General information

1) Molecular confirmation of gene trap insertion site by RT-PCR
2) Cell culture media and reagent preparation
3) Thawing and growing feeder-independent ES cell lines
4) X-gal staining ES cells
5) Sub-cloning gene trap cell lines
6) Genotyping mice

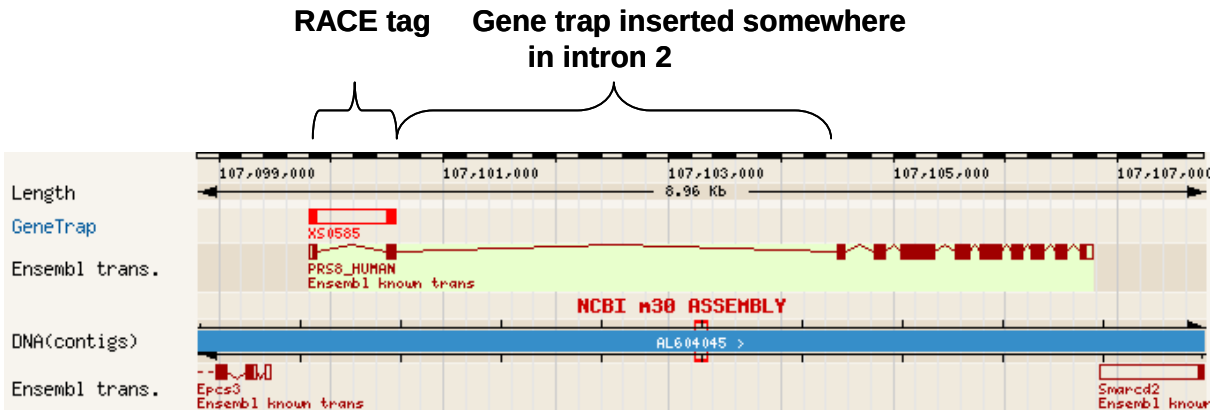
---

1) Molecular confirmation of gene trap insertion site by RT-PCR

Upon thawing a cell line from the Sanger Institute Gene Trap Resource (SIGTR), we strongly recommend that you perform RT-PCR to confirm that the cell line contains the insertion in your gene of interest. This section describes how to choose primers for RT-PCR and how to interpret the results.

The majority of our insertions are within the introns of target genes, therefore, RT-PCR is the easiest method to check that the gene trap vector has inserted in the correct target gene. The following figure shows an Ensembl screen shot of the SIGTR gene trap XS0585, which has an insertion in intron 2 of *Prs8*:

Figure 1: Ensembl view of XS0585

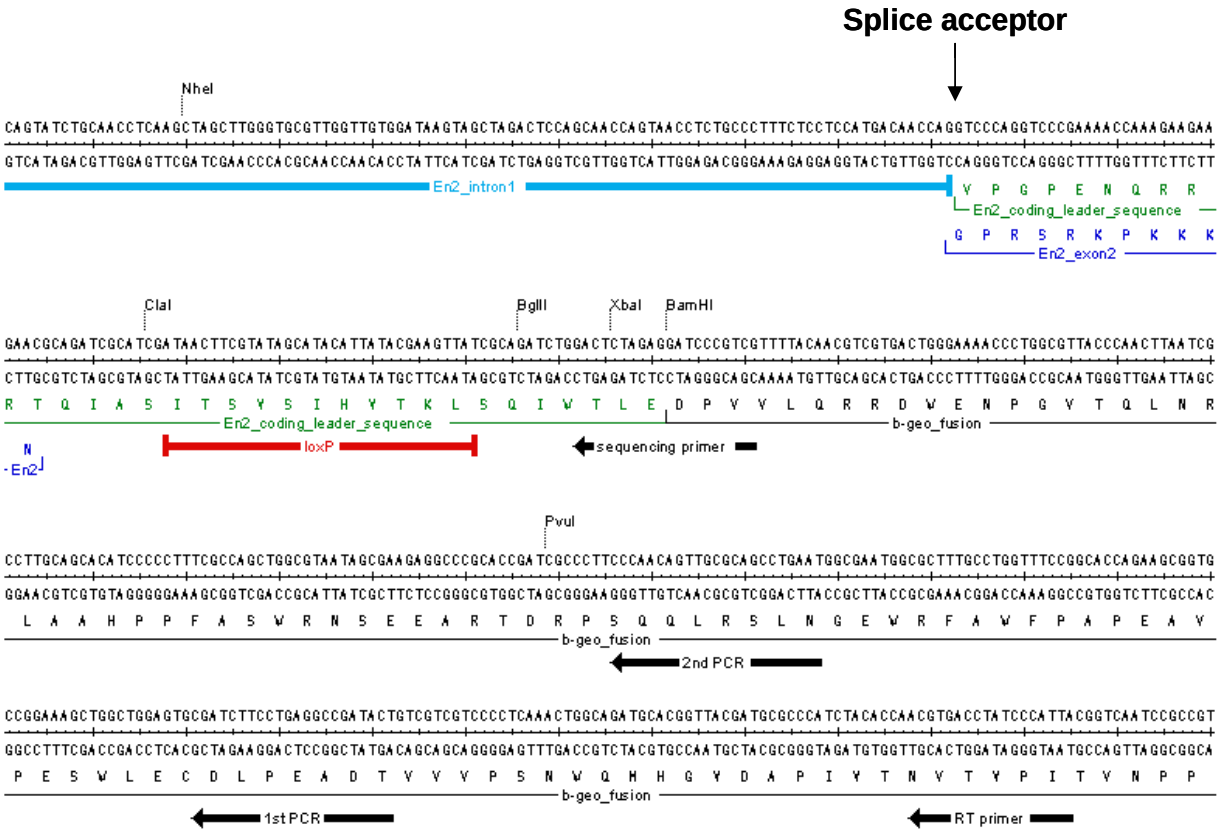


There are a couple of points to note from this example:

1. It is common for the sequence tag generated by 5'RACE to span multiple exons, in this case exons 1 and 2. However, one exon is usually enough to localise a gene trap event to a specific gene.
2. The insertion point is located somewhere within the intron immediately downstream of the end of the sequence tag. In this example the gene trap vector has inserted within the 3.9 kb intron 2.

