

# ***Rack1 Cas9-KO Strategy***

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**Reviewer:**

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**2020-1-7**

# Project Overview

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**Project Name**

***Rack1***

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**Project type**

**Cas9-KO**

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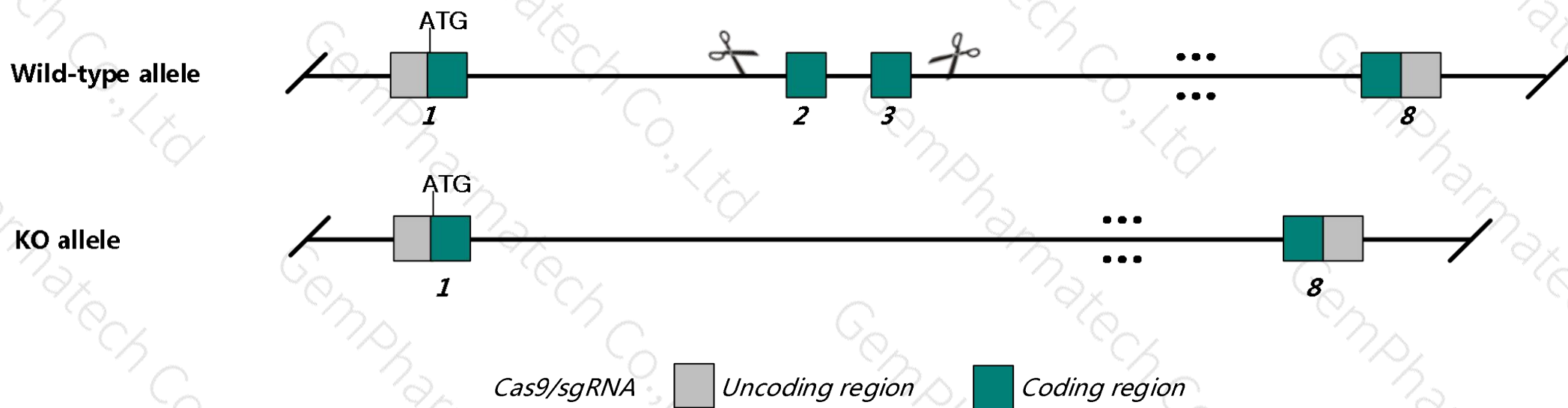
**Strain background**

**C57BL/6JGpt**

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# Knockout strategy

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



# Technical routes

- 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. Cas9 and gRNA 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.

- 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

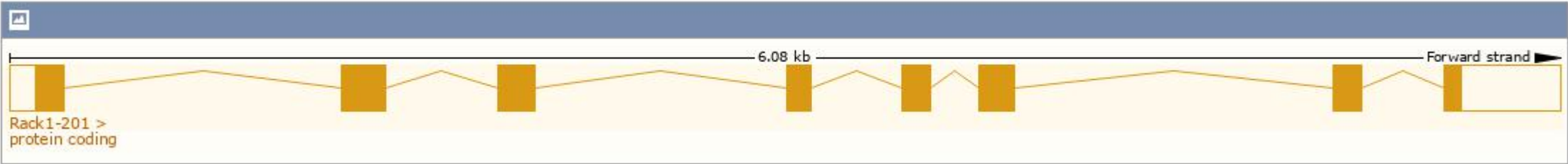
Official Symbol	Rack1 provided by <a href="#">MGI</a>
Official Full Name	receptor for activated C kinase 1 provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:101849</a>
See related	<a href="#">Ensembl:ENSMUSG00000020372</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<a href="#">Mus musculus</a>
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 <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information ( Ensembl )

