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Genetically engineered models (GEMS)

Sord Knock-out Rat

MODEL	Sord Knock-out Rat
STRAIN	NTac:SD-Sord^{em1Env}
LOCATION	U.S.
AVAILABILITY	Cryopreserved
NCBI GENE ID	24788
UPSTREAM CRISPR SEQUENCE	ttgtgtgtttactggtagtaGGG
DOWNSTREAM CRISPR SEQUENCE	gctccccttctcggctgttaAGG

CHARACTERISTICS/HUSBANDRY

- Deletion of 17kb region of rat Sord gene encompassing coding regions of all isoforms
- This multiple exon deletion strategy eliminates the possibility that small fragments of the gene would still lead to expression of N- or C-terminally truncated protein fragments
- Sorbitol dehydrogenase protein is absent in the brain, spinal cord and sciatic nerves of homozygous rats as determined via Western Blot[1]
- Background strain – Sprague Dawley

ZYGOSITY GENOTYPE

Will be recovered from frozen as homozygous breeders

RESEARCH USE

- Charcot-Marie-Tooth disease
- SORD deficiency
- Axonal degeneration
- Ballooned myelin sheaths
- Hereditary motor peripheral neuropathy
- Sorbitol dehydrogenase

ORIGIN

This Sord KO rat was developed for the Hereditary Neuropathy Foundation at Inotiv's genetic engineering laboratory in St. Louis, MO. This line continues to be maintained by Inotiv as a cryopreserved offering.

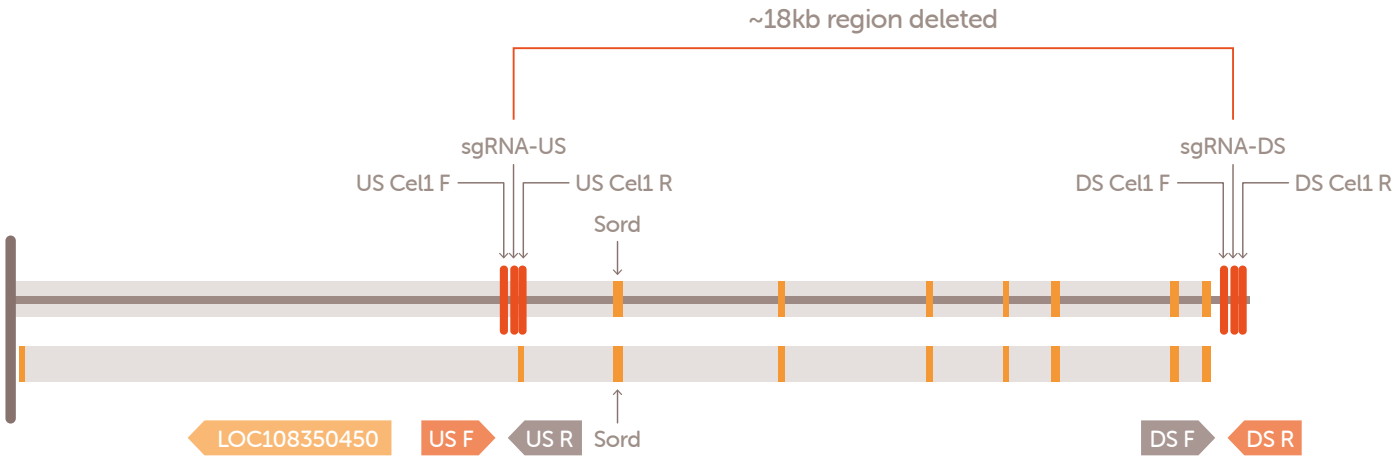
DESCRIPTION

This model carries a 17kb deletion of the Sord rat gene on Chromosome 3. Biallelic Sord mutations in humans leads to the most common form of recessive hereditary neuropathy. Loss of expression in this gene results in high sorbitol accumulation in cells and serum.[1]

[1] *Rebelo AP, Abad C, Dohrn MF, Li JJ, Tieu EK, Medina J, Yanick C, Huang J, Zotter B, Young JI, Saporta M, Scherer SS, Walz K, Zuchner S. SORD-deficient rats develop a motor-predominant peripheral neuropathy unveiling novel pathophysiological insights. Brain. 2024 Sep 3;147(9):3131-3143. doi: 10.1093/brain/awae079. PMID: 38538210.*

GENOTYPING SCHEMATIC

Two CRISPR sgRNAs were designed to cleave together in order to generate a ~18kb deletion between the target sites. Primers flanking each sgRNA site were designed to test individual NHEJ activity, as well as paired together with (red arrows) to screen for deletion mutations between the two target sites.



GENOTYPING PROTOCOL

Upstream Wildtype Allele detection
US F: 5'- GTGGTGGTGTGTTGAGCAAT
US R: 5'- AGGCCAACACCAGCAGATAC
Expected band size: 477bp

Downstream Wildtype Allele detection
DS F: 5'- GCTCTGCCTCAGCTCAGAAT
DS R: 5'- ACTGCAGCCCAAGTTACACC
Expected band size: 415bp

Large Deletion between sgRNAs
US F: 5'- GTGGTGGTGTGTTGAGCAAT
DS R: 5'- ACTGCAGCCCAAGTTACACC
Expected band size: ~399bp

**Typically use 1 minute extension time per 1kb of sequence.*
DNA template was extracted using Epicentre® QuickExtract™ solution.
PCR products were resolved on a 1% agarose gel.

PCR REACTION SETUP

Reaction Components	Volume (µl)	Final Concentration
DNA template	1	--
10 µM Forward Primer	5	1 µM
10 µM Reverse Primer	5	1 µM
Hot-Start Go Taq® (M5 122)	25	1X
ddH_2O	14	--
	50 µl reaction	

PCR REACTION SETUP

Cycle Step	Temp (°C)	Time	
1	95	2 min	
2	95	45s	
3	55	1 min	X35 cycles
4	72	2 min	
5	72	5 min	
6	4	∞	