LENTIVIRAL PRODUCTION FOR PCRISPRi DUAL GUIDE mDA NEURON LIBRARY

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

Created: Feb 27, 2024

ABSTRACT

This protocol outlines the production of lentiviral supernatant from a CRISPRi Library plasmid. The plasmid is transfected into HEK293T cells and used for CRISPRi perturbation in both human pluripotent stem cells (hPSCs) and hPSC-derived dopaminergic neural cells. To enhance the viral titer, the virus is concentrated using the Lenti-X concentrator from Takara-Bio following collection of the viral supernatant from HEK 293T cells.

ATTACHMENTS

LENTIVIRAL PRODUCTION FOR pCRISPRi dual-guide mDA Neuron Library.docx

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PROTOCOL integer ID: 95846

Keywords: ASAPCRN, Lentiviral Production, CRISPRi machinery, dopaminergic neuron differentiation, Perturb-Seq

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MATERIALS

	A	B	C
	MATERIAL	COMPANY	CATALOG
	T25 Flask	Corning	430639
	15ml polypropylene centrifuge tubes	Falcon	352096
	5ml serological pipettes	Corning	4487
	10ml serological pipettes	Corning	4488
	DNA Low-bind tubes 1.5ml	Eppendorf	022431021
	DMEM High glucose	ThermoFisher Scientific	11965092
	FBS	Bovogen	2008A
	Lipofectamine 3000	ThermoFisher Scientific	L3000015
	psPAX2	Addgene	12260
	pCAG-VSV-G	Addgene	35616
	Transfer Plasmid (Plasmid of interest) pCRISPRi dual-guide mDA Neuron Library	Prepared by Robert Weatherhitt Lab at Garvan	NA
	Opti-MEM I Reduced Serum Medium	ThermoFisher Scientific	31985062
	Lenti-X Concentrator	Takara-Bio	631231

1. **Reagent Composition for HEK293T culture media**

	A	B
	REAGENT	AMOUNT
	DMEM (1X)	450ml
	10% FBS	50 ml

1. Aliquot the media into 50 ml tubes and store it at 4C.
2. Do not use Pen-Strep in your media for transfection protocol.

Day 0: Seeding HEK 293T

1 Seed HEK 293T cells into T25 flasks in 5ml of HEK culture media at a cell seeding density of 1.5x10^6 cells/flask.

Day 1: Transfection OF CRISPRi plasmid pool

2 After 24 hours, check whether the HEK293T cells are at least 60% confluent and proceed with transfection and bring the Opti-MEM to room temperature.

3 In TUBE A, mix the following reagents. Make this volume of reagent per T25 flask.

	A	B
	TUBE A	AMOUNT IN ul
	Lipofectamine 3000	15
	Opti-MEM	235
	Total	250

4 In TUBE B, mix the following reagents. Make this volume of reagent per T25 flask.

	A	B
	TUBE B	AMOUNT
	psPAX2	4ug
	VSV-G	2ug
	pCRISPRi dual-guide mDA Neuron Library	4ug
	P3000	20ul
	Opti-MEM	X ul
	Total	250 ul

5 Transfer tube B contents to tube A, mix gently by pipetting and incubate for 10 mins at room temperature.

6 Replace the media in the T25 flask with fresh 5 ml of HEK culture media during incubation.

7 Add the whole mix (~500ul) gently, drop by drop, making sure to cover the entire area of the T25 flask.

8 Gently swirl the media in the flask to ensure the proper distribution of the packaging mixture, and incubate the flask in the incubator for 24 hours.

Day 2: Media change

9 After 24 hours, aspirate the media and gently add 5 ml of fresh HEK culture media

> **Note**
>
> Caution: HEK293T cells tend to lift off easily, so be extremely careful while changing the media and add the media on the opposite wall of the flask where the cells are not attached.

10 Return the flask to the incubator.

Day 3: Harvesting the supernatant and concentrating the virus

11 Harvest the lentivirus-containing supernatant into 15 ml tubes.

12 Centrifuge the supernatant at 500 g for 10 mins.

13 Transfer the clarified supernatant (5 ml) into a sterile 15 ml falcon tube.

14 Depending on the volume of the supernatant, combine 1 volume of Lenti-X Concentrator with 3 volumes of clarified supernatant.

	A	B
	VOLUME OF CLARIFIED SUPERNATANT	VOLUME OF CONCENTRATOR
	x = 5 ml	x/3 = 1.6 ml

15 Mix by gentle inversion.

16 Incubate mixture overnight at 4C.

Day 4: Centrifuge and Resuspension of pellet

17 Centrifuge the concentrated virus at 1500g for 45 mins at 4C.

Note

After the centrifugation, an off-white pellet will be visible.

18 Carefully remove the supernatant without disturbing the pellet. The residual supernatant can be removed with a pipette tip or by brief centrifugation at 1500g.

19 Gently resuspend the pellet in 1/10th of the original volume (500ul) with DMEM.

Note

The pellet will be sticky at first but will suspend quickly.

20 Detailed protocol for concentration for the virus can be found in this link: Lenti-X™ Concentrator Protocol-at-a-Glance