

Infection of Target Cells with Lentivirus

Biohazard Concerns

Lentivirus is a modified HIV virus and although unable to replicate in a host, it must be handled with caution. When working with these viruses, work only in a BL2+ designated hood or viral vector room. All handling, storage and disposal of biohazard waste must be in accordance with Institute rules and regulations, OSHA, EPA and MWA.

Materials:

- 1.) 6-well CellBIND Tissue Culture Microplate (Fisher # 08-757-214)
- 2.) Puromycin (Fisher # ICN10055225)
- 3.) Polybrene (Hexadimethrine bromide – Sigma #H9268)
- 4.) Target Cell Line
- 5.) DMEM Media (ATCC #30-2002)
- 6.) FBS (ATCC #30-2020)
- 7.) PenStrep (ATCC #30-2300)
- 8.) 50ml Centrifuge Tubes (Fisher # 07-203-510)
- 9.) 15mL Centrifuge Tubes (Fisher # 05-538-53F)
- 10.) 1.5mL RNase Free Microfuge Tubes (Fisher # NC9445663)

Notes:

Every 6-well plate experiment should include:

- 1.) pLKO empty vector control well (in duplicate)
- 2.) pLKO vector with shRNA construct well (in duplicate)
- 3.) A control well with no infection (in duplicate)

Before Starting:

- 1.) Make DMEM media with 10% FBS and 1% PenStrep
- 2.) Prepare a Puromycin stock at 1ug/ul with sterile water. Filter and aliquot into 1.5mL microfuge tubes containing 500ul each, and store at -20C.
- 3.) Prepare a Polybrene stock at 4ug/ul with sterile water. Filter and aliquot into 1.5mL microfuge tubes containing 100ul each, and store at -20C.

Procedure:

Day Before Transduction:

- 1.) Split cell line to be infected at proper density in a 6-well tissue culture plate in 2ml of DMEM media containing 10% FBS and 1% Pen-Strep. Seed the proper number of plates for the number of experiments being run. Be sure to include the proper controls and replicates.
 - a.) SW620 cells should be seeded at 5×10^5 cells per well and infected at an MOI of 10.
 - b.) SW480 cells should also be seeded at 5×10^5 cells per well and infected at an MOI of 10.
- 2.) Incubate in a 37°C incubator at 5% CO₂ overnight undisturbed.

Note: This does not need to be done in a virus room at this point.

Day of Transduction:

- 1.) Remove concentrated, frozen lentivirus from -80°C freezer and thaw at room temperature in the virus room.
- 2.) While virus is thawing, warm DMEM media containing 10% FBS, 1% PenStrep.

Note: An infectious viral titer needs to be determined prior to transduction. Use this to calculate the amount of virus to add for the proper MOI.
- 3.) Once the virus is fully thawed, transfer the proper amount for infection to a fresh 1.5mL microfuge tube.
- 4.) Bring up the volume in the tubes to 1mL with OptiMEM media if necessary.
- 5.) Add 2ul of Polybrene (at a stock of 4ug/ul) to the 1mL of virus/media for a final concentration of 8ug/mL.
- 6.) Bring the target cells into the virus room and aspirate the media.
- 7.) Add the 1mL of media containing virus to the cells.

Note: You do not want to disturb or stress the cells. Be careful when adding the media.

Note: Be sure to mark which wells in the plate have received which shRNA.

- 8.) Swirl the plate gently to mix and cover the cells. Place the plate in the virus incubator at 37°C and 5% CO₂ overnight.

1 Day After Transduction:

- 1.) Remove the virus media and replace with normal DMEM media supplemented with 10% FBS and 1% PenStrep.
- 2.) Place plate back in the virus incubator and incubate at 37°C and 5% CO₂.

3 Days After Transduction:

- 1.) Remove the plates of target cells from the virus incubator and remove the media.
- 2.) Replace the media on the cells with 2ml of DMEM media supplemented with 10% FBS, 1% PenStrep and the proper concentration of Puromycin.

Note: The concentration of Puromycin is going to be different with each cell line as some are more sensitive to the drug than others. It is crucial to do a kill curve with each cell line and pick the lowest concentration of Puromycin that will completely kill non-selected cells within 7-10 days.

- a.) SW480 and SW620 cells need 15ug/mL Puromycin. To 25mL of DMEM media, add 375ul of Puromycin (stock concentration of 1ug/ul) for a final concentration of 15ug/mL.

One to Three Weeks Later:

- 1.) Watch the cells carefully over the next 1-3 weeks, passaging the cells and changing the media as necessary. Be sure each media change or passage contains Puromycin. Once the cells are recovering well from the Puromycin selection (depending on transduction efficiency, many cells may be killed off and it can take a while to grow the transduced cells), you may transfer them to a 10cm dish for further propagation.
- 2.) When the control plate/wells of cells (no infection) is completely dead, then the Puromycin has done its job and only infected cells remain.
- 3.) Let the infected cells grow to the desired density in the presence of Puromycin. It is generally a good idea to grow the cells under Puromycin for at least a few days after the control cells are dead, just to be safe.
- 4.) Harvest cells.

Note: shRNA transduction is stable! After 2-3 weeks of Puromycin selection, your cell line is considered stable for expressing the transduced shRNA. If desired, cells may be frozen for future use.

- 5.) Extract RNA from cells and run microarray and/or qRT-PCR experiments.