Production of retrovirus for transient or stable transduction of gene into cell line

Plasmids needed:

1. cDNA encoding protein of interest cloned into pQCXIP retroviral expression vector (S-AD-5E in database; frozen bacterial stocks stored at positions 2G & 3H in glycerol strain box AD, -80°C freezer).
   - If protein tag is required, pQCXIP vector with a V5 N-terminal tag (S-AD-7F) is stored in position 8I in glycerol strain box AD, -80°C freezer. In order to be in-frame, the 5’ ATG of the cloned gene has to be directly after or a multiple of 3 dNTPs downstream of the pQCXIP PacI site (TTAATTAA). The V5 tag can be detected with mouse anti-V5 (AbD serotec, MCA1360GA) stored position 5A RW box 1, fridge 1 (A-AC-6G).
2. pVSV-G – retroviral envelope expressing plasmid (S-AD-4E, frozen bacterial stocks stored at positions 9F & 2H in glycerol strain box AD, -80°C freezer)

1. Co-transfect GP2-293 cells (70% confluent, 10 cm plate) with 5 µg pQCXIP containing gene of interest and 3 µg pVSV-G (use total of 4.5 ml OPTI-MEM and 60 µl lipofectamine).
2. Following 5-6 hours incubation of cells with transfection mix at 37°C, top up the plate with 10 ml DMEM medium + 10% FCS (no pen/strep).
3. Incubate plate at 37°C for 48-72 hours and then harvest the medium containing the retrovirus.

Transduction of target cells

1. Grow target cell line in 24 or 6 well plate to 30-50% confluence.
2. Remove medium from target cells and add retrovirus-containing medium.
3. Add polybrene to 2 µg/ml.
4. Spin plate at 1,000 x g for 30 minutes.
5. Incubate cells overnight with retrovirus-containing medium unless cells are really particular about their medium requirements, in which case, remove retrovirus-containing medium and replace with cell medium.
6. Incubate for 1-2 days.
7. For stable transduction - Add puromycin to 2-5 µg/ml depending on how resistant the cell line is to the antibiotic (determine this by kill curve performed on un-transduced cells).
8. Incubate plate, occasionally replacing the puromycin containing medium until all un-transduced cells are dead, leaving only stably transduced cells that “should” be expressing the gene of interest.