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Production of lentiviruses in Lenti-X cells

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ASAP Collaborative Rese…

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We use this protocol and it's working

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Abstract

This is a protocol to produce lentivirus in a HEK cell line selected to produce high-titer virus.

Attachments

958-2489.docx

15KB

Materials

- Opti-MEM™ Reduced Serum Medium **Thermo Fisher Scientific Catalog #**31985062
- X-tremeGENE™ HP DNA Transfection Reagent **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**6366244001
- Lenti-X™ Concentrator **Takara Bio Inc. Catalog #**631231
- Lenti-X™ 293T Cell Line **Takara Bio Inc. Catalog #**632180

Equipment

Mlllex®-GP Filter Unit (Sterile)	NAME
Filter	TYPE
Mlllex®	BRAND
SLGP033RB	SKU
https://www.merckmillipore.com/IN/en/product/Mlllex-GP-Filter-Unit-Sterile,MM_NF-SLGP033RB	LINK

- psPAX2 **addgene Catalog #**12260
- pMD2.G **addgene Catalog #**12259
- DMEM, high glucose, pyruvate **Thermo Fisher Catalog #**11995073 supplemented with 10% FBS (Fetal Bovine Serum **Gibco - Thermo Fischer Catalog #**10270106) and 1X Pen/Strep (Penicillin-Streptomycin (10,000 U/mL) **Gibco - Thermo Fisher Catalog #**15140122)
- 1X PBS PBS, pH 7.2 **Thermo Fisher Scientific Catalog #**20012068
- Appropriate transfer vector

Transfection

1 Plate Lenti-X cells 2.5 x 10^6/10 cm 24:00:00 before transfection. 1d

2 Warm OptiMEM, X-tremeGENE HP DNA Transfection Reagent, and DNA to warm to Room temperature.

3 Add 1 mL OptiMEM to a tube.

4 Add plasmid DNA to the OptiMEM, gently pipetting to mix.

	A	B	C
	psPAX2	0.65 pmol	4600 ng
	pMD2.G	0.36 pmol	1400 ng
	Transfer vector	0.82 pmol	

5 Add X-tremeGENE HP DNA Transfection Reagent to OptiMEM/DNA (4 µL per 1 µg of DNA).

6 Incubate for 00:15:00 at Room temperature. 15m

7 Add OptiMEM/DNA/X-tremeGENE to the plate of cells dropwise, then gently swirl the plate.

8 The day after transfection, replace the medium with

Collection 3d 1h 35m

9 72:00:00 after transfection, collect the medium in a 15 ml conical. 3d

10 Centrifuge 500 x g x 00:05:00 , then pass the supernatant through a .22 µm filter. 5m

11 Add 3 mL of LentiX concentrator to the supernatant, invert 6 times to mix, then incubate Overnight at 4 °C . 45m

12 Centrifuge at 1500 x g for 00:45:00 at 4 °C . 45m

13 Remove the supernatant and resuspend the pellet in 120 µL ice cold PBS.

14 Make aliquots in multiples of 14 µL and freeze at -70 °C .