Antibiotic selection kill curve

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 antibiotic selection kill curve (please refer to guidelines for function titer and MOI). For fluorescent markers, please see the note at the end of this guideline.

Some cells have a natural resistance to antibiotics (which is especially true for puromycin) and will need to be evaluated using an antibiotic titrating assay to determine the optimal concentration for selection during any screen.

During transfection or transduction (infection), there are cells that have not received plasmid (transfection) or virus (transduction). This is especially true for pooled screens transduced at a multiplicity of infection (MOI) of 0.3 leaving 70% of the cells un-transduced.

Selection should be applied to remove the un-transduced cells. The lowest concentration of antibiotic that provides adequate selection needs to be determined for the actual experimental cell line.

The optimal antibiotic concentration will kill the cells rapidly (2 - 4 days). This is particularly important for screens involving essential genes that may be selected against prior to the experiment.

Puromycin and blasticidin have a similar range of concentration that is toxic to most cell lines. So, the same kill curve can usually be used for both.

Dilutions and volumes required for establishing optimal puromycin concentration:

Volume of Puromycin Stock Solution Added (µl)	Total Volume of Media plus Antibiotic per 24 Well (µl)	Final Concentration (µg/ml)
0	500	0
0.2	500	0.5
0.4	500	1
0.6	500	1.5
0.8	500	2
1	500	2.5
1.2	500	3
1.6	500	4
2	500	5
3	500	7.5
4	500	10

Note: For single or multiple construct screens where an antibiotic cannot be used, and not all the cells are positive for fluorescence, FACs sorting will have to be done. In the case of single constructs, multiple colony picking can be carried out prior to analysis.