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 CO₂ 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
Two harvesting options (A&B)

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

A. Add 300 \( \mu l \) 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 \( \mu l \) PBS/1 mM EDTA to each well and place on a shaking platform at room temp for 15 minutes. Prepare eppendorf tubes with 300 \( \mu l \) 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 \( \mu l \) 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.