

Recql4 Cas9-KO Strategy

Designer: Xingkai Xiao

Reviewer: Xiangli Bian

Design Date: 2024-4-8

Overview

Target Gene Name

- *Recql4*

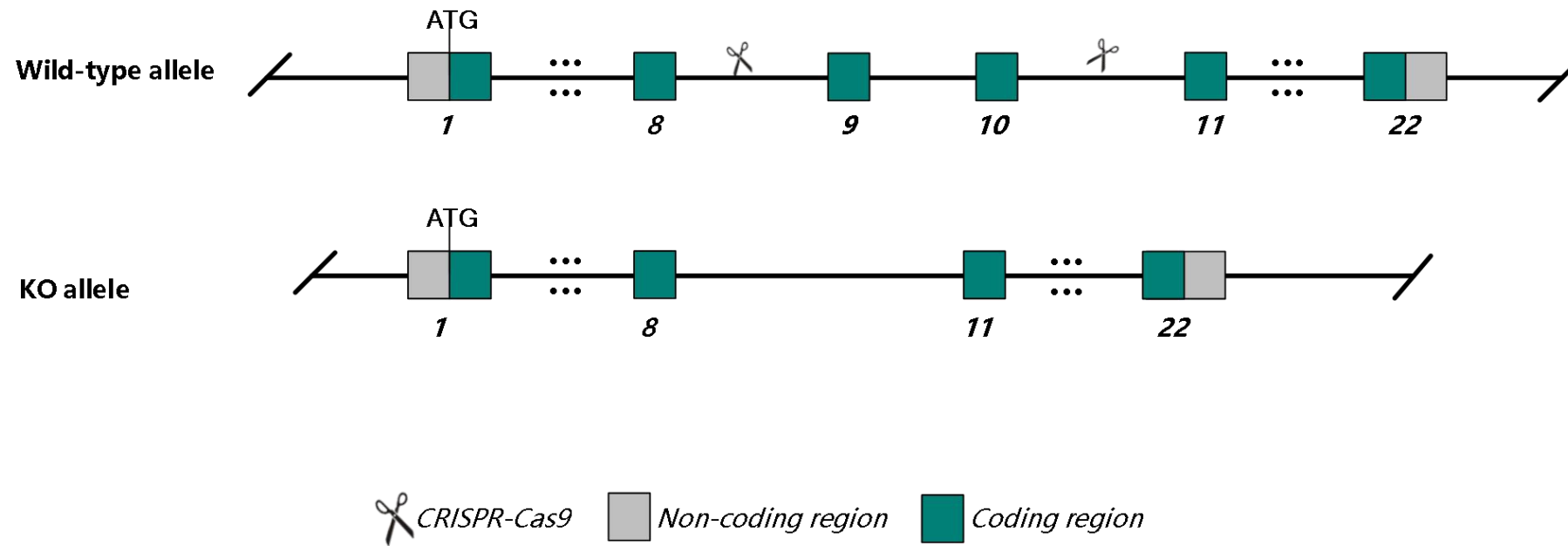
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy

