Akr1b3 Cas9-CKO Strategy

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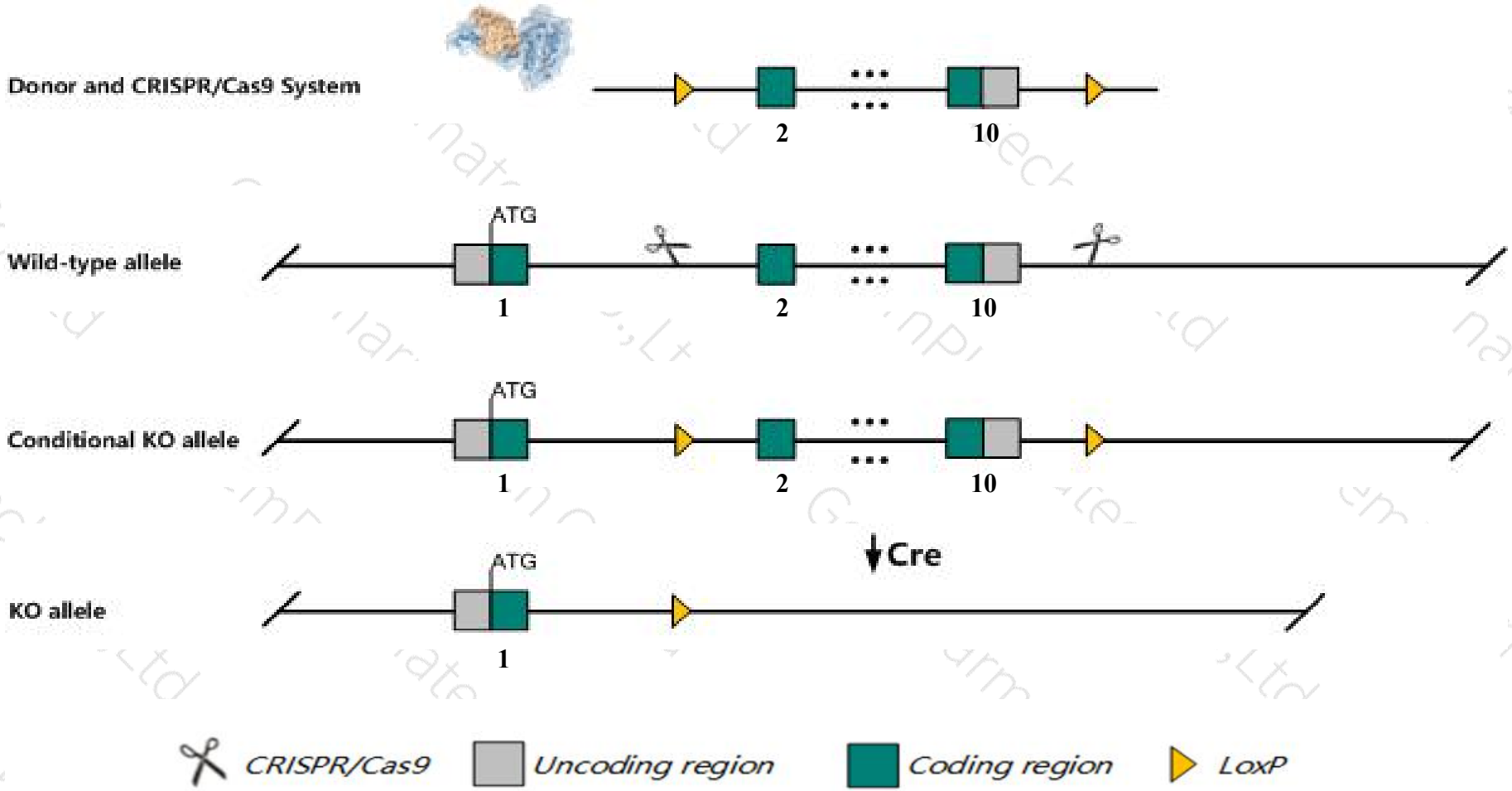
Project Overview

Project Name	***Akr1b3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Akr1b3* gene. The schematic diagram is as follows:



Technical routes

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- The *Akr1b3* gene has 6 transcripts. According to the structure of *Akr1b3* gene, exon2-exon10 of *Akr1b3-201* (ENSMUST00000102980.10) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Akr1b3* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ According to the existing MGI data,Homozygous mutation of this gene results in increased drinking, increased urination, and dilation of the renal tubules.

