

Publication shows value of Cryopreserved Mutant Mouse Bank, Life Sciences Division

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- “Efficient gene-driven germ-line point mutagenesis of C57BL/6J mice,” E. J. Michaud et al. *BMC Genomics* 6:164 (2005)
- The Cryopreserved Mutant Mouse Bank was shown to be a valuable gene-driven approach for functional annotation of the mammalian genome and for generation of mouse models for humans.

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Efficient gene-driven germ-line point mutagenesis of C57BL/6J mice

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Abstract

Background: Analysis of an allelic series of point mutations in a gene, generated by N-ethyl-N-nitrosourea (ENU) mutagenesis, is a valuable method for discovering the full scope of its biological function. Here we present an efficient gene-driven approach for identifying ENU-induced point mutations in any gene in C57BL/6J mice. The advantage of such an approach is that it allows one to select any gene of interest in the mouse genome and to go directly from DNA sequence to mutant mice.

Results: We produced the Cryopreserved Mutant Mouse Bank (CMMB), which is an archive of DNA, cDNA, tissues, and sperm from 4,000 G₁ male offspring of ENU-treated C57BL/6J males mated to untreated C57BL/6J females. Each mouse in the CMMB carries a large number of random heterozygous point mutations throughout the genome. High-throughput Temperature Gradient Capillary Electrophoresis (TGCE) was employed to perform a 32-Mbp sequence-driven screen for mutations in 38 PCR amplicons from 11 genes in DNA and/or cDNA from the CMMB mice. DNA sequence analysis of heteroduplex-forming amplicons identified by TGCE revealed 22 mutations in 10 genes for an overall mutation frequency of 1 in 1.45 Mbp. All 22 mutations are single base pair substitutions, and nine of them (41%) result in non-conservative amino acid substitutions. Intracytoplasmic sperm injection (ICSI) of cryopreserved spermatozoa into B6D2F1 or C57BL/6J ova was used to recover mutant mice for nine of the mutations to date.

Conclusions: The inbred C57BL/6J CMMB, together with TGCE mutation screening and ICSI for the recovery of mutant mice, represents a valuable gene-driven approach for the functional annotation of the mammalian genome and for the generation of mouse models of human genetic diseases. The ability of ENU to induce mutations that cause various types of changes in proteins will provide additional insights into the functions of mammalian proteins that may not be detectable by knockout mutations.



The Cryopreserved Mutant Mouse Bank (CMMB) is an archive of DNA, cDNA, tissues, and sperm from 4,000 ENU-mutagenized mice. Mutations can be identified in any gene in the genome. Genes of interest are amplified by PCR from the 4,000 DNA or cDNA samples and screened for mutations by Temperature Gradient Capillary Electrophoresis and DNA sequence analysis. Live mice are reconstituted from frozen sperm for examination of gene function.

- The CMMB is being used to annotate the functions of mouse genes that are orthologous to human genes on chromosomes 5, 16, and 19.
- Numerous external users and collaborators benefiting from the CMMB.
- Two new sources of funding at ORNL based on success of CMMB:
 - “Systems Biology of the Mammalian Cilium: A Cellular Organelle Essential for Human Health and Development
ORNL internal LDRD funds
PI: E. J. Michaud
 - “University of Alabama at Birmingham Affiliated Recessive Polycystic Kidney Disease Core Center”
NIH/NIDDK P30 grant
PI: L. M. Guay-Woodford (UAB); Co-PI: E. J. Michaud
- Additional NIH grants with a CMMB component are pending from collaborators