

# **Prrg4 Cas9-CKO Strategy**

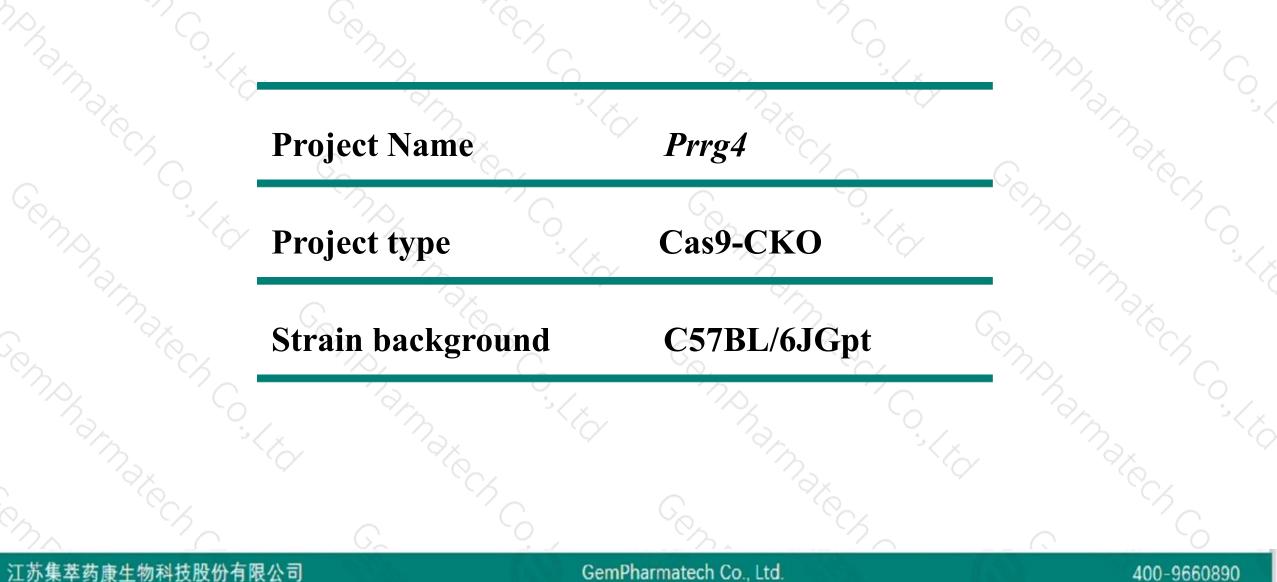
Designer: Xueting Zhang

Reviewer: Daohua Xu

Design Date: 2020-5-14

### **Project Overview**





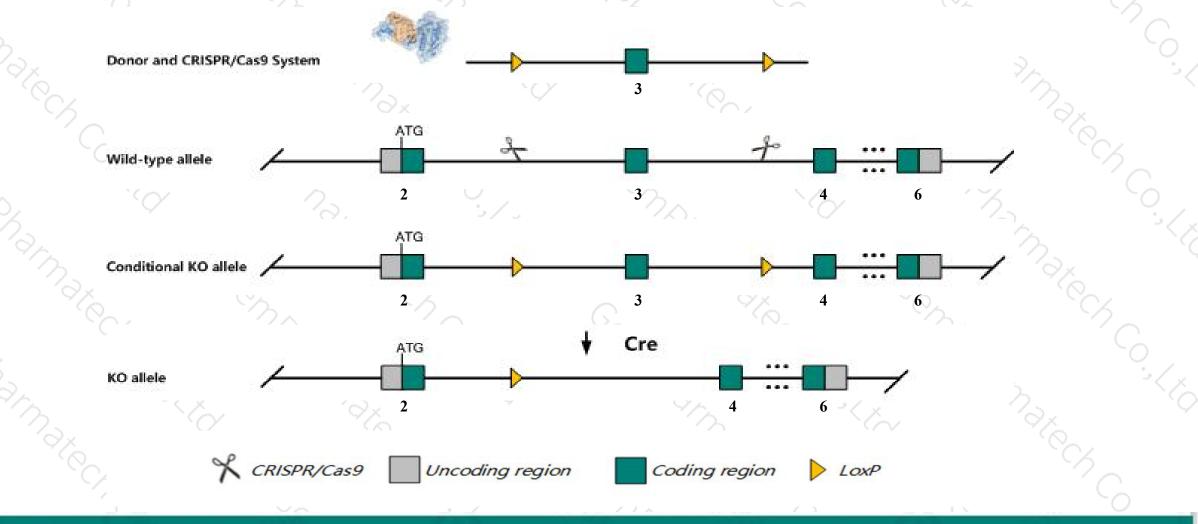
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### **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Prrg4 gene. The schematic diagram is as follows:



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The Prrg4 gene has 2 transcripts. According to the structure of Prrg4 gene, exon3 of Prrg4-201 (ENSMUST00000028593.10) transcript is recommended as the knockout region. The region contains 164bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Prrg4* gene. The brief process is as follows:CRISPR/Cas9 system 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 and cell types.

