Lentiviral infection of ES cells

Cell lines:

- AK7 ES cells
- SNL feeder cells (STO cell line that produces LIF). Can use standard MEFs, but will need to add exogenous LIF.

Lentivirus is prepared and titered as described in Rubinson et al. 2003..

ES cell culture: ES cells are cultured according to standard procedures. Cells are routinely split 1:10 to 1:12 when discernable colonies developed on feeders (usually 0.5 to 1.0 x 10^6 cells on a 60 mm dish). Low passage, actively growing cells work best for infection (less than 12-13 passages).

Infection: ES cells (often used one 60 mm dish, but could definitely use less) are trypsinized in .25 % trypsin for 5 minutes at 37 degrees. Colonies are broken up by pipeting the cell suspension up and down for at least 40 times (a sterile plugged glass pipet works well for this). Cells are spun down at 1000 g for 3 minutes and resuspended in fresh ES cell media. Cells are then plated onto a gelatinized dish (100 or 60 mm) and placed in incubator for 30 minutes to allow the feeder cells to settle and adhere. After 30 minutes, the media (with ES cells) is removed and cells counted. Approximately $1x10^5$ cells are used per infection. We often set up three or more infections with varying concentrations of virus. The infections are conducted in single-cell suspension in a 5ml FACS tube for 12-16 hours (overnight). After this time, each infection is plated onto 1-2 100 mm dishes with irradiated feeders. The number of cells plated is critical because you must be able to make out individual colonies for picking after 7-9 days – too many cells plated and individual colonies will be impossible to pick, too few and individual colonies will not grow well and you reduce the chances of identifying a good clone.

Picking: Colonies are picked by mouth aspiration using standard techniques. Colonies can be picked "blindly" or by screening for GFP expression using a flourescent microscope. If picking blind, a large number (>100) colonies should be picked. Colonies are trypsinized in 30-50 ul of 0.25 Trypsin for 5 minutes at 37 and then broken up by pipetting up and down 30-40 times. This (hopefully) single cell suspension is added one well of a 96 well dish with irradiated feeders. ES cells are allowed to attach for 4 to 6 hours, and than the media is changed to remove all traces of trypsin.

Clone Maintenance/Selection: Media is changed at least once a day, twice for mature, actively growing clones. After 3-5 days (depends on rate of growth), clones are split onto either two 12 well or two 24 well dishes, one with feeders and one without (but coated with gelatin). Cells grown on the feeders are expanded and frozen down (and possibly used for injection based on FACS results). Cells grown without feeders are expanded and screened by FACS to look at levels of GFP expression. High expressing, homogenous clones are used for blastocyst injection.