Retroviral Infection

Incubate target cells with the conditioned producer cell supernatants in the presence of polybrene by a plate centrifugation method as described below. Infection of the target cells can begin as early as 48 hr after starting producer cell transfections, with the first batch of harvested fresh virus.

- 1. Twenty-four hr before beginning infection, seed wells in a 6 X 35 mm multiwell plate with sufficient target cells such that they are no more than 40% confluent the following day when infection begins. For our vascular smooth muscle cells, we typically inoculate each well with \sim 150,000 cells.
- 2. For infection, either thaw a frozen retroviral supernatant or filter a freshly collected aliquot. Exhange the target cell growth media by adding 2 ml of retroviral supernatant to each well. Mix in 20 μl of a 100X polybrene stock solution per well (100X stock = 0.8 mg/ml of hexadimethrine bromide; Sigma H-9268; dissolved in phosphate buffered saline, filter sterilized and stored at –20C in aliquots).
- 3. Incubate the plates at 32C for 15 min. During this time, equilibrate a refrigerated bench top centrifuge to 32C by running the rotor with buckets at 2500 rpm to generate frictional heat, setting the temperature dial to 40C. We use a Beckman model GS-6R centrifuge equipped with a GH 3.8 swing bucket rotor.
- 4. Place the cell culture plates in suitable microplate carrier buckets and centrifuge at 2500 rpm for 30 minutes at 32C. Most cells seem to tolerate this force, but may be damaged if less then 2 ml of media per well is used.
- 5. After the spin, aspirate the retroviral media and replace with fresh growth media. Incubate the cells at 37C until the next infection cycle.
- 6. Infections can be repeated successively at 12 hr intervals, timed to fresh retroviral supernatant harvesting. Some cells may require only one round of infection, whereas others may require three or more rounds to achieve the highest possible infection efficiency.

Typically, the cells can be used directly for experiments if desired 24 to 48 hours after the last infection cycle. However, they likely are undergoing cell stress due to the foreign RNA sequence, which can complicate many parameters. To avoid this, allow several days time for chromatin integration, after which the stress should subside. More often, they are expanded after infection by sub-culture, and treated appropriately for any selection strategies before performing experiments.