TIGM

TEXAS INSTITUTE FOR GENOMIC MEDICINE

BRINGING YOUR RESEARCH TO LIFE

From hypothesis to publication, TIGM has the knockout resources and services to help you reach your research goals.

Knockout Resources

Easy access to more than 620,000 cell lines representing over 13,000 genes

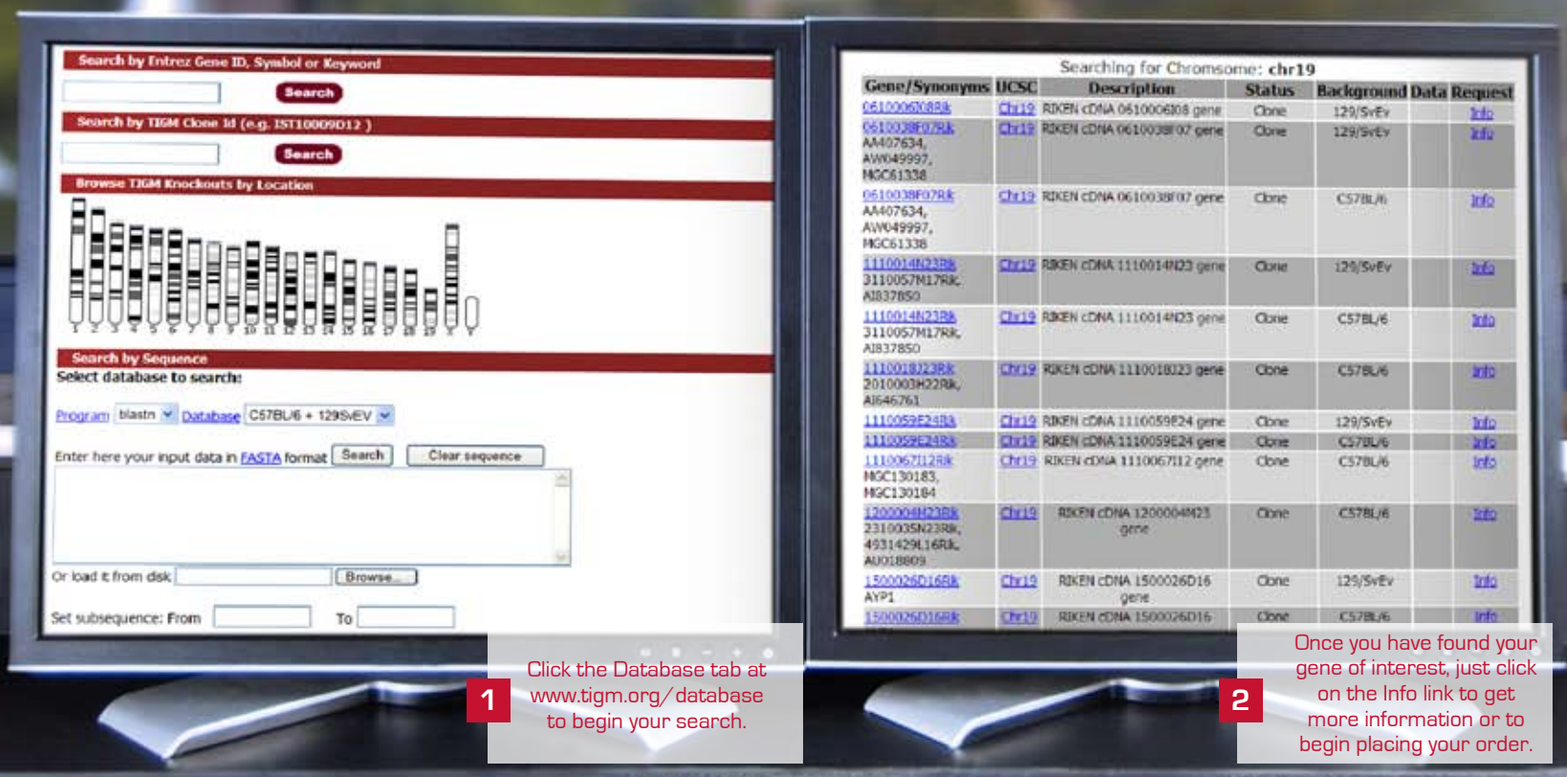
The Texas Institute for Genomic Medicine (TIGM) is an essential resource for academic researchers looking to procure genetically engineered knockout mice and mouse embryonic stem (ES) cells faster and with favorable intellectual property (IP) terms. Our resources include the world's largest gene trap library of ES cells in the C57BL/6 mouse strain and access to the largest library of ES cells in the 129 SvEvBrd mouse strain.