In the figure below, the two PCR primers used in our RACE protocol are shown relative to the position of the splice acceptor site of the vector. Either one of these primers can be used in an RT-PCR reaction in combination with a gene-specific primer.

Figure 2: En2 leader sequence and start of β-geo

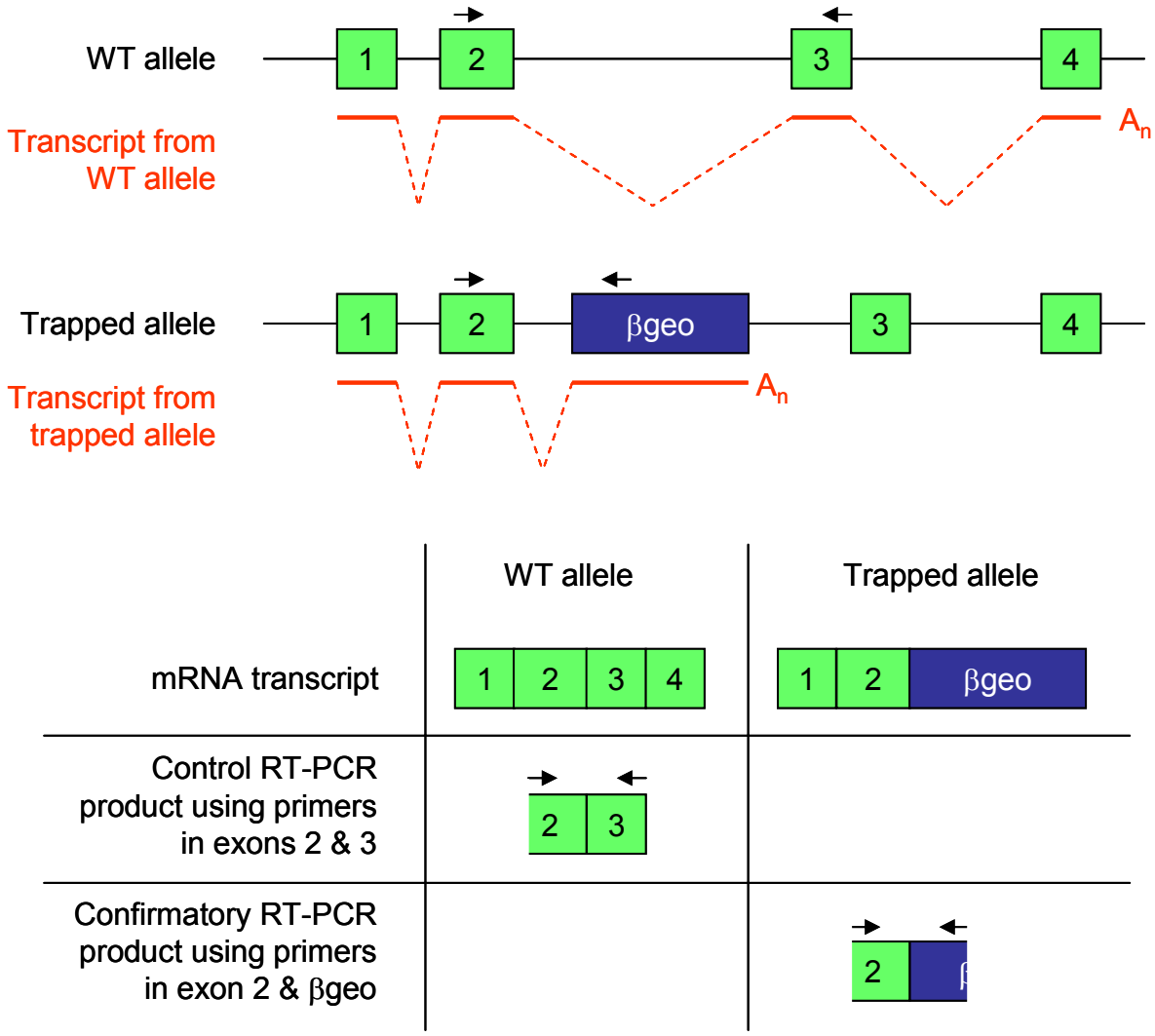


The vector-specific primers used for 5'RACE and RT-PCR are shown below:

Primer name	Length of vector portion of RT-PCR product (bp)	Sequence (5' → 3')	T_m (°C)
1st PCR	327	AGTATCGGCCTCAGGAAGATCG	57
2nd PCR	246	ATTCAGGCTGCGCAACTGTTGGG	59

The RT-PCR reaction is designed to detect the fusion transcript generated by splicing of endogenous gene exons upstream of the insertion site to the gene trap vector. Thus, a sense strand, gene-specific primer is needed and should contain cDNA sequence 5' to the insertion point and be of reasonable length for efficient amplification and resolution by gel electrophoresis. Consider the situation shown below:

Figure 3: RT-PCR in wild type and trapped loci



In this example, a primer is chosen in exon 2 of a theoretical gene that has a gene trap insertion somewhere in intron 2. The primer is 100bp from the 3' end of exon 2 and so this primer in combination with the β-geo primer used for the 2nd PCR is expected to generate a 346 bp RT-PCR product. As a positive control, a primer to exon 3 is used in combination with the exon 2 primer (Note: these primers are also useful to genotype mice, see below). If the expected fragments are not obtained in your assay, please contact us through info.genetrap@sanger.ac.uk and we will advise you further.

