Mrgprd Cas9-CKO *Strategy*

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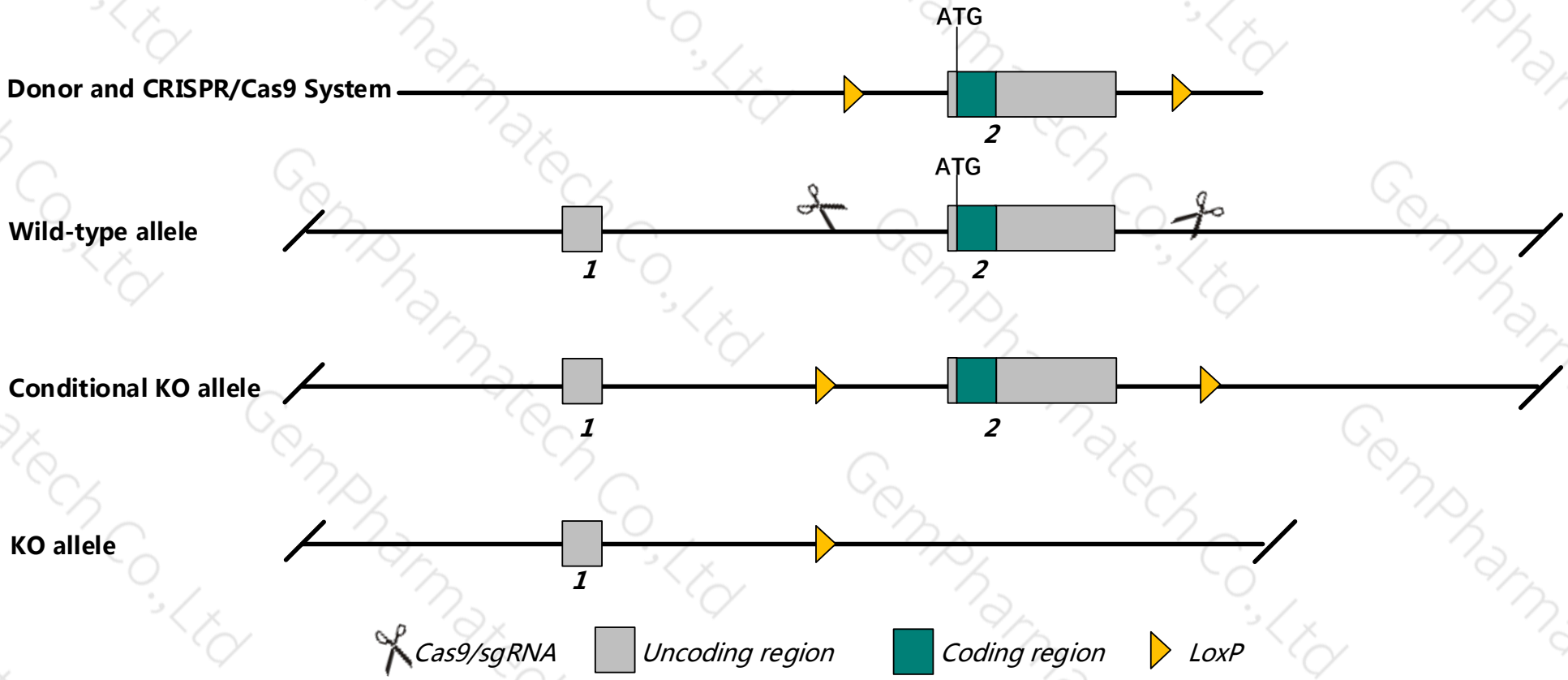
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Mrgprd* gene has 1 transcript.According to the structure of *Mrgprd* gene, exon2 of *Mrgprd*-201 (ENSMUST00000062163.7) transcript is recommended as the knockout region.The region contains all coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Mrgprd* 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/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 was 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.

Notice

- According to the existing MGI data，mice homozygous for a knock-out allele exhibit decreased sensitivity to cold, heat, and mechanical stimuli. Mice for another null allele exhibit dilated cardiomypathy and cardiac dysfunction.

- The *Mrgprd* gene is located on the Chr7. 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）

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Mrgprd MAS-related GPR, member D [*Mus musculus* (house mouse)]

Gene ID: 211578, updated on 21-Aug-2019

Summary

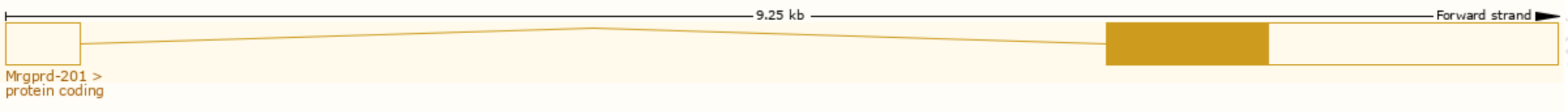
Official Symbol	Mrgprd provided by MGI
Official Full Name	MAS-related GPR, member D provided by MGI
Primary source	MGI:MGI:3033142
See related	Ensembl:ENSMUSG00000051207
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Mgrd; MrgD; TGR7; Gm499
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information（Ensembl）

