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We use this protocol and it's working

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Lentivirus production in HEK293FT with calcium-phosphate method

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ABSTRACT

To produce lentivirus, HEK293FT cells are transfected with lentiviral vector and packaging plasmids using thecalcium-phosphate method.

Day1

1 Seed 6 million HEK293FT in a 10cm plate with 10 ml complete medium (DMEM+10%FBS+1% pen-strep+1% MEM-NEAA)

Day 2

2 Add 25 µl from 10 mM Chloroquine (Final Concentration:25µM) to 9 ml of complete medium. (To avoid lysosomal degradation of the transfected DNA)

3 While preparing the plasmid mix, keep the plates inside the incubator.

4 In 2ml tubes, prepare the transfection mixture in the following order:

	Ingredients	Stock solution Conc. / mass
	pCMV-VSV-G	4µg
	psPAX2	7.5µg
	Lentiviral Vector	10µg
	ddH2O	variable
	Total value: 437.5 µl	
	2M CaCl2 (ide-cold)	62.5µl

Transfection mix for Lentivirus production.

5 500 µl of ice-cold HBS-HEPES buffer saline is added to the mix in a slow dropwise manner.

6 The Solution was further mixed by passing the tube on a tube rack for 30-45 seconds. until the mixture is full of bubbles.

7 After 5-7 minutes at RT the mixture was added to the plate drop by drop and with a circular motion to cover the surface of the plates.

8 After 6 hours, the medium was changed.

Day 4

9 The medium was collected 48 hours post-transfection and filtered through 0.45 µm filter

10 The lentivirus-containing medium was aliquoted and stored at -80°C for later use.