Stamatatos Lab
Bulk Viral Infection in PBMCs
Contact: Zane Kraft at zane.kraft@seattlebiomed.org

Day 1
Isolate PBMCs and activate them with PHA for three days

Day 3
Count PBMCs
Spin the PBMCs at 2000rpm for 5 minutes and aspirate the supernatant
Resuspend cell pellet in RPMI w/ 20U/mL IL-2 at 10x10^6 cells /mL
Aliquot 30 mLs of cells into a T225cm^2 flask
Add polybrene (2 ug/ml) to each tube
Incubate for 30 minutes at 37^{o}C
Spin down the cells, 2000 rpm for 5 minutes, and aspirate the supernatant
Resuspend the cell pellet in 10mL of virus stock
Incubate at 37^{o}C for 3 hours (gently mix cells every hour)
After 3 hours, spin down the cells at 2000 rpm for 5 minutes and aspirate the supernatant
Resuspend the cells in 100 mL of complete RPMI w/ 20 U/mL of IL-2
(this returns the cells to 3x10^6 cells/mL)
Transfer to a T225cm^2 flask and incubate at 37 ^{o}C

Day 7, 10, 14, and 17
Take out 30-50 mL of bulk infection
Spin at 2000rpm for 5 minutes
Aliquot the supernatant into cryotubes (1 mL each) store at –80 ^{o}C
Resuspend the cells in tube in 30-50 mL of complete RPMI w/ 20 U/mL IL-2 and return them to the flask
Repeat on days 10, 14 and 17 (Collect all supernatant on day 17)

NOTE: The viral innoculum used should only have been passaged once in PBMCs – primary isolates cannot be repeatedly passaged