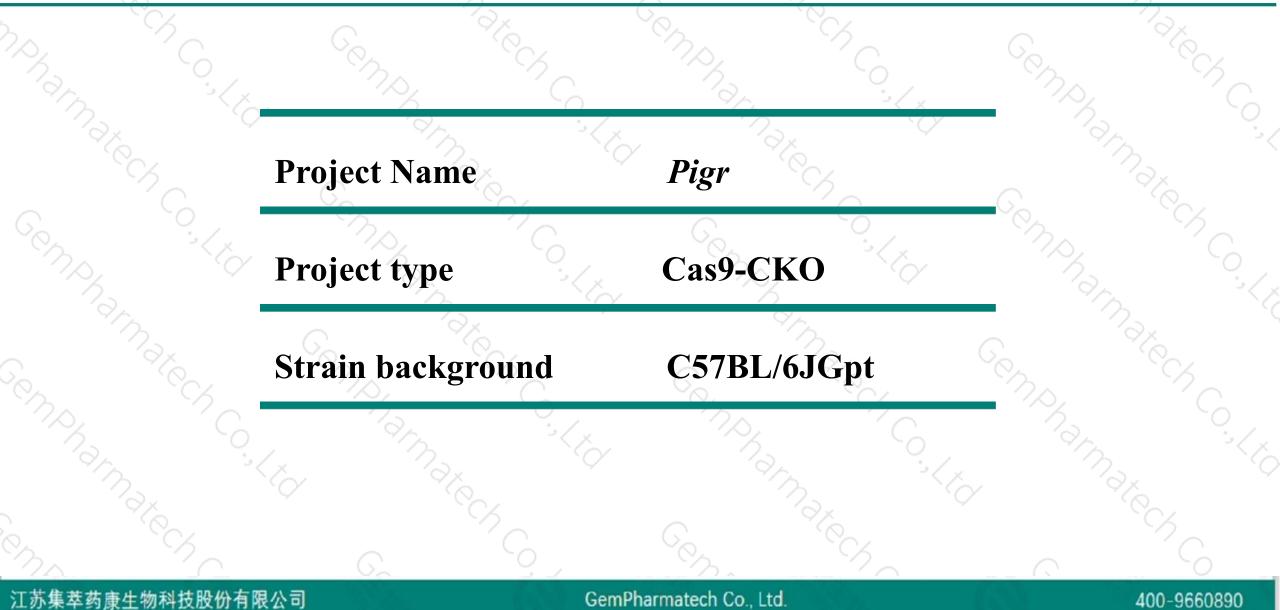


Pigr Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2019-11-24

Project Overview



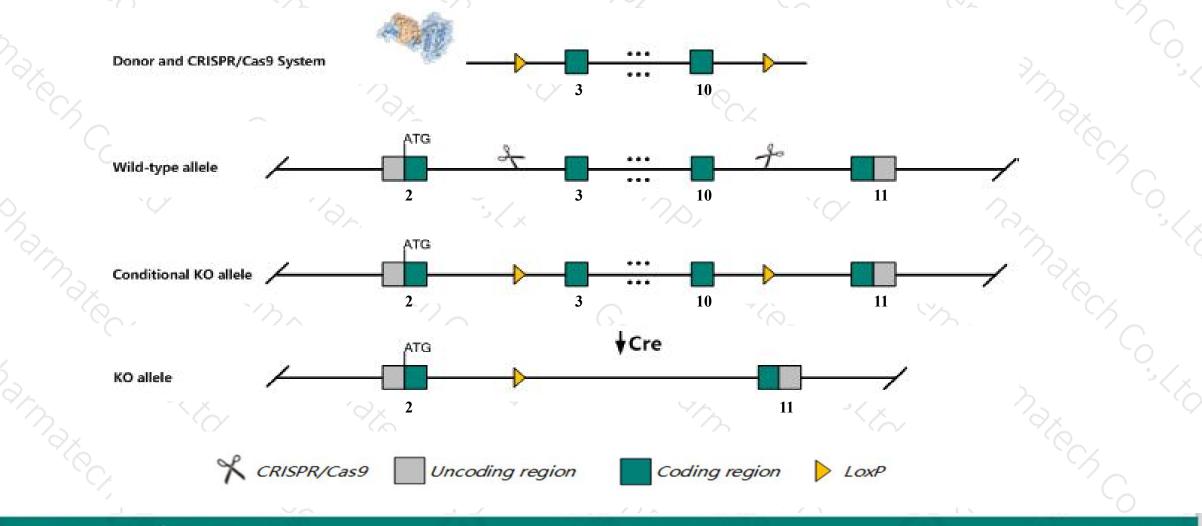


Conditional Knockout strategy



400-9660890

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



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The Pigr gene has 3 transcripts. According to the structure of Pigr gene, exon3-exon10 of Pigr-201 (ENSMUST00000027675.13) transcript is recommended as the knockout region. The region contains 2177bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Pigr* 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.



- According to the existing MGI data, Nullizygous mice show impaired transpithelial transport of dimeric IgA, increased serum IgA levels and mucosal leakiness. Studies of one null allele show increased susceptibility to mycobacterial infections while another allele causes impaired clearance of the protozoan parasite Giardia.