INTELLECTUAL PROPERTY

Mice and ES cell clones are available through a simple material transfer agreement (MTA) which can be reviewed from TIGM's Web site. Researchers are the sole owners of the intellectual property generated through the use of mice and clones obtained from TIGM.

ABOUT TIGM

Created in 2005, TIGM is an independent, non-profit research center that uses advanced technologies to discover breakthroughs in science and medicine. TIGM's mission is to help accelerate the pace of scientific discoveries, pioneer the development of life-changing medical breakthroughs, and advance personalized medicine.



1 Click the Database tab at www.tigm.org/database to begin your search.

2 Once you have found your gene of interest, just click on the Info link to get more information or to begin placing your order.

Online Database From your computer to your bench

TIGM has an extensive, user-friendly database accessible via the Internet. It allows you to search through the C57BL/6 and 129SvEvBrd gene trap libraries and inquire about a specific line.

YOU CAN SEARCH BY:

- Entrez Gene ID
- Keyword
- Sequence in FASTA format
- Gene symbol
- Chromosome

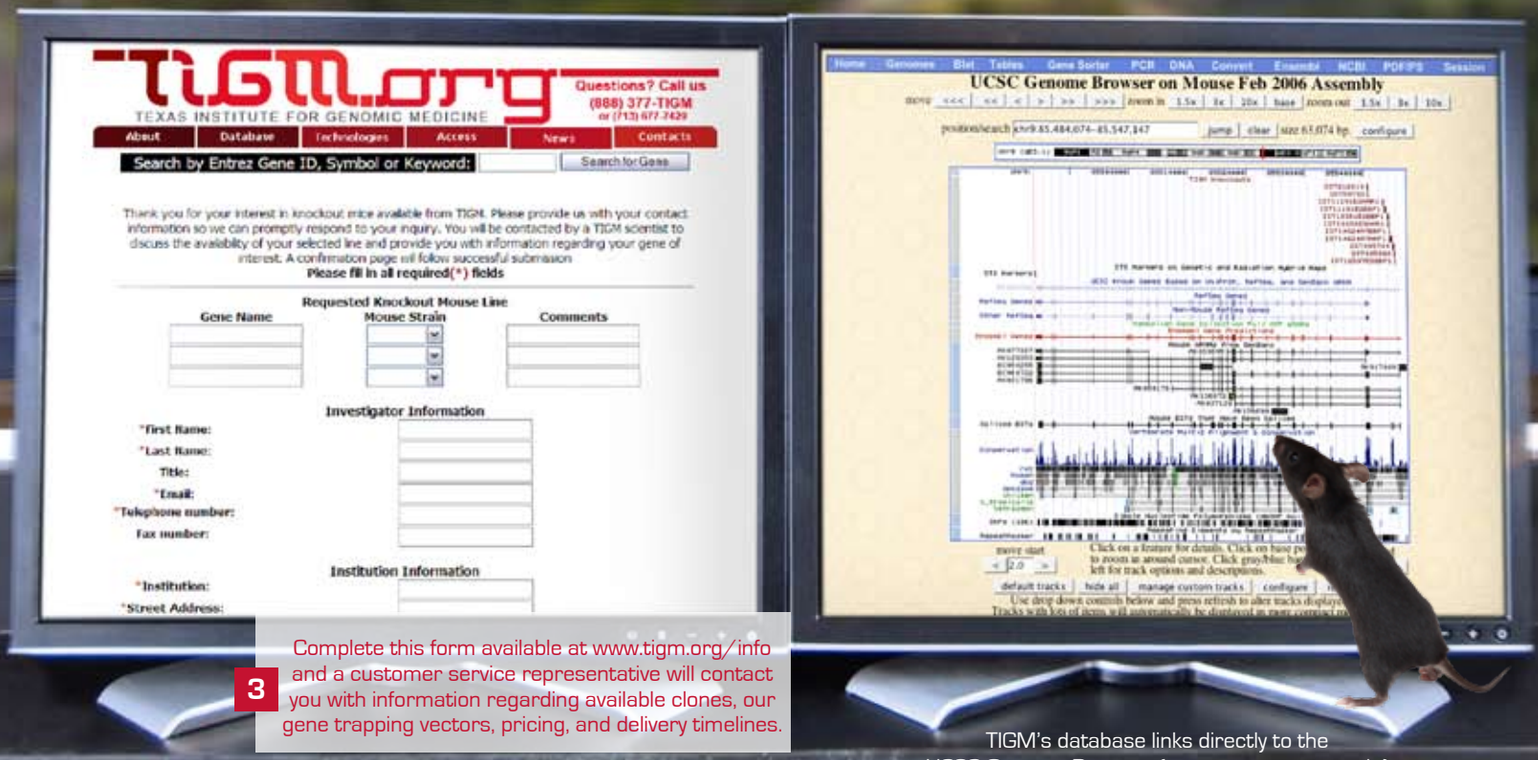
C57BL/6 GENE TRAP LIBRARY

TIGM operates a gene trap library—a premier knockout mouse ES cell resource—that contains approximately 350,000 cell lines in the C57BL/6 mouse background. This library contains mutated ES cell clones representing more than 10,000 genes.

129SvEvBrd GENE TRAP LIBRARY

TIGM has access to a privately held 129SvEvBrd gene trap library. This library contains more than 270,000 sequence-tagged ES cell clones in the 129SvEvBrd mouse strain representing mutations in over 9,000 genes. The National Institutes of Health (NIH) has obtained rights to a subset of these lines, thus allowing TIGM to make them available for distribution to academic researchers on a subsidized basis.

Together, these established resources provide unparalleled coverage of the mouse genome with over 620,000 cell lines representing over 13,000 genes.



3 Complete this form available at www.tigm.org/info and a customer service representative will contact you with information regarding available clones, our gene trapping vectors, pricing, and delivery timelines.

TIGM's database links directly to the UCSC Genome Browser (www.genome.ucsc.edu) and allows you to view the sequence tags generated from our vector insertion sites. Click the TIGM KNOCKOUTS link at the top of the UCSC genome browser window to view all of TIGM's available clones.

