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Rapid targeted genome modification in mice, rats and rabbits

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The rat, rabbit, zebrafish, and pig have long been important experimental models in multiple fields of study. Unlike the mouse, efficient gene targeting in these species has remained a near impossibility with researchers forced to rely on random methods of mutagenesis. The zinc finger nuclease technology is a well-established tool for targeted manipulation of genomes and has been utilized extensively for a broad array of in vitro applications. We have now taken this technology and applied it in vivo. Data will be presented on the creation and characterization of targeted "knockout" mice, rats, and rabbits where key genes have been removed from the genome. Using zinc fingers we have created rat models such as a p53 knockout rat, a suite of drug transporter knockout rats, and a suite of knockout rats that model Parkinson's disease. Furthermore, data will be presented on the addition ("knock-in") of genes, in a targeted manner, into the rodent genomes as well as the creation of conditional knockouts and humanized models.

Homologous recombination-based gene targeting using *Mus musculus* embryonic stem cells has greatly impacted biomedical research. This study presents a powerful new technology for more efficient and less time-consuming gene targeting in mice using embryonic injection of zinc-finger nucleases (ZFNs), which generate site-specific double strand breaks, leading to insertions or deletions via DNA repair by the nonhomologous end joining pathway. Three individual genes, multidrug resistant 1a (*Mdr1a*), jagged 1 (*Jag1*), and notch homolog 3 (*Notch3*), were targeted in FVB/N and C57BL/6 mice. Injection of ZFNs resulted in a range of specific gene deletions, from several nucleotides to >1000 bp in length, among 20–75% of live births. Modified alleles were efficiently transmitted through the germline, and animals homozygous for targeted modifications were obtained in as little as 4 months. In addition, the technology can be adapted to any genetic background, eliminating the need for generations of backcrossing to achieve congenic animals. We also validated the functional disruption of *Mdr1a* and demonstrated that the ZFN-mediated modifications lead to true knockouts. We conclude that ZFN technology is an efficient and convenient alternative to conventional gene targeting and will greatly facilitate the rapid creation of mouse models and functional genomics research.

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