Drug curves are done to ascertain the quantity of drug (antibiotic) to use to select stable cell lines or lines after viral transduction. Like antibiotics that select for plasmids in bacteria, plasmids usually have promoters and resistance genes to select for stable transductants in eukaryotic cells. How to do a drug curve:

1. Do a drug curve:

* Example: Plate out 1 X 105 cells per well in two to three six well plates (depending on the number of concentrations of drug you would like to test). [The density of cells affects a drug curve. Note that drug curves are done on untransfected/untransduced cells to ascertain how the cell line reacts to the drug. Once a drug concentration is determined, use the same number of cells per well and then treat the transduced or transfected cells with the antibiotic.
* Treat duplicate wells in columns as illustrated in the following diagram:

Control-No Antibiotic

Antibiotic-1

Antibiotic-2

Antibiotic-3

Antibiotic-4

Antibiotic-5

* Example: Treat one column with no antibiotics as a control (cells should remain viable in this well over the time course of this experiment). Treat the other columns with your drug of choice with increasing concentrations of drug, eg column 1- no drug control, column 2-100 ug/ml hygromycin, column 3-200 ug/ml hyg, column 4-400 ug/ml hyg, column 5-600 ug/ml hyg, column 6-800 ug/ml hyg, column 7-1000 ug/ml hyg.
* Some common drug ranges are:

a. Blasticidin typical range: 2-10 μg/ml

b. Hygromycin typical range: 25-1000 μg/ml

c. G418 (neo/kan) typical range: 50-1000 μg/ml

d. Zeocin typical range: 50-1000 μg/ml

e. Puromycin typical range: 0.25-5 μg/ml

* Observe plates daily. You should see robust cell growth of the untreated wells and a diminution of cell growth over the concentrations tested.
* You want to choose a concentration of drug at which there is minimal, minimal, cell growth (eg 1-3% cells surviving) but not extensive cell death (100% cell death should be seen at the highest concentration tested).

1. Transfect your gene of choice and a selection marker (either on the same plasmid or on separate plasmids) or transduce cells with your lentivirus. Put cells in media without the drug and incubate 48 h to allow expression of drug resistance gene. Plate out cells at the same density that was used for the drug curve. (Cell density affects drug selection so everything should be done just like drug curve). (This protocol can be adapted to a different size plate or 96 well plates as long as the number of cells per well are modified per well and tested per the drug curve.)
2. Every 3 days change media and add fresh drugs. Score the cells daily by noting the confluency. After 2-3 doses of drug you should have an answer as to the appropriate amount of drug to use for selection. For a more quantitative result, use a proliferation assay (like Roche, WST-1 reagent) for assessing cell survival.
3. For stable cell lines, screen by IHC or western blotting for expression of your gene of interest. Continue to expand cells and freeze clones as soon as possible. Subclone by limiting dilution 3X’s to be sure you have a clonal population of cells and screen that the cells are still producing your gene of interest frequently.

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