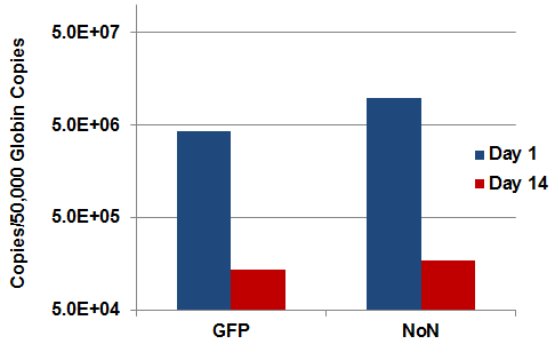
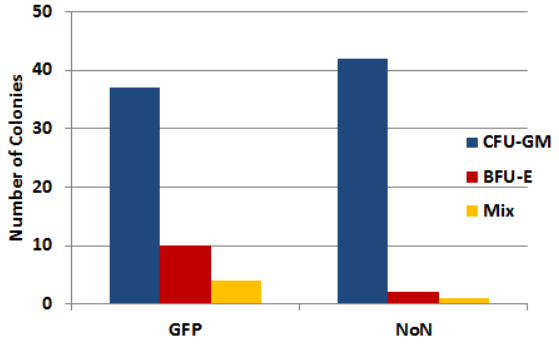
A. Pellets



B. Progenitors



Bone marrow CD34+ cells were thawed and plated at 10^5 cells/cm^2 in non-tissue culture-treated 25 cm^2 flasks that had been coated with 4 µg/cm^2 of the RetroNectin (Takara Bio Inc., Kyoto, Japan). Pre-stimulation was performed overnight in X-Vivo 15 serum-free medium (Lonza, Hopkinton, MA) supplemented with 2 mM L-glutamine, 100 units/mL penicillin, and 100 mg/mL streptomycin, and containing 100 ng/mL each of the recombinant human cytokines: thrombopoietin (TPO), Flt3-Ligand (Flt3-L), and stem cell factor (SCF) (R&D Systems, Minneapolis, MN, USA). Cells were resuspended in fresh cytokine-containing medium and transduced in separate portions with the SIV-GFP or NoN vector supernatant at the final vector concentration of $8x10^7$ TU/ml, with protamine sulfate at 4 µg/ml (American Pharmaceutical Partners, Inc., Schaumburg, IL, USA). After 2 hours, the medium volume was doubled and cells were incubated at 37°C with 5% CO_2 for 6 – 8 hours. A second aliquot of each SIV vector supernatant was added to the cells and incubated overnight. Cells were washed, and resuspended in PBS with 1% serum. Cell counts and viability were determined using trypan blue exclusion. Aliquots of cells were plated for methylcellulose colony assays (including GFP+ colony counts), quantitative PCR analysis (Day 1 and 14), flow cytometry, and examination under a microscope for GFP expression and scoring. Results showed an approximate 30-50-fold decline in the GFP or NoN copy numbers in transduced CD34+ cells over the 14-day culture period (Fig. A). No significant differences were observed in the number of progenitor colonies between SIV-GFP and SIV-NoN (Fig. B). Greater than 75% of the colonies derived from CD34+ cells transduced with SIV-GFP tested positive for the transgene sequence by PCR, whereas only 15% of the colonies derived from cells transduced with SIV-NoN were positive. Approximately 30% showed GFP expression when cells transduced with SIV-GFP were evaluated for transgene expression by flow cytometry.