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Generation of stable cell lines via retroviral or lentiviral transduction

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1 Works for me

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ABSTRACT

The ability to stably express a protein of interest in cells is critical to study its function. Here, we describe a protocol to generate stable cell lines using a retrovirus system that can be used for a variety of mouse and human cell lines. Our protocol includes the production of retroviruses encoding the transgene of interest in HEK293FT and the subsequent transduction of the cell line that is intended to stably express the protein of interest. The same protocol can also be used to generate stable cell lines using a lentivirus system. It should be noted that when using this method, the transgene of interest will be randomly integrated into the cell genome.

ATTACHMENTS

[ius5bgdrf.docx](#)

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KEYWORDS

HEK293FT , Lentiviral transduction, Stable cell lines

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References:

Bozena Szulc, Paulina Sosicka, Dorota Maszczak-Seneczko, Edyta Skurska, Auhén Shauchuk, Teresa Olczak, Hudson H. Freeze and Mariusz Olczak (2020). Biosynthesis of GlcNAc-rich N- and O-glycans in the Golgi apparatus does not require the nucleotide sugar transporter SLC35A3. *Journal of Biological Chemistry* 295 (48), 16445-63. ISSN 0021-9258. <https://doi.org/10.1074/jbc.RA119.012362>.

MATERIALS TEXT

Cell lines

- HEK293FT for virus packaging and propagation (Invitrogen™. Catalog# R70007).
- HEK293 cells.

Note: This protocol can be used to stably express a protein of interest in a variety of mouse and human cell lines.

Plasmids:

Retrovirus plasmid construct (pBABED vector) or Lentivirus plasmid construct (pLVX or pLJC5 vector) with the gene encoding the protein of interest inserted. As an example for this protocol, we used pBABED-SLC35A2-2xFLAG (DU72094 available at MRCPPU Reagents and Services <https://mrccpureagents.dundee.ac.uk>). The plasmid should contain an antibiotic selection cassette for the selection of successfully transduced cells (hygromycin, in this case). pCMV VSV-G. Retrovirus envelope plasmid. (Cell Biolabs. Catlaog# RV-110).

Note: Use Lenti-X HTX Packaging system (Clontech. Catalog# 631247) for Lentivirus based construct.

- pCMV Gag/Pol. Retrovirus Gag/Pol plasmid. (Cell Biolabs. Catalog# RV-111).

Note: Use Lenti-X HTX Packaging system (Clontech. Catalog# 631247) for Lentivirus based construct.

Note: We purify plasmids using a QIAGEN HiSpeed® Plasmid Maxi kit following the manufacturer's protocols and ensure sterile reagents are used and mixtures prepared in tissue culture hood to avoid contamination.

Media and Reagents:**Growth Media (for HEK293FT and HEK293 cells):**

- [DMEM, high glucose, no glutamine](#) **Thermo**
- **Fisher Catalog #11960085**
- [Fetal Bovine Serum](#) **Sigma**
- 10% (v/v) **Aldrich Catalog #F7524** (Batch# BCBW6817)
- [L-Glutamine \(200mM\)](#) **Thermo Fisher**
- 1% (v/v) **Scientific Catalog #25030024**
- [Penicillin-Streptomycin](#) **Gibco - Thermo**
- 1% (v/v) **Fisher Catalog #15140122**

Selection Media (for HEK293 cells after retroviral/lentiviral transduction):

Growth Media with 500 ug/ml Hygromycin (InvivoGen. Catalog# ant-hg-5).

Transfection media (for HEK293FT cells):

☒ Opti-MEM™ I Reduced Serum Medium Gibco - Thermo

Fischer Catalog #31985062

☒ DPBS no calcium no magnesium Gibco - Thermo

Fischer Catalog #14190169

☒ PEI MAX® - Transfection Grade Linear Polyethylenimine Hydrochloride (MW

40000) Polysciences Catalog #24765-1

☒ Polybrene Infection/Transfection reagent Merck Millipore

Sigma Catalog #TR-1003-G

Equipment:

Incubator with FPI-sensor system and display controller MB1 (BINDER GmbH. Model: CB150. Power Output: 1.40kW, 230V, 6.1 Amp). This incubator has CO2 and O2 control.

Consumables:

▪ 10 cm

☒ Nunc™ Cell Culture/Petri Dishes, 56.7cm2, Nunclon Delta treated, lid, vent Thermo

Fisher Catalog #172931

☒ 15 ml centrifuge tubes greiner bio-

▪ one Catalog #188271

▪ Standard 1 mL

☒ PIPETTE TIPS 100- 1000 µL BLUE SUITABLE FOR EPPENDORF STERILE 60 PIECES PER RACK greiner bio-

one Catalog #686271

and 200 µL

☒ PIPETTE TIP 10 - 100 µL SUITABLE FOR EPPENDORF 96 PIECES / ST RACK greiner bio-

one Catalog #685261

respectively.

- Syringe filter (0.45 µm . Sartorius, Item # ST16537-Q)
- Syringes (10 mL) (Medicina. REF# IVS10. LOT# 19111004)

Packaging SLC35A2-2xFLAG plasmid into a Retrovirus system

1

Note 1: The same protocol can be used to package the gene of interest into a Lentivirus system.