### Notice



- The Prrg4 gene is located on the Chr2. 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)**



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#### Prrg4 proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane) [Mus musculus (house mouse)]

Gene ID: 228413, updated on 13-Mar-2020

#### Summary

Prrg4 provided by MGI
proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane) provided by MGI
MGI:MGI:2442211
Ensembl:ENSMUSG0000027171
protein coding
VALIDATED
Mus musculus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Muridae; Murinae; Mus; Mus
9930111118Rik, TMG4
Biased expression in placenta adult (RPKM 2.9), bladder adult (RPKM 1.7) and 14 other tissues See more
human all

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### **Transcript information (Ensembl)**



#### The gene has 2 transcripts, all transcripts are shown below:

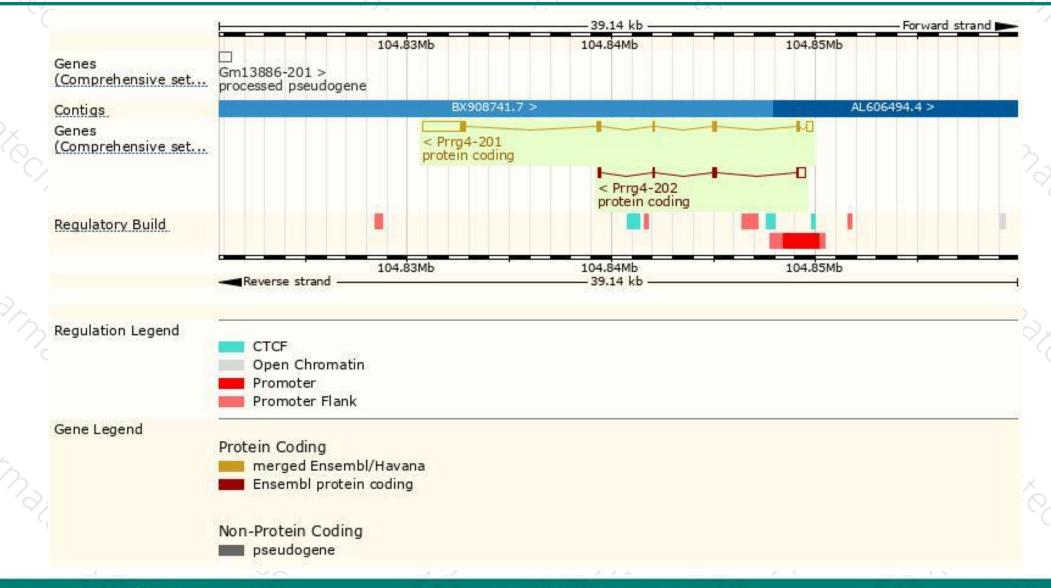
Name         Transcript ID         bp         Protein         Biotype         CCDS         UniProt           Prrg4-202         ENSMUST000001268241         075         26aa         Protein coding         CCDS16493         04KL73 QBBCh6         TSL1 GENCODEDBasic APPEnDisa system to annotate attempting to the system to annotat	Flags ripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
	ipts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
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	Ale Constant
The strategy is based on the design of <i>Prrg4-201</i> transcript, the transcription is	s shown below:
< Prrg4-201 protein coding	- M

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### **Genomic location distribution**





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### **Protein domain**



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ENSMUSP00000028 Transmembrane heli Low complexity (Seg) Cleavage site (Sign										-	30
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<u>Pfam</u>			International Action of the second	COLUMN TWO IS NOT THE OWNER.	amic acid-ric						
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PROSITE patterns			Ga	mma-carb é	xyglutamic a	icid-rich (Gl	LA) domain				5
PANTHER	PTHR24251	:SF15									
	PTHR24251		-		- 51						
Gene3D			Coagulation	factor-like,	Gla domain :	superfamily	e 2				
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Scale bar	0	20 40	60	80	100	120	140	160	180	200	226
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If you have any questions, you are welcome to inquire. Tel: 400-9660890



