

Rb1 Cas9-CKO Strategy Rohalanakoch Co.

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Project Overview



Project Name Rb1

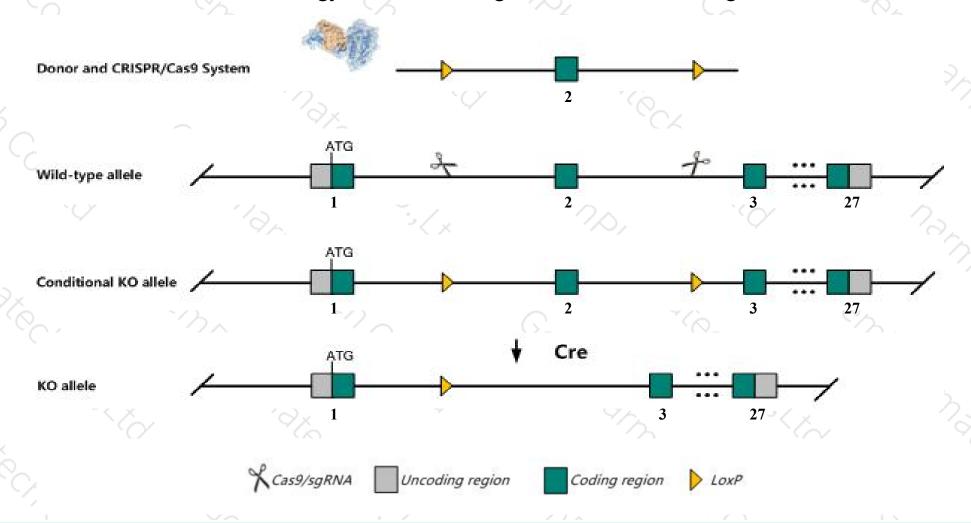
Project type Cas9-CKO

Strain background C57BL/6J

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Rb1* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Rb1* gene has 6 transcripts. According to the structure of *Rb1* gene, exon2 of *Rb1-201*(ENSMUST00000022701.6) transcript is recommended as the knockout region. The region contains 127bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Rb1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.
- The flox mice was 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, Homozygotes for targeted mutations exhibit abnormalities of the neuronal and hematopoietic systems and die in utero. Heterozygotes may develop pituitary tumors associated with loss of the normal allele.
- ➤ The *Rb1* gene is located on the Chr14. 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)



Rb1 RB transcriptional corepressor 1 [Mus musculus (house mouse)]

Gene ID: 19645, updated on 29-Oct-2019

Summary

☆ ?

Official Symbol Rb1 provided by MGI

Official Full Name RB transcriptional corepressor 1 provided by MGI

Primary source MGI:MGI:97874

See related Ensembl: ENSMUSG00000022105

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 Rb; pRb; Rb-1; pp105

Expression Ubiquitous expression in liver E14 (RPKM 8.6), whole brain E14.5 (RPKM 8.3) and 24 other tissues See more

Orthologs human all

Genomic context



Location: 14 38.73 cM; 14 D3

See Rb1 in Genome Data Viewer

Exon count: 28

Annotation release	Status	Assembly	Chr	Location	
108	current	GRCm38.p6 (GCF_000001635.26)	14	NC_000080.6 (7319285873325951, complement)	
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	14	NC_000080.5 (7359530973725598, complement)	

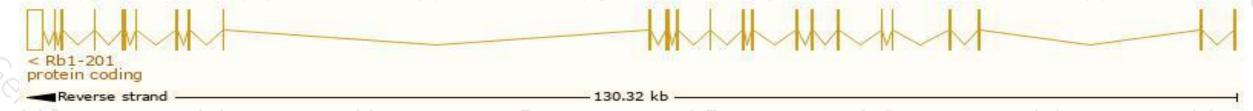
Transcript information (Ensembl)



