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LENTIVIRAL TRANSDUCTION OF 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 transduction of human pluripotent stem cells (hPSCs), including induced pluripotent stem cells (iPSCs) or human embryonic stem cells (hESCs). In this protocol, concentrated lentiviral supernatant with a Multiplicity Of Infection (MOI) ranging from 0.1 to 0.3 (equivalent to 10% to 30% Blue Fluorescent Protein (BFP) positive cells) is combined with E8 Flex media and added to adherent H9 CRISPRi dCAS9 cells in 48-well plates. Subsequent centrifugation (spinfection) is employed to ensure efficient transduction. Transduction efficiency is assessed by determining the percentage of cells expressing Blue Fluorescent Protein (BFP) using Fluorescence Activated Cell Sorting (FACS).

ATTACHMENTS

LENTIVIRAL TRANSDUCTION OF HUMAN PLURIPOTENT STEM CELLS_.docx

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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
	DPBS	Thermo Fisher Scientific	14040133
	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 COMPOSITION

	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 Add 60 ul of VTN-N to 6 ml of DPBS.

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

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

4 Seed $3x10^4$ cells/cm^2 in a 48 well plate with E8 flex media and RevitaCell after dissociating the cells with accutase.

5 Incubate the cells overnight at 37ºC with 5% CO2 and 20.9% O2.

Day 1: Transduction of hPSCs with Lentiviral CRISPRi library supernatant

6 The H9 dCAS9 CRISPRi cells were transduced with the pooled CRISPRi library to harvest genomic DNA (gDNA), after calculating the exact volume of viral supernatant needed to achieve the desired MOI (multiplicity of infection).

7 Prepare 15 ml tubes with E8 flex media and concentrated lentiviral supernatants for a MOI of 0.1-0.3.

8 Add 200 ul/well of the virus media cocktail with the cells from step i.

9 To increase the transduction efficiency, centrifuge the plate at 300g for 20 minutes at 25ºC.

10 Incubate the cells at 37°C with 5% CO2 and 20.9% O2 for 16-18 hours.

Day 2: Replace media

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

12 Return the plate back to the incubator.

Day 3: FACs Sort

13 Aspirate the spent media.

14 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

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

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

17 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.

18 Centrifuge the cells at 300 g for 4 minutes.

19 Aspirate the spent media gently without disturbing the pellet.

20 Resuspend the cells in 300 ul of FACs buffer.

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

22 Sort the H9 CRISPRi cells to obtain 10- 30% BFP positive cells and collect the cells in E8 flex media.

23 Centrifuge the cells at 300 g for 4 mins.

24 Aspirate the spent media to obtain a cell pellet to be frozen or freshly used for DNA extraction.