➢This strategy knocks out the target gene and knocks out part of the intron of the *Gm14546*.

➢ The *Akr1b3* gene is located on the Chr6. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

➢ This Strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Akr1b3 aldo-keto reductase family 1, member B3 (aldose reductase) [Mus musculus (house mouse)]

Gene ID: 11677, updated on 3-Feb-2019

Summary

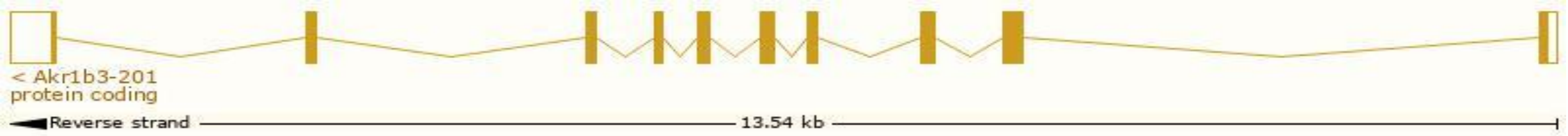
Official Symbol	Akr1b3 provided by MGI
Official Full Name	aldo-keto reductase family 1, member B3 (aldose reductase) provided by MGI
Primary source	MGI:MGI:1353494
See related	Ensembl:ENSMUSG00000001642
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	ALR2, AR, Ahr-1, Ahr1, Akr1b1, Aldor1, Aldr1
Expression	Broad expression in bladder adult (RPKM 290.1), heart adult (RPKM 93.4) and 24 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

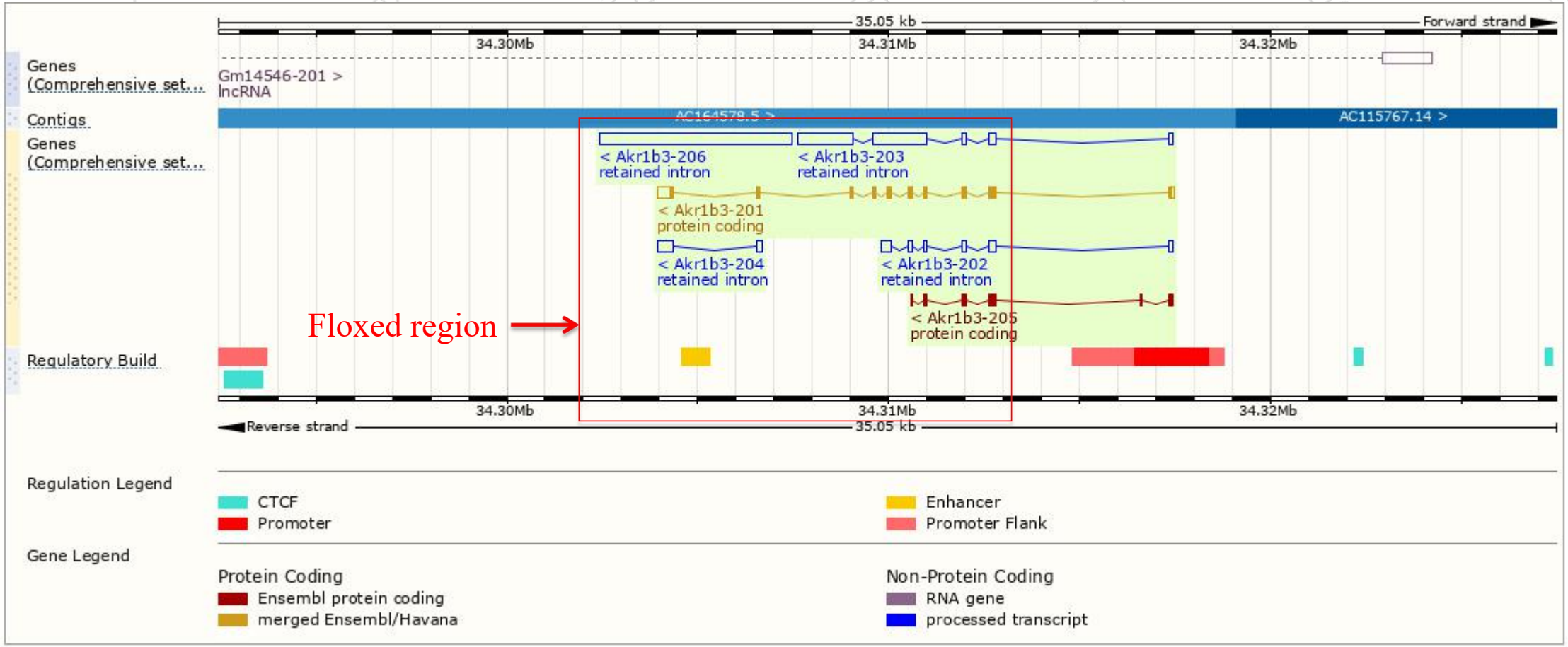
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Akr1b3-201	ENSMUST00000102980.10	1387	316aa	Protein coding	CCDS19990	P45376 Q3UDY1	TSL:1 GENCODE basic APPRIS P1
Akr1b3-205	ENSMUST00000154655.1	583	176aa	Protein coding	-	D3YVJ7	CDS 3' incomplete TSL:5
Akr1b3-206	ENSMUST00000201392.1	5024	No protein	Retained intron	-	-	TSL:NA
Akr1b3-203	ENSMUST00000136559.7	3254	No protein	Retained intron	-	-	TSL:2
Akr1b3-202	ENSMUST00000126991.1	845	No protein	Retained intron	-	-	TSL:3
Akr1b3-204	ENSMUST00000142761.1	554	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Akr1b3-201* transcript,The transcription is shown below