- The Pigr gene is located on the Chr1. 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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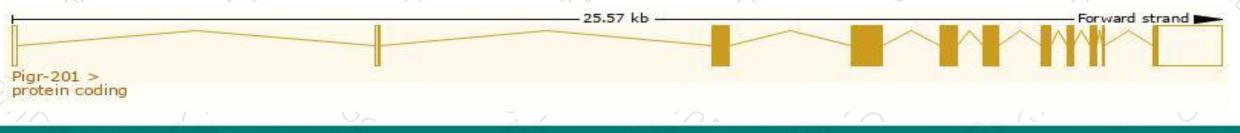
Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pigr-201	ENSMUST00000027675.13	3849	<u>771aa</u>	Protein coding	CCDS15260	070570	TSL:1 GENCODE basic APPRIS P1
Pigr-202	ENSMUST00000133792.7	784	<u>211aa</u>	Protein coding	-8	D3Z2D3	CDS 3' incomplete TSL:3
Pigr-203	ENSMUST00000137782.1	740	<u>200aa</u>	Protein coding	28	D3YVM4	CDS 3' incomplete TSL:3

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

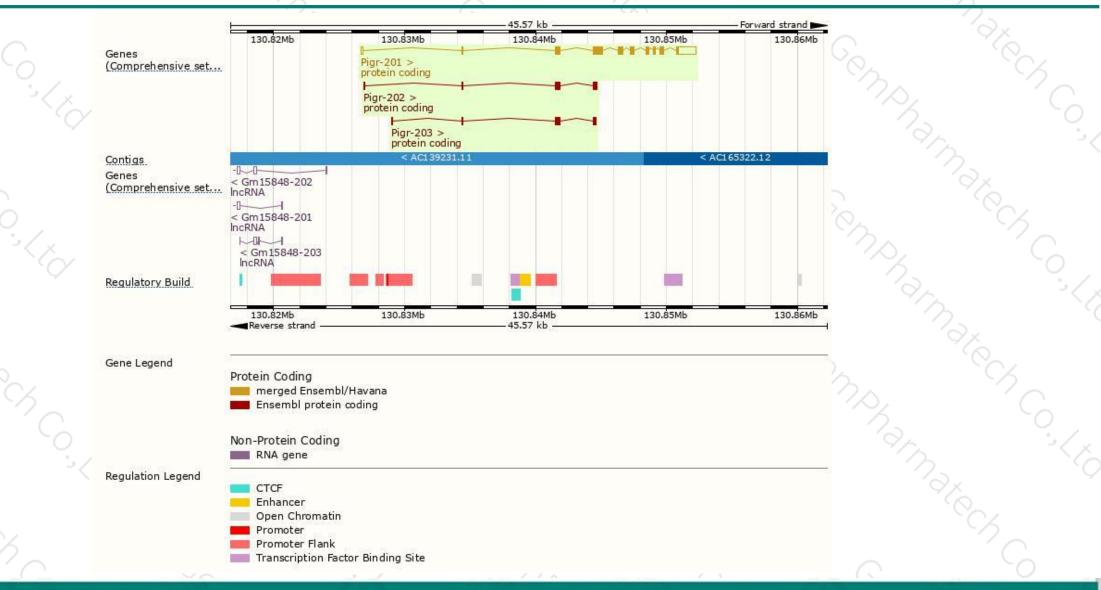


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Genomic location distribution



