

Genotyping BayGenomics Mice

1. Introduction

The gene-trap vectors used within BayGenomics contain a splice-acceptor sequence upstream of a reporter gene, β -*geo* (a fusion of β -galactosidase and neomycin phosphotransferase II)¹. These vectors insert randomly into introns. The resulting insertional mutation creates a fusion transcript containing sequences from exons upstream to the insertion joined to the β -*geo* marker. As a result, the identity of the trapped gene can easily be determined by 5' rapid amplification of cDNA ends (5' RACE) followed by automated DNA sequencing. The sequence tags posted on the BayGenomics website have been trimmed of any "contaminating" vector sequences.

2. Confirming the identity of the trapped gene

The identity of the trapped gene should be confirmed. BayGenomics frequently confirms the identity of clones by repeat DNA sequencing. However, this process takes time, and the results are frequently not back until after the ES cell clone has been mailed off. However, the identity of the ES cell clone can be assessed by RT-PCR. First, use reverse transcriptase to prepare cDNA from RNA isolated from either the BayGenomics ES cell clone or from tissue from a mutant mouse. Then perform a PCR reaction with a forward primer corresponding to sequences within the

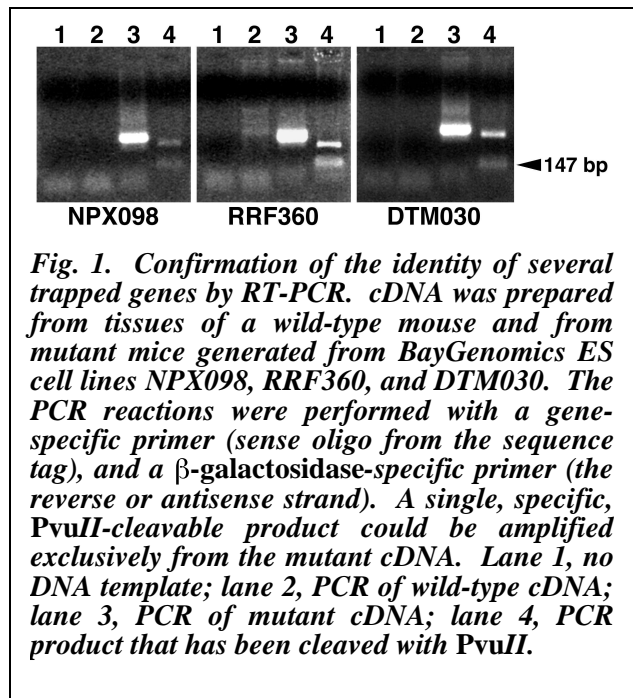


Fig. 1. Confirmation of the identity of several trapped genes by RT-PCR. cDNA was prepared from tissues of a wild-type mouse and from mutant mice generated from BayGenomics ES cell lines NPX098, RRF360, and DTM030. The PCR reactions were performed with a gene-specific primer (sense oligo from the sequence tag), and a β -galactosidase-specific primer (the reverse or antisense strand). A single, specific, PvuII-cleavable product could be amplified exclusively from the mutant cDNA. Lane 1, no DNA template; lane 2, PCR of wild-type cDNA; lane 3, PCR of mutant cDNA; lane 4, PCR product that has been cleaved with PvuII.

¹ Stryke, D., Kawamoto, M., Huang, C. C., Johns, S. J., King, L. A., Harper, C. A., Meng, E. C., Lee, R. E., Yee, A., L'Italien, L., Chuang, P. T., Young, S. G., Skarnes, W. C., Babbitt, P. C., and Ferrin, T. E. (2003) *Nucleic Acids Res.* **31**, 278–281

BayGenomics sequence tag and the reverse primer from the β -galactosidase reporter (5'-GAC AGT ATC GGC CTC AGG AAG ATC G-3').

This PCR reaction should yield a single band—exclusively with cDNA from mutant cells or mouse tissues. The PCR product should not be present in cDNA from wild-type ES cells and tissues. The identity of the amplified band can be further substantiated by cleaving the PCR product with *PvuII*; *PvuII* cleaves 147 bp from the sequences encoding -gal (Fig. 1).