RT-PCR Protocol

RNA preparation

1. Grow cells to confluence in a single well of a 6-well plate.
2. Lyse the cells with 1 ml Trizol reagent. Pass the lysate through a pipette several times.
3. Incubate the homogenized samples for 5 minutes at 15 to 30°C.
4. Add 0.2 ml of chloroform per ml of Trizol and cap sample tubes securely.
5. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30°C for 2 to 3 minutes.
6. Centrifuge the samples at no more than 12,000*g* for 15 minutes at 2 to 8°C.
7. Transfer the colourless upper aqueous phase to a fresh tube.
8. Precipitate the RNA from the aqueous phase by mixing with 0.5 ml isopropyl alcohol. Incubate samples at 15 to 30°C for 10 minutes and centrifuge at no more than 12,000*g* for 10 minutes at 2 to 8°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.
9. Remove the supernatant. Wash the RNA pellet once with at least 1 ml of 75% ethanol (prepared using RNase-free water). Mix the sample by vortexing and centrifuge at no more than 7,500*g* for 5 minutes at 2 to 8°C.
10. Briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 minutes). Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility.
11. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60°C.

RT reaction

1. Before performing the RT reaction, heat 5 μg of total RNA in a 10μl volume at 65°C for 5 to 10 minutes and then quench on ice.
2. Set up the following components in a 1.5 ml Eppendorf tube:

    10.0 μl heat denatured RNA
    3.0 μl 10 x PCR buffer
    2.5 μl 10mM dNTPs
    6.0 μl 25mM $MgCl_2$
    1.0 μl random primers (1.0 μg)
    0.5 μl SuperScript II reverse transcriptase
    17.0 μl water

!!!Warning: do not use DEPC-treated water for the RT reaction as DEPC will inhibit the RT and PCR reactions!!!

3. Leave the samples at 25°C for 10 minutes then incubate at 42°C for 1 hour.
4. Denature the cDNA at 95°C and place on ice.

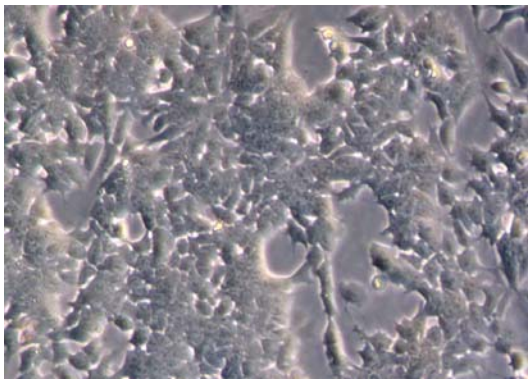
PCR reaction

1. Set up the following components in a 0.5ml PCR tube:
   6.0 µl cDNA product
   1.5 µl 10 x PCR buffer
   0.2 µl Taq polymerase
   0.5 µl primer 1 (100 ng)
   0.5 µl primer 2 (100 ng)
   10.3 µl water
2. Perform PCR with 30 cycles of denaturation: 30 seconds at 95°C; annealing: 45 seconds at 60°C; and extension 60 seconds at 72°C.

2) ES cell culture

Feeder-independent ES cells, derived from either the 129Ola or 129SvEv strain of mice, were used to generate each gene trap cell line. These cells are cultured on gelatinised plates in the presence of LIF.

Figure 4: Feeder-independent ES cells



If you are unaccustomed to culturing ES cells without feeders, we recommend that you culture these cells on feeders as normal.

Cell culture media and reagents

PBS:
Dulbecco's phosphate-buffered saline (PBS) without calcium and magnesium (Invitrogen cat. # 14190-094).

β-mercaptoethanol stock solution:
Add 70 µl of β-mercaptoethanol (Sigma cat. # M-7522) to 20 ml of distilled, deionized water (Invitrogen cat. # 15230-071). Filter sterilize and store at 4°C for up to 2 weeks.

ES cell medium:
1 x GMEM medium (Sigma cat. # G5154) supplemented with 2 mM glutamine (Invitrogen cat. # 25030-024), 1 mM sodium pyruvate (Invitrogen cat. # 11360-039), 1 x nonessential amino acids (Invitrogen cat. # 11140-035), 10% (v/v) fetal bovine serum (Invitrogen cat. # 10270-106), a 1:1000 dilution of β-mercaptoethanol stock solution, and 500-1000 units per ml of leukocyte inhibitory factor (LIF; Chemicon).

Freezing medium:
Add DMSO (dimethyl sulphoxide, tissue-culture grade, Sigma cat. # D-2650) to ES cell medium to a final concentration of 10% (v/v). Filter sterilize. Make fresh before use.

Trypsin solution:
Add 100 mg of EDTA tetrasodium salt (Sigma cat. # E-6511) to 500 ml of PBS. Filter sterilize and add 10 ml (for 1 x) or 20 ml (for 2 x) of 2.5% trypsin solution (Invitrogen cat. # 15090-046) and 5 ml of chicken serum (Invitrogen cat. # 16110-082). Store in 20 ml aliquots at -20°C. (Note: 2.5% trypsin solution should be aliquoted and stored at -20°C to avoid multiple freeze-thawing cycles.)

0.1% gelatin:
Add 25 ml of 2% bovine gelatin solution (Sigma cat. # G1393) to 500 ml of PBS. Store at 4°C.

Geneticin (G418; Invitrogen 11811-031):
Dissolve powder in the appropriate volume of PBS to make a 125 mg/ml stock solution (active concentration). For example, using 5g of powder with an active concentration of 740 μg/mg, this would require:

$$\left(\frac{5000mg}{125mg/ml}\right)\times\left(\frac{740\mu g}{1000\mu g}\right)$$

i.e. 29.6 ml of PBS. Filter sterilize and store at -20°C. For a 175 μg/ml working solution, add 0.784 ml to each bottle (560 ml) of ES cell medium. (Note: The concentration of Geneticin should be titrated to determine the minimum concentration that will kill non-transfected ES cells in 5 days.)

3) Thawing and growing feeder-independent ES cell lines

You will receive duplicate vials of the requested cell line shipped on dry ice. Upon arrival, they should be stored in liquid nitrogen. Each vial contains 2 x 10^6 cells, which should be plated on to a well of a 6-well plate. The cells should reach confluence in 2 or 3 days.

