

## **The construction and analysis of a mouse gene-trap mutant resource created in the C57BL/6N genetic background.**

Here we report the first large-scale use of a C57BL/6 ES cell line in the production of a library of mutagenized ES cell clones for generating knock-out mice. We have used high-throughput gene-trapping with retroviral vectors in mouse C57BL/6N ES cells to generate a library of more than 480,000 mutated ES cell clones. We generated a tractable and unique sequence tag from 73% of the clones that was subjected to an automated inverse genomic PCR-based direct-sequencing protocol. As of today, TIGM resource contains over 270,000 sequence-tagged ES cell clones suitable for producing gene knockouts. Each mutant clone is identified by a genomic sequence tag representing the exact insertion site, allowing accurate prediction of mutagenicity and enabling direct genotyping of mutant alleles. Mutations have been identified in over 10,000 genes and show a bias toward the first gene intron.

Mutant clones demonstrated moderate performance in blastocysts microinjections. The average injection success rate of individual clones of the library was about 64%. On a clone by clone basis, the average germline transmission rate achieved from each of the C57BL/6N clone projects was 43% for coat color transmission.

The trapped ES cell lines, which can be requested from the Texas A&M Institute for Genomic Medicine (TIGM; [www.tigm.org](http://www.tigm.org)), are readily available to the scientific community.

Session, Date & Time:

**Modeling Disease, Wednesday November 5, from 13.45-14.30  
22<sup>nd</sup> Annual Mammalian Genome Conference, Prague, Czech Republic**