3. Identifying where the insertional mutation occurred

The BayGenomics cell line's web page links directly the sequence tag to the coding sequences of the trapped gene that are available at the GenBank (NCBI) or the Mouse Genome Informatics (The Jackson Laboratory) websites. From either of these sites it is straightforward to retrieve genomic sequences as well, since they both link out to LocusLink (<http://www.ncbi.nlm.nih.gov/LocusLink>). (Of note, for some BayGenomics cell lines, it is actually possible to link out directly to LocusLink.) Then, align the coding and the genomic sequences at <http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html> by entering the corresponding accession numbers in the designated fields. Doing so will allow the determination of the structure of your gene of interest (namely, the number and size of exons and introns there are in that specific gene). Then, aligning the sequence tag (copy and paste the tag sequence in the designated window) and the cDNA makes it possible to determine which exon(s) are upstream to the insertion, and thereby in which intron the insertion occurred.

4. Genotyping of BayGenomics mice

BayGenomics mice can be genotyped by PCR, Southern blot, or DNA Dot blot.

4.1. Genotyping by PCR

The insertion site can be determined precisely with various commercial inverse PCR kits. In the “lucky” event that the vector inserted in a short intron (~1 kb or less), it is possible to genotype

BayGenomics mice by PCR, without knowing the exact site of insertion. The wild-type allele will be detectable by designing a forward primer in the exon immediately upstream to the insertion, and a reverse primer in the exon immediately downstream to the insertion. (Of note, under standard PCR conditions it will be impossible to amplified the mutated allele this way since the vectors used in BayGenomics are at least 8kb long.) The mutated allele will be detected using the same forward primer than above, and by designing a reverse primer in the vector. We have used with success either of the following reverse primers:

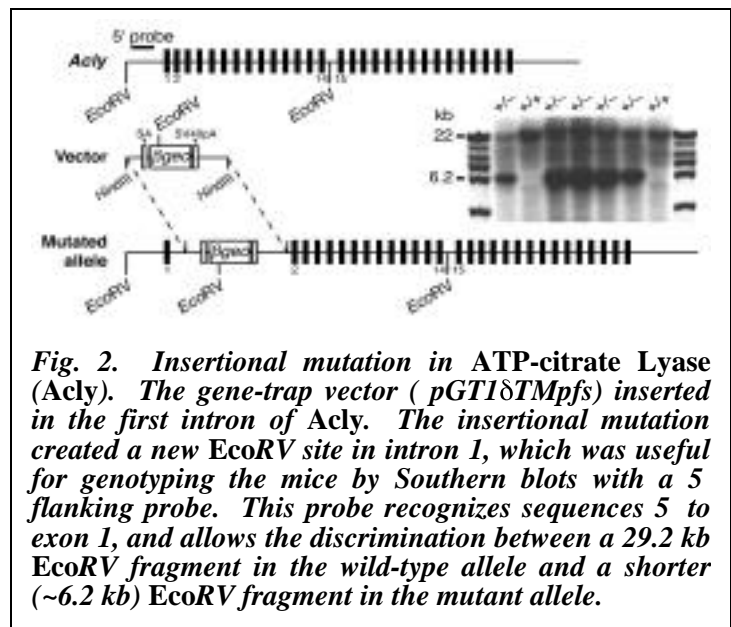
5 -CAC TCC AAC CTC CGC AAA CTC-3

5 -CGT GTC CTA CAA CAC ACA CTC CAA CC-3

The BayGenomics vector sequences are readily accessible from the BayGenomics web site, should you need to design your own reverse primer.

4.2. Genotyping by Southern blot

There are two straightforward ways of genotyping BayGenomics mice by Southern blot. All is needed is the complete sequence of the trapped intron, and the sequence of the vector used to trap your gene-of-interest. One approach is to choose a restriction endonuclease that will cut in the vector but not in the trapped intron.



For example, there is a single *EcoRV* site in the vector backbone. In this case, a probe recognizing sequences 5' to the trapped intron will detect the wild-type allele and the shorter mutated allele (Fig. 2).