ES cells are frozen in medium containing 10% DMSO. Since DMSO can induce the differentiation of ES cells, we advise thawing the cells late in the day and changing the medium the following morning to minimize the effects of residual DMSO. Cells should be grown in ES cell medium supplemented with 150 μg/ml G418 and kept under selection for at least 2 passages prior to freezing additional stock.

ES cells are routinely passaged every 2 days and the medium is changed on alternate days. Thus, ES cells require daily attention. In our experience, feeder-independent ES cells grow rapidly and quickly acidify the medium, turning it yellow. Allowing the cells to acidify the medium (by not changing the media every day or by passaging the cells at too low a dilution) will cause the cells to undergo crisis, triggering excess differentiation and cell death, after which their totipotency cannot be guaranteed. Plating cells at too low a density, insufficient dispersion of cells during passage, or uneven plating can cause similar problems, as the cells will form large clumps before reaching confluence and the cells within these clumps will differentiate or die. Germline transmission is significantly reduced in cells that have been mistreated, even when they appear healthy at the time of injection.

ES cell culture Protocol

Thawing SIGTR ES cells

1. Coat a single dish of a 6-well plate with 0.1% gelatin and aspirate off immediately before use.

!!!Warning: Loosen cap of cryovial immediately upon removal from liquid nitrogen or vials are likely to explode!!!

2. Thaw ES cells (vial contains approx. 2×10^6 cells, equivalent to 1/2 a confluent 6-well) in a 37°C water bath and add to 10 ml of pre-warmed ES cell medium**.** Mix well by swirling.
3. Pellet the cells by spinning for 3 minutes at 1200 rpm in a bench-top clinical centrifuge.
4. Aspirate off medium and gently resuspend cells in 5 ml of pre-warmed medium.
5. Transfer cell suspension to the 6-well plate and grow at 37°C in a humidified 7% CO_2 incubator.
6. Change medium the following day to remove dead cells and residual DMSO.

Passage and expansion of ES cell cultures

1. For a confluent well of a 6-well plate, aspirate medium off and wash two times with 5 ml of pre-warmed PBS.
2. Cover cells with 0.5 ml of 1× trypsin solution and return to 37°C incubator for 2 minutes or until cells are uniformly dispersed into small clumps.
3. Add 4.5 ml of medium to inactivate the trypsin.
4. Add 1 ml (~1×10^6 cells) to a freshly gelatinized 25 cm^2 tissue culture flask.

Freezing ES cells

1. Trypsinize a confluent 25 cm^2 flask of cells (approximately 1×10^7 cells) as described above.
2. Collect trypsinized cells in 9 ml of medium and pellet for 3 minutes at 1200 rpm.
3. Aspirate off medium and resuspend cell pellet in 2.5 ml of freshly prepared freezing medium. Aliquot 0.5 ml of cells into five cryotubes.
4. Freeze the vials at -80°C overnight and transfer to liquid nitrogen for long-term storage.

4) X-gal staining

In majority of cases, gene trap insertions should express detectable levels of the β-galactosidase reporter in ES cells and/or in differentiated cells in the culture. No staining could indicate that the endogenous gene is expressed at levels below detection in ES cells or that the fusion transcript does not encode a functional β-galactosidase fusion product. For example, insertions that capture the N-terminal signal sequence of a secreted or membrane protein are inactive (Skarnes *et al.*, (1995)). We recommend staining ES cells with X-gal to check if β-galactosidase is expressed in your cell line and the purity of your cells (see below). The cells do not need to be confluent for this analysis.

X-gal staining Protocol

Reagents

0.1M phosphate buffer (pH 7.3) for X-gal staining:
Dissolve 3.74 g monobasic sodium phosphate, (NaH_2PO_4-H2O; FW = 137.99*) and 10.35 g dibasic sodium phosphate (Na_2HPO_4; FW = 141.96*) in 1 litre water.
*Note: the FW will differ depending on degree of hydration

Fix buffer for X-gal staining:
This is 0.1M phosphate buffer (pH 7.3) supplemented with 5 mM EGTA (Sigma cat. # E4378), pH 7.3, 2 mM $MgCl_2$ and 0.2% glutareldahyde (Sigma cat. # G-7776). This can be stored at 4°C for up to 4 months.

Wash buffer for X-gal staining:
This is 0.1M phosphate buffer (pH 7.3) supplemented with 2 mM $MgCl_2$, and can be stored at 4°C indefinitely.

X-gal staining buffer:
This is 0.1M phosphate buffer (pH 7.3) supplemented with 2 mM $MgCl_2$, 5mM potassium ferrocyanide ($K_4Fe(CN)_6$-$3H_20$ Sigma cat. # P-9287) and 5mM potassium ferricyanide ($K_3Fe(CN)_6$ Sigma cat. #P-8131). It should be stored at 4°C and is light sensitive. Before use, add X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) to a final concentration of 1 mg/ml and filter sterilize to minimize the formation of crystals in the staining reaction.

X-gal stock solution (50mg/ml):
Add 10 ml dimethylformamide (BDH cat. # 103224J) to a 500 mg bottle of X-gal. Store at –20°C.

Fix and Wash times**

Monolayer cells: Fix 15 min, Wash 2 x 5 min
Frozen sections: Fix 15 min, Wash 2 x 5 min
Embryos (up to 8.5 d): Fix 15 min, Wash 3 x 5 min
Embryos (9.5 to 11.5 d): Fix 30 min, Wash 3 x 15 min
Embryos (12.5d and beyond): Cryostat section, Fix 15 min, Wash 2 x 5 min

**Note: for staining whole embryos or small pieces of tissue, include 0.01% deoxycholate and 0.02% NP-40 in both Wash and Staining buffers (improves penetration of stain). Embryos/tissues should be rocked gently during staining. Frozen sections can be counterstained using Nuclear Fast Red.

