UMMS RNAi Core Facility Protocol: Infection using TRC or pGIPZ Viral Supernatant

The following protocol works well with most commonly used cancer cell lines. However, be aware that some cells, particularly primary cells, are extremely sensitive to Polybrene. It is therefore a good idea to pre-determine the most suitable concentration of Polybrene to be used in the infection.

Reagents

Cells of interest to be infected

Cell culture medium

Viral supernatant (purchased from the RNAi Core Facility, or produced from pGIPZ/TRC plasmid DNA [see accompanying protocol])

Polybrene, 1 µg/µl (Sigma)

Puromycin (various sources such as Sigma and Clonetech)
The ideal concentration of puromycin should be pre-determined based on the cell line.

Method

Day 1: Plate $1x10^5$ to $1.25x10^5$ cells per well in a 6-well plate.

Day 2: Aspirate the medium and infect cells with 250 to 500 µl viral supernatant. Add fresh medium to a final volume of 1 ml. Add 10 µl (or pre-determined optimized amount) of 1 µg/µl Polybrene.

Day 3: Re-feed the cells with fresh medium.

Day 4: Start puromycin selection or check the cells under a fluorescence microscope for GFP expression (if using the pGIPZ system).

After 5 to 7 days of puromycin selection, the cells are ready to use in assays.