Another approach is to cut with an enzyme that cuts once (ideally) in the trapped intron but not in the vector.

For example, there are no *SpeI* sites in the vector backbone. It is necessary to test probes recognizing sequences 5' or 3' to the trapped intron, since it is not possible to know *a priori* whether the vector inserted before or after the restriction site for *SpeI*. In this case, the appropriate probe will detect the wild-type allele and a longer mutant allele.

These strategies will prove difficult to use if:

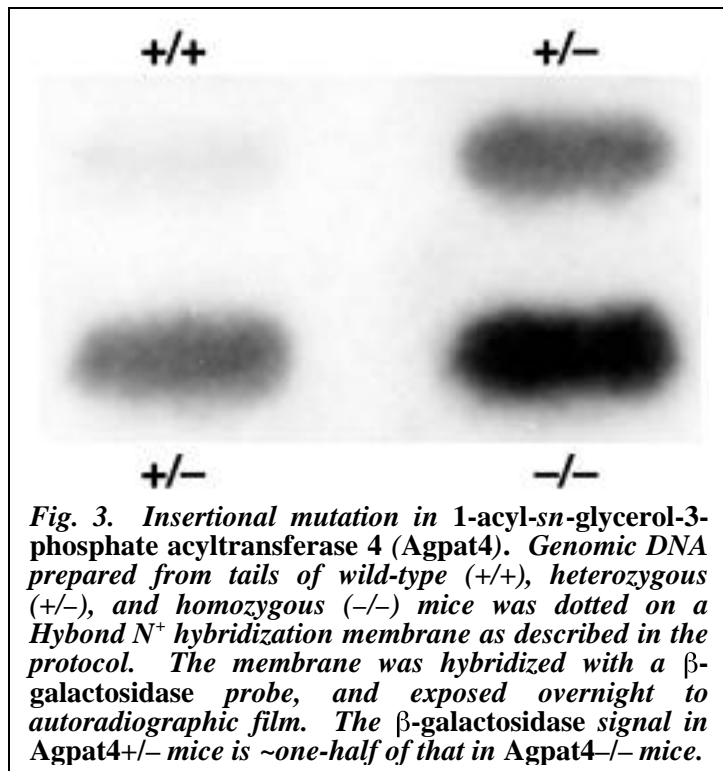
—the complete sequence of the trapped intron is not available in the current databases (very unusual); or

—the vector inserted in a large intron. In this case, the chances of finding an enzyme that will not cut, or cut only once in such intron, are much lower.

4.3. Genotyping by DNA Dot blot

Should genotyping by PCR or Southern not work out, it will *always* be possible to genotype BayGenomics mice with a DNA dot blot. The protocol for this technique is posted on the website at <http://baygenomics.ucsf.edu/protocols/genotyping.html>.

It is essential to follow scrupulously the described protocol, as it has been optimized to ensure that the amount of



DNA loaded on the membrane is saturating. This will allow the discrimination between heterozygous and homozygous knockout mice (Fig. 3).

We have used the following β -galactosidase probe with success:

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TTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGG
AGCGCCGAAATCCCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAG
CAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCC
GTTGCTGATTTCGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGA
TGGTGCAGGATATCCTGCTGATGAAGCAGAACAACCTTAACGCCGTGCGCTGTTTCGCATTATCCGAACCAT
CCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGG
CATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGA
ATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCG
CTAATCACGACGCGCTGTATCGCTGGATCAAATCTGTTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGC
GGAGCCGACACCACGGCCACCGATATTATTTGCCCGATGTACGCGC
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This probe was amplified by PCR, from genomic DNA extracted from BayGenomics mouse tail, with the following primers:

5 -TTA TCG ATG AGC GTG GTG GTT ATG C-3

5 -GCG CGT ACA TCG GGC AAA TAA TAT C-3

Dot blotting constitutes a fast, easy, and sure way of genotyping *any* BayGenomics mouse.

Remember, it is also possible to determine the exact site of insertion of the vector by inverse PCR with commercially available kits. We will soon post our new and improved protocols for inverse PCR.