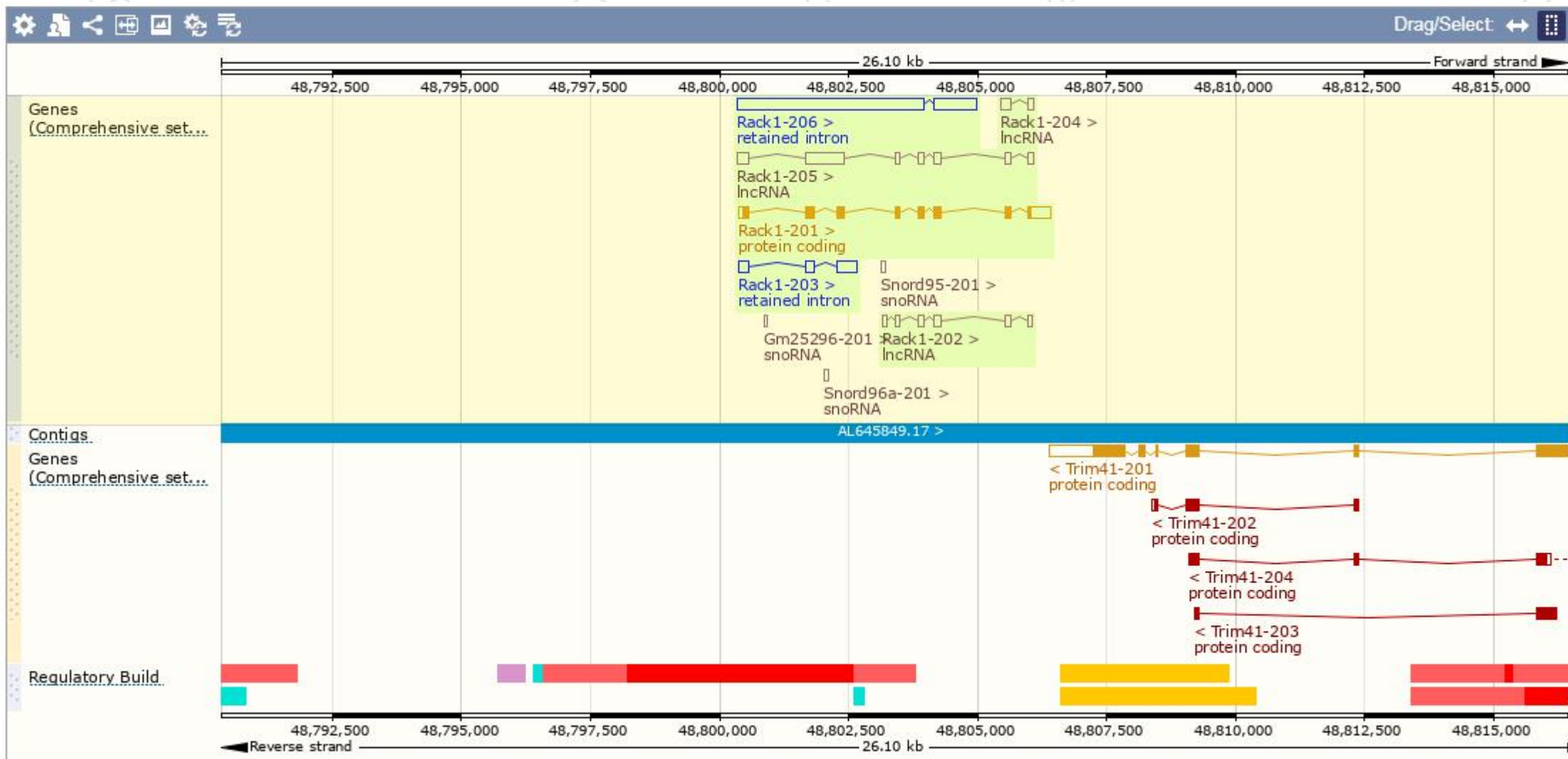
The gene has 6 transcripts, and all transcripts are shown below:

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

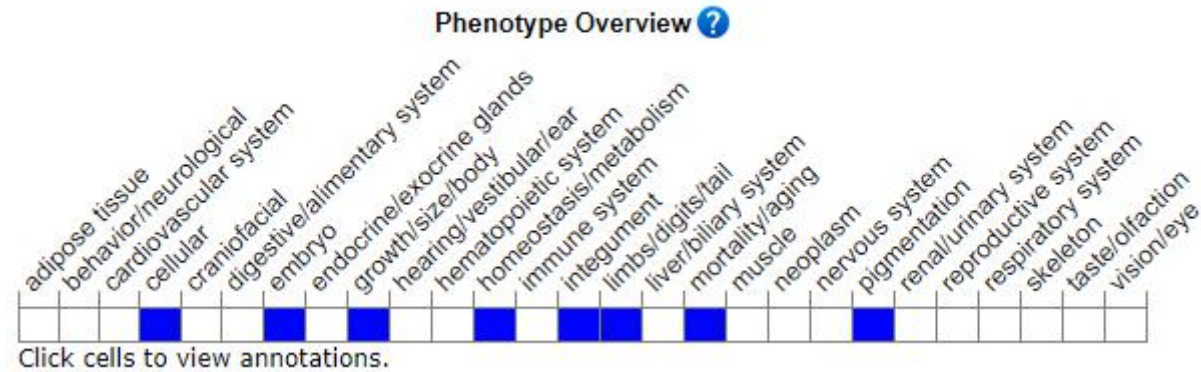




# Protein domain



# 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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