

Rack1 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Rack1

Project type

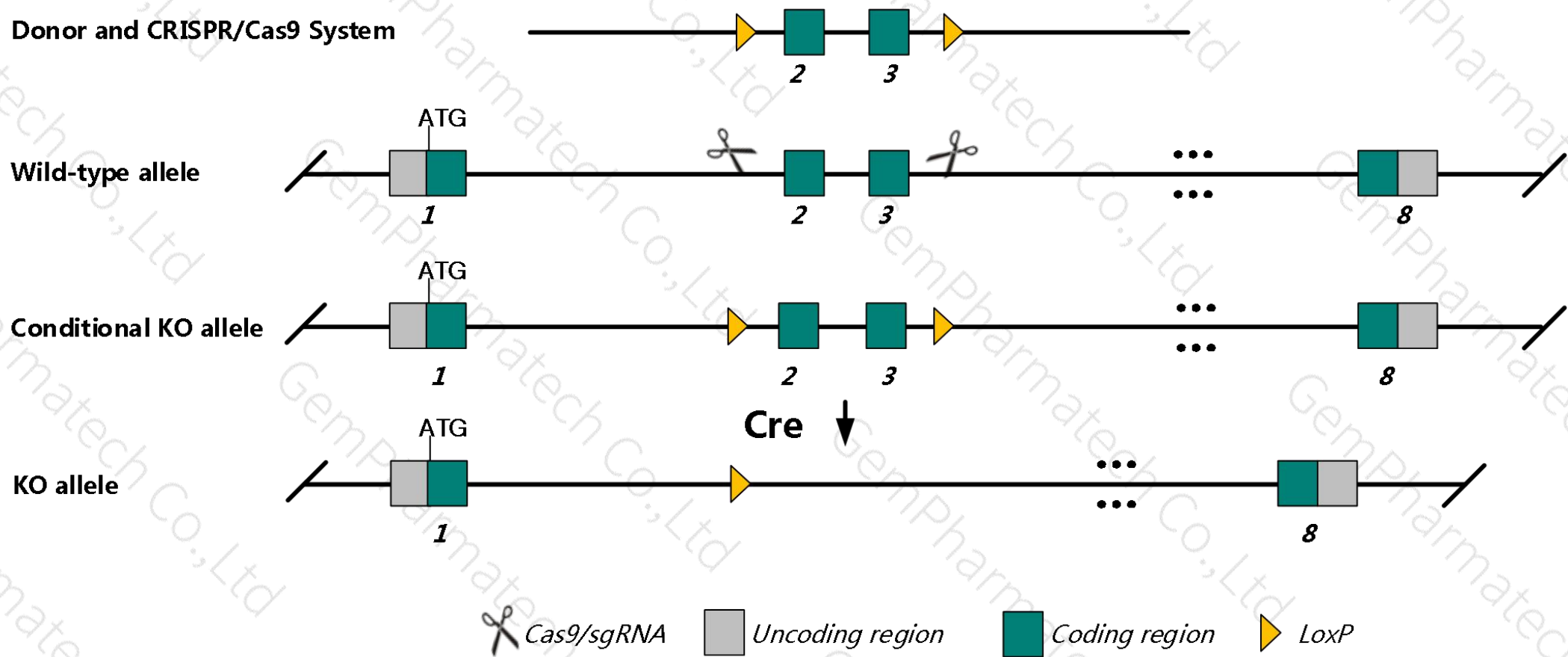
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rack1* gene. The schematic diagram is as follows:



- The *Rack1* gene has 6 transcripts. According to the structure of *Rack1* gene, exon2-3 of *Rack1*-201 (ENSMUST00000020640.7) transcript is recommended as the knockout region. The region contains 320bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rack1* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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 or cell types.