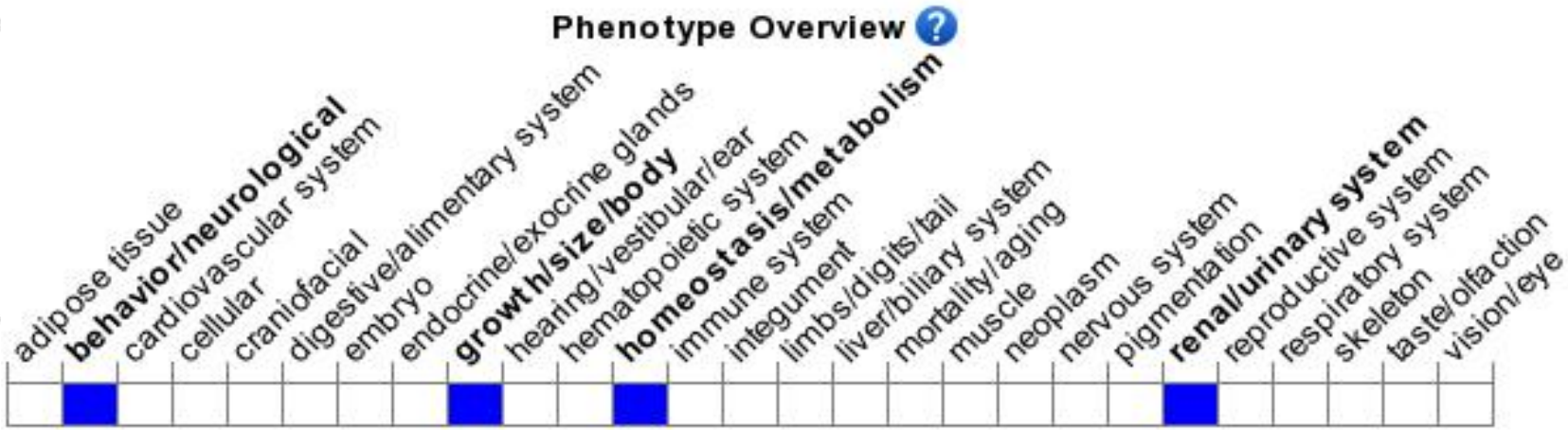


Genomic location distribution



Protein domain

Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,Homozygous mutation of this gene results in increased drinking, increased urination, and dilation of the renal tubules.