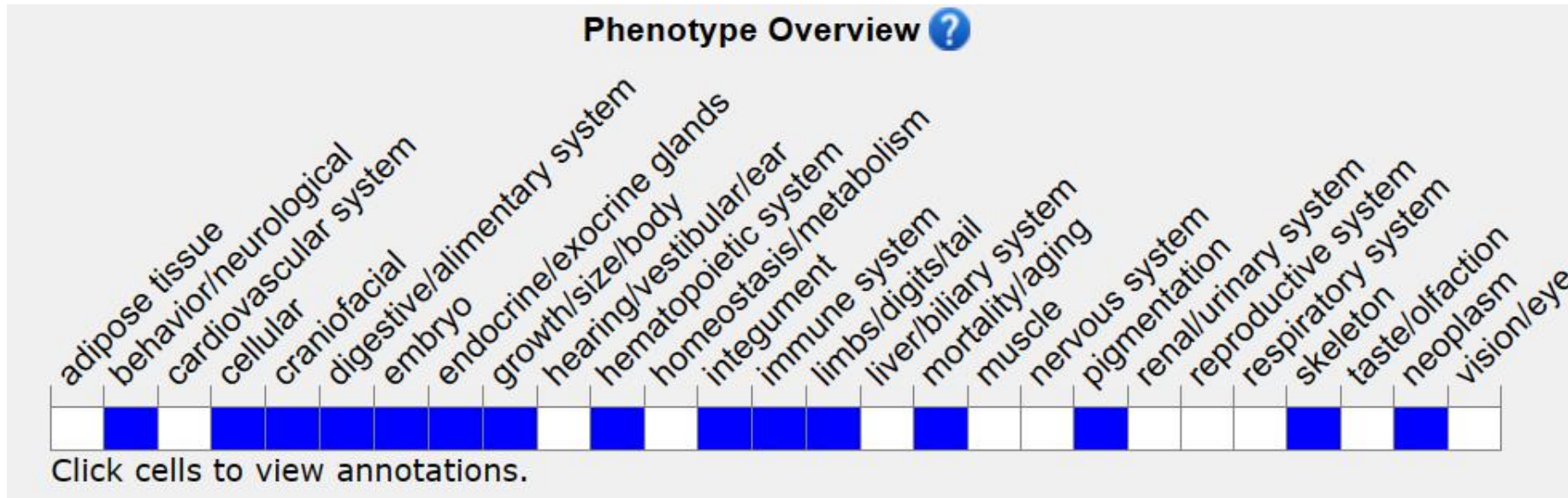


Schematic representation of CRISPR-Cas9 engineering used to edit the *Recql4* gene.

Technical Information

- The *Recql4* gene has 4 transcripts. According to the structure of *Recql4* gene, exon 9-10 of *Recql4*-201 (ENSMUST00000036852.9) is recommended as the knockout region. The knockout region contains 230bp of coding sequence. Knocking out the region may result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Recql4* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

MGI Information



Homozygous loss of exons 5-8 causes embryonic death. Deletion of exon 13 causes neo- and postnatal lethality, stunted growth, skin, hair and bone defects, tissue hypoplasia and tooth dysgenesis. Mice lacking exons 9-13 show palate and limb defects, aneuploidy, poikiloderma and cancer predisposition.

<https://www.informatics.jax.org/marker/MGI:1931028>

Gene Information

Recql4 RecQ protein-like 4 [*Mus musculus* (house mouse)]

[Download Datasets](#)

Gene ID: 79456, updated on 3-Apr-2024

Summary

Official Symbol	Recql4 provided by MGI
Official Full Name	RecQ protein-like 4 provided by MGI
Primary source	MGI:MGI:1931028
See related	Ensembl:ENSMUSG00000033762 AllianceGenome:MGI:1931028
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	RecQ4
Summary	Predicted to enable DNA binding activity and DNA helicase activity. Acts upstream of or within several processes, including negative regulation of sister chromatid cohesion; positive regulation of cell population proliferation; and skeletal system morphogenesis. Predicted to be located in chromosome, telomeric region. Predicted to be active in chromosome; cytoplasm; and nucleus. Is expressed in several structures, including Harderian gland; alimentary system; immune system; male reproductive gland or organ; and nervous system. Used to study Rothmund-Thomson syndrome. Human ortholog(s) of this gene implicated in Baller-Gerold syndrome; Rothmund-Thomson syndrome; and rapadilino syndrome. Orthologous to human RECQL4 (RecQ like helicase 4). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in liver E14.5 (RPKM 9.5), liver E14 (RPKM 8.4) and 28 other tissues See more
Orthologs	human all
NEW	Try the new Gene table Try the new Transcript table

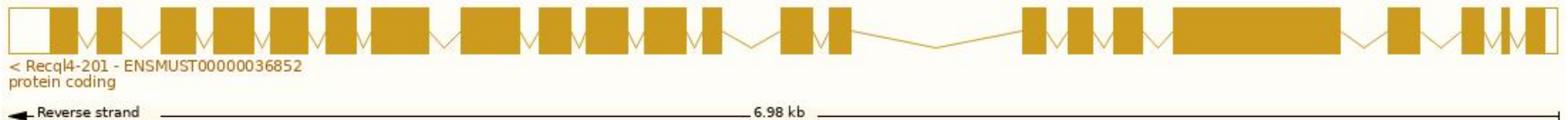
<https://www.ncbi.nlm.nih.gov/gene/79456>

