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**

$10\mu$L viral stock + 10mL Hi-5 cells $\rightarrow 10^3$ dilution

$13\mu$L $10^3$ dilution + 13mL Hi-5 cells $\rightarrow 10^6$ dilution

$1.3mL$ $10^6$ dilution + $11.7mL$ Hi-5 cells $\rightarrow 10^7$ dilution

etc. for $10^8$ and $10^9$ dilutions

Plate the $10^6$, $7$, $8$ and $9$ 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°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 $\times$ dilution of plate $\times$ 0.1mL/well

**Virus Cloning**

$10\mu$L viral stock + 10mL Hi-5 or SF-9 cells $\rightarrow 10^3$ dilution

$1mL$ $10^3$ dilution + $9mL$ Hi-5 or SF-9 cells $\rightarrow 10^4$ 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°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.