- According to the existing MGI data , Embryos homozygous for a hypomorphic allele lack early egg cylinders and die at gastrulation. Heterozygotes show a transient growth deficit, a white belly spot and hypopigmented tail and paws, while embryonic fibroblasts show a reduction in PMA- and insulin-stimulated translation.
- The *Rack1* gene is located on the Chr11. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Rack1 receptor for activated C kinase 1 [*Mus musculus* (house mouse)]

Gene ID: 14694, updated on 5-Jan-2020

Summary

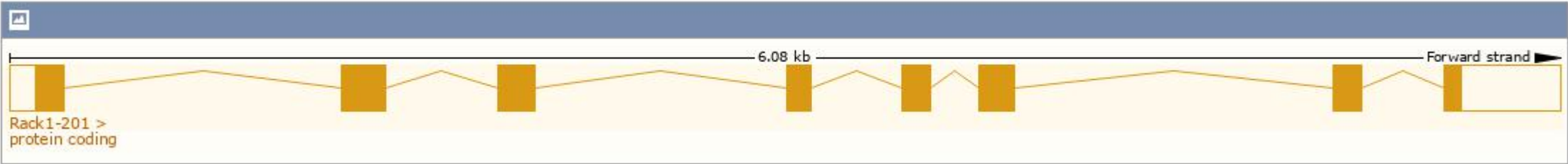
Official Symbol	Rack1 provided by MGI
Official Full Name	receptor for activated C kinase 1 provided by MGI
Primary source	MGI:MGI:101849
See related	Ensembl:ENSMUSG00000020372
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	p205; Gnb2l1; GB-like; AL033335; Gnb2-rs1
Expression	Ubiquitous expression in ovary adult (RPKM 1476.2), thymus adult (RPKM 1056.2) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

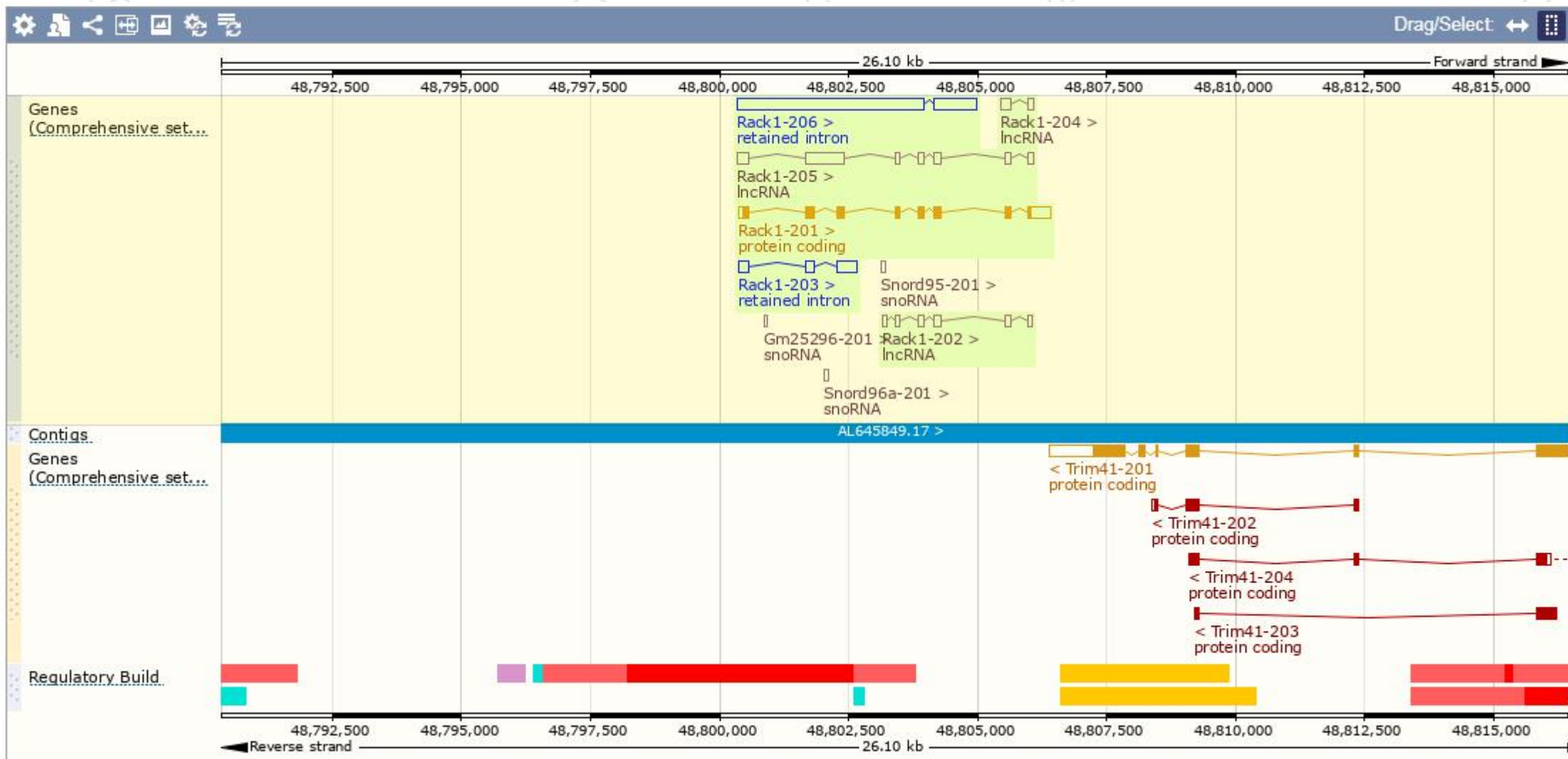
The gene has 6 transcripts, and all transcripts are shown below:

