University of Pittsburgh Cancer Institute
Lentiviral Core Facility
Recommended Protocol

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Recommended Protocol
HIV Lentivirus Transduction (attached cells)

Summary: HIV-based lentivirus particles are prepared by transfection of 4 plasmids into 293-FT cells [Dull T et al. "A third-generation lentivirus vector with a conditional packaging system" J Virol. 1998 Nov;72(11):8463-71 and Rubinson DA, Dillon CP, Kwiatkowski AV, et al. A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference" Nature Genetics 2003;33(3):401-6].

Virus particles are isolated from the cell culture supernatant and may be stored at -80˚C or used immediately for transduction of the target cell. As per current University of Pittsburgh IBC/rDNA protocols, this virus preparation may be used following BSL-2 bio-safety procedures.

Day 1:
Seed your target cells. Target cells should be 50% confluent on the day of infection/transduction after seeding. We recommend using a 60 mm dish or 6-well plates for the procedure below. Larger plates will require a larger volume.

Day 2: (some cells may need to be seeded for 48 hours before transduction)

1) Prepare the following dilutions on ice in sterile tubes:

Well #	Vol. DMEM (ml)	Vol. Virus (ml)	Polybrene (µl)
1 (negative control)	2.0	0	2
2 (+virus)	1.0	1.0	2

(The concentration of the stock solution of polybrene is 8 mg/ml, which is diluted down to 8 µg/ml when added to the virus. For example, 3 µls of the polybrene is added to 3 mls of the Lentivirus stock)

2) Aspirate media from each well.
3) Add Lentivirus dilution to each plate as in table.
4) Incubate at 32˚C/ 5% CO_2 overnight (16-18 hours).
(If you do not have a 32˚C incubator, 37˚C will suffice).

Day 3:
Replace Lentivirus transduction solution with fresh growth media. **Now incubate at 37˚C.**

Days 6-9 Analyze cells for GFP expression on microscope or protein expression by extract preparation and immunoblot. Alternatively, split cells and enrich for infected cells by selection (puromycin or GFP as appropriate for the vector system).

It may take at least 48-96 hrs for the transgene/shRNA to express.