Relative transduction efficiency (functional titer)

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 relative transduction efficiency (please refer to guidelines for selection and MOI).

Functional titer must be determined using the experimental cell line to ensure optimal transduction during the screen. The functional titer is the number of viral particles, or transducing units (TU), able to transduce the target cell line per volume. This is measured in TU/ml. Cell type, media components, and viral production efficiency influence functional titer and needs to be calculated for every batch of virus produced. A functional titer also needs to be calculated when using a new cell line.

The functional titers for the viral particles provided with the screening library C of A are determined in HEK293T cells. However, many cell lines have lower or higher transduction efficiencies than HEK293T. The relative transduction efficiency is a measure of the ratio of the number of HEK293T cells transduced to the number of experimental cell line cells transduced given the same volume of viral particles. Once this ratio is determined, it can be applied to the remaining tubes of viral particles. This allows the functional titer to be determined without consuming the viral particles that are needed for the screen.

Transduction optimization should be done with the provided non-targeting control (NTC) viral particles.
If extensive optimization is required, additional NTC may be purchased as viral particles from Transomic.

HEK293T cells may be used for troubleshooting. If needed, repeat the protocol with the experimental cell line and HEK293T

Functional titer calculations:

Functional titer of non-targeting control (NTC) viral particles in experimental cell line:

$$(Number\ of\ colonies\ ) \times (dilution\ factor) \div 0.025\ ml = \frac{TU}{ml}\ functional\ titer\ in\ experimental\ cell\ line$$

Relative transduction efficiency of experimental cell line:

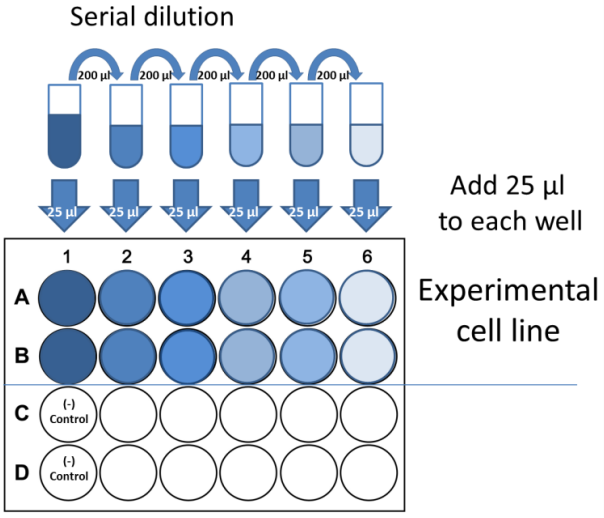
$$(NTC\ titer\ in\ HEK293T\ cells\ [provided\ in\ C\ of\ A]) \div\ (functional\ titer\ of\ NTC\ in\ experimental\ cell\ line) = relative\ transduction\ efficiency$$

Functional titer of pooled screening library viral particles in experimental cell line:

$$(Pool\ titer\ in\ HEK293T\ cells\ [provided\ in\ C\ of\ A]) \div\ (Relative\ transduction\ efficiency) = Functional\ titer\ of\ Pool\ screening\ library\ viral\ particles\ in\ experimental\ cell\ line$$

Schematic for titration and dilution table:

The following can be used consecutively or together to determine the titer and the relative transduction efficiency in HEK293T cells and the experimental cell line.



Tube	Viral particles	Dilution medium	Dilution factor
1	20 µl (from titer aliquot)	980 µl	50
2	200 µl (from Tube 1)	800 µl	250
3	200 µl (from Tube 2)	800 µl	1250
4	200 µl (from Tube 3)	800 µl	6,250
5	200 µl (from Tube 4)	800 µl	31,250
6	200 µl (from Tube 5)	800 µl	156,250

Example:
If the control virus has a titer of 2 x 10^8 TU/ml (Refer to the C of A for the exact titer of your lot of viral particles.) , then the expected number of fluorescent colonies in the titering assay using HEK293T would be:

Tube	1	2	3	4	5	6
Dilution	1/50	1/5	1/5	1/5	1/5	1/5
Diluted titer TU/ml	4,000,000	80,000	160,000	32,000	6,400	1280
Volume used to transduce cells (ml)	0.025	0.025	0.025	0.025	0.025	0.025
Fluorescent colonies expected	100,000	20,000	4,000	800	160	32

If the experimental cell line has an average of 100 colonies in well A4 and B4 then the functional titer for the experimental cell line is calculated as follows:

100 colonies X 6,250 ÷ 0.025 ml = 2.5 X 10^7 TU/ml

Since the functional titer of the control in HEK293T cells = 2 x 10^8 (provided on the C of A) the experimental cell line is transduced 8 times less efficiently. The relative transduction efficiency is calculated as follows:
2 x 10^8 TU/ml (functional titer in HEK293T) ÷ 2.5 X 10^7 TU/ml (functional titer in experimental cell line) = 8
The relative transduction efficiency can be used to calculate the functional titer of the pooled shRNA viral particles in the experimental cell line. If the pool shRNA viral particles functional titer in HEK293T cells = 4 x 10^8 (provided in the C of A) then the function titer in the experimental cell line is calculated as follows:

4 x 10^8 TU/ml (functional titer in HEK293T) ÷ 8 (Relative transduction efficiency)
= 5 x 10^7 TU/ml (functional titer in experimental cell line)