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Knockin Rat Model Production by Germline Gene Targeting in Sperm Stem Cells

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Rat models provide critical infrastructure for biomedical research aimed at elucidating cellular and molecular mechanisms that impact drug addiction. We previously reported efficient knockout rat model production by CRISPR/Cas9-mediated germline gene targeting in donor spermatogonial stem cells (i.e. sperm stem cells) *via* the non-homologous end-joining DNA repair pathway. Here, we demonstrate efficient CRISPR/Cas9-mediated homology-directed DNA repair (HDR) within fully functional spermatogonial stem cell lines by targeted insertion of a transgene construct (~12 kb knockin) into the *Rosa26* locus of Brown Norway, Sprague Dawley, Long Evans and Fischer 344 rats. Given high success rates at targeting the rat's *Rosa26* locus by HDR in spermatogonia, we proceeded to conduct studies to verify the effectiveness of our spermatogonial gene manipulation protocols for making gene-specific modifications to a wider assortment of target alleles within the rat's germline by HDR. We consistently observed precisely targeted insertion of relatively small (1 bp knockin) or relatively large (2.5, 4.6, 4.8, 5.3, 5.6 kb knockins) sequence modifications in 6 distinct protein-coding genes within Brown Norway (n=3 targeting constructs) and Sprague Dawley (n=3 targeting constructs) spermatogonial lines. Sequence verified targeted germline modifications were effectively transmitted from donor spermatogonia to F1 rat progeny by breeding recipient males (sterile-testis complementation) with wildtype females to generate novel knockin rat model strains. Thus, we demonstrate robust experimental tractability of donor spermatogonial lines for vertically transmitting precisely targeted genomic modifications made by HDR directly within the rat's germline DNA.