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Section 1: Overview - What is MOI and why calculate it?

MOI (multiplicity of infection) 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, you can choose a higher MOI 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.

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) for your specific cell line
3. Is this an individual construct experiment or a pooled screen?
4. Multiplicity of Infection (MOI) calculated based on your cell line, not the titering cell line.

Once the relative transduction efficiency is known for the experimental cell line versus the titering cell line (see separate guideline for determining transduction efficiency), the following calculation will determine the amount of viral particles that need to be added to a certain number of cells (individual construct experiments):

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.

Section 2: MOI for Pooled Screening

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 for a pooled screen is 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: Based on the Poisson distribution, at an MOI of 0.3, the majority 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.

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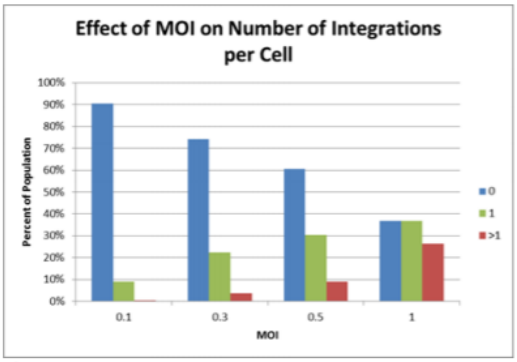

The Poisson distribution as related to MOI.

For pooled screens, after taking relative transduction efficiency, fold representation, and number replicates into account:

- Number of viral integrants needed:
  - Number of constructs 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

Section 3: Calculating volume of viral particles for a given MOI (Individual constructs)

Calculate the total number of transducing units (TU_{total}) that would be added to a well for a given MOI with the following equation:

$$TU_{total} = (MOI \times Cell\ Number)/Viral\ titer\ (TU/\mu l)$$

Where:

- MOI = the desired MOI in the well (units are TU/cell)
- Cell number = number of cells in the well at the time of transduction

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For example, if the experiment requires:

- MOI of 10 (highest MOI)
- Cell density of 10,000 cells per well at time of transduction
- Viral Titer is 1 x 10^7 TU/ml

Then, TU_{total} per well is calculated:

TU_{total} = (10 TU/cell) x (10,000 cells/well)] / 1 x 10^4 TU/µl = 10 µl of viral stock/well.

Therefore, the volume of viral particles with a titer of 1x10^7 TU/ml required for an MOI of 10 is 10 µl per well.

Protocol for determining optimal MOI

This protocol provides a basic outline of the transduction process. The following should be optimized prior to transduction:

- Transduction media: % Serum, Polybrene µg/ml
- Time exposed to transduction media: hours or overnight
- Selection media: µg/ml appropriate antibiotic

Materials

- Experimental cells
- Complete media for experimental cell line
- Serum free media for each cell line
- 24-well tissue culture plate
- Lentiviral particles
- Sterile Microcentrifuge tubes
- Polybrene
- Doxycycline (for inducible vectors ONLY)

Equipment

- Automatic pipette /Pipette-aid (for tissue culture)
- Pipette (for dilutions and handling of viral particles)
- Disposable or autoclaved tissue culture pipettes
- CO_2 cell culture incubator at 37°C
- Fluorescent microscope with appropriate filter

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Protocol

The following protocol represents the standard procedure followed for determining optimal MOI in HEK293T cells. Cell numbers, serum and polybrene concentrations, times, and culture conditions are likely to be different for the experimental cell line. The optimal conditions for a target cell line can be determined using the protocol for functional titer and transduction optimization (Section 1).

1. Plate cells 18-24 hours prior to transduction in a 24 well plate with complete media. Plate at a density of cells so that the cell confluency ranges between 30 and 40% at the time of transduction.
2. Incubate overnight with 5% CO_2 at 37°C.
3. Prepare viral particles:
    a. Set up 8 sterile microcentrifuge tubes and label two tubes each with MOI. For example: 1, 2, 5, and 10.
    b. Add 50 µl of medium containing 1% serum and appropriate level of Polybrene.
    c. Add the volume of viral stock that corresponds to the MOI (use the calculation above for determining volume for the desired MOI).
    d. Bring volume in each tube up to 100 µl with medium containing 1% serum and appropriate level of Polybrene.
    e. Mix well by gently pipetting up and down (10 - 15 times) without creating bubbles and discard the tip.
    f. Incubate for 10 minutes at room temperature.
4. While viral particles are incubating, remove media from cells in each well.
5. Add to each well 125 µl of 1% serum media containing NO Polybrene.
6. After the 10-minute incubation, transfer all (100 µl) of virus from tubes to the corresponding wells (225 µl final volume) for a total of 8 wells (two well for each MOI). The remaining wells (without viral particles) should be evaluated as negative controls.
7. Rock plate gently a few times to distribute the viral particles across the well.
8. Incubate overnight with 5% CO_2 at 37°C (12-24 hours).
9. Replace the viral supernatant with complete media
    a. **For inducible vectors ONLY**, include 1.0 - 2.0 µg/ml doxycycline for one set of MOIs (induced) and no doxycycline for the other set (non-induced)).
10. Incubate cells in culture for 72-96 hours.
11. Using a fluorescent microscope, assess fluorescent expression in the wells.
    a. **For inducible vectors**: Choose an MOI that results in a high level of induction (bright fluorescent expression in the induced set) and low level of leakiness (no or faint fluorescent expression in the non-induced set).

    Note: levels of fluorescent protein expression will vary greatly across a culture due to random integration of lentiviral vectors into regions of the chromosomes with varying levels of transcriptionally active and non-active states.