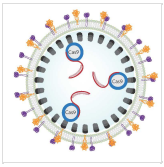

Nanoblade production

maxime.smits [1], Simone Giovannozzi[1], Louise Medaer[1]

[1]KU Leuven

Jun 16, 2021

1 *Works for me* Share This protocol is published without a DOI.

center for molecular medicin KU Leuven

maxime.smits

ABSTRACT

Nanoblades are virus-like particles that are able to transiently deliver a Cas9 protein together with a gRNA, without integration of DNA in the genome.

PROTOCOL CITATION

maxime.smits , Simone Giovannozzi, Louise Medaer 2021. Nanoblade production. **protocols.io** https://protocols.io/view/nanoblade-production-bvubn6sn

KEYWORDS

Nanoblades, Virus-like particles

CREATED

Jun 15, 2021

LAST MODIFIED

Jun 16, 2021

PROTOCOL INTEGER ID

50787

GUIDELINES

Production of Virus-like particles is always 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 TEXT

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)
- HEK293T's

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 STARTING

- Desinfect the flow with 70 % Ethanol before starting any work there.
- Always wear to 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
GAG-POL (MLV-gag-pol Els Verhoeyen**)	6,38 µG	31,9 µG
BIC-Cas9 (BIC-SpCas9 Els Verhoeyen**)	6,38 µG	31,9 µG
Crizi-sgRNA* (Crizi Els Verhoeyen**)	6,38 µG	31,9 µG
VSV-G (VSVG Els Verhoeyen**)	1,59 µG	7,95 µG
BaevR (hCMV-BaevR Less ClaI-HpaI**)	1,59 µG	7,95 µG

*This plasmid changes, depending on the gRNA
**plasmid name from the database

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 557 µL (1 PD) or 2785 µL (5 PD)

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

A	B	C
	1 PD	5 PD
Linear PEI	245,5 µL	1227,5 µL
PBS	311,0 µL	1555,0 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 cold 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 Nanoblades can be stored at -80 °C