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Lentiviral transduction of iPSCs with sgRNAs and sgRNA libraries

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1 Works for me dx.doi.org/10.17504/protocols.io.8dfhs3n

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Tian et al (2019). CRISPR Interference-Based Platform for Multimodal Genetic Screens in Human iPSC-Derived Neurons. Neuron pii: S0896-6273(19)30640-3. [Epub ahead of print] PubMed PMID: 31422865.

Lentiviral transduction of iPSCs with sgRNAs and sgRNA libraries.pdf

MATERIALS

NAME	CATALOG #	VENDOR
DMEM, high glucose	11965092	Thermo Fisher Scientific
Opti-MEM™ I Reduced Serum Medium	31985070	Thermo Fisher Scientific
TransIT®-Lenti Transfection Reagent	MIR 6600	Mirus Bio
Lentivirus Precipitation Solution	VC125	ALSTEM Cell Advancements

MATERIALS TEXT

12 ml Luer Lock Syringe
Syringe
NORM-JECT ® 4100.X00V0

Filter, 0,45 µm
Sterile Syringe Filter
Millex SLHV033RB

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Day 0: Seeding

1 18 – 24 hours before transfection, seed 293T cells into a 6 well plate or other format with a density that will make the cells **80 – 95 %** confluent on the day of transfection. Refer to a seeding chart if necessary to seed appropriate density.

2 

Incubate overnight.

Day 1: Transfection

3 Change 293T media with fresh DMEM.

4 Warm *Trans*IT-Lenti Reagent to **Room temperature** .

5 Vortex gently before using.

6 Gather Opti-Mem, DNA, and packaging mix and refer the table below for the recommended amount of reagents to add based on the format of 293Ts seeded. **Amounts refer to each well of a plate.**

Typically for individual sgRNAs, 2 wells of a 6 well plate per sgRNA will produce enough Lentivirus particles.

For sgRNA libraries (containing up to 50,000 elements), a 15 cm dish can be used. This can be scaled down for smaller libraries.

Culture vessel	48-well plate	24-well plate	12-well plate	6-well plate	10-cm dish	T75 flask	15-cm dish
Surface area	1.0 cm^2	1.9cm^2	3.8 cm^2	9.6 cm^2	59 cm^2	75 cm^2	145cm^2
Complete growth medium	263 µl	0.5 ml	1.0 ml	2.0 ml	10 ml	15 ml	30 ml
Opti-Mem serum-free medium	26 µl	50 µl	100 µl	200 µl	1.0 ml	1.5 ml	3.0 ml
Transfer DNA (1 µg/µl stock)	0.13 µl	0.25 µl	0.5 µl	1.0 µl	5 µl	7.5 µl	15 µl
Packaging DNA Premix (1 µg/µl stock)	0.13 µl	0.25 µl	0.5 µl	1.0 µl	5 µl	7.5 µl	15 µl
TransIT-Lenti Reagent	0.78 µl	1.5 µl	3 µl	6 µl	30 µl	45 µl	90 µl

7 Add Opti-MEM into a sterile tube.

8 In another tube, mix Packaging DNA Premix and DNA.

9 Add the DNA mix to the Opti-MEM and mix gently.

10 Add *Trans*IT-Lenti Reagent to the mixture and mix gently.

11 Incubate for **00:10:00** for transfection complexes to form.

12 Add all of the *Trans*IT-Lenti:DNA complex mixture to the 293Ts dropwise and gently swirl to mix.

13 Incubate for 2 days. If a fluorescent marker is included in your DNA, you can check if cells are making virus by checking fluorescence after 24 hours.

Day 3: Harvest

14 With a 12 ml syringe, take up the media/supernatant of the cells.

15 Put a 0.45 µm filter on the syringe and filter the supernatant into a fresh 15 ml conical tube.

Change the filter if it becomes hard to push. Do not push too hard that bubbles are coming out.

16 Add 1:4 ratio of cold viral precipitation solution (e.g. 0.25 mL viral precipitation solution for 1 mL of viral supernatant).

17 Mix well by pipetting up and down 10x.

18 Incubate the viral supernatant at **4 °C** for at least **04:00:00** and up to 3 days but no more than 3 days.

19 Cool down the centrifuge to 4 °C .

20 

Spin down viral supernatant for 00:30:00 at 1500 x g .

21 The pellet will contain the virus.
Resuspend the pellet with 1 ml of your media of choice.

22 Virus can be aliquoted and stored at -80 °C for long term or 4 °C for short term (a few days).

Flash freezing the virus particles in liquid nitrogen may increase the retention of their potency.

Transduction with virus

23 Seed iPSC cells so that they will reach **50 %** confluency the next day.

24 Add virus to cells. The amount to add depends on how concentrated the virus is (adding ¼ or ½ of the total produced virus to cells is generally sufficient, see below for typical infection amounts).

step case

sgRNA library (15 cm dish)

A library prepared from a 15 cm dish typically infects 10 million iPSCs in one matrigel-coated T175 flask using 50 % of the produced virus.

25 Check next day for fluorescence by microscopy and the next time they are passaged by flow cytometry to check transduction efficiency.

26 For sgRNA constructs including puromycin resistance, add 0.8 ug/ml puromycin to select for cells with the sgRNA until they are at least **80 %** confluent (typically within 2 passages).

step case

Individual sgRNAs (2 wells of a 6-well plate)

Individual sgRNAs prepared from 2 wells of a 6-well plate typically infect 0.5 million iPSCs in one matrigel-coated well of a 6-well plate using 25 % of the produced virus.

25 Check next day for fluorescence by microscopy, and the next time they are passaged by flow cytometry, to check transduction efficiency.

26 For sgRNA constructs including puromycin resistance, add 0.8 ug/ml puromycin to select for cells with the sgRNA until they are at least **80 %** confluent (typically within 2 passages).