To search for your gene of interst, **visit www.tigm.org/database**

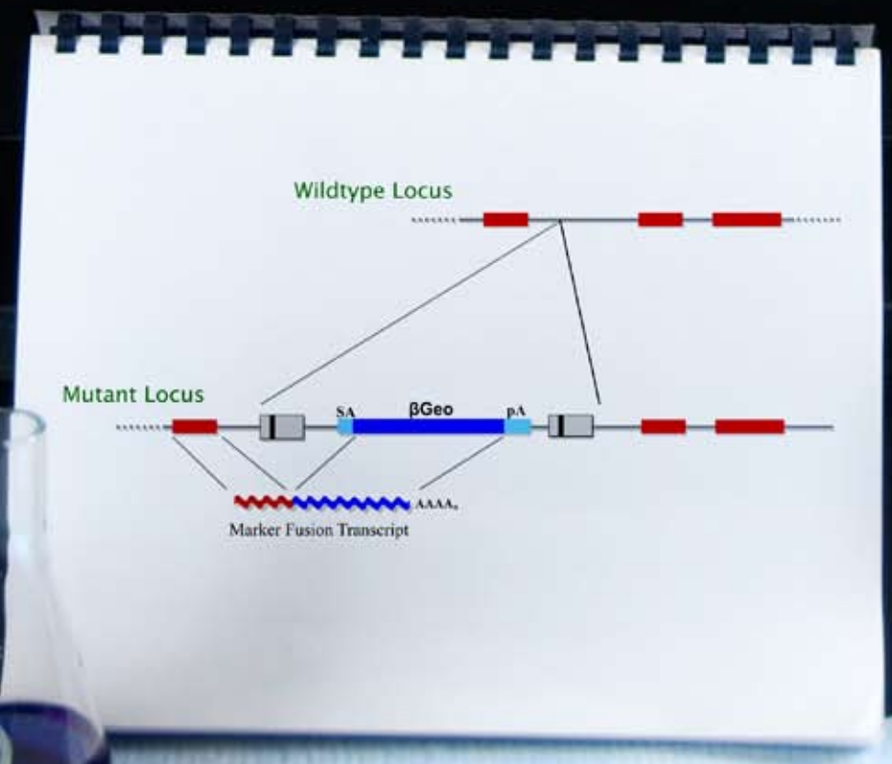
Gene Trap Technology

Access to the world's largest gene trap library

Two ES cell libraries are currently available from TIGM: 129SvEv and C57BL/6. These libraries utilize gene traps for gene inactivation. The retroviral gene trap vectors include a promoterless marker/reporter gene downstream of a splice acceptor sequence. The retrovirus inserts a single copy per locus, with no rearrangement of flanking sequences.

GENE TRAP VECTORS USED IN C57BL/6 LIBRARY*

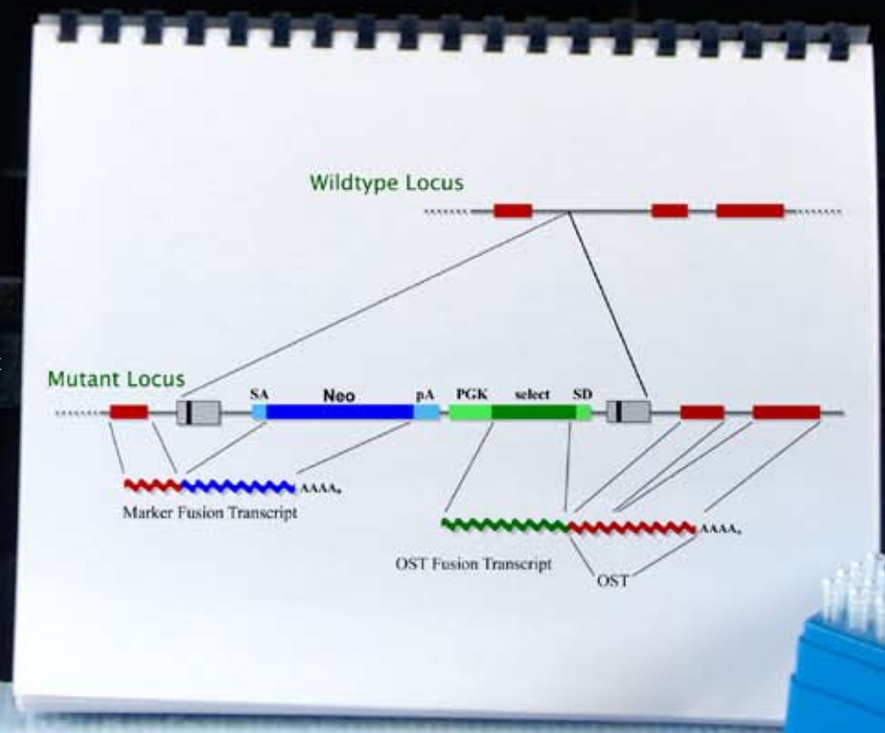
The retroviral vectors used to generate the C57BL/6 library contain a splice acceptor sequence (SA) followed by the 5' selectable marker ß-geo, a functional fusion between the ß-galactosidase and neomycin resistance genes, for identification of successful gene trap events. The ß-geo marker also allows for in vivo expression studies of the trapped gene through in situ hybridization or immunohistochemistry. High-throughput gene-trapping with retroviral vectors has been used in mouse C57BL/6 ES cells to generate a library of approximately 350,000 mutated ES cell clones.



