Gene trapping can be performed in a number of ways. Some labs use plasmid constructs and other use retroviral constructs. Some constructs are designed to be expressed only when integrated into genes that are being transcribed and others can integrate and be transcriptionally silent until activated upon cell differentiation. Some gene traps are designed to integrate into the 5' introns of genes, whereas others are designed to be polyA traps. Some labs use gene traps only for sequence identification, whereas others use them for gene mutation and study in vivo.

Please note that a gene trap is not the same as an enhancer trap or a promoter trap.

You should note that plasmid constructs can have multiple tandem insertions in one site or more than one insertion site in the genome, whereas replication deficient retroviral gene traps insert only once per cell.

Plasmids should be linear and bacterial sequences should be removed because they may lead to transcriptional silencing.

Although most gene trapping has been performed in embryonic stem cells, any cell type that is easily grown in culture can be used.

Electroporation of cells with gene trap plasmids containing neomycin resistance genes

1) Aspirate medium from cells. Wash twice with 1xPBS.
2) Add 1 ml 0.05% Trypsin/EDTA to cells, incubate 5 min. at 37°C.
3) Add 3 ml 15% FBS to cells and repipet to break up clumps.
4) Pellet cells in a clinical centrifuge. Resuspend $1x10^7$ cells in 1 ml 15% FBS.
5) Add 900 ul of cell suspension to 10 ug of gene trap DNA. Use 100 ul as nontransfected control.
6) Incubate on ice for 15 minutes. Gelatinize ten 100mm tissue culture plates.
7) Place cell/DNA suspension in sterile 0.4 cm electroporation cuvette.
8) Electroporate at 500 µFarads, 250 Volts using a GenePulser (Biorad) or another comparable instrument.
9) Add electroporated cells to 100 ml normal medium and seed onto 10 gelatinized plates. Begin selection with 400 mg/ml G418 (Gibco/BRL or other supplier of tissue culture reagents) in normal medium on the next morning.
10) Selection should be complete and colonies should be visible after 10 days.
11) The untransfected control should have no colonies.
12) For gene traps containing hygromycin resistance genes, use 100 ug/ml for selection and 50 ug/ml for maintenance.

Infection of cells with retroviral gene traps.

1. A suitable retroviral gene trap vector should be used such as ROSAβ-geo or ROSAβ-gal. The virus is packaged by a cell line such as GP+E86 into viral particles, and secreted into the surrounding medium. This medium is filtered through a 0.2 μm filter prior to use.
2. Viral titer is determined by feeding virus producing cells with fresh media for 16 hours shortly before reaching confluency. Cells are seeded at a density of $5x10^5$ cells per well of a 6 well dish and incubated overnight.
3. Serial dilutions of medium containing viral particles are used to infect the cells that are selected with 0.4 mg/ml G418 24 hours after infection.
4. Colonies are counted after 7-10 days of selection after staining with 2% methylene blue in water for a few minutes.
5. For infection with the retroviral gene trap, 4 ml of pretitered virus ($5x10^3$ cfu/ml) in the presence of 4 mg/ml Polybrene (Sigma) is placed over cells plated at a density of 10^6 cells per 100 mm tissue culture dish.
6. After 20 hours, the medium is replaced with fresh medium for one day.
7. On the following day, infected cells are selected on medium containing the
8. antibiotic G418 (0.4mg/ml; Gibco/BRL) for 7-10 days.
9. During this period, noninfected cells die and detach from the plate; infected cells generate colonies 7-10 days after infection.