells for virus titration in a separate T175 flask. Then virus titration can be started immediately after harvest of the virus, i.e. Day 3.

## Lentivirus production [Day 1: transfection of cells] 15m

28 Check to see how confluent cells are [should preferably be 75% - 90%].

29 Make sure you have all calculations done prior to starting, including the volumne of PEI to be used [102 µm - 132 µm].

30 Mix packaging vectors [pMDL, psRev and pMD2G] and transfer vector in a 50 ml tube with serum free media [DMEM+P/S] or PBS, so that the total volumne [including PEI] becomes 3.6 ml.

31 Add PEI and mix by vortexing for a few seconds.

32 Incubate the DNA/PEI mix at Room temperature for 00:15:00 . 15m

33 Change media in flasks and add 16.2 mL fresh complete media .

34 Add 1.8 mL transfection mix to each T175 flask, mix gently over cells by tilting the flask.

## Lentivirus production [Day 3: Harvest]

35

**Note**

Perform this section 45 hours after transfection.

**Safety information**

Start using Virkon and double gloves. Rinse everything in Virkon when used [flasks, pipettes etc.]. When finished, turn off incubator and turn on UV.
Please consult with your institution´s biosafety officer for specific instructions for handling and disposal during and after production.

36 Check that they are alive and if they have the right flurophore [if applicable].

37 Collect media from the two flasks for each virus into one 50 ml tubes.

38 Spin the supernatant at 800 x g, 4°C, 00:10:00 .

39 While tubes are in the centrifuge, prepare in hood the requirements for the next step. Also, rinse the now empty culture flasks with Virkon and discard.

Note

- Beckman centrifuge tubes [Label!]
- Spare tube if required to balance
- 45 µm filters
- 50 ml syringes
- Centrifuge canisters
- Beaker of Virkon

40 Remove the 50 ml tubes from centrifuge, and in the hood, pour the supernatant into open syringe with attached filter over the labelled Beckman UC tube. After each virus/50 ml tube, change the syringe and filter. Ensuring that the discarded syringe and filter are placed and rinsed in Virkon.

41 Put Beckman UC tubes into their chambers using forceps.

42 To avoid collapsing the tubes, fill up sample tubes with media. Fill up counterweight tubes with DPBS.

43 Balance the weight of the tubes carefully, down to 5 ug difference.

44 Clean everything in the hood thoroughly. Any plastics that have been used must be rinsed with Virkon before discarding. Spray pipettes, racks, pipette gun etc. with ethanol. Clean hood surfaces with Virkon first, then with ethanol.

45 Turn off the hood fully and then put the UV on.

## Ultracentrifuge

2h 5m

46 Put down rotor, spin it.

Note

You don´t have to screw.

47 Put down all 6 chambers into centrifuge [you do not have to weigh the empty ones].

48 Check settings of centrifuge:

- 19500 x g, 4°C, 02:00:00
- Enter to save
- Ensure Acceleration is set to max
- Ensure Deceleration is set to max

49 Wait until centrifuge reaches full speed before leaving.

50 Set alarm for when centrifugation is done, don´t let the tubes stand longer.

51 Deceleration of centrifuge takes approx. 00:05:00 . After it reaches 3000G, you can release the vacuum to increase the speed of deceleration. 5m

52 Take out Beckman UC tubes using forceps, and pour out liquid into Virkon.

53 Place tuned upside down on paper to dry, be careful to remove all liquid, also from edges and walls.

54 Take a bottle of cold PBS from freezer and resuspend pellet in 80 µL [75 ul - 100 ul is ok]

55 Seal tubes with Parafilm, vortex and put into fridge, leave them there for at least 02:00:00 - but preferably Overnight . 2h

56 Aliquot virus [10 ul/tube] and store -80 °C .

57 Viruses can now be titrated and/or used.