MOI calculation and optimization

There are three important considerations when infecting an experimental cell line:

1. Selection (either by antibiotic resistance or fluorescence)
2. Relative transduction efficiency (functional titer)
3. Multiplicity Of Infection (MOI)

This guideline deals with MOI (please refer to guidelines for selection and functional titer).

MOI is the number of viral particles that can infect each cell in the tissue culture vessel. This can range anywhere from 0.1 to 10, 20, 30x etc.

For a single construct, one can go higher to achieve a high infection rate, however always take the potential toxicity of a viral infection on the cell into consideration.

For multiple constructs (such as a pool), it is important to stay at an MOI=0.3 to enable result deconvolution and avoid ambiguity of the results.

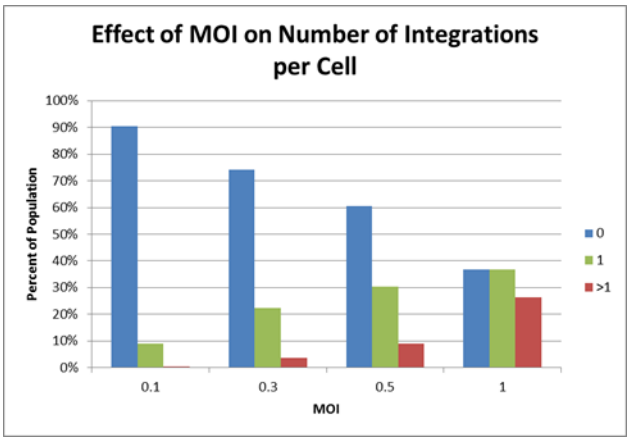
Once the relative transduction efficiency is known for the experimental cell line versus the titering cell line, the following calculation will determine the amount of viral particles that need to be added to a certain number of cells:

Assuming the titer (functional titer) of the viral particles is 1 x 10^7 TU/ml (thus 1 x 10^4 TU/µl etc.):

- *for 1 x 10^7 cells…1 ml of the viral suspension needs to be added to achieve an MOI=1.*

As mentioned above, it is essential to use an MOI=0.3 when doing pooled screens.
In a pooled screen, cells should be transduced at an MOI of 0.3 to maximize the number of cells with a single integration and limit the number of cells needed at transduction. For example, assuming the titer (functional titer) of the viral particles 1 x 10^7 TU/ml (thus 1 x 10^4 TU/µl etc.), for 1 x 10^7 cells, 300 µl of the viral suspension needs to be added to achieve an MOI=0.3.

Note: an MOI=0.3 is based on the Poisson distribution which dictates that at MOI=0.3, 70% of the cells remain un-transduced (uninfected). At an MOI 0.3 or less, greater than 95% of infected cells are predicted to have a single integration and is therefore recommended for pooled screening. Selection should be applied at this point to remove the un-transduced cells.



The Poisson distribution as related to MOI.

After taking relative transduction efficiency, fold representation, and number replicates into account:

- *Number of viral integrants needed:*
  - *Number of shRNA in the pool x Fold representation = Number of integrants needed*

- *Number of cells needed at transduction:*
  - *Number of integrants needed ÷ MOI = Number of cells needed at transduction*

For Example:
In a pooled screen, cells should be transduced at an MOI of 0.3 to maximize the number of cells with a single integration and limit the number of cells needed at transduction. Transducing a pool of 500 shRNA at 1000-fold representation will require 5 x 10^5 transduction units (TU) and approximately 1.5 x 10^6 cells to achieve an MOI of 0.3.
Calculate as follows:

500 shRNA x 1000 fold representation = 5 x 10^5 TU
5 x 10^5 TU / 0.3 MOI = 1.5 x 10^6

Prior to your screen, confirm that there is sufficient volume of viral particles for the biological replicates and the representation needed for your experimental design. The total number of TU needed for the experiment and titer will be required to calculate the volume needed for the experiment. Refer to the titer calculated in previous sections.

- *Number of transducing units:*
  - *Representation x shRNA per pool x Biological replicates = Total TU needed for experiment*

- *Total volume of virus needed for experiment:*
  - *TU for experiment ÷ Functional Titer in Experimental Cell Line (TU/ml) = Volume (ml) of virus needed for the experiment*