**Titer Virus**

1. Plate $4x10^5$ 293.T cells/well in a 6-well plate 12-24 hours prior to titering. It is helpful to have an additional well as a negative control that you mock infect with D10+polybrene but without virus.
2. Make a stock solution of D10 with 8μg/ml polybrene.
3. Generate a 10-fold dilution series of virus in the D10+polybrene. Using 1.5mls/well you should have 1μl, .1, .01, .001, .0001, and .00001μΛ οφ ϖιρυσ/ωελλ.
4. Incubate at 37 degrees O/N. Replace media with fresh D10.
5. At least 48 hours after infection trypsinize cells for FACS analysis. (Trypsinize, inactivate with media, spin, and resuspend in cold PBS).
6. FACS analyze for EGFP expression and record the percentage of cells that are EGFP positive.
7. Use a well that has between .1% and 10% of cells expressing EGFP to determine titer.

Sample calculation assuming 1% infection from the well with .01μl of virus.
.01 (percentage of cells that are EGFP positive) x 4 x $10^5$ = 4 x $10^3$ positive cells.
4 x $10^3$ x 100 (dilution factor) = 4 x $10^5$ viral particles/ul
In general you should have at least 5 x $10^5$ viral particles/ul for embryo infections.