University of Pittsburgh Cancer Institute
Lentiviral Core Facility
Recommended Protocol

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Recommended Protocol
HIV Lentivirus Transduction (suspension 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:

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	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)

*or you may use your normal cell growth media.

2) Carefully pellet 125,000 cells in a 15 ml tube (1500xg, 5 min). Remove media and suspend cells with the virus solution (2 ml) as indicated in the table. Transfer to a 60 mm dish.

3) Incubate at 32˚C/ 5% CO_2 overnight.

Day 2:
Carefully pellet 125,000 cells in a 15 ml tube (1500xg, 5 min). Remove media and suspend cells with fresh growth media, transfer to a 60 mm dish. **Now incubate at 37˚C.**

Days 5-8: Analyze cells for GFP expression on microscope or protein expression by extract preparation and immunoblot.

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