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Transduction of Microglia-like cells (iMG) with lentivirus

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We use this protocol and it's working

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Abstract

iPSC-derived microglia are an important tool for studying the role of microglia and neuro-immune alterations during development, adulthood, and aging, both in health and disease. However, microglia are resistant to DNA delivery, so following this protocol, transduction with lentiviruses is facilitated by the SIV-encoded protein Vpx, which degrades SAMHD1, a restriction factor that prevents reverse transcription of lentiviral RNA. Co-delivery of Vpx packaged in virus-like particles (VLPs) leads to ~89% transduction in iMGs, compared with ~4% of iMGLs with lentivirus alone (Dolan, Therrien et al 2023). This protocol is used in combination with the iPSC-derioved microglia protocol (10.17504/protocols.io.ewov11rw2vr2/v1)

Protocol materials

- TransIT®-LT1 Transfection Reagent **Mirus Bio Catalog #**MIR 2304
- Opti-MEM **Life Technologies Catalog #**31985070
- Lenti-X Concentrator **Takara Bio Inc. Catalog #**631232
- DMEM-F12 medium **Gibco - Thermo Fisher Scientific Catalog #**31331093

Before starting

1 This protocol is designed for transduction of microglia at Day 35 of differentiation. Because of this stress, transduced cells need stabilization in the maturation process; therefore, it is recommended to perform desired experiments at final time-point Day 42 instead of Day 40.

	A	B
	Vpx (plasmid #132928)	Addgene; https://www.addgene.org/132928/
	VSV-G (plasmid #8454)	Addgene, https://www.addgene.org/8454/
	psPAX2 (plasmid #12260)	Addgene; https://www.addgene.org/12260/

Plasmids needed

Procedure for lentivirus production in HEK293T cells:Untitled section

5d 13h 20m

2 Day 1: Seed 6 × 10^5 HEK293T cells/well in 6-well plates in DMEM with 10% FBS without antibiotics.

> Note
>
> Number of cells at plating might need to be adjusted depending on cell growth

3 Day 2: Transfection

30m

Follow the **TransIT® -LT1 transfection protocol** for HEK293T cells

- Warm the TransIT-293 reagent at room temperature and vortex gently before use.
- Place 250 µl of Opti-MEM **Life Technologies Catalog #**31985070 per well in a sterile tube.

- Add 3 µg of plasmid DNA per well to the respective tube (see tables below for details).
- Pipet gently to mix all the components completely.
- Add 8 µL of TransIT®-LT1 Transfection Reagent **Mirus Bio Catalog #**MIR 2304 Reagent to the diluted DNA mixture and pipet gently again.
- Incubate at room temperature for 00:30:00 .
- Distribute the complexes to cells in complete growth medium drop-wise, rock the plate and transfer in normoxic incubator (20% O2, 5% CO2, 37 °C).

	A	B
	Component	*µg of plasmid DNA/well*
	Vpx (pSIV3) plasmid	2.6
	VSV-G plasmid	0.4

For producing viral-like particles loaded with Vpx protein in 6-well plate (for 1 well containing 2 mL media)

	A	B
	Component	*µg of plasmid DNA/well*
	Transfer plasmid	1.6
	VSV-G plasmid	0.4
	psPAX2 plasmid	1

For producing target gene lentivirus in 6-well plate (for 1 well containing 2 mL media)

Note

In order to have an efficient transfection, it is important to mix well the reagents both in the tube and in the well.

4 Day 3: 18:00:00 after transfection, change media to 2 mL Opti-MEM **Life Technologies Catalog #**31985070 18h

5 Day 4: 24:00:00 to 72:00:00 after transfection collect the virus and concentrate 4d

5.1 Collect virus 5m

- Harvest viral supernatant and collect in a 15 ml conical tube.
- Centrifuge viral supernatant at 300 x g, 00:05:00 to remove any HEK293T cell in the supernatant. Alternatively, filter through a 0.45 µm filter.

Note

Determine the best incubation time post-transfection for each virus type. The optimal incubation time is generally 24–72 hours, but will vary depending on the goal of the experiment, nature of the plasmid used, and cell doubling time.

5.2 Concentrate the virus, if necessary 18h

- Follow the **Lenti-X™ Concentrator Protocol** for viral concentration
- Transfer supernatant to a new tube. Add 1/3 of the volume of Lenti-X Concentrator **Takara Bio Inc. Catalog #**631232 to the viral supernatant and incubate at least 18:00:00 4 °C

Note

Concentration of the lentivirus is only required for viruses with large viral genomes. Viruses with small genomes are produced at higher titers and may not need to be concentrated.
Vpx particles do not need to be concentrated.

6 Day 5: Finalize virus concentration 45m

- Centrifuge the tubes at 1500 x g, 4°C, 00:45:00 to spin down the lentivirus with Lenti X concentrator.
- Resuspend the virus in 1/10th of the original volume with iMG medium or DMEM-F12 medium **Gibco - Thermo Fisher Scientific Catalog #**31331093 .
- VPX particles and lentiviruses can be stored frozen at -80 °C .

Note

Avoid cycles of thawing and refreezing, so aliquot desired amount of Vpx particles and viruses and only thaw prior using it.

6.1 Lentivirus titration:
For efficient microglia transduction, the amount of virus is 53 ng of P24/10,000 cells. In order to determine the virus titer, follow the **Lenti-X p24 Rapid Titer Kit User Manual.**

Note

Vpx cannot be measured by p24 lentivirus assay. In our hands, usually, 10 µl of Vpx/10,000 cells allows robust infection of microglia.

iMG Transduction

1d 14h

7 Transduction is done on iMG at Day 35 of differentiation.

- If using a multi-well plate, adjust volume across the wells (96-well plate to 50ul) so it is equal across the plate. Keep the conditioned media to use on Day 36.
- Based on the virus titer obtained, calculate the volume needed to have 53 ng P24/10,000 cells + 10ul of Vpx.
- Combine Vpx particle and lentivirus , gently mixing doing up and down.
- Add appropriate volume to each well.

8 Day 36 of differentiation: 18:00:00 - 20:00:00 after infection 1d 14h

- Remove media in each well.
- Add 50 µL of the conditioned medium collected on Day 35 and 50 µL of Mg basal differentiation medium supplemented with Day 30 growth factors (refer to the tables below).

9 Day 38, 40: Feed iMG

- Add 50ul of fresh Mg basal differentiation medium supplemented with Day 30 growth factors

10 Day 42: Assess cells

Note

All experiments reported in Dolan, Therrien et al. (2023) were performed on day 42, 7 days after transduction. This time point allows iMG to recover after transduction and show normal morphology and transcriptional profile. Timing can vary depending on the experiments.