Protocol to achieve stable Lentiviral shRNA-mediated target gene knockdown using Puromycin Selection

**Reminder pLKO.1 plasmid may be used for traditional, transient transfection if preferred but lentiviral infection is far superior.*

Lentiviral infection consists of the following steps:

Day 0 - Seed cells at appropriate density

- ~75% confluency if Puromycin selection is not utilized (growth rate dependent)
- A list of cell lines lentiviral infection has been achieved can be found at: http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/learning-center/lentivirus-cell-line.html

Day 1 - Add lentivirus to cells in growth media containing polybrene

- The use of polybrene will increase infection efficiency: 2-10µg/ml
- Note: polybrene can be toxic to some cells
  (*Sigma: H9268 Hexadimethrine bromide or P9620 Puromycin dihydrochloride*)
  *Using a minimal volume and increasing MOI can help achieve greater infectivity.
  *Additional reagents such as Sigma's ExpressMag bead system may help if infectivity is problematic.
- All lentiviral procedures should be carried out in accordance with biosafety requirements of the host institution.

Day 2 - Remove media and replace with fresh growth media

----------*If not using Puromycin stop here and allow 48-72 hr for gene knockdown*

Day 3 - Remove media and replace with media containing puromycin

- May need to wait an addition 24 hr for cells to robustly express Puromycin resistant gene (cell type dependent)
- Use (2-10µg/ml) Puromycin Dihydrochloride (*Sigma #P8833*), concentration will vary based on cell type; protamine sulfate can be used if polybrene is toxic to cells
- Puromycin death curve may be needed prior to lentiviral infection
- Allow 2-3 days for Puromycin selection (concentration and cell type dependent) and remove media containing dead cells

Day 5 – Remaining cell population should contain shRNA of interest

- Perform experiment <u>or</u> passage to continue stable batch cell line <u>or</u> select single cells to generate clonal cell lines, lentivirus is stably integrated.

Additional lentiviral protocols may be found at Sigma's website:
http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/learning-center/mission-protocols.html

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