RESOLUTE

Lab protocols to study Solute Carrier Transporters

Generation of Knockout-Overexpression cell lines

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Protocol description

Protocol for the generation of knockout (KO) overexpression (OE) cell line pools via lentiviral transduction.

Materials

Biological materials:	▪ Envelope plasmid pMD2.G (Addgene, 12259) ▪ Packaging plasmid psPax2 (Addgene, 12260) ▪ Lentiviral plasmid expressing HA-Strep-tagged codon-optimized cDNA (modified from pCW57.1, Addgene #41393) ▪ HEK293T cells ▪ Knockout cell line to be transduced
Reagents:	▪ Lipofectamine 3000 (ThermoFisher, L3000001) ▪ Blasticidin (Invivogen, ant-bl-5b) ▪ Opti-MEM (ThermoFisher, 31985070) ▪ Polybrene (Sigma-Aldrich, H9268)
Equipment:	▪ Sterile tissue culture hood, general lab equipment

Reagents setup

5 mg/ml stock solution of Polybrene

Dissolve 50 mg of Polybrene in 10 ml of ddH20 and sterilize through a 0.22-µm filter. Make 500 µl aliquots of the solution and store at -20°C.

Procedure

- **Transfection - Day 1**

1. Seed HEK293T cells in 100 µl DMEM supplemented with FBS at a density of 15000 cells/well on a 96 well plate

- **Transfection - Day 2**

2. Check HEK293T cells (should be 50-70% confluent)
3. Prepare transfection reagents:
4. Lipofectamine MM (scale up according to planned transfections, add 10% extra)

	µl per reaction
Optimem	7.15
Lipofectamine 3000	0.35

Total volume	7.5

5. Let the Lipofectamine MM incubate for 10 min at RT
6. DNA MasterMix (MM) (scale up according to planned transfections, add 10% extra)

	µl per reaction
Optimem	4.8
pMD2.G (100 ng/ul)	0.3
psPax2 (100 ng/ul)	0.5
P3000 Reagent	0.3
Total volume	6.0

7. Pipette 6 µl of DNA MM per reaction into clean PCR strips or plates
8. Add 1.5 µl of overexpression plasmid (concentration: 50-100 ng/µl) to 6 µl of DNA MM
9. Mix 6 µl of Lipofectamine MM per reaction with each DNA MM
10. Incubate at RT for 5-15 minutes
11. Add 10 µl of mix to HEK293T cells (keep pipette tips in medium while dispensing but do not touch the bottom of the wells)

- **Transfection - Day 3**

12. Add 120 µl fresh medium to transfected HEK293T cells (pipette very slowly to avoid detachment of HEK293T cells)

- **Transfection - Day 4**

13. Transfer 200 µl of the virus-containing medium to a safe-lock tube and immediately freeze at -80°C

- **Transduction - Day 1**

1. Seed the cell line to be transduced at $1.5x10^4$ cells/well in a 96 well plate (Note: add one more well than needed as a negative control)

- **Transduction - Day 2**

2. Thaw the safe-lock tube with the virus-containing medium at RT

3. Change the medium of the cell line to 100 µl of medium with 10 µg/ml polybrene
4. Add 100 µl of thawed lentivirus to the cells

- **Transduction - Day 3**

5. If transduced cells are confluent: split transduced cells and transfer 200 µl to a 24 well plate
6. If not confluent: change medium on the cells

- **Transduction - Day 4**

7. Change medium to selection medium (10 µg/ml Blasticidin)
8. If cells are still on a 96 well plate, transfer to a 24 well plate and start Blasticidin selection the next day

- **Transduction - Day 5-12**

9. Monitor cells and split when reaching 80-100%. Keep under Blasticidin selection. Change medium every 3-4 days depending on confluency

- **Transduction - Day 13-15**

1. Observe negative control (untransduced cells), remove Blasticidin from transduced cells if all cells in neg. control are dead
2. Monitor cells and expand when reaching 80-100% confluency (after at least 10 days of selection)
3. Expand cells to 10 or 15 cm dishes (depending on how many freeze stocks are needed).

Additional notes

Data/reagent availability

https://re-solute.eu/resources/reagents

References

Please write to contact@re-solute.eu in case of questions or errors.