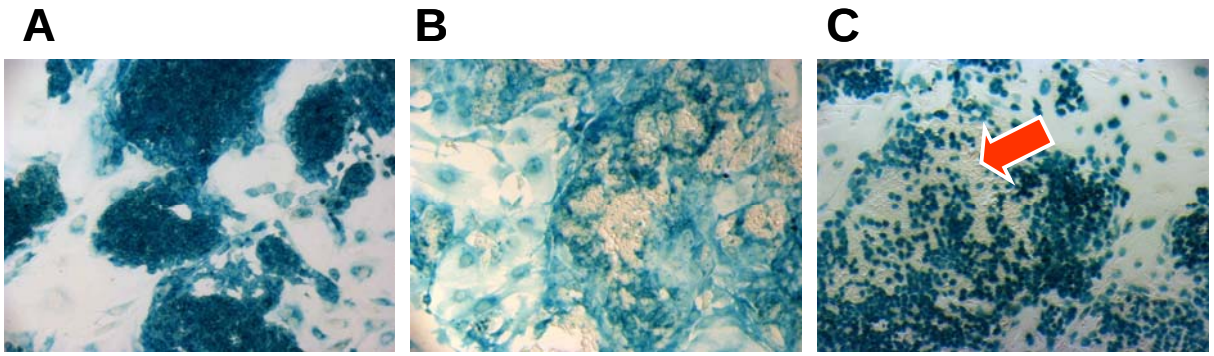
Staining ES cell cultures in multiwell plates

1. Aspirate off ES cell medium and replace with X-gal Fix buffer.
2. Fix the cells for 15 minutes at room temperature.
3. Wash twice (5 minutes each) with X-gal Wash buffer.
4. Add X-gal staining buffer containing 1 mg/ml X-gal (just enough to the cover cells)
5. Place plate inside a humidified chamber, cover, and incubate overnight at 37°C
6. The next day, aspirate off the staining solution, and add Wash buffer.
7. For long term storage, add Fix buffer, wrap in parafilm and store at 4°C.

5) Sub-cloning ES cells

Sub-cloning the ES cell line is recommended if you suspect the cultures do not contain a pure population of the gene trap cell line. X-gal staining (as described above) is useful for determining if the ES cell line is contaminated with wild-type ES cells or another gene trap cell line. Example A below shows a culture of a pure cell line where X-gal staining is uniform in undifferentiated and differentiated cells. Example B is also a pure cell line that shows uniform expression, but only in differentiated cells. Example C is a cell line with probable wild-type ES cell contamination (red arrow) in which only a subset of undifferentiated cells stain with X-gal. To sub-clone the ES cell line, simply plate between 500 - 1000 cells of a well-trypsinized single cell suspension onto triplicate gelatinised 10 cm cell culture dishes. Grow 2 of the plates for 7 days in medium containing 150 μg/ml G418 and 1 plate in medium without G418, changing the medium every 2-3 days. When colonies are visible by eye, stain one of the two plates grown in G418 and the plate grown without G418 with X-gal. A mixture of white and blue colonies on plates grown in the absence, but not in the presence, of G418 indicates wild-type ES cell contamination. Pick several colonies from the second plate grown in G418, expand and re-confirm by RT-PCR. A mixture of blue and white colonies or colonies with different patterns of X-gal expression on plates grown in the presence G418 indicates a mixture of gene trap cell lines. Pick enough colonies to ensure that both cell lines will be isolated, expand and confirm the correct cell line by RT-PCR.

Figure 5: Examples of X-gal staining



If you are uncertain as how to interpret the staining pattern in your cell line, please send an image of the staining pattern to info.genetrap@sanger.ac.uk.

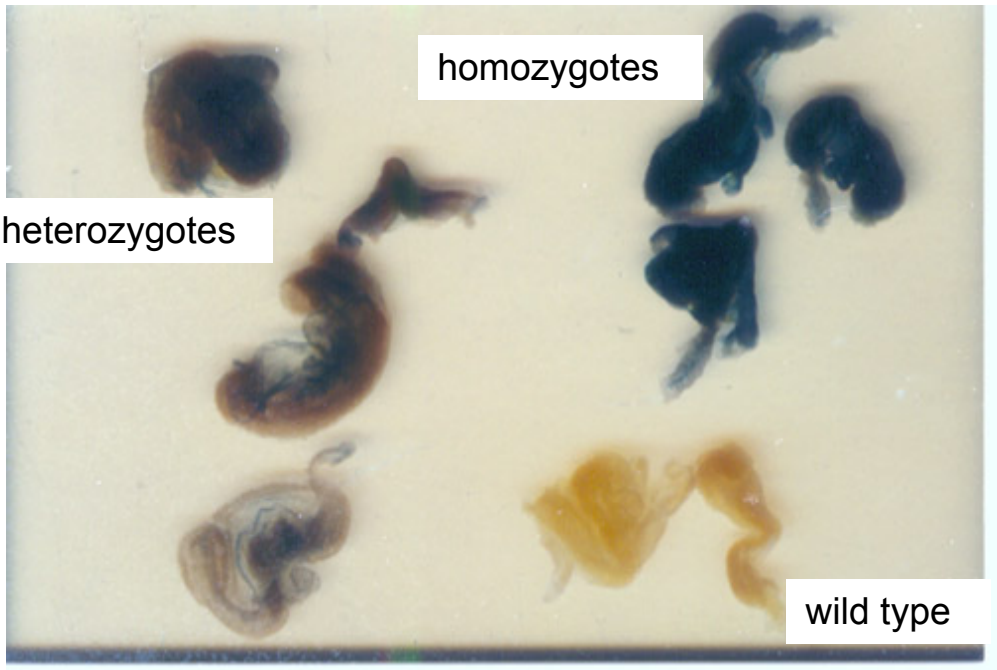
6) Genotyping protocols

Following successful germline transmission, it is advisable to develop a genotyping protocol for identifying mice heterozygous and homozygous for the insertion. Heterozygous animals can be easily identified by PCR from genomic DNA using primers in lacZ or neo, however, homozygous animals are more difficult to genotype by PCR without first characterizing the insertion site (real-time PCR is an option, although this is costly and may not be reliable). Unlike gene targeted alleles where the disrupted allele is well-defined, gene trap insertions can occur anywhere within an intron. Below are several strategies that we commonly use to genotype mutant embryos and mice:

6.1) X-gal staining embryos/tissues

Each gene trap insertion places the βgeo reporter under the control of the endogenous gene and therefore X-gal staining can be used to characterize the pattern of expression of the target gene during embryonic development and in adult tissues. β-galactosidase expression is also a reliable method for genotyping animals or embryos given the fact that homozygous cells/tissues/embryos stain more intensely than heterozygous samples. Below is an example of a litter of embryos from a heterozygous intercross that was stained with X-gal, showing non-stained (wild-type), intermediate (heterozygous) and strongly stained (homozygous) embryos in the expected Mendelian ratio of 1:2:1. X-gal staining of cryostat sections are recommended for adult tissues and embryos (12.5 dpc – neonates).

