**Infection of adherent cells**

1. Seed 5x104 cells per well (24 well plate) 1 day before infection.
2. On the day of infection, remove medium. Add 250 ul of viruse supernatant of appropriate dilutions (see’ virus preparation’ below).
3. Incubate for 2 hr.
4. Discard the supernatant. Add 250 ul fresh medium.
5. Analyze infected cells after 72 hr.

If applicable

1. **After 72 hr,** puromycin selection. Take out medium, and put into tube. Add 500μl fresh medium to the wells. Spin the tube at 1200rpm for 5 minutes. Resuspend pellet in 500μl medium containing puromycin. Put these 500μl back to the well.
2. **On the next day** get out the medium of the well which is undergoing puromycin election, add 500μl fresh medium.

P.S. Concentration + timing of puromycin selection may vary from cell to cell

**Infection of suspension cells**

1. Change medium of the suspension cells 1 day before the expt. To ensure log phase growth.
2. On the day of infection, collect cells and count.
3. Aliquot 1x105 cells per capped centrifuge tube. Spin at 4000rpm for 2 min.
4. Remove supernatant, and add 250 ul virus of appropriate dilutions (see “virus preparation”). Mix by pipetting.
5. Incubate for 2 hr at 37c (incubator).
6. After 2hr, add 1 ml fresh medium.
7. Pellet cells as in step. Remove supernatant.
8. Add 0.5 ml of fresh medium to each tube, and transfer the cells to a 24- well plate.
9. Analyze after 72hr

If applicable

1. **After 72 hr** puromycin selection. Take out medium, and put into tube. Add 500μl fresh medium to the wells. Spin the tube at 1200rpm for 5 minutes. Resuspend pellet in 500μl medium containing puromycin. Put these 500μl back to the well.
2. **On the next day** get out the medium of the well which is undergoing puromycin election, add 500μl fresh medium.

Alternative protocol

1. Do a cell count (0.1x106 cells best for transduction)
2. Add cells to eppendorf tube
3. Centrifuge cells @ 2000 rpm for 5 minutes
4. Remove supernatant & resuspend pellet with 250μl of virus suspension
5. Put solution in 24 well plate
6. Centrifuge @ 2000 rpm for 1 hour (24oC)
7. Remove supernatant (place pipet tip at corner of well and draw up liquid very slowly)
8. Resuspend pellet in 0.5ml of cell culture medium.
9. Incubate at 37oC, 5% CO2

P.S. Concentration + timing of puromycin selection may vary from cell to cell

Virus preparation

1. virus stock ( concentrated by ultrafuge or pooled supernatant ) must be centrifuged to remove debris and filtered before use.
2. Dilute virus stock to appropriate concentration for titration.
3. Add polybrene stock\* to each virus solution at a final concentration of 8 ug/ml.
4. Use 250 ul of the virus solution( virus with polybrene) to infect cells.

\*polybrene stock: 1mg/ml, filtered and kept at -20c.

**EX. Cppt2E (40%)**

Titre = 1.62x108 IU/ml

Want MOI of 20 IU/cell

Infect: (0.5x106 cells)(20 IU/cells) = 1.0x107 IU

So (1.0x107 IU) / (1.62x108 IU/ml) = 61.7μl of conc. virus (in a final volume of 250 ul)

So to make 300μl of diluted virus (for MOI 20) = 74μl conc. virus + 2.4 μl polybrene + 223.6 μl culture media