Preparation for Retroviral vector

Generating retrovirus particles

1. Plate 293T cells to 10 cm plates with a 1:8~10 split so that they are ~60% confluent the day of transfection.
2. Transfect retroviral vector into 293T cells with PEI:

   10 µg retroviral vector
   3 µg Gag/pol
   1 µg NFκB
   0. 1~1 µg VSVG

3. Dilute DNAs above into 500 µl OptiMEM, invert to mix and incubate 5 min at r.t.
4. Dilute 40 µl PEI into 500 µl OptiMEM in the other tube, invert to mix and incubate 5 min at r.t.
5. Combine diluted DNAs and diluted PEI and incubate 25 min at r.t.
6. During incubation replace the medium of plates into 5 ml DMEM without FBS
7. Add 1 ml combined mixture dropwise onto the cells and incubate plate for 16~24 h at 37˚C in a CO_2 incubator.*
8. Replace the medium with 5 ml DMEM contains10% FBS and 50 mM HEPES (complete media) and incubate for 24 h.
9. Collect medium contains virus and replace with fresh growth complete medium every 24 hours. Store collected medium at 4˚C.
10. Centrifuge collected media at 1,800 rpm for 10 min at 4˚C and filtrate the supernatant through 0.80µm filter unit.
11. Centrifuge the supernatant at 38,000 rpm for 2.5 h at 4˚C
12. Remove supernatant completely and suspend the pellet in TNE buffer.
13. Soak viral pellet in the buffer O/N at 4˚C.
14. Transfer 50 µl aliquot into 1.5 ml centrifugation tubes and store at -70˚C

* Add 1 ml combined mixture dropwise onto the cells and incubate plate for 4~5 h at 37˚C in a CO_2 incubator. Add DMEM contains 20% FBS and incubate o/n.

Titeration of infectious units of retrovirus

1. Ensure you have enough BJAB cells for the number of titers you will run (need ~ $3x10^5$) per titer. Resuspend cells to a concentration of $5x10^4$ cells/50µL ($1x10^3$ cells/µL)

2. Add 200 µl RPMI 1640 medium contains10% FBS and 50 mM HEPES (complete medium) to each well of 24 well plate.
3. Add 50 µl of virus stock solution to first well and mix well by pipetting (3~4 times).
4. Take 50 µl medium out from the first well and add to next well. Repeat for a total of 3 to 5 dilutions
5. Discard 50 µl of medium out of last well.
6. Add 50 µl of BJAB cells (1 x 10^6/ml) to each well (final: 5 x 10^4 cells/well)
7. Mix well by pipetting and film edges with parafilm and rock for 1 h at 4˚C.
8. Add 750 µl complete media and incubate for 48 h~72 h at 37˚C in a CO_2 incubator.
9. Count the number of fluorescent signal-positive cells in each well with hemocytometer
10. Calculate Infectious units using the well with 25~50% fluorescent signal-positive cells (Scan each row for the well that contains the cells that are 50% fluorescent-positive. Count fluorescent-positive cells from the 50% positive well as well as the one about or below. For each well, count ~100 cells.)

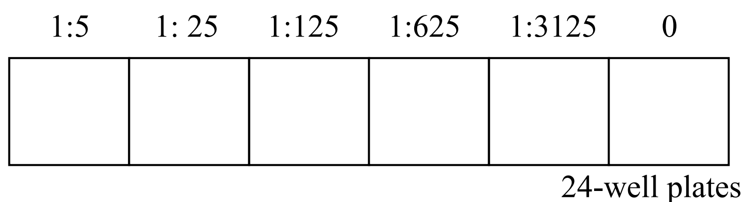
Possion distribution

Infectious units (Particle/ml)

= [(-ln % fluorescent signal-negative cells)(number of infected cells)(dilution factor)] x 1000

Volume of virus stock solution

* number of infected cells = 5 x 10^4
* Volume of virus stock solution = 50 µl
* dilution factor



Infection of Retrovirus into Mammalian cells

Infection into B cells

1. Suspend cells for a final concentration of 1~5 x 10^6/ml in RPMI1640 medium contains 10% FBS, 50 mM HEPES (and 125~250 µg/ml polybrene, if required).
2. Add virus solution and rock for 1 h at 4˚C.

3. Centrifuge cells and replace into fresh growth medium.
4. Incubate the cells for 48~72 h at 37˚C in a CO_2 incubator.

Infection into adherent cells

1. Plate the cells the day before of infection so that cells are ~50% confluent the day of infection.
2. Remove the medium and replace into DMEM contains 10% FBS, 50 mM HEPES, and 16 μg/ml polybrene (not necessary for 293 and HeLa cells).
3. Add virus solution and rock for 1 h at 4˚C.
4. Remove virus and replace with fresh growth medium.
5. Incubate cells for 48~72 h at 37˚C in a CO_2 incubator.