Protocol 3 – Lentivirus transduction into target cell lines

The following protocol using is based upon the Addgene protocol found at: http://www.addgene.org/tools/protocols/plko/#E

Lentiviral particles can efficiently infect a broad range of cell types, including both dividing and non-dividing cells. Addition of puromycin will allow you to select for cells that are stably expressing your shRNA of interest.

Materials

Hexadimethrine bromide (polybrene)*	Sigma-Aldrich: #H9268
Protamine Sulfate*	MP Biomedicals: #194729
Puromycin*	Sigma-Aldrich: #P8833
Target cells	Varies based on your experiment
Culture media for target cells	Varies based on your experiment
Materials for assay	Varies based on your experiment

Detailed protocols for preparing polybrene, protamine sulfate, and puromycin are located in the "Appendix".

Determining the Optimal Puromycin Concentration
Each cell line responds differently to puromycin selection. Addgene strongly recommends that you determine the optimal puromycin concentration for your cell line before initiating your experiment.

Day 1:

A. Plate target cells in ten 6 cm plates and grow at 37° C, 5% CO2 overnight.

Day 2:

B. The target cells should be approximately 80-90% confluent.

C. Dilute puromycin in the preferred culture media for your target cells. The final concentration of puromycin should be from 1-10 µg/mL in 1 µg/mL increments.

D. Label plates from 1-10 and add appropriate puromycin-containing media to cells.

Days 3+:

E. Examine cells each day and change to fresh puromycin-containing media every other day.

F. The minimum concentration of puromycin that results in complete cell death after 3-5 days is the concentration that should be used for selection in your experiments. (You may wish to repeat this titration with finer increments of puromycin to determine a more precise optimal puromycin concentration.

Protocol for Lentiviral Infection and Selection

Day 1:

A. Plate target cells and incubate at 37°C, 5% CO2 overnight.

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Day 2:

B. Target cells should be approximately 70% confluent. Change to fresh culture media containing 8 µg/mL polybrene.
   Polybrene increases the efficiency of viral infection. However, polybrene is toxic to some cell lines. In these cell lines, substitute protamine sulfate for polybrene.
C. Add lentiviral particle solution from step E. For a 6 cm target plate, add between 0.05-1 mL virus (add ≥0.5 mL for a high MOI, and ≤0.1 mL for a low MOI). Scale the amount of virus added depending on the size of your target plate.
   MOI (multiplicity of infection) refers to the number of infecting viral particles per cell. Addgene recommends that you test a range of MOIs to determine the optimal MOI for infection and gene silencing in your target cell line.
D. Incubate cells at 37°C, 5% CO2 overnight.

Day 3:

E. Change to fresh media 24 hours after infection.
   If viral toxicity is observed in your cell line, you may decrease the infection time to between 4 – 20 hours. Remove the virus-containing media and replace with fresh media. Do not add puromycin until at least 24 hours after infection to allow for sufficient expression of the puromycin resistance gene.
F. To select for infected cells, add puromycin to the media at the concentration determined in step E.2.
   Addgene recommends that you maintain one uninfected plate of cells in parallel. This plate will serve as a positive control for the puromycin selection.

Days 4+:

G. Change to fresh puromycin-containing media as needed every few days.
H. Assay infected cells. The following recommendations are guidelines for the number of days you should wait until harvesting your cells. However, you should optimize the time based on your cell line and assay:

Published Articles

Khvorova A et. al. 2003. Functional siRNAs and miRNAs exhibit strand bias. Cell 115:209-216. (PubMed)

Moffat J et. al. 2006. A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. Cell 124:1283-1298. (PubMed)

Naldini L et. al. 1996. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272:263-267. (PubMed)

Schwarz DS et. al. 2003. Asymmetry in the assembly of the RNAi enzyme complex. Cell 115:199-208. (PubMed)

Stewart SA et. al. 2003. Lentivirus-delivered stable gene silencing by RNAi in primary cells. RNA 9(4):493-501. (PubMed)

Zufferey R et. al. 1997. Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. Nat Biotechnol 15(9):871-5. (PubMed)

Zufferey R et. al. 1998. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. J Virol 72(12):9873-80. (PubMed)

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