Lentivirus infection and general screening plan—Suspension cells

Prepping for screening

-Ensure you have enough media for screen
-If using spinner flasks, wash carefully and autoclave prior to infection day (can also grow up un-infected cells in these flasks)
-Test small scale (~6-well plate size) transfection and infection to estimate infectivity. Goal infection rate is 20-50% infected cells, as measured by %BFP+ 2-3 days after infection.
-If using a drug/toxin selection, test concentrations and dosing protocols in as close to the screen conditions as possible (e.g. test in spinner flasks if planning to use, as these can affect dosing significantly). See the screening principles document for more information.
-Scale up culture to reach the number of cells you need on infection day.

5 days prior to infection

Seed 15cm plates for mega transfection protocol. Each plate yields ~30mL virus, so calculate the number of plates based on the volume of virus you will need on infection day. The volume will depend on your cell line and packaging efficiency, as tested above. The quantities listed below are typical numbers we use in K562 cells. In addition, these quantities are for a single replicate. We typically do screens in duplicate, maintaining two independent cultures starting from the infection.

Day 0 Infection

1. Harvest virus as described in transfection protocol.
2. Centrifuge a number of cells equal to ~1000x the size of the library and aspirate media.
3. Resuspend cells in 0.5 mL virus-containing media per million cells. Add 8ug/mL (final conc.) polybrene. For an example genome-scale screen, resuspend 240 million cells in 120 ml of virus-containing media and then add polybrene stock to get final conc.
4. Aliquot cell/virus mixture to 6-well plates (~5-6 mL per well), and spin 1000x g for 2hr at 33C.
5. Resuspend cells from the 6-well plates, centrifuge cells, and aspirate virus-containing media.
6. Resuspend cells carefully with 2 mL fresh media per million cells in static or spinner flask.

Day 1

Check cell number and split or expand as necessary (see Day 5), maintaining cells at $0.5x10^6$/mL each day. Some BFP fluorescence may be visible but will not accurately represent infection rate.

Day 2 Start puromycin selection

Check infection by BFP % using flow cytometry. Split or expand cells as

necessary, then start puromycin selection with final concentration of 0.75 ug/mL. Typically, 2 days of puro selection at this dose will enrich the population to 80-90% infected, the target range for T0. This allows you to monitor the fraction of uninfected cells throughout the course of the screen.

Day 3
Continue puro selection, assuming the dose from Day 2 has been fully metabolized and applying a fresh dose at 0.75ug/mL final concentration.

Day 4
Recover cells by diluting with puro-free media or by centrifuging cells and resuspending in fresh media.

Day 5 “T0”
To start the screen, you will need to harvest cells, with the number of infected cells equal to 1000x the size of the library, for **each** of the following groups:

- The untreated/DMSO-treated control culture
- Each drug-treated condition
- Freezer stocks for sequencing sample prep. At least two stocks are recommended to insure against any issues that may arise in sample prep.

For a genome-scale screen with one drug condition, this requires $240x10^6$ cells infected at 85% (for $200x10^6$ infected cells) each for two cultures and two freezer stocks, or almost 10^7 cells total.

For the screening cultures, seed cells into flasks and dilute to $0.5x10^6$/mL. Throughout the screen, maintain cells at this number of infected cells at a minimum and at the same volume if possible. For any drug-treated conditions, begin treatment today.

For freezer stocks, centrifuge enough cells for at least 2 1000x stocks, aspirate media, and resuspend in freezing media (FBS with 10% DMSO). Aliquot cells into cryotubes (either multiple 1mL tubes or one 5mL tube per stock) and slow-freeze at -80C.

Days 6+
Each day, measure cell concentration, viability (just for your reference, not used for growth calculations), and BFP% for each culture, and split to maintain cells at $0.5x10^6$/mL. Use these daily measurements to populate the accompanying spreadsheet “screening measurements.xlsx”. You can freeze the remaining cells from splits as midpoint samples if you want to measure cell responses at multiple points of the screen or to protect against contaminations arising late in the screen.

With drug-treated cells, the day-to-day culturing depends on your dosing protocol and cell health—recently treated cells may not require any splitting, while cell populations that have recovered from treatment will return to the untreated

growth rate and can be then treated again or harvested depending on the selection pressure exerted by preceding treatments (see below).

Ending the screen

There is no one optimal time for ending a screen. As the screen progresses, there is a trade-off between increasing the selective pressure on the population (thus improving signal-to-noise) and saturating the dynamic range. Each day of course also increases reagent cost. Our general rule of thumb is to measure cell doublings as above, and aim for at least 6 doubling differences between treated and untreated cells, or 10 cell doublings if only culturing an untreated sample (i.e. for essential gene screens). Treated cells should be allowed to recover to almost untreated growth rate before harvesting.

Freeze at least 2 1000x library cell stocks as above, expanding the culture prior to the endpoint day if necessary to have enough cells.