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Production of lentiviruses in Lenti-X cells

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Protocol status: Working

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

Milllex®-GP Filter Unit (Sterile)	NAME
Filter	TYPE
Milllex®	BRAND
SLGP033RB	SKU
https://www.merckmillipore.com/IN/en/product/Milllex-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 Fisher Scientific Catalog #10270106**) and 1X Pen/Strep ( Penicillin-Streptomycin (10,000 U/mL) **Gibco - Thermo Fisher Scientific Catalog #15140122**)
- 1X PBS  PBS, pH 7.2 **Thermo Fisher Scientific Catalog #20012068**
- Appropriate transfer vector



Transfection

- 1 Plate Lenti-X cells $2.5 \times 10^6/10$ 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 μ L per  1 μ g 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 .

