

Lentivirus production (Organelle Lab)

See <https://www.addgene.org/viral-vectors/lentivirus/lenti-guide/>

	2 nd generation	3 rd generation
Transfer (Lentiviral plasmid)	pLVX, and others, GFP control (#19319, 1203), sgRNA and Cas9 (#52916, 1201)	
Packaging plasmid(s)	psPAX2 (#12260, 1202)	pRSV-REV (#12253, 959) pMDLg/pRRE (#12251, 958)
Envelope plasmid	pVSVG (pMD2.G, #12259, 742, 960)	

Preparation of lentivirus particles (using 2nd generation system) and establishing stable lines

Make sure P2 regulation and protocols

Day0

Plate 293T cell line into T75 flask

Day1

Transfection

A293T cell line (~85% confluent, T75)

↓ replace media, 8ml DMEM+10%FBS

↓ Transfection

mix below, incubate for 15min, r.t.

500μl Serum free DMEM

↓ +x μl pVSVG 4.5μg (box14#742)

↓ +x μl packaging plasmid 9μg (box24#1202)

↓ +x μl lentiplasmid 12μg

↓ +60μl 1mg/ml PEI Max

↓ +transfection mixture above, 37°C, O/N

Day2

Discard media, and change to 8ml DMEM-10%FBS @37°C

Day3

Collect media (Sup1), and add 8ml DMEM-10%FBS @37°C

Check GFP expression if possible, then if lower than 30%, redo transfection

Plate cells for stable line in 6well

Day4

Collect media (Sup2), combine Sup1+Sup2

↓

Centrifuge pooled Sup 1+2 @1000rpm, 1min

↓

Filter through 0.45 μ m pore (ex. Millipore, #SE1M003M00)

↓

Optional (if you need higher titer viruses)

↓ add 1/3 volume of Lenti-X™ Concentrator (Clontech, #631231)

4oC, 30min~O/N

Centrifuge, 1500 g, 4oC, 45min

Collect ppt and resusp w/ 2~4ml culture medium

↓

Add 2~4ml + 1.2~2.4 μ l Polybrene (10mg/ml stock, filtered) to cells in 6well

↓

Spin 6well plate w/ tape tightly @1700rpm, 60min, r.t. →37oC

Day5

Split cells and culture cells with drug (puro 2, hyg 800) for selection

Day6

Split and/or change media (puro 0.8, hyg400)

Day7

Split and/or change media (puro 0.8, hyg400)

Day8 or 9

Split and/or change media (puro 0.8, hyg400)

Plate cells for immunofluorescence to check target gene expression

Keep culture cells until they stably grow, make frozen stocks

Media for A293T: Should be warmed @37°C
DMEM+10%FBS

Polybrene 10mg/ml filtered stock@-20°C (Millipore)
Use @0.6mg/1ml
←might be toxic for some cell lines,
use Protamine Sulfate

Optimization of drug conc. for selection
Don't just add drugs to cells in culture
Add drugs when cells splitting
Expression level should be dependent on drug conc.

Drug conc. for HeLa Puro 2→0.8µg/L
Hug 800→400mg/L

Virus sup storage For short term @4°C
For longer term @-20°C