UT Southwestern Medical Center

TITLE: Generation of Knockout Mice Using CRISPR/Cas9 Genome Editing in Spermatogonial Stem Cells
INVENTORS: Douglas Anderson, Eric Olson, Huanyu Zhou
TECHNOLOGY: Biologicals
UTSD: 3049

SUMMARY: This invention is a method to rapidly and easily generate germline mutations in the mouse genome, which can be utilized to generate loss-of-function alleles to study gene function. This approach uses lentiviral transducton or electroporation of plasmid DNA into the testis of a male mouse to deliver Cas9 and guide-RNA(s) to spermatogonial stem cell (SSCs). Direct genome editing in SSCs can result in the production of mature sperm carrying modified alleles. Subsequent mating of the male mouse containing the edited sperm with a wild type female can result in the generation of offspring carrying the modified allele on the paternal chromosome. Further mating and intercrosses can be performed to generate mice homozygous for the modified allele.

If transduction with a lentivirus is used, insertion of the lentiviral genome carrying CRISPR/Cas9 machinery in the paternal DNA can further result in transmission and expression in the fertilized zygote, which can modify the maternal allele to generate homozygous loss-of-function alleles. Thus, this method can allow for the generation of knockout mice in a single generation.

Alternatively, lentiviral transducton or electroporation of plasmids encoding only the CRISPR guide RNA can be performed in male mice globally expressing Cas9 protein (Cas9 expressed from the ROSA26 locus), which could improve transduction efficiency and ease of producing high titer lentivirus. This approach can also be utilized to generate conditional knockout alleles, which is difficult using current CRISPR/Cas9 technologies. Further, the multiplex nature of CRISPR editing (Cas9 can be targeted to many different loci by using a library of unique guide-RNAs) and the ability to transduce a vast number of individual spermatogonial stem cells in a single testis, allows for a single male mouse to generate a vast number of uniquely edited sperm. Thus, mating of the edited mouse with multiple wild type female mice can result in the generation of a library of uniquely modified offspring in a single step.

Please contact the Office for Technology Development for more details:

Phone: 214-648-1888
Email: TechnologyDevelopment@utsouthwestern.edu

Please reference UT Southwestern Case Number: **3049**