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LENTIVIRAL TITRATION FOR HUMAN PLURIPOTENT STEM CELLS

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Protocol status: In development
We are still developing and optimizing this protocol

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ABSTRACT

We have developed a protocol for lentiviral titration of human pluripotent stem cells (hPSCs), including induced pluripotent stem cells (iPSCs) or human embryonic stem cells (hESCs). Concentrated lentiviral supernatants are added at various dilutions to adherent hPSCs in 48-well plates. Subsequent centrifugation, known as spinfection, ensures high efficiency in transduction. Transduction efficiency is quantified by determining the percentage of cells expressing Blue Fluorescent Protein (BFP) using Fluorescence Activated Cell Sorting (FACS).

ATTACHMENTS

LENTIVIRAL TITRATION FOR HUMAN PLURIPOTENT STEM CELLS_.docx

PROTOCOL integer ID: 95918

Keywords: ASAPCRN, Lentiviral Titration, Human Pluripotent Stem Cells , CRISPRi

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MATERIALS

	A	B	C
	MATERIAL	COMPANY	CATALOG
	48 well TC treated plate	Falcon	353078
	15ml polypropylene centrifuge tubes	Falcon	352096
	5ml serological pipettes	Corning	4487
	10ml serological pipettes	Corning	4488
	DNA Low-bind tubes 1.5ml	Eppendorf	022431021
	P1000 tip	Neptune	BT1250
	FBS	Bovogen	2008A
	Dulbecco Phosphate Buffer Saline (DPBS)	Thermo Fisher Scientific	14190-250
	E8 Flex media Kit	Thermo Fisher Scientific	A2858501
	RevitaCell Supplement(100x)	Thermo Fisher Scientific	A2644501
	Accutase	StemCell Technologies	7922
	Vitronectin-N (VTN-N)	Thermo Fisher Scientific	A14700

REAGENT

	A	B
	FACS Buffer (PBS +2% FBS)	
	REAGENT	VOLUME IN mL
	PBS	49
	FBS	1

Day 0: Coating wells with VTN-N and seeding hPSCs

1 Coat 100 ul per well in a 48-well plate.

2 Incubate the plate at room temperature for an hour and the plate is ready to be used.

3 Seed 3x104 cells/cm2 in a 48 well plate with E8 flex media and RevitaCell after dissociating the cells with accutase.

4 Incubate the cells overnight at 37°C with 5% CO2 and 20.9% O2.

Day 1: Titration of hPSCs with Lentiviral CRISPRi library supernatant

5 Prepare 15 ml tubes with E8 flex media and concentrated lentiviral supernatants in serial dilutions in the 48 well plate in the following manner.

Note

Make sure to mix well by gentle pipetting. Change tips after making up each dilution. Titration was done in triplicates.

	A	B	C	D	E	F	G	H	I	J	K
	DILUTION										
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
	Media(ul)	600	600	600	600	600	600	600	600	600	600
	Viral supernatant(ul)	600	600 of 1/2	600 of 1/4	600 of 1/8	600 of 1/16	600 of 1/32	600 of 1/64	600 of 1/128	600 of 1/256	600 of 1/512

Table: 1 Serial dilution of concentrated lentiviral supernatant to determine lentiviral titer in TU/mL

6 Aspirate the spent media with RevitaCell.

7 Add 200 ul /well for each viral dilution with the cells.

8 Incubate the cells at 37ºC with 5% CO2 and 20.9% O2 for 16-18 hours.

Day 2: Replace the media

9 Aspirate the viral supernatant media gently and immediately add maturation media.

10 Return the plate back to the incubator.

Day 4: FACs Analysis

11 Aspirate the spent media.

12 Wash the cells 10 times with DPBS to remove the viral particles from the lentivirus transduced hPSCs.

Note

Be very gentle while doing the washes as the cells tend to lift off during the wash step.

13 Add 100 ul accutase and incubate the cells for 10 mins in the incubator.

14 Note: Ideally the hPSCs should dissociate as single cells.

15 Neutralize the accutase with E8 flex media and collect the cells into 1.5ml eppendorf tubes.

> **Note**
>
> Use a P1000 tip to pipette the cells up and down to break them into single cell suspension.

16 Centrifuge the cells at 300 g for 4 minutes.

17 Aspirate the spent media gently without disturbing the pellet.

18 Resuspend the cells in 300 ul of FACs buffer.

19 Transfer the cells with the FACs buffer into FACs tubes.

20 Analyze the cells through flow cytometry to determine BFP positive cells.

21 The Multiplicity of Infection (MOI) for CRISPRi screen was quantified as the 0.1-0.1 or 10-30% of BFP-positive cells to ensure one gRNA enters one cell.

Calculating the TU/ml

22 **Method 1: Calculating using dilution Factor**

- T= (NxFxD)/Vt

Where
T= Titer, (TU/mL)
N= Number of cells transduced
F= Fraction of cells with fluorescence
D= Dilution Factor
Vt= Transduction volume in mL

Method 2: Calculating using volume of virus

- T= NxFxVv

Where
T= Titer, (TU/mL)
N= Number of cells transduced
F= Fraction of cells with fluorescence
Vv= Virus volume

Detailed calculation for lentiviral titration for the virus can be found in the following link:
https://www.addgene.org/protocols/fluorescence-titering-assay/

Calculating Virus volume for required MOI

23 For Perturb seq, to restrict the viral integration in such a way that one virus infects one cell, we keep the MOI between 0.1-0.3
Calculating the virus volume, for MOI (0.1-0.3)

MOI=(T x Vv)/N

Where
T= Titer, (TU/mL)
N= Number of cells transduced
Vv= Virus volume

Detailed protocol for calculating MOI can be found in the following link:
https://info.abmgood.com/multiplicity-of-infection-moi