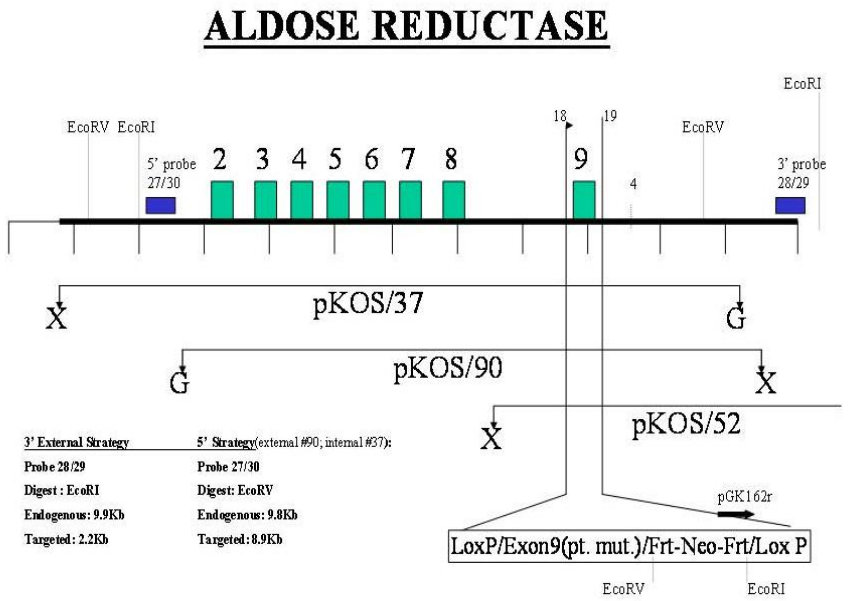
Existing model(MGI)

Export: Text File Excel File

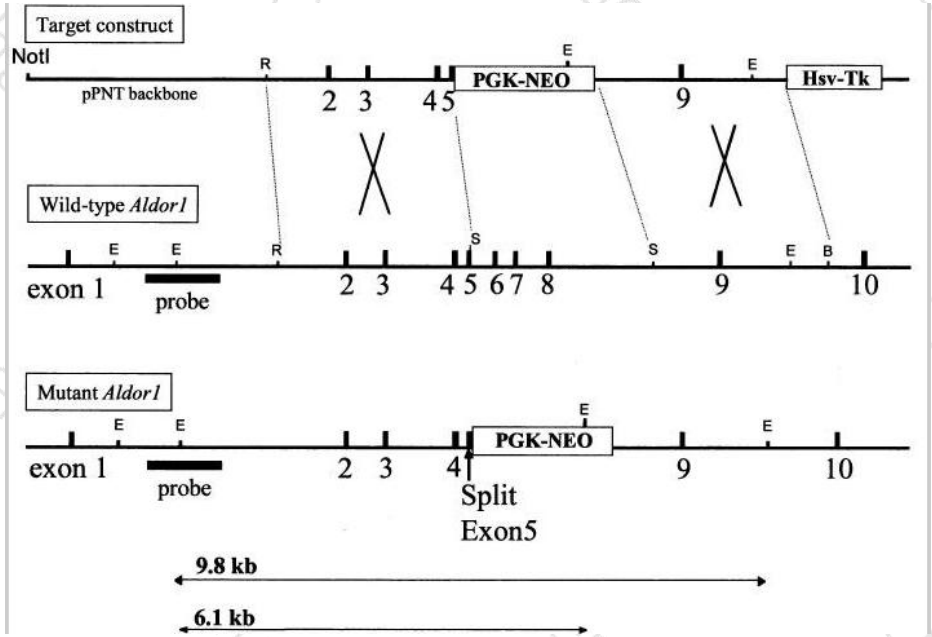
Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems	Human Disease Models
Akr1b3$^{tm1.1Kgab}$ aldo-keto reductase family 1, member B3 (aldose reductase); targeted mutation 1.1, Kenneth H Gabbay	6	AR^{-}	Targeted (Null/knockout)	homeostasis	
Akr1b3^{tm1Lex} aldo-keto reductase family 1, member B3 (aldose reductase); targeted mutation 1, Lexicon Genetics	6		Targeted (Null/knockout)		
Akr1b3^{tm1Skc} aldo-keto reductase family 1, member B3 (aldose reductase); targeted mutation 1, Sookja K Chung	6	Aldor1^{-}, AR^{-}, AR-KO	Targeted (Null/knockout)	behavior, homeostasis, renal/urinary	
Akr1b3^{tm1Tona} aldo-keto reductase family 1, member B3 (aldose reductase); targeted mutation 1, Toshimasa Onaya	6	Akr1b1^{-}	Targeted (Null/knockout)	growth/size/body, homeostasis, renal/urinary	
Akr1b3$^{tm1(KOMP)Wtsi}$ aldo-keto reductase family 1, member B3 (aldose reductase); targeted mutation 1, Wellcome Trust Sanger Institute	6		Targeted (Null/knockout, Reporter) **(Cell Line)**		
Akr1b3$^{tm1a(EUCOMM)Hmgu}$ aldo-keto reductase family 1, member B3 (aldose reductase); targeted mutation 1a, Helmholtz Zentrum Muenchen GmbH	6		Targeted (Conditional ready, Null/knockout, Reporter) **(Cell Line)**		
Akr1b3$^{tm1e(EUCOMM)Hmgu}$ aldo-keto reductase family 1, member B3 (aldose reductase); targeted mutation 1e, Helmholtz Zentrum Muenchen GmbH	6		Targeted (Null/knockout, Reporter) **(Cell Line)**		

