Aug 05, 2025 Version 2

Lentiviral vector production V.2

Version 1 is forked from Nanoblade production

DOI
https://dx.doi.org/10.17504/protocols.io.81wgbw1rygpk/v2

maxime smits[1], Joris Van Asselberghs[1]

[1]KU Leuven

center for molecular me...

Joris Van Asselberghs
KU Leuven

DOI: https://dx.doi.org/10.17504/protocols.io.81wgbw1rygpk/v2

Protocol Citation: maxime smits, Joris Van Asselberghs 2025. Lentiviral vector production. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbw1rygpk/v2Version created by **Joris Van Asselberghs**

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

Created: July 09, 2025

Last Modified: August 05, 2025

Protocol Integer ID: 222075

Keywords: Nanoblades, Virus-like particles, lentiviral vector production lentiviral vector, lentivirus, type of retrovirus, retrovirus, gene delivery into cell, genetic material into the host cell, gene delivery, term transgene expression, host cell, genetic material,

Abstract

Lentiviral vectors are derived from lentiviruses, a type of retrovirus, and are widely used for gene delivery into cells. They are particularly valuable because of their ability to transduce both dividing and non-dividing cells, and their capacity to integrate their genetic material into the host cell's genome, leading to stable and long-term transgene expression.

Guidelines

Production of lentiviral vectors are being performed in a Bio Safety Lab 2 (BSL-2 lab)

BSL-2: Here, it is permitted to work with organisms that cause diseases. However, the lab is only accessible to people who work there and know the procedures. Doors are always closed during work and windows cannot be opened. The entire area is set up for efficient cleaning and disinfection. All waste is disinfected.

Materials

Materials

- Sterile 1,5 mL Eppendorf tubes
- Sterile 50 mL Falcon tubes
- Petri-dishes (PD)
- Pipetboy
- Sterile pipets (5 mL, 25 mL)
- Micro-pipets
- Sterile Pipet tips (100 µL, 1000 µL)
- 0,45 µM filter
- Sterile syringes
- Sterile vivaspin membranes
- spectrophotometer/Nanodrop
- Incubator
- Microscope

Reagents

- DMEM medium 2% FCS
- OptiMEM
- Gentamycin
- Linear PEI
- PBS
- Plasmids (transfer, envelop, packaging)
- HEK293

Safety warnings

- Be careful not to mix bleach with alcohol-derived reagents. The key ingredient in household bleach is sodium hypochlorite. Sodium hypochlorite reacts with ethanol, isopropyl alcohol, and other types of alcohol to make chloroform ($CHCl_3$), hydrochloric acid (HCl), and other compounds, such as dichloroacetate or chloroacetone.

Before start

- Desinfect the flow with 70 % Ethanol before starting any work there.
- Always wear two sets of gloves and a sterile lab coat.
- Desinfect bottles and tip boxes with 70 % Ethanol, before entering the flow and after the work is finished.
- Desinfect gloves regularly with 70 % Ethanol.
- Waste is collected in a beaker filled with bleach.

DAY 0

1 **SEEDING Petri-dishes (PD)**

1.1 Seed HEK293T cells in a 10 cm PD in DMEM 2% FCS + 500 µL Gentamycin

1.2 Incubate on 37 °C at 5% CO_2

DAY 1

2 **TRANSFECTION of transfer, packaging, and envelop plasmids**

2.1 Before starting, measure the DNA concentration and the purity of the plasmids in the molecular lab.

2.2 Make a DNA mixture in an 1,5 mL tube of the following plasmids:

	A	B	C
		1 PD	5 PD
	transferplasmid	12,74 µG	63,7 µG
	packaging plasmid	6,36 µG	31,8 µG
	envelope plasmid	3,18 µG	15,9 µG

2.3 Write down the total volume of the DNA mixture

2.4 Transfer the DNA mixture in a 50 mL Falcon tube in the vector lab, under the flow

2.5 Add autoclaved PBS to the mixture with a final volume of 559,6 µL (1 PD) or 2798 µL (5 PD)

2.6 Dilute the linear PEI (323 mg/L) with autoclaved PBS:

	A	B	C
		1 PD	5 PD
	Linear PEI	245,6 µL	1228 µL
	PBS	255,0 µL	1275 µL

2.7 Add the diluted linear PEI to the DNA mixtures **GENTLY**, and incubate for 5 to 10 minutes on RT

2.8 Add 5 mL (1 PD) or 25 mL (5 PD) of medium (DMEM 2% FCS + 500 µL Gentamycin or OptiMem + 500 µL Gentamycin) to the DNA-PEI mixtures **GENTLY** and mix

2.9 Check the seeded HEK293T cells under the microscope for confluency

2.10 Remove the medium on the HEK293T cells and add the transfection medium. Normally there is enough for 6 mL of medium per PD

2.11 Incubate on 37 °C at 5% CO_2

2.12 Leave the medium flask at RT

DAY 2

3 **REMOVING TRANSFECTION MEDIUM**

3.1 Remove the Transfection medium and replace it with RT medium. 5 mL per PD should be sufficient

3.2 Incubate on 37 °C at 5% CO_2

3.3 Also store the medium flask at 37 °C

DAY 3

4 **1st HARVEST**

4.1 Collect the supernatant from the PD using a 0,45 µM syringe and store at 4 °C

4.2 Add Pre-warmed medium to the cells.

4.3 Incubate on 37 °C at 5% CO_2

DAY 4

5 **2nd HARVEST**

5.1 Collect the supernatant from the PD using a 0,45 µM syringe and store at 4 °C

5.2 Desinfect the PD by spraying 70% Ethanol or bleach on the cells, before throwing them in the yellow bin

5.3 The supernatant of both harvest days will be concentrated via vivaspin

1. First wash the vivaspin membrane by adding 10 mL of PBS and spinning down at 3000g for 3 minutes

2. Add the supernatant to the vivaspin membrane and spin down at 3000g, until there is about 0,5 - 1,0 mL remaining

5.4 Make aliquots of the desired size and freeze them in the -80°C ultrafreezer.

5.5 IMPORTANT REMARK: do no re-freeze aliquots! With every freeze-thaw cycle you have a loss of lentiviral vector functionality