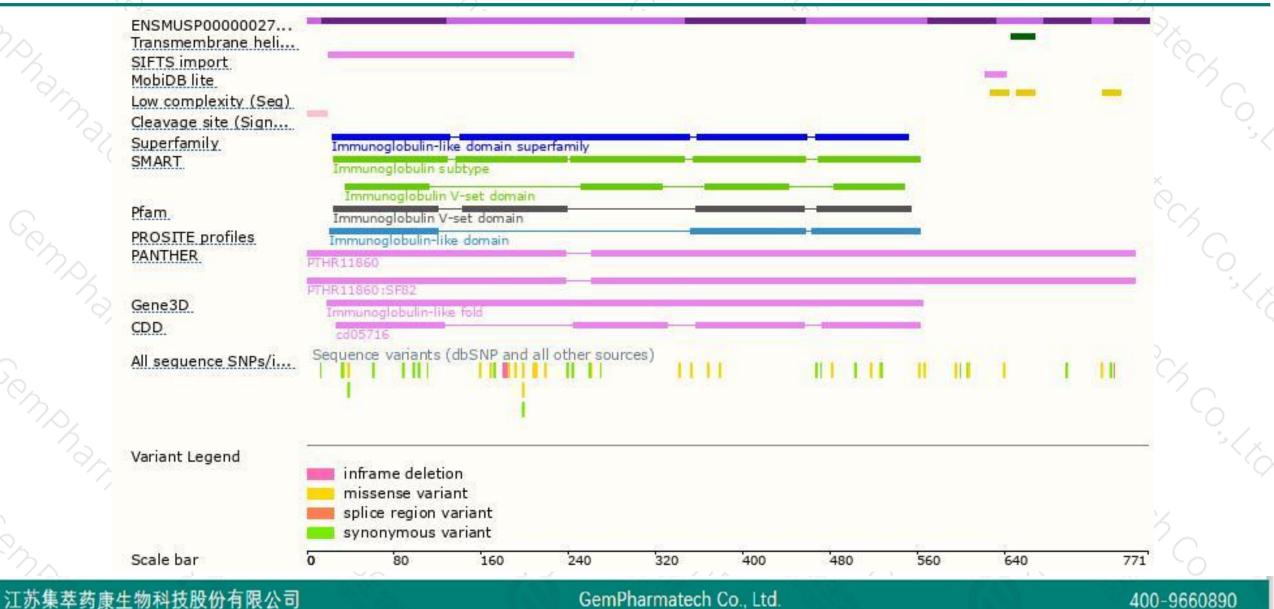


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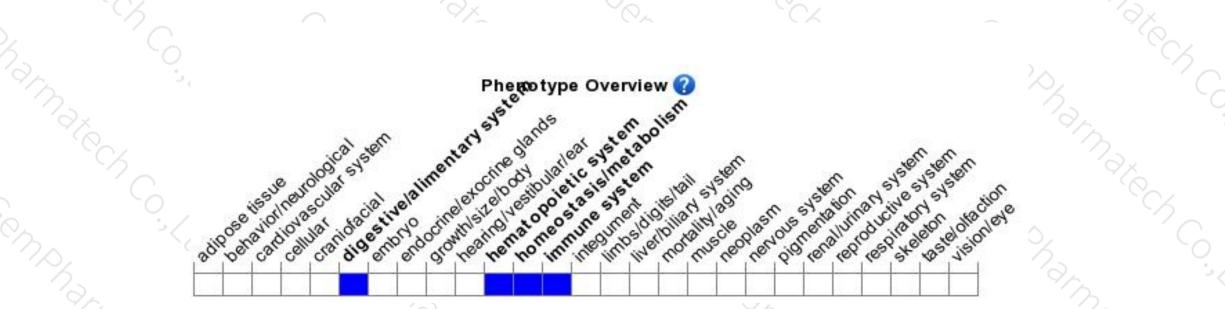
Protein domain





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, Nullizygous mice show impaired transpithelial transport of dimeric IgA, increased serum IgA levels and mucosal leakiness. Studies of one null allele show increased susceptibility to mycobacterial infections while another allele causes impaired clearance of the protozoan parasite Giardia.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