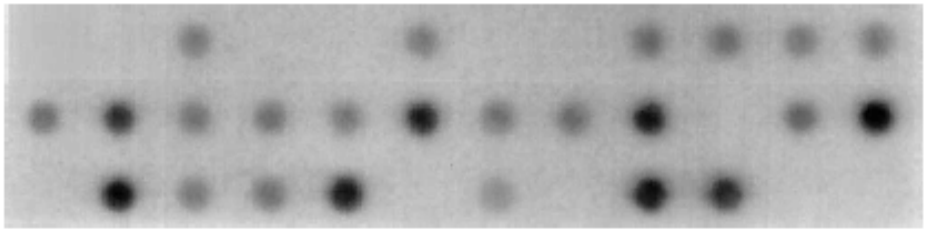
Figure 6: X-gal staining of intercross litter at 9.5 dpc



6.2) Dot blot hybridisation

Dot blot hybridization of genomic DNA prepared from tail biopsies with a lacZ probe is useful for genotyping animals. As shown in the figure below, homozygous animals, which carry two copies of the vector, are easily distinguishable from their heterozygous litter mates. The reliability of this method depends on obtaining uniform signals and thus it is critical to apply enough DNA to saturate the membrane. ***The amount DNA sample applied to the dot blot manifold must be determined empirically***. The following protocol is a convenient one-tube method for preparing genomic DNA from a 1.5 cm tail biopsy which is then applied to Hybond N+ (Amersham) membrane using a BioRad 96-well dot blot apparatus. We recommend using non-radioactive detection methods, however, radiolabelled probes work equally well.

Figure 7: Sample dot blot



If you do not have a plasmid that contains bgal, a 680 bp β-galactosidase probe can be conveniently generated by PCR. Use the primers shown below to amplify the probe from genomic DNA extracted from any mouse heterozygous for a gene trap insertion.

Primer 1: 5' - TTA TCG ATG AGC GTG GTG GTT ATG C

Primer 2: 5' - GCG CGT ACA TCG GGC AAA TAA TAT C

Dot blot protocol

1. Prepare fresh tail buffer by dissolving proteinase K powder (Sigma P-0390 or P-6556) in 10 mM Tris-HCl (pH 8), 100 mM NaCl, 50 mM EDTA (pH 8), and 0.5% SDS to a final concentration of 1 mg/ml.
2. Add 0.1 ml of 5 M NaCl to digested samples while tubes are still warm and vortex at high speed for 10 seconds. Add 0.5 ml of chloroform and vortex as before. Spin in microfuge at full speed for 15 minutes.
3. Transfer 50 µl of the aqueous (top) phase to a 96-well plate. Denature DNA by adding 150 µl of 0.53 M NaOH and incubating for 30 minutes at 37°C.
4. Prepare Hybond N^+ hybridization membrane by prewetting briefly in water and soaking in 0.4 M NaOH for 10 minutes. Wet one piece of Whatman paper in 0.4 M NaOH and place on the manifold. Lay membrane on top, assemble the apparatus, and briefly apply vacuum to empty wells of excess liquid.
5. Apply samples and wait for 30 minutes before applying vacuum.
6. Gently vacuum samples through manifold. Disassemble apparatus and wash membrane in 150 mM sodium phosphate buffer containing 0.1% SDS at 65°C for 1 hour.
7. Prehybridize membrane for 10 minutes at 65°C in 20 ml Church and Gilbert prehybridization/hybridization buffer (0.5 M sodium phosphate buffer, 7% SDS, and 2.5 mg/ml fraction V bovine serum albumin (Sigma A-3803).
8. Replace with 20 ml prehybridization/hybridization buffer containing denatured random-prime-labeled βgal probe and hybridize at 65°C overnight.
9. Wash filter three times 30 minutes at 60°C in 30 mM sodium phosphate buffer containing 0.1% SDS and expose filter to autoradiographic film.

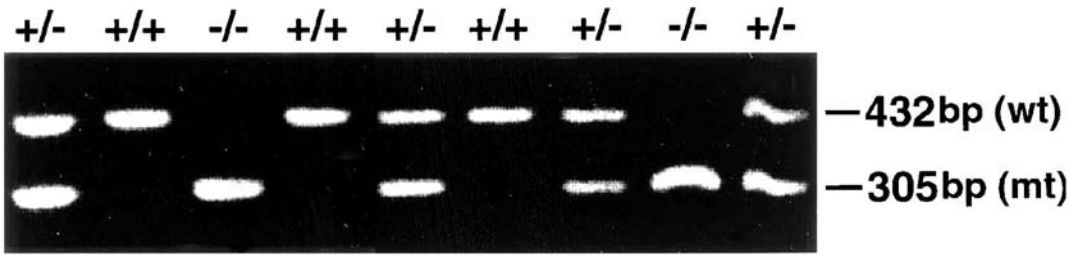
Non-radioactive detection methods also work well (e.g. Amersham "Gene Images" kit; RPN 3500)

This protocol is based on Skarnes (2000).

6.3) RT-PCR genotyping

Using the RT-PCR primers developed for molecular confirmation of the gene trap insertion (see above) are equally useful for genotyping embryos/tissues. However, it is essential to prepare RNA from a tissue or embryonic stage that expresses the trapped gene. X-gal staining described above is a useful method to determine which tissue(s)/embryonic stage(s) express the trapped gene. Due to the sensitivity of RT-PCR, it is important to determine the minimum number of cycles required to produce products that are visible by gel electrophoresis. Below is an example of an RT-PCR assay on intercross progeny carrying an insertion in neuropilin 1.