http://www.informatics.jax.org/allele/summary?markerId=MGI:1353494&alleleType=Targeted

References



Generation of a Mouse Aldose Reductase Knock-out An aldose reductase (*Akr1b*) knock-out mouse was separately generated in the course of developing a knock-in mouse with enhanced aldose reductase activity.[4] The mutations (V280L and C298V) are in exon 9, which was flanked with LoxP sites. The LoxP sites allowed the entire exon 9 to be excised with disruption of the gene when the homozygote animals were bred with a homozygous Cre1 recombinase mouse (obtained from Dr. Heiner Westphal, National Institutes of Health). The resulting $AR^{-/-}$ knock-out mouse strain was repeatedly bred with C57BL/6J mice to generate an F10 backcross with a uniform C57BL/6J background. An ARKO/GRKO double knock-out model was generated by cross-breeding the two F10 backcross knock-out strains. The generation schemata are shown in supplemental Fig. S2.



The *Aldor1* gene has been previously cloned and characterized (14). The *Aldor1* gene was disrupted in ES cells by homologous recombination with the transforming DNA containing the *Aldor1* gene where exons 5, 6, 7, and 8 were replaced by the neomycin-resistant gene (Fig. 1a). Two independent $Aldor1^{-/-}$ ES cell lines were established from which two *ALR2*-deficient mouse lines (ARD1 and ARD2) were generated. Breeding of heterozygous founder mice produced wild-type heterozygous and homozygous progeny, as determined by Southern blot analysis (Fig. 1b), at a ratio consistent with the 1:2:1 Mendelian inheritance. Heterozygous ($Aldor1^{+/-}$) and homozygous ($Aldor1^{-/-}$) ALR2-deficient mice were normal in appearance, and their body weights were comparable to those of their wild-type littermates. Northern and Western blot analysis showed, respectively, that ALR2 mRNA and protein are absent in $Aldor1^{-/-}$ mice and reduced in $Aldor1^{+/-}$ mice to about half of that found in the wild type (Fig. 1c and d). $Aldor1^{-/-}$ mice have no sorbitol in their kidneys (data shown below), the only tissue where sorbitol is detectable in normal mice. These results indicate that $Aldor1^{-/-}$ mice are indeed deficient in ALR2 and that ALR2 is the major enzyme responsible for the synthesis of sorbitol in the kidney.

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If you have any questions, you are welcome to inquire.

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