

Lentiviral Transduction

The MISSION™ TRC shRNA libraries are lentiviral based shRNA vector collections for use in gene knockdown studies. This protocol describes the use of MISSION TRC shRNA Lentiviral Particles and provides a system for long-term silencing and phenotypic observation. The following protocol has been developed for high-content screening in 96-well plates with stable selection through puromycin.

Materials

Solutions

Hexadimethrine Bromide (2 mg/ml stock solution) Product No. [H9268](#)
Puromycin (10 mg/ml stock solution) Product No. [P9620](#)

shRNA

- MISSION TRC shRNA Lentiviral Particles Target Set (2-15 μ l per shRNA construct)
- Negative Control shRNA Lentiviral Particles
 - pLKO.1-puro Control (Product No. [SHC001V](#))
 - shRNA Non-Target Control (Product No. [SHC002V](#))
- Positive Control shRNA Lentiviral Particles
 - JAK1 Control Lentiviral Particles (*Individual Construct*) TRCN0000003102

Media

Minimum essential medium containing 10% fetal calf serum or optimized growth media specific for cell line

Special Equipment

96-well cell culture treated plates

Cells

Exponentially growing cultures of mammalian cells
Cells should be no more than 70-80% confluent before transduction

Detection of Knockdown

- Western Blot
 - Antibody against protein of interest (www.sigma-aldrich.com/antibodyexplorer) Anti-JAK1 antibody (Product No. [J3774](#))

- qRT-PCR
Primers, probes, and PCR mix
- Phenotypic Assay
cell-based, enzymatic, or array based assays can be used

⚠ The lentiviral particles should be treated as Risk Group Level 2 (RGL-2) organisms. Follow all published RGL-2 guidelines for handling and waste decontamination. Pipettes and media should be decontaminated in 10% bleach for at least 20 minutes before disposal. Use extra caution when using lentiviral particles that express shRNA targeting genes involved in cell cycle control, such as tumor suppressors.

Method

Day 1. Add 1.6×10^4 cells in fresh media to the number of wells needed for each construct in a 96-well plate. Duplicate or triplicate wells for each lentiviral construct and control should be used. Incubate 18-20 hours at 37°C in a humidified incubator in an atmosphere of 5-7% CO₂.


ⓘ Growth rate of cells vary greatly. Adjust the number of cells plated to accommodate a confluency of 70% upon transduction. Also account for the length of time the cells will be growing before downstream analysis when determining the plating density.

Day 2. Remove media from wells. Add 110 µl media and Hexadimethrine bromide (final concentration 8 µg/ml) to each well. Gently swirl the plate to mix.


⚡ Hexadimethrine bromide enhances transduction of most cell types. Some cells, like primary neurons, are sensitive to hexadimethrine bromide. Do not add hexadimethrine bromide to these types of cells. If working with a cell type for the first time, a hexadimethrine control only well should be used to determine cell sensitivity.

Add 2-15 µl of lentiviral particles to appropriate wells. Gently swirl the plate to mix. Incubate 18-20 hours at 37°C in a humidified incubator in an atmosphere of 5-7% CO₂.


ⓘ When transducing a lentiviral construct into a cell line for the first time, a range of volume or MOI should be tested. 2, 5, 10, and 15 µl of lentiviral particles per 1.6×10^4 cells or MOIs of 0.5, 1, and 5 should be used to determine the optimal transduction efficiency and knockdown for each cell line. Transduction efficiency can be optimized using the pLKO.1-puro Control Transduction Particles ([SHC001V](#)).

 Cells may be incubated for as little as 4 hours before changing the media containing lentiviral particles. Overnight incubation may be avoided when toxicity of the lentiviral particles are a concern.

Day 3. Remove the media containing lentiviral particles from wells. Add fresh media to a volume of 120 μl to each well.


 For cell types that do not strongly adhere to the plate, 100 μl of media may be removed and replaced with 100 μl fresh media.

Day 4. Remove media from wells. Add fresh media containing puromycin.

 The appropriate concentration of puromycin for each cell type is different. If the concentration for the desired cell type is unknown, a titration experiment must be performed. Typically, 2-10 $\mu\text{g/ml}$ are sufficient to kill most untransduced mammalian cell types.

Day 5 and on. Replace media with fresh puromycin containing media every 3-4 days until resistant colonies can be identified.

Pick a minimum of 5 puromycin-resistant colonies and expand each clone to assay for knockdown of the target gene.

 Due to the random integration of the lentivirus into the host genome, varying levels of target gene knockdown may be seen with different puromycin resistant colonies. Testing a number of colonies will allow the optimal degree of knockdown to be determined.

A variety of phenotypic, enzymatic, or gene expression assays may be performed. The desired assay should be optimized prior to the high-content screen with both negative and positive controls.

Additional notes:

Multiplicity of Infection (MOI) is the number of transducing lentiviral particles per cell. It is highly recommended that for each new cell type to be transduced, a range of MOI be tested.

To calculate: (total number of cells per well) x (desired MOI) = total transducing units needed (TU)

(total TU needed) / (TU/ml reported on C of A) = total ml of lentiviral particles to add to each well

Puromycin titration (kill curve) should be performed when working with a new cell type.

1. Plate 1.6×10^4 cells into wells of a 96-well plate with 120 μ l fresh media.
2. The next day add 500 – 10,000 ng/ml of puromycin to selected wells.
3. Examine viability every 2 days.
4. Culture for 10 – 14 days. Replace the media containing puromycin every 3 days.
5. The minimum concentration of puromycin that causes complete cell death after 3-5 days should be used for that cell type.