Figure 8: Results of RT-PCR genotyping



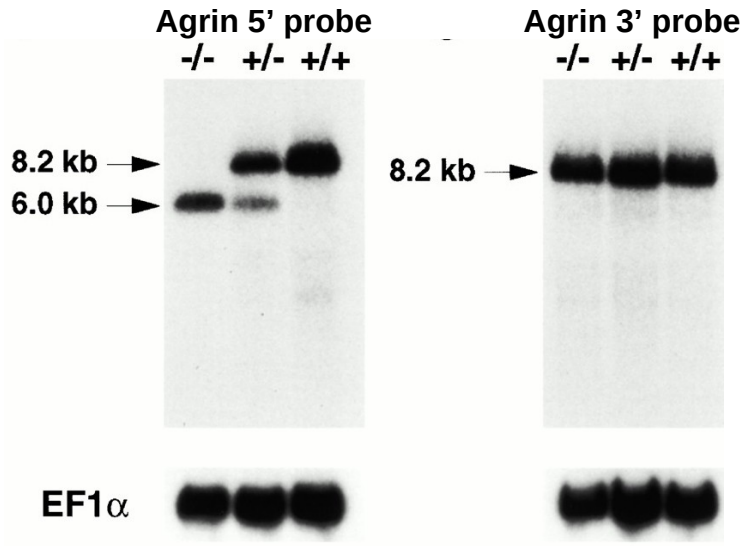
Taken from Chen *et al.,* (2000).

6.4) Northern blot analysis

Northern blot analysis is highly recommended to establish if the gene trap insertion has effectively mutated the gene at the site of insertion. A cDNA probe to sequences downstream of the insertion site is needed. We recommend using a 0.5-1.5 kb probe that contains the 3' untranslated region (3'UTR) of the gene which can be amplified directly from RNA prepared from ES cells or from genomic DNA.

In most cases, wild-type mRNA transcripts should not be detectable by Northern blot analysis of homozygous RNA. However, there are cases where wild-type transcripts might be detected with probes 3' to the insertion. On the blot shown overleaf, the gene trap insertion has interrupted one of the two isoforms of Agrin initiated at alternative promoters. Due to the similarity in the size of the two isoforms, it appears as if the gene was not disrupted by the insertion using a 3' probe common to both transcripts. However, using a 5' probe, it is clear that the insertion eliminates expression of one of the two isoforms. Therefore, if wild-type transcripts are detected in homozygous samples, it is advisable to consider the possibility of multiple isoforms and to perform RT-PCR or ribonuclease protection with the appropriate probes.

Figure 9: Northern blot analysis of insertion in agrin gene



Taken from Burgess *et al.,* (2000).

6.5) Southern blot (RFLP) analysis

The most reliable method for genotyping mice is by Southern blot analysis, where gene-specific probes are used to detect a restriction fragment length polymorphism (RFLP) caused by the insertion of the vector.

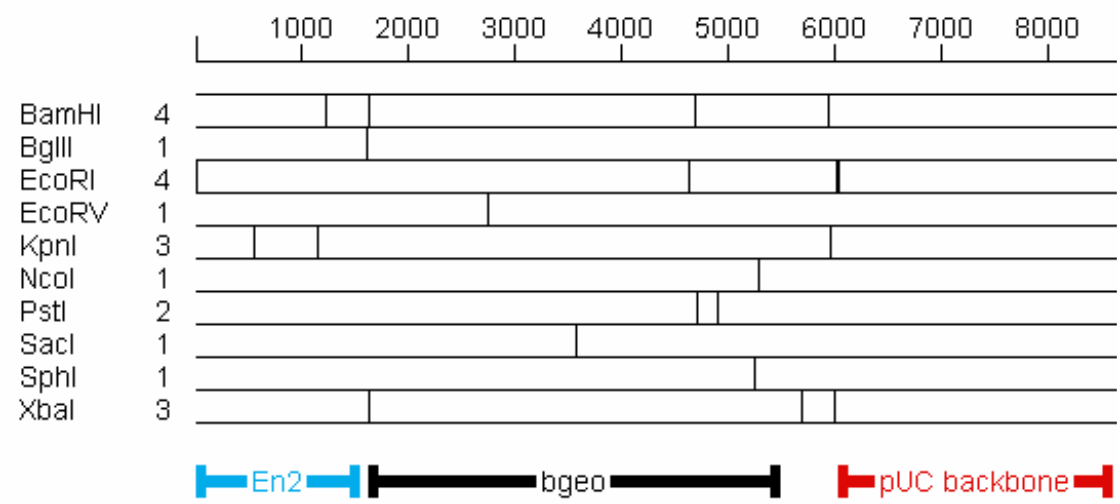
Probe choice

The first consideration is the probe(s) to be used. If the insertion has occurred in an intron that is less than 10kb in length, we recommend using the control RT-PCR product (see Part 1 above) as a probe. If the intron is larger than 10kb, we recommend the use of probes within the intron, the number of which depends on the length of the intron. For an intron of 10*n* kb, *n*-1 probes are needed (e.g. 3 probes for a 40 kb intron). To find suitable probes that detect a unique site in the genome, it is important to run the intron sequence through RepeatMasker (http://www.repeatmasker.org/), and then design PCR primers to generate probes in the 300 – 500 bp size range. It is also worth checking these probes on a test blot to be sure the probes are unique.

Restriction endonuclease (RE) choice

There are a number of restriction endonuclease sites that cut within the gene trap vector that are useful for RFLP analysis. We recommend choosing several of these for the digestion of genomic DNA for the gene trap cell line and a control cell line (either wild-type cells or another gene trap cell line with an insertion in an unrelated gene).

