

Retrovirus transient transfection of 293GPG packaging cells

1. Trypsinize 293GPG cells from a confluent 10 cm dish and quench in 8 ml 293GPG media in a 50 ml conical tube. Each 10 cm plate will yield three 6 cm dishes for transfection. Spin cells for 5 min at RT to remove trypsin, and resuspend pellet in 293GPG media(4ml/6 cm dish to be plated). Plate cells the evening prior to transfection. On the day of transfection, the cells should be 80-90% confluent.

Note: to avoid clumping of cells after plating, vigorously pipette cells with 2 ml pipette against the dish 2-3X. Do not overtrypsinize as this will damage cells and also cause clumping.

2. Prepare DNA for transfection as follows:
In a 6ml snap-cap tube (Falcon 2063), mix 4ug DNA (CsCl double-banded or Qiagen prepared) and 16 μ l Plus reagent and bring up to 150 μ l with DMEM (no serum etc). Mix well by flicking tube and incubate at RT for 15 min.
3. Add to the DNA/Plus mixture, 150 μ l of diluted lipofectamine (12 μ l lipofectamine/138 μ l DMEM). Mix well by flicking tube and incubate at RT for 15 min.
4. During DNA/lipofectamine incubation, remove tetracycline-media from 293GPG cells completely, washing with 2 ml DMEM, and replacing with 2 ml DMEM. Take care not to add media directly to cells as this will disrupt the GPG cells which are loosely adherent. Return cells to incubator.

5. At the end of incubation, aspiration media from 293GPG cells(one plate at a time), add 1.5 ml DMEM to DNA/lipofectamine mixture and overlay on cells by adding to side of dish. Incubate for 7-8 hours.
6. At the end of 7-8 hour incubation, overlay 2 ml 293 media on each dish. Replace the following day with 2.5 ml 293 media and harvest three successive 24 hour viral supernatants(24-48 hrs, 48-72 hrs and 72-96hrs). To harvest virus use a 3ml syringe with an 18 g needle and filter supernatant through a 0.45 μ M acrodisc (Gelman 4184) into a 15 ml Falcon tube. Sups can be stored at 4C while harvesting virus. For long term storage, freeze at -85°C .
7. For transfection and infection control, use U3nlsLZ control plate transfected with U3nlsLZ should be X-gal stained at 48 hours post-transfection (transfection efficiencies of 20-25% roughly correspond to a titer of 10^6 cfu/ml and are adequate for most purposes. However, transfection efficiencies of $>50\%$ can be achieved with this protocol). The 24-48 hour supernatant harvest from the U3nlsLZ plate should be used to infect NIH 3T3 cells to confirm viral titer. Plate 1×10^5 NIH 3T3 cells in a 35mm well and infect with 1 ml supernatant for 8 hours. Replace with 3T3 media and X-gal stain 48 hours post-transfection. $>80-90\%$ X-gal positive cells would be expected for 293GPG transfection efficiencies of $>20\%$.

293GPG media:

DMEM(Gibco 11965-050)

10% IFS (inactivated fetal bovine serum)

1XMEM Sodium Pyruvate(Gibco 11360-013)

2 mM L-Glutamine

50 μ /ml Pen-Strep

1 µg/ml tetracycline(Sigma T-7660)

2 µg/ml puromycin(Sigma P-7255)

0.3 mg/ml G418

293 media:

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1XMEM Sodium Pyruvate(Gibco 11360-013)

2 mM L-Glutamine