Retrovirus Infection Protocol

Day 1 Plate packaging cells with construct at 50-75% confluence. After plating replace medium with fresh media without antibiotics.

Plate cells to be transduced at 25-40% confluence.

Day 2 Collect media from packaging cells and pass through a 0.45µM syringe filter. Dilute, depending on the viral titer, the virus stock with medium. Add polybrene to a final concentration of 4ug/mL from 4mg/mL stock (kept at 4°C). For a 6-well or 60mm plate use 1mL, for a 100mm plate use 4mL, for a 150mm plate use 12mL. Remove medium from cells to be transduced and add the virus/polybrene solution.

Incubate at 37°C for 1-2 hours.

Add growth medium with polybrene 4ug/mL to bring up to usual plate volume and continue incubating at 37°C for 5 more hours or overnight.
Remove medium from cells and rinse once with fresh medium, then add fresh growth medium without polybrene.

Day 3 At this point the cells can be split into several plates, using a series of dilutions to ensure selected colonies that are well isolated, or passaged as usual.

Day 4 If selection is necessary change media to selection media containing the appropriate antibiotic; for example fibroblasts infected with PA317-neo use G418 at 400µg/ml; for fibroblasts infected with PA317-hygro use hygromycin B at 100 units/ml. Selection will usually take from 7-10 days for G418and 5-7 days for hygromycin B.

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