Note 2: All the following steps should be performed under sterile conditions in a CATEGORY 2 biological safety cabinet.

Grow HEK293FT cells to 50-60% confluency in Growth media in a 10 cm Petri Dish.



2 Prepare a transfection mix in a sterile 1.5 ml Eppendorf tube, containing:

- 3.8 µg pGag/Pol plasmid
- 2.2 µg pVSVG plasmid
- 6 µg pBABE-SLC35A2-2xFLAG plasmid

-  **300 µL** OptiMem

3 

Prepare PEI mixture in a sterile 1.5 ml Eppendorf tube, containing:

-  **20 µL** ^(M)**1 mg/mL** PEI Max 40K dissolved in distilled water.
-  **300 µL** OptiMem.

4 

5m

Incubate each mixture (from steps 2 and 3) separately for ~  **00:05:00** at  **Room temperature** , then combine.

5  

30m

Mix by vortexing and incubate at  **Room temperature** for  **00:30:00** .

6 

Add the mixture dropwise to the cells from step 2 using a P1000 sterile pipette.




7 


1d


Incubate cells at  **37 °C** for  **24:00:00** .

8 

1d

Replace media with  **10 mL** of fresh Growth Media and incubate cells for a further  **24:00:00** at  **37 °C** .



9 Collect the culture media from step 8 (that now contains the retroviruses) and pass through a  **0.45 µm** syringe filter.

Note: The retrovirus infection media from step 9 can be used immediately (as described below) or can be stored at  **-80 °C** for subsequent use.

Retrovirus infection (Transduction) and Selection of cells stably expressing SLC35A2-2xFLAG


2w 2d

10  

Mix  **5 mL** of retrovirus infection media from step 9 with  **5 mL** of fresh Growth Media in a sterile 15 ml Eppendorf tube.

11 

Add Polybrene (^(M)**10 mg/mL** stock dissolved in MilliQ water, sterile filtered) to a final concentration of ^(M)**10 µg/ml** .

12 Gently add to a  **10 cm** plate of HEK293 cells (or any cell line of interest) at ~60% confluency.

13 


1d

Incubate at 37°C for 24:00:00.

14 

1d

Change media to Growth Media and incubate for another 24:00:00 at 37°C .

15 To select cells stably expressing SLC35A2-2xFLAG, replace media with  10 mL of freshly prepared Selection Media.

Note 1: Cells that have not been infected should be included as a control for the efficiency of the selection agent.

Note 2: Cells that have not been successfully transduced should start dying 24 hr after the addition of selection media.

16 Change Selection Media every 24 hr for 72:00:00 - 120:00:00 to remove dead cells. After 5 days, cells stably expressing SLC35A2-2xFLAG should have reached 100% confluency. ^{1w 1d}

17 Cells can now be passaged and plated for experiments, or frozen down for long term storage in liquid nitrogen (Freezing media: growth media added with 10% v/v DMSO).

Note: Cells should be grown in Selection Media only.

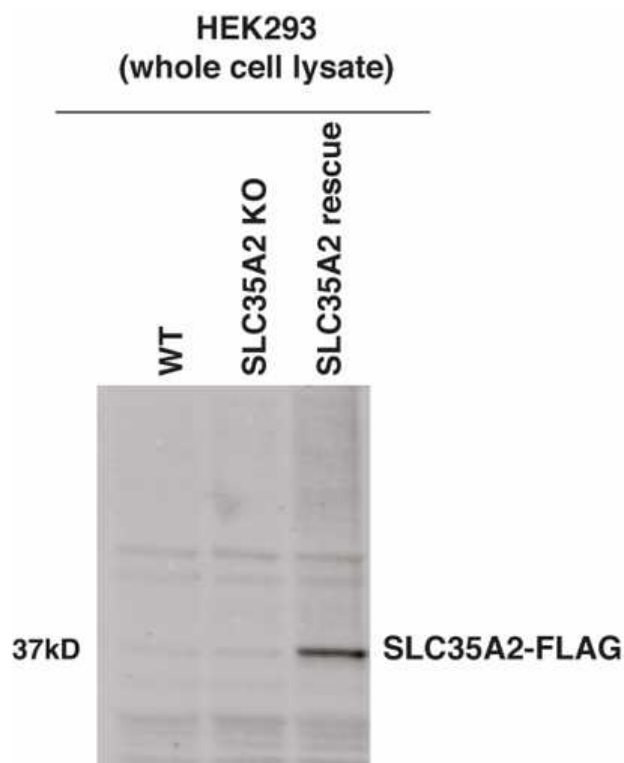


Figure 1: Verification by immunoblotting analysis of the expression of SLC35A2-FLAG that was re-expressed in SLC35A2 knock-out (KO) HEK293 cells using the method described here. Whole cell lysate was prepared from HEK293 cells that are SLC35A2 wildtype (WT), SLC35A2 knock-out (SLC35A2 KO) and SLC35A2 rescue. The lysate was immunoblotted with anti-FLAG antibody (Sigma, Catalog# F1804, RRID:AB262044) to detect the stable expression of SLC35A2-FLAG reintroduced by retrovirus transduction.