Transcript Information

The gene has 4 transcripts, the transcript are shown below:

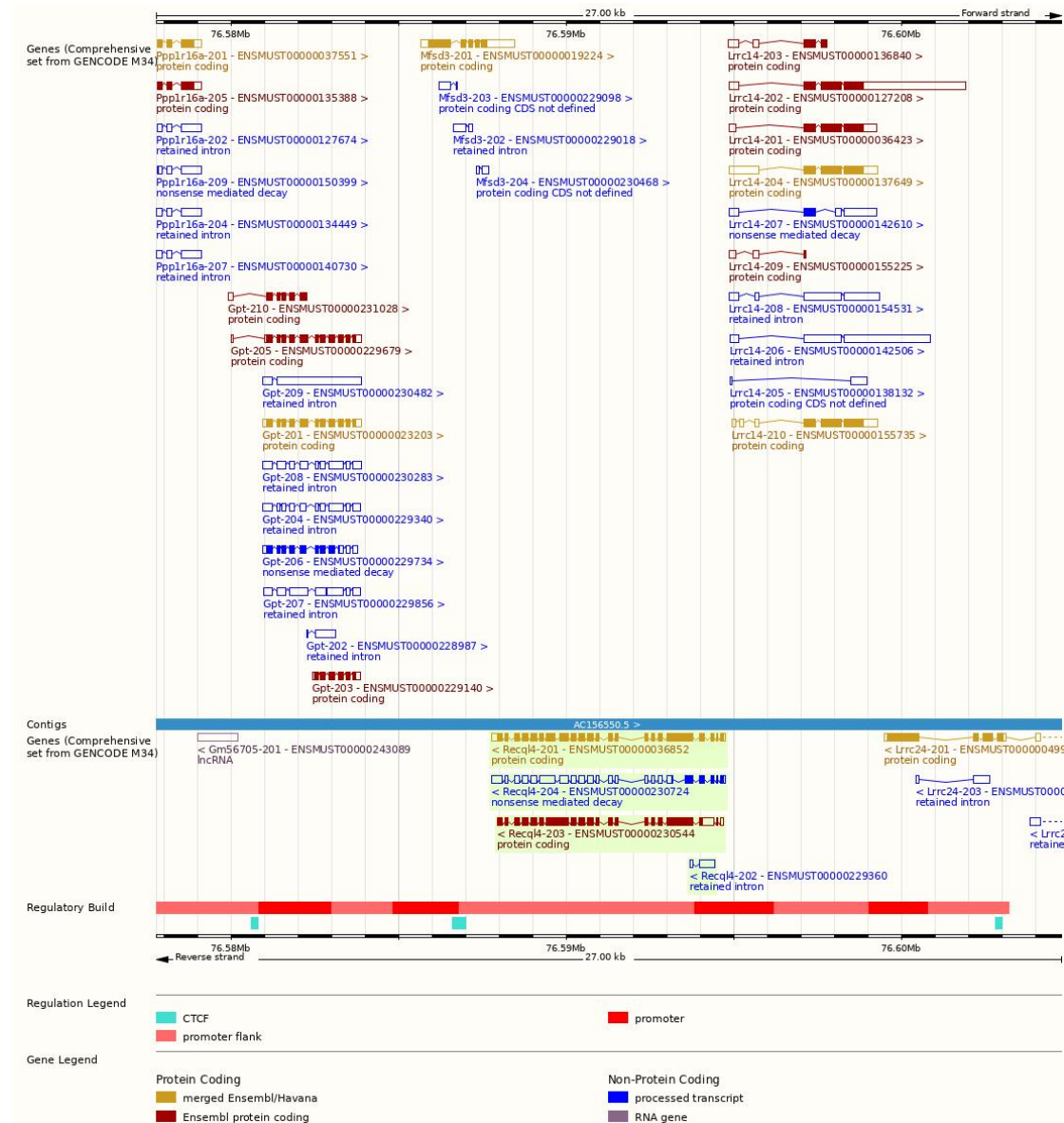
Show/hide columns (1 hidden)							Filter	
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags	
ENSMUST00000036852.9	Recql4-201	3896	1216aa	Protein coding	CCDS27588	A0A0R4J0J3	Ensembl Canonical	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000230544.2	Recql4-203	3984	1173aa	Protein coding		A0A2R8W710	GENCODE basic	
ENSMUST00000230724.2	Recql4-204	3592	195aa	Nonsense mediated decay		A0A2R8W6H5	-	
ENSMUST00000229360.2	Recql4-202	503	No protein	Retained intron		-	-	