The vectors produce incorrect splicing of the target gene such that all exons downstream of the insertion site are not expressed. Analysis of non-embryonic lethal mouse lines demonstrates that gene-trap insertions within both exons and introns of the gene of interest lead to the disruption of the endogenous mRNA transcript in all cases. Of these, >96% show complete absence of WT message, with the remaining hypomorphic lines showing an average reduction in mRNA levels of 91.6%, as measured by quantitative PCR [1]. These data demonstrate that intragenic insertion efficiently disrupts gene transcription in vivo and can be used to reliably predict mutagenicity before mouse production.

GENE TRAP VECTORS USED IN 129SvEv LIBRARY*

In the 129SvEv library, the retroviral vectors contain a splice acceptor sequence (SA) followed by a promoterless selectable marker Neo with a polyadenylation signal (pA). Insertion of the retroviral vector into an expressed gene leads to the splicing of the endogenous upstream exons into this cassette to generate a fusion transcript. The vectors also contain a promoter that is active in ES cells [such as that of the mouse phosphoglycerate kinase (Pgk) gene] followed by a first exon [such as that of the Bruton's Tyrosine Kinase (Btk) gene] upstream of a splice donor (SD) signal. Splicing from this signal to the exons downstream of the insertion gives rise to a fusion transcript that can be used to generate a sequence tag (OST) of the trapped gene by 3' RACE [2]. High-throughput gene-trapping with retroviral vectors has been used in mouse 129SvEv ES cells to generate a library containing more than 272,000 mutated ES cell clones.



* PGK, phosphoglycerate kinase-1 promoter; SD, splice donor sequence; SA, splice acceptor sequence; Neo, neomycin phosphotransferase gene; ß-geo, galactosidase/neomycin phosphotransferase fusion gene; pA, polyadenylation sequence; Btk, first exon of the murine Bruton's tyrosine kinase gene. References: 1 .Zambrowicz, B.P. et al., 2003. Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 100(24):14109-14114. 2. Zambrowicz, B.P. et al., 1998. Disruption and sequence identification of 2,000 genes in mouse embryonic stem cells. Nature. 392(6676):608-611.

Resources and Services

We create your knockouts so you can concentrate on your research

TIGM goes through a step-by-step process with each order to ensure quality and to make sure you receive what you need for your research.

ES CELL CLONE ORDERS

Quality control procedures for ES cell clones:

- qPCR for Neo to ensure one copy of gene trapping vector is present in the genome
- qPCR for SRY to ensure there is no loss or degradation of the Y chromosome
- iPCR to ensure the gene trapping vector is in the correct location and orientation
- Assays to ensure cultures are free of mycoplasma, fungi and bacteria contamination

For standard ES cell clone orders, TIGM will supply:

- Two vials, each containing approximately $6.0x10^6$ ES cells
- Validated genotyping protocols

KNOCKOUT MOUSE ORDERS

Quality control procedures for mice:

- The clones are QCed as shown above before microinjection
- Tail samples are genotyped with wild type and mutant strategies specific for the insertion site before the animals are shipped out

FOR STANDARD KNOCKOUT MOUSE ORDERS, TIGM WILL SUPPLY:

- Four (4) heterozygous mice, including at least one (1) breeding pair
- Validated genotyping protocols
- Animal health data

TIGM ALSO PROVIDES THE FOLLOWING SERVICES:

- Blastocyst injection
- Pronuclear microinjection
- Rederivation of transgenic lines
- Embryo transfer
- Colony maintenance
- Colony expansion
- Preliminary phenotyping
  - Clinical chemistry assays
  - Pathology assays
  - Histology assays

WANT TO KNOW MORE?

TIGM

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The scientists and technicians at TIGM pride themselves on providing personal service and attention to your needs.