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

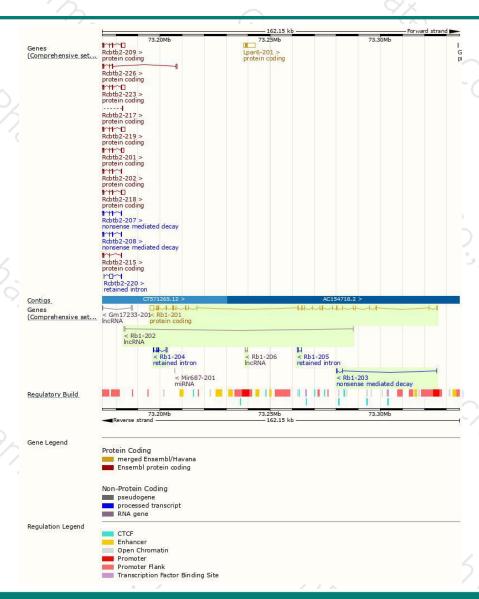
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rb1-201	ENSMUST00000022701.6	4656	<u>921aa</u>	Protein coding	CCDS27267	P13405	TSL:1 GENCODE basic APPRIS P1
Rb1-203	ENSMUST00000164624.1	324	<u>47aa</u>	Nonsense mediated decay	19 1	E9Q5C5	TSL:5
Rb1-204	ENSMUST00000168495.1	946	No protein	Retained intron	N L	-	TSL:2
Rb1-205	ENSMUST00000169002.1	355	No protein	Retained intron		20	TSL:3
Rb1-202	ENSMUST00000163932.1	411	No protein	IncRNA	1.5	-	TSL:5
Rb1-206	ENSMUST00000170967.1	408	No protein	IncRNA	8-	18	TSL:5

The strategy is based on the design of *Rb1-201* transcript, The transcription is shown below



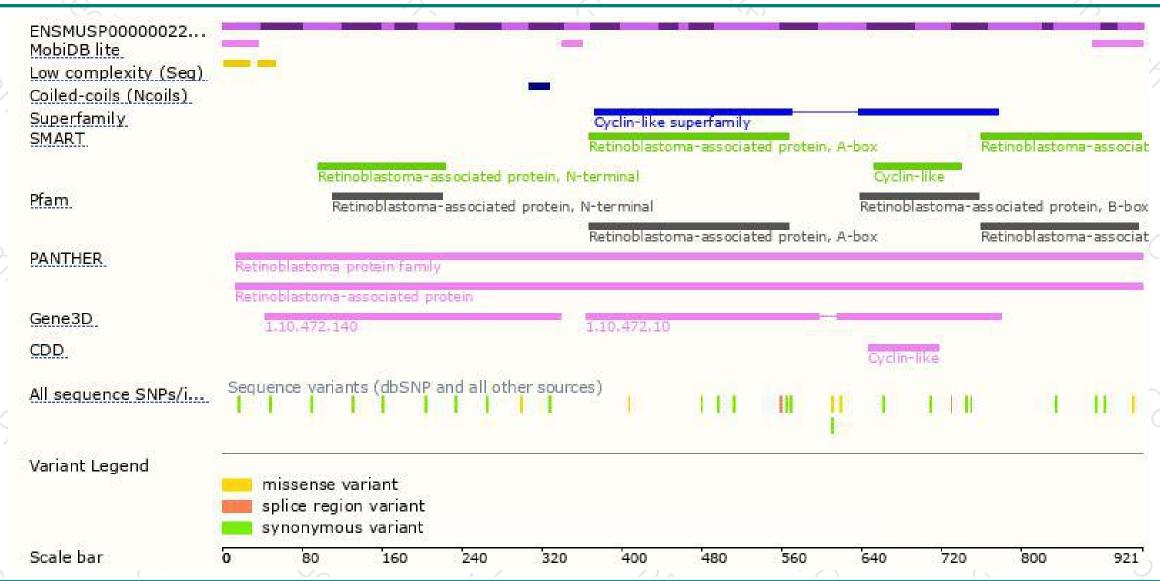
Genomic location distribution





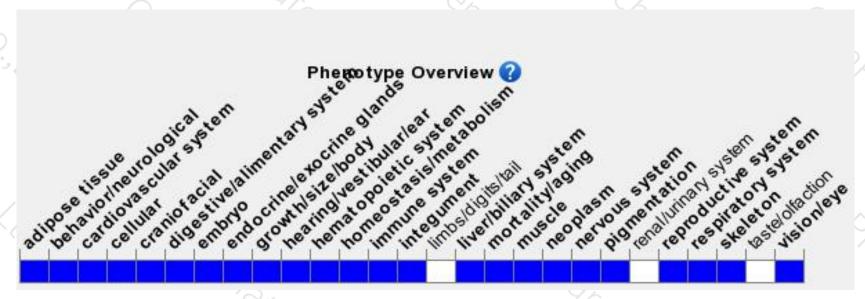
Protein domain





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, Homozygotes for targeted mutations exhibit abnormalities of the neuronal and hematopoietic systems and die in utero. Heterozygotes may develop pituitary tumors associated with loss of the normal alleles.



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

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