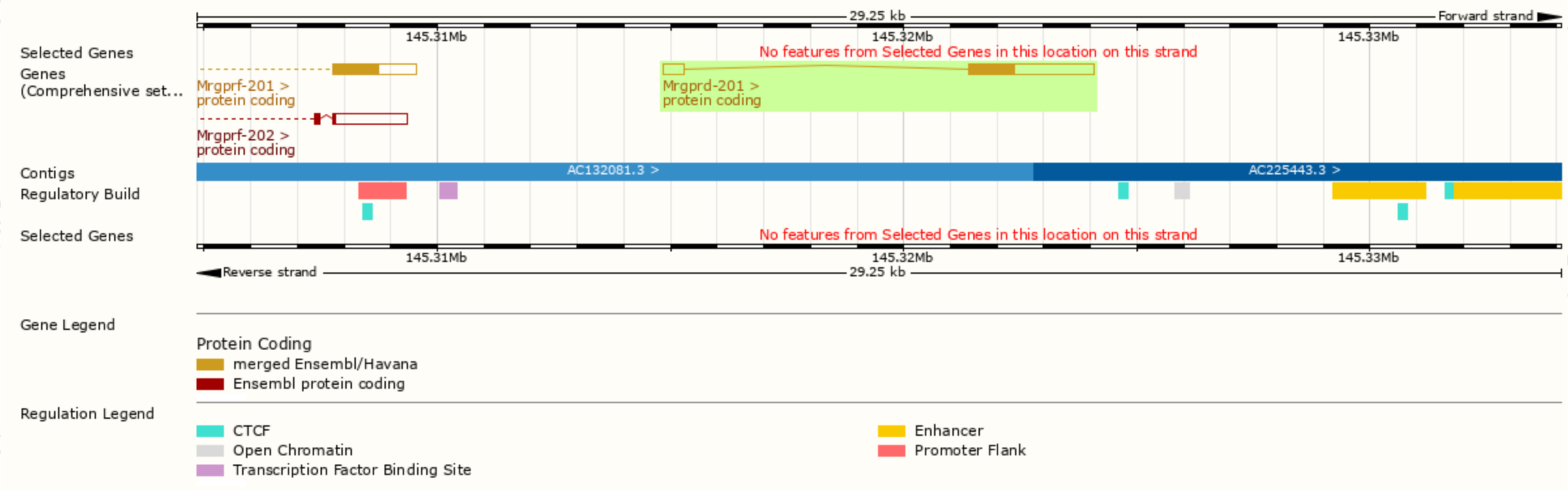
The gene has 1 transcript, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mrgprd-201	ENSMUST00000062163.7	3144	321aa	Protein coding	CCDS22057	Q5UCB4 Q91ZB8	TSL:1 GENCODE basic APPRIS P1

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

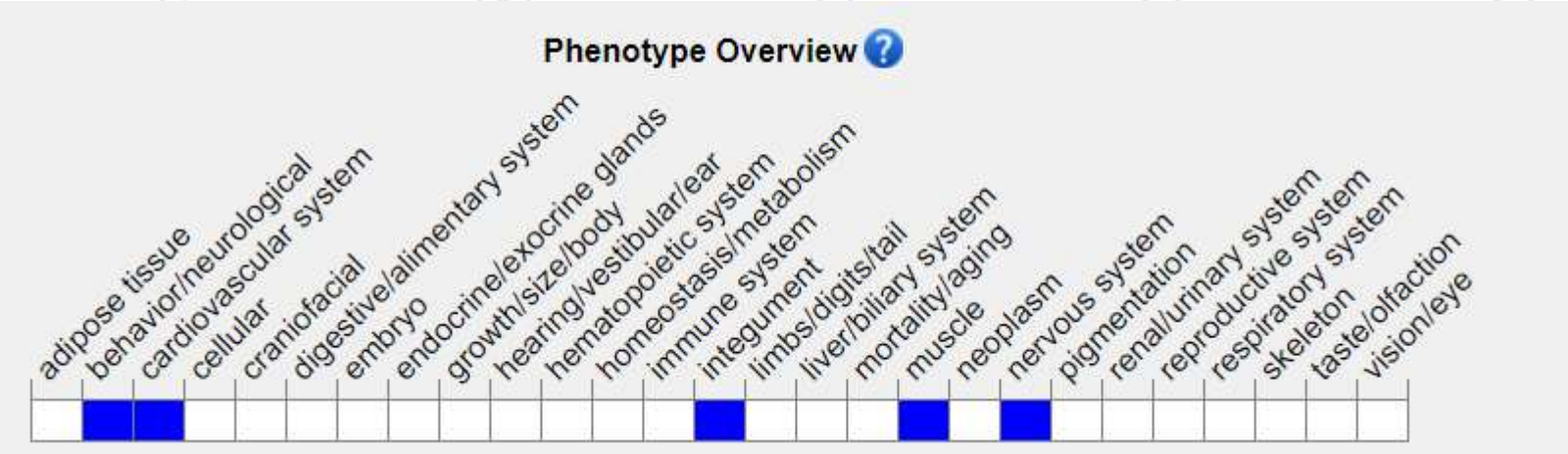


Genomic location distribution



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/) .

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

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