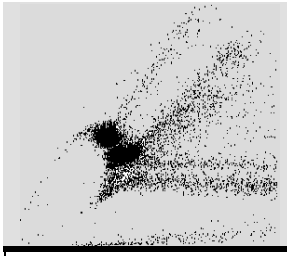

THE GOODELL LABORATORY

Author	Karen Lin	July 30, 2005
Title	**Titering MSCV Viruses with 3T3 Cells**	
Introduction	This protocol is to titer an ecotropic viral vector expressing GFP with FACS analysis of GFP expression. Target cell-lines and read-out of titering may be varied when distinct packaging method and viral construct are used.	
Materials	***Transduction Medium for 3T3 cells:*** DMEM (Invitrogen, 11965-092) 10% Calf Serum (Colorado Serum Co., Cat# 1334) 1X Pen/Strep 4 ug/ml polybrene (=Hexadimethrine bromide, Sigma, H9268) ***Culture Medium for 3T3 cells:*** DMEM (Invitrogen, 11965-092) 10% Calf Serum 1X Pen/Strep **24-Well Tissue Culture Plates**	
Protocol		*Notes*
1.	**Day0** (1) Plate 5x 10^4 3T3 cells/well in a 24 well plate with 1 ml culture(C) medium. (2) Incubate 37^{o}C overnight.	keep healthy cell stock. Because cells will be under a huge stress from virus infection, cells for tittering should be of healthy condition when loading viruses.
2.	**Day1** (1) Replace medium with 950ul transduction (T) medium (2) Thaw virus rapidly in 37^{o}C waterbath. (3) Make 10 fold dilution series of each virus, and apply 50ul of diluted sample onto cell culture. 10^0 = non-diluted thawed supernatant	Viral particles should be freshly thawed right before the infection.

	10^{-1} = 6 ul of non-diluted thawed supernatant + 54 ul T medium 10^{-2} = 6 ul of 10-1 + 54 ul T medium 10^{-3} = 6 ul of 10-2 + 54 ul T medium 10^{-4} = 6 ul of 10-3 + 54 ul T medium Leave one well for no virus control	
3.	**Day2 (24 hours after transduction)** Replace media with culture medium	
4.	**Day3 (48 hours after infection)** Trypsinize 3T3 cells, FACS analysis for GFP expression to evaluate titer For a well of ~1-10% expression in GFP **Titer = (%) GFP x (#) cell plated x dilution factor / 0.05 ml = viral particles/ml**	Typically with a decent virus production, we find the 1-10% eGFP bracket at the 10^{-2} dilution.

Note:

1. A freeze-thaw cycle would decrease the virus titer by ~10 fold. Therefore, it is very important to set an aliquot of viral supernatant for virus titering when harvesting viral particles out of 293T cells (the production of retrovirus).
2. Typically, for retroviruses produced from a 8kb vector, the titer is about 5 x 10^6 virus/ml. For a virus from a 7kb vector, the titer can reach 1x10^7 virus/ml.
3. For a good transduction of hematopoietic stem/progenitor cells, it is recommended to use virus titer no less than 2x10^6 virus/ml. It is empirical that we found a larger volume of viral supernatant (exceeding a quarter of the total transduction volume) will decrease the transduction efficiency. For example, with a transduction of 5x10^5 cells (in 1ml transduction medium), it is highly recommended to have a virus titer of no less than 2x10^6 virus/ml.
4. If the titering results are not satisfying, it usually means the transfection reactions for virus production is not good. I would go back trouble-shooting the transfection efficiency.