Friday the 24th, October, 06 Hiro Suwanai

Titration of Lentivirus

##All procedure must be done at BL2 and all used materials must be bleached until cells are prepared for facs##

Materials
6 well plate
Concentrated virus
293 FT cells (4x10^5cells/well): Prepare healthy cells
Polybrene
cDMEM media for 293T cells:
10% heat-inactivated FCS
1% Sodium Pyruvate
1% L-glutamine

day 1: Dilution of Virus and infection to 293FT cells
1. Plate 4x10^5 293FT cells/2 ml media/well in 5 wells. You will need 4 wells/virus plus one well for a negative control.
2. 6hours later, check under a microscope to be sure that cells have adhered to the plate.
3. Add 1ul polybrene/well (10 mg/ml stock solution. Final concentration is 5ug/ml)

4. Make serial dilutions of virus in 1.5 ml eppendorf tubes as follows:

1x: VIRUS STOCK (usually 10-15 ul aliquot)	⇄ 5 ul
1/10x: 5 ul virus + 45 ul cDMEM	⇄ 5 ul
1/100x: 5 ul 1/10x virus + 45 ul cDMEM	⇄ 5 ul
1/1000x: 5 ul 1/100x virus + 45 ul cDMEM	

4. Add serial dilutions of virus to pre-plated 293FT cells, 5 ul/well.

Mix gently. Wrap plate with Saran Wrap and Spin 2000 rpm at 32 C for 90 minutes. Incubate 37C for 48 hours exactly in order to make reproducible data.

Day3: Flow Cytometry and calculation of virus titer
1. Put plate on ice after exact 48 hours of 37C incubation.
2. Carefully wash cells 1x with PBS.
3. Trypsinize attached cells by adding 100 ul 0.05% Trypsin-EDTA solution.
4. Quench Trypsin by adding 1 ml/well FACS Buffer. Resuspend cells, wash 2x. wash buffer 2x and filter it. Run flowcytometry to detect GFP positive cells.

Calculation of virus titer

Take numbers from data between 1-10% infection rate.
For example,

if infection rate is 90% at1st row (2.5ul of virus/ml), 55% at 2nd row (0.25ul of virus/ml), 5.00% at 3rd row (0.025ul of virus/ml) and 0.50% at 4th row (0.0025ul of virus/ml),,,,
use 5.00% at 3rd row and calculate

4x10^5 cells x5.0%/100= 2x10^4 postive cells
2x10^4cells/0.025ul=8x10^5 viral particles/ul

It means,,,, also,,,,

Titer in IU/mL =
{(# cells at starting time)*(dilution factor)*(percent infection)} / (vol virus solution added expressed in mls)

{(4x10^5 cells/ml)*(100 times dilution)*(5.0%/100)} / (0.0025ml) = 8.0 x 10^8IU/ml

In general, you should have at least 5.0x10^8IU/ml for embryo infections.