

## Virus Titration and Virus Cloning

You need  $3 \times 10^4$  SF-9 cells/mL or  $2 \times 10^4$  Hi-5 cells/mL, in TMN/FH medium. For a titration, 60mL is sufficient and for a cloning, 80mL is needed.

### Titration

10uL viral stock + 10mL Hi-5 cells →  $10^3$  dilution  
13uL  $10^3$  dilution + 13mL Hi-5 cells →  $10^6$  dilution  
1.3mL  $10^6$  dilution + 11.7mL Hi-5 cells →  $10^7$  dilution  
etc. for  $10^8$  and  $10^9$  dilutions

Plate the  $10^{6, 7, 8}$  and  $10^9$  dilutions.

Use a new pipet for each transfer of virus, to avoid carry-over of virus.

Using a repeating syringe with a “special” needle, plate 1x96-well plate for each dilution, 100uL/well. Incubate at  $27^\circ\text{C}$ , in a plastic bag (prevents evaporation in the non-humidified incubator).

Wait 10 days, and read the results. To identify an infected well vs. a non-infected well, look at the number of cells (much fewer in infected), the size of the cells (many mega-cells in infected) and absence of nuclear membrane in infected.

To calculate the titer in Units/mL (approximately)  
#infected wells X dilution of plate X 0.1mL/well

### Virus Cloning

10uL viral stock + 10mL Hi-5 or SF-9 cells →  $10^3$  dilution  
1mL  $10^3$  dilution + 9mL Hi-5 or SF-9 cells →  $10^4$  dilution  
etc. for  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$  and  $10^9$  dilutions

If you have an ELISA to detect your protein, using SF-9 cells is OK. It is difficult to tell which wells are infected when you use SF-9 cells. If you will have to assess your infection visually, it is better to use Hi-5 cells for cloning.

Use a new pipet for each transfer of virus, to avoid carry-over of virus.

Using a repeating syringe with a “special” needle, plate 48 wells of a 96-well plate at 100uL/well, for each dilution. Also plate 48 wells of SF-9 cells alone. This procedure requires 4 plates. Incubate at  $27^\circ\text{C}$ , in a plastic bag (prevents evaporation in the non-humidified incubator).

The clones to test are from the plates with fewer than 100% infected wells. Choose the clones to save from the plate with 30% or fewer positive wells. The number of positive wells is usually the same as the number of infected wells. The usual test is an ELISA. Clones may be tested between days 7 - 10.