pLX ORF Screening Assay Development for HTS

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Arrayed Screening Standard protocol

Day 0-1	Seed cells
Day 1	Add lentivirus to cells in growth media containing polybrene or protamine sulfate (optional: centrifuge cells to promote infection)
Day 1 - 2	Remove media and replace with fresh growth media
Day 2+	Select for infected cells with media containing blasticidin
Day 4+	Assay infected cells

Early/Infection Optimization:

1. test polybrene concentration,
2. titrate blasticidin,
3. test cell density

1. **Polybrene or Protamine Sulfate** is used to facilitate lentiviral infection. However, some cell types are sensitive to higher concentrations or longer time periods in polybrene. Expose cells to media containing 0-4-6-8-16 μg/mL polybrene. To determine polybrene toxicity, assess cell growth and cell morphology for several days following 24 hour and 2 hour polybrene exposure. Compare to no polybrene exposure.

2. ***Blasticidin*** *concentration should be optimized.* Titrate blasticidin in the range of 0-30ug/ml to determine find the concentration where uninfected cells are killed. T*ypical concentrations range from 5-15 ug/mL.*

3. **Cell density :** test several cell densities e.g. 96 well plates: 500 – 10,000 cells/well; 384 well plates: 100-1,000 cells per well), and assess confluency at 3-4-5-6 days

Example Experiments:

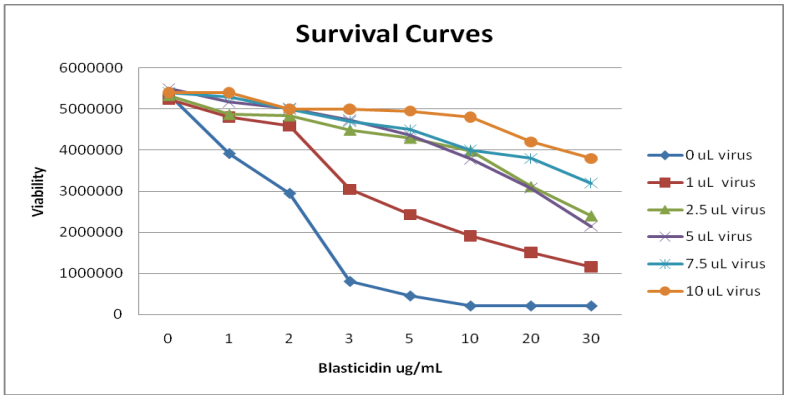
	1	2	3	4	5	6	7	8	9	10	11	12
A	500	1000	1500	2000	3000	4000	5000	6000	7000	8000	9000	10000
B	500	1000	1500	2000	3000	4000	5000	6000	7000	8000	9000	10000
C	500	1000	1500	2000	3000	4000	5000	6000	7000	8000	9000	10000
D	500	1000	1500	2000	3000	4000	5000	6000	7000	8000	9000	10000
E	500	1000	1500	2000	3000	4000	5000	6000	7000	8000	9000	10000
F	500	1000	1500	2000	3000	4000	5000	6000	7000	8000	9000	10000
G	500	1000	1500	2000	3000	4000	5000	6000	7000	8000	9000	10000
H	500	1000	1500	2000	3000	4000	5000	6000	7000	8000	9000	10000

Seed at several densities

infect with test virus (ORF-GFP,-HcRed, -BFP, -lacZ, -Luciferase)
0 – 1 - 2 – 5 – 7.5 – 10ul virus
select +/- blasticidin (0-1-2-3-5-10-20-30 ug/ml)

		1	2	3	4	5	6	7	8	9	10	11	12
		0 uL		1 uL		2 uL		5 uL		7.5 uL		10 uL virus	
A	0												
B	1												
C	2												
D	3												
E	5												
F	10												
G	20												
H	30												

Example survival curves: effect of viral titer on assay. Analyze data to assess whether increasing amounts of virus affect the screening results. For example, does increased virus lead to decreased cell counts/cell viability?



Assay Optimization:

Assay timeline: seed cells, infect with test virus select +/- blasticidin, assay at different timepoints. Different proteins/transcripts display different stabilities. In general, and if applicable to your assay, we aim to assess phenotypes 3-5 days post-infection.

Assay reproducibility: seed cells, infect at least 2 plates with one volume of viral stocks, grow under screening conditions (e.g. +/- blasticidin), assay. Include uninfected cells in parallel. Analyze data for distribution and variation – test various forms of the data for normal distribution (e.g. raw data, background-subtracted, log transformed); compute standard deviation, %CV.

Automation: test robotics during all screening steps: seeding, infection, media change, end point.

Pre-Screen

Prescreen using a sampling of screening set virus plates with optimized HTS protocol.
Test ~4 virus infection volumes for 2-4 library (96 well) plates in the screening set, which are selected to represent the range of titer in the screening set. The idea of the prescreen is to run a small number of plates in the exact HTS conditions before beginning the large scale screen; this also determines the amount of virus to use in the final screen.

Primary screening

Screening batches are limited to 60 plates per infection day. Exceptions may be made for simpler assays or experienced screeners.