Real Time Identification of Induced Point Mutations that Cause Phenotype

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The unbiased character of random germline mutagenesis has led to many surprises in biology. But historically, it has been a slow process, limited by the speed of genetic mapping. To assign functions to genes far more efficiently, we have developed a fully automated system for genetic mapping. Our approach is to identify all ENU induced mutations in G1 mice, and to genotype the mutated sites in all G3 mice prior to screening for phenotype. Approximately 600 pre-genotyped G3 mice are produced weekly in 20 pedigrees containing 20-50 G3 animals. Each pedigree bears an average of 70 mutations. Hence, about 1,400 mutations are examined in depth for their ability to cause phenotype(s). Screening data from these animals, probing more than 30 independent aspects of immune function, are uploaded to a computer. This triggers a search for linkage using recessive, additive, and dominant models of inheritance. Instances of linkage are immediately accessible for analysis, and in many instances, the causative mutation can be determined instantly, irrespective of novelty. Both qualitative and quantitative phenotypes can be mapped, and the number of phenotypes emanating from a pedigree does not complicate the analysis. As multiple alleles of individual genes accumulate within the growing database of mutations and phenotypes, they are combined into “superpedigrees,” which can give overwhelming evidence of causation, or alternatively, can exonerate loci suspected of participation in a particular biological process. Our system is also capable of detecting complex phenotypes, occasionally observable in pedigrees derived from ENU mutagenized mice. It permits precise estimates of genome saturation. At present writing, 42,838 allelic variants of 15,185 genes have been tested in screens applied to 20,197 mice from 721 pedigrees. The mutation sites have been queried 2,512,078 times in total to detect phenotypic effects. Hundreds of mutations affecting genes with known immunological function have been implicated as causative of phenotype, and hundreds of genes not previously known to be involved in immunity have been implicated as well.