Show/hide columns (1 hidden) Filter							
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rack1-201	ENSMUST00000020640.7	1447	317aa	Protein coding	CCDS24585	P68040	TSL:1 Gencode basic APPRIS P1
Rack1-206	ENSMUST00000142269.1	4456	No protein	Retained intron	-	-	TSL:1
Rack1-203	ENSMUST00000136703.1	766	No protein	Retained intron	-	-	TSL:2
Rack1-205	ENSMUST00000139959.7	1542	No protein	lncRNA	-	-	TSL:5
Rack1-202	ENSMUST00000125166.1	637	No protein	lncRNA	-	-	TSL:3
Rack1-204	ENSMUST00000136849.1	322	No protein	lncRNA	-	-	TSL:2

The strategy is based on the design of *Rack1*-201 transcript, The transcription is shown below



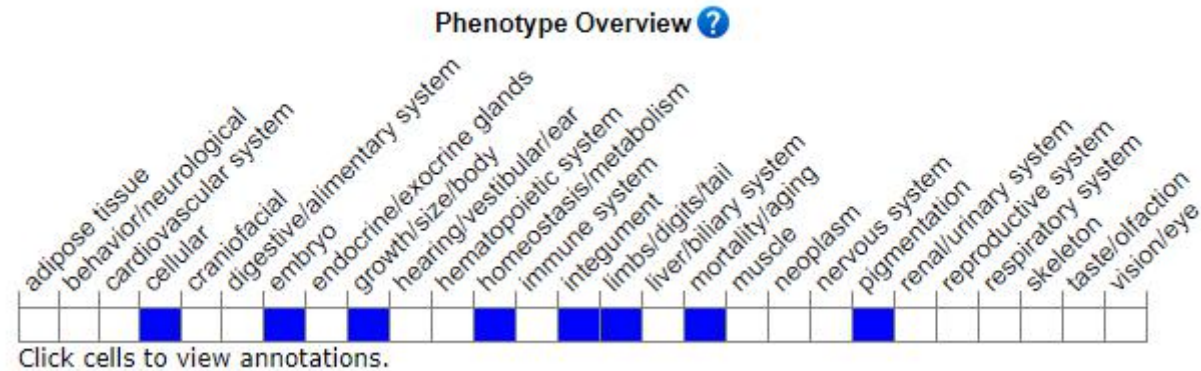
Genomic location distribution



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, Embryos homozygous for a hypomorphic allele lack early egg cylinders and die at gastrulation. Heterozygotes show a transient growth deficit, a white belly spot and hypopigmented tail and paws, while embryonic fibroblasts show a reduction in PMA- and insulin-stimulated translation.

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