The strategy is based on the design of *Recql4*-201 transcript, the transcription is shown below:



Source: <http://asia.ensembl.org/>

Genomic Information



Protein Information

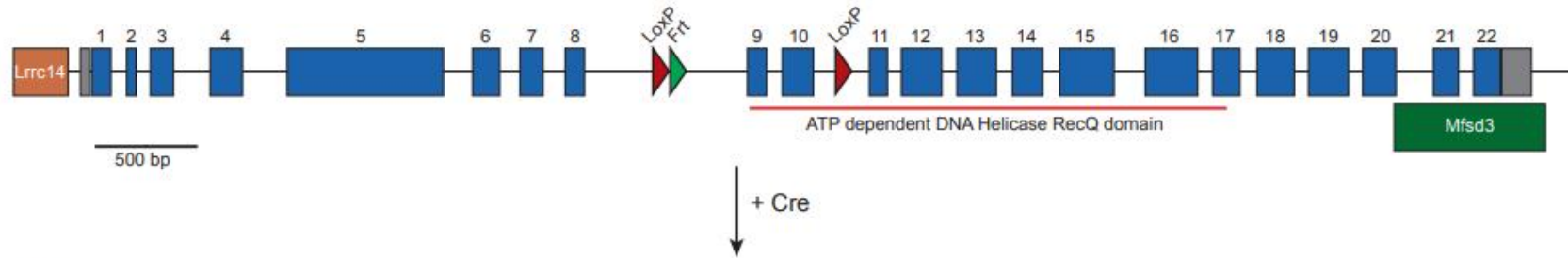


Important Information

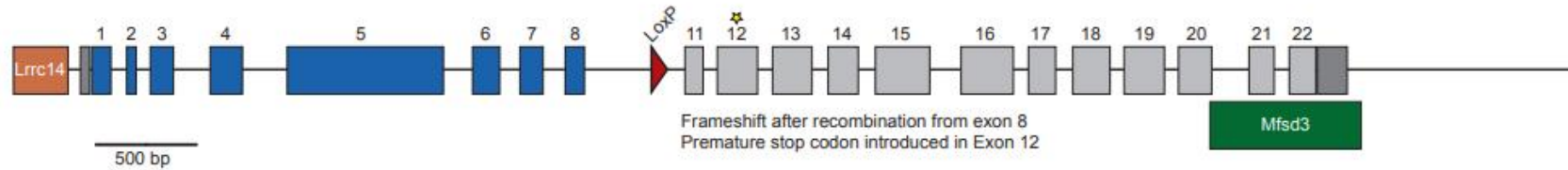
- Homozygous loss of exons 9-10 causes embryonic death^[1].
- The 5' of *Recql4* will retain 480 aa, the risk is unknown.
- The knockout region is 2.7kb away from 3' of *Mfsd3*, which may affect the regulation of *Mfsd3*.
- The knockout region is 2.6kb away from 5' of *Lrrc14*, which may affect the regulation of *Lrrc14*.
- *Recql4* is located on Chr 15. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Reference

B. *Recql4* Conditional Allele (C57BL/6-*Recql4*^{tm2272Arte})



C. *Recql4* Recombined KO allele



[1]The Rothmund-Thomson syndrome helicase RECQL4 is essential for hematopoiesis. J Clin Invest. 2014 Aug;124(8):3551-65. DOI: 10.1172/JCI75334