Care and use of GHOST (3) HIV indicator cells (18feb98)

Please expand and freeze down early (no more than 10-15) passages of these cells as their phenotype changes over passaging.

Maintenance medium:

High glucose DMEM + 10% FCS (any cheap brand) + pen/strep
500 μg/ml G418
100 μg/ml Hygro (reduce to 50 μg/ml if cells appear too sensitive) & for all coreceptor encoding cells (i.e. NOT PARENTAL) add
1 μg/ml puromycin

Note to previous users of GHOST (34) cells: GHOST (3) cells express a uniformly high level of human CD4 and are significantly more sensitive than the analogous (34) cells in infection assays. However, one disadvantage of the (3) line is that they express a detectable, albeit weak, level of endogenous CXCR4 on their cell surface. This corresponds to an increased susceptibility to CXCR4-tropic virus infection on all GHOST (3) cell lines. Nonetheless, the GHOST (3) cells transduced with exogenous CXCR4 are 10-20 fold more sensitive in infection assays with CXCR4-tropic virus than the other GHOST (3) pools.

Typical infection protocol for GFP analysis

All HIV/SIV handling should adhere to standard BL-3 protocols

Day 0, Infection preparation:

Seed 2.5 x 10^4 cells per well of a 12 well plate the day before infection
Cells can be plated in nonselective medium for single round infection exps

Day 1, Virus infection:

Apply virus in the presence of 20 μg/ml polybrene to enhance infection efficiency. Cells are sensitive to DEAE/Dextran.
Preferably, infections should be performed in a total volume of 300 μl per well of a 12-well plate. After 2 hr incubation in a 37°C humidified CO_2 chamber, virus and polybrene should be replaced with 1 ml media.
Alternatively, infections can be performed in 0.5 ml total volume overnight. Replace virus-containing medium the next day.

48 hr post-infection, Harvest and Analysis:

A sample infection time course is attached. hGFP fluorescence indicating positive infection is depicted along the abscissa (FL1-H). Cells can be analyzed as little as 24 hr after infection; however the mean GFP fluorescence of the positively infected cells will be only 10-fold greater than mock infected cells. 48 hr post-infection, the mean GFP fluorescence intensity is greater than 20-fold over background. At the same time there is no discernable difference in the percentage of positively infected cells between 24 and 48 hr suggesting that the GFP read-out reflects a single-round infection dynamic. At 72 hr post-infection, the increase in mean GFP fluorescence of the infected cells is minimal compared to 48 hr. However, with a replication

competent virus stock, two problems may arise. If the stock applied is of high titer, considerable cell death will be apparent beyond 48 hrs reducing the number of detectable positive cells. In contrast, if cells are harvested significantly more than 48 hr after challenge with a low titer virus, virus spread will manifest in a greater number of infected cells which will complicate experiments designed to determine or normalize virus titers.

Two harvesting options (A&B)

First, wash infected cells on the plate 1x with PBS (no ions)

A. Add 300 μl trypsin to each well, incubate at 37°C for no more than 5 minutes, and prepare 1.5 ml eppendorfs containing 1 ml of any media with serum to kill trypsin. After trypsin incubation, break up remaining cell clumps in the 12-wells and transfer trypsinized cells to medium-containing eppendorfs and spin cells in eppi fuge at 7,000 rpm for 30 secs. Remove media, wash cells with PBS (no ions) once, and spin again. Re-suspend cell pellet in 4% paraformaldehyde.

B. Wash cells once more with PBS (no ions). Add 300 μl PBS/1 mM EDTA to each well and place on a shaking platform at room temp for 15 minutes. Prepare eppendorf tubes with 300 μl of 4% paraformaldehyde. With a blue tip, vigorously pipette to remove infected cells and place into eppendorfs with para fixative, vortex. Final concentration of 2% paraformaldehyde is sufficient.

Remove from BL-3 and keep on ice or at 4°C for at least 1 hr. If sensitivity isn't an issue, cells can be maintained, light-protected, this way for up to 48 hrs - the GFP is very stable. Fixation with paraformaldehyde will kill the virus. Outside the BL-3, it's still a good idea to handle all previously infected cell samples with gloves and decontaminate anything used in manipulating the samples (e.g. Pasteurs, FACS machine intake, etc.) with 10% bleach or EtOH.

After incubation on ice, spin cells in eppi fuge at 7,000 rpm for 30 secs, remove fixative.
Resuspend cells in 200 μl PBS/2% serum.
Analyze by FACS for GFP expression. Expect an approx 20-fold shift in mean GFP-fluorescence of infected cells over non-infected.

Controls to consider:

1) Mock infected cells of each cell type tested.
2) Challenge each cell type tested with a HIV/VSV-G or HIV/Ampho Env pseudotype to demonstrate cells are healthy and transducible.
3) Challenge of GHOST parental cells with every virus being tested.