Figure 10: Restriction map of gene trap vector



Strain differences

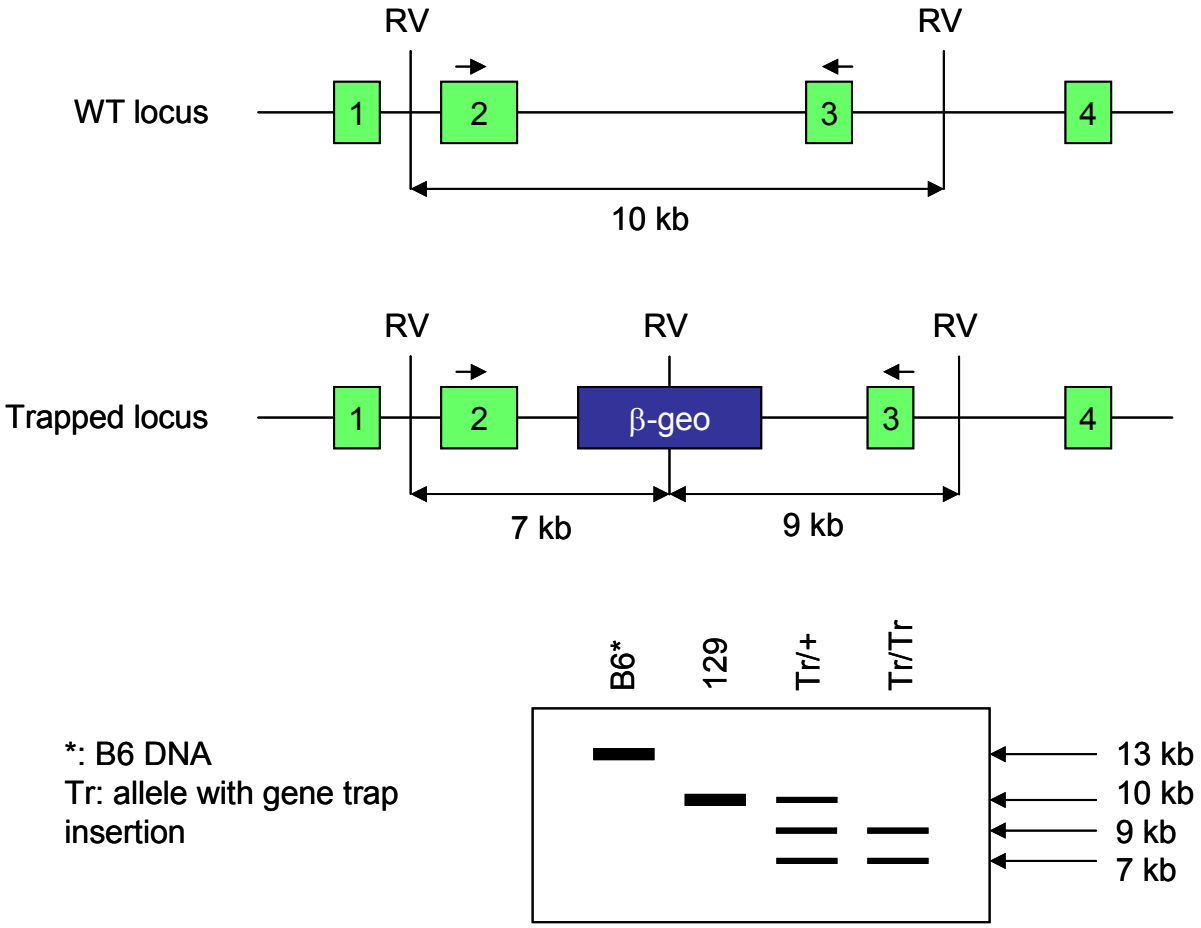
The mouse genomic sequence available from Ensembl is based on the C57BL/6 strain of mice. Beware that some of these sites are polymorphic between C57BL/6 and the 129 (ES cell) strains. These strain differences can potentially confuse the genotyping of mice if the animals are bred to strains other than 129. Therefore, an important control for the RFLP analysis is to include a DNA sample of the other strain of mice used in your crosses (e.g. C57BL/6).

Theoretical example

Because the vector can insert at any point along the length of the intron, it is not possible to predict the size of the novel fragment created by the insertion of the vector. One is simply looking for any deviation in the size of the restriction fragment using a given restriction endonuclease.

Consider the theoretical example shown overleaf. Based on the sequence tag, all we know is that the insertion has integrated somewhere in intron 2. Using the control RT-PCR product (across exons 2 and 3), a 10kb fragment is detected in wild type 129 DNA and a 13 kb polymorphic fragment in the C57BL/6 strain. Insertion of the gene trap vector introduces a new EcoRV site into the locus, such that 2 bands (7 and 9 kb) are detected on a Southern blot of EcoRV-digested genomic DNA from the gene trap cells. Thus with this probe, heterozygous animals will show 3 bands (10, 9 and 7 kb) whereas homozygous animals will show only 2 bands (7 and 9 kb) that are diagnostic of the mutant allele. If the mice are crossed into C57BL/6 strain, we expect to see a 13 kb band representing the wild-type C57BL/6 allele in some of the animals.

Figure 11: Theoretical Southern blot



References

Burgess RW, Skarnes WC, Sanes JR. Agrin isoforms with distinct amino termini: differential expression, localization, and function. J Cell Biol. 2000 Oct 2;151(1):41-52.

Chen H, Bagri A, Zupicich JA, Zou Y, Stoeckli E, Pleasure SJ, Lowenstein DH, Skarnes WC, Chedotal A, Tessier-Lavigne M. Neuropilin-2 regulates the development of selective cranial and sensory nerves and hippocampal mossy fiber projections. Neuron. 2000 Jan;25(1):43-56.

Skarnes WC, Moss JE, Hurtley SM, Beddington RS. Capturing genes encoding membrane and secreted proteins important for mouse development. Proc Natl Acad Sci U S A. 1995 Jul 3;92(14):6592-6.

Skarnes WC. Gene trapping methods for the identification and functional analysis of cell surface proteins in mice. Methods Enzymol. 2000;328:592-615.