

Cnksr1 Cas9-CKO Strategy

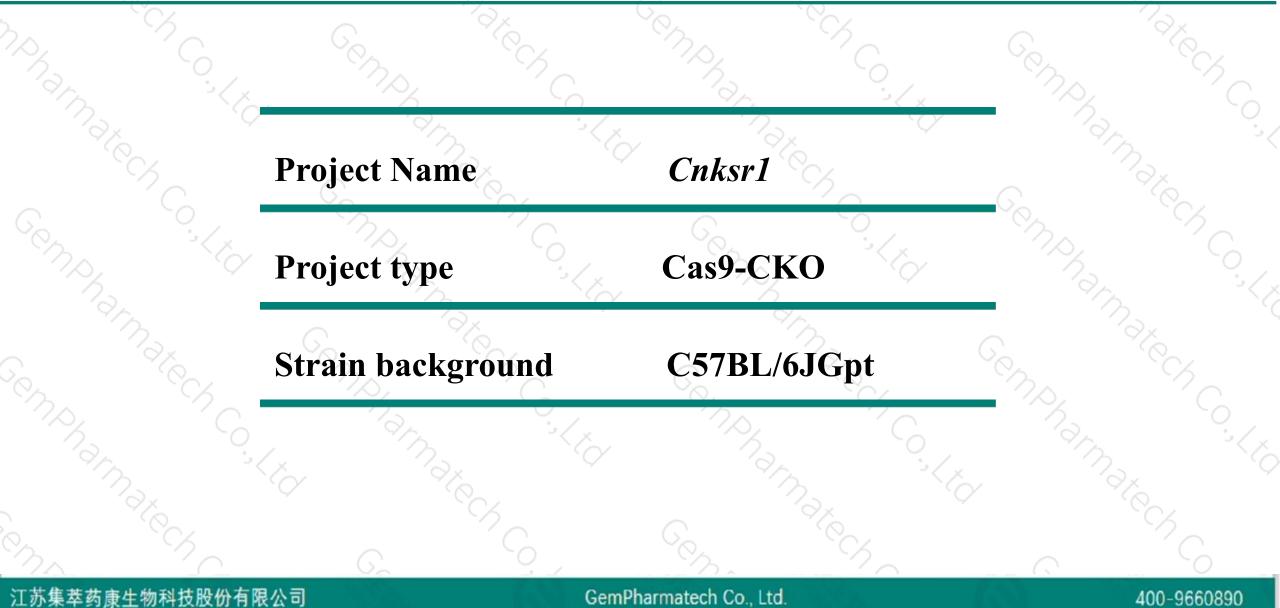
Designer: Reviewer:

Design Date:

Huan Wang Huan Fan 2020-2-18

Project Overview

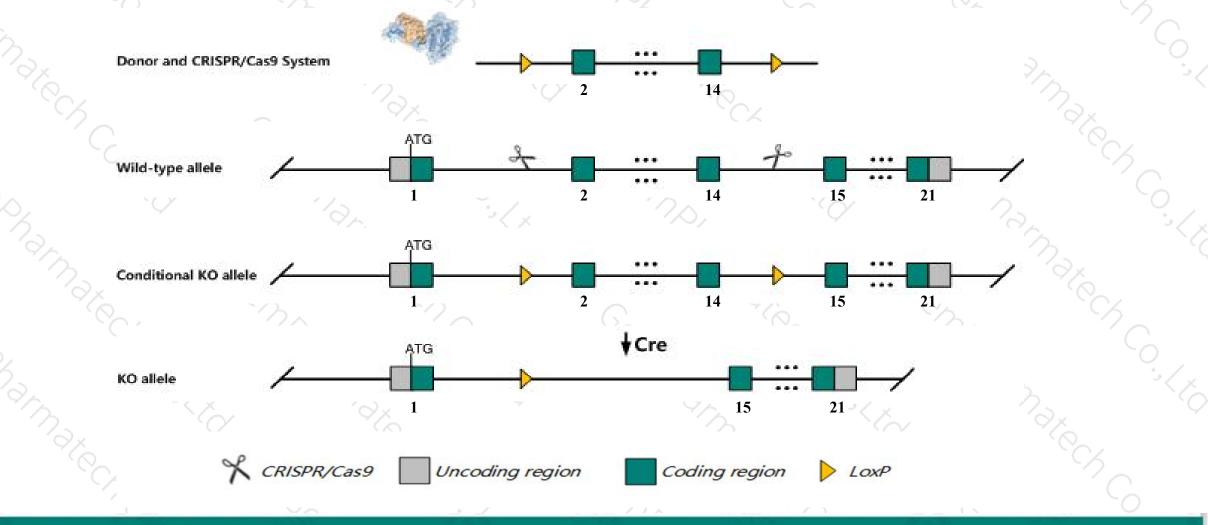




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Cnksr1* gene. The schematic diagram is as follows:



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The Cnksr1 gene has 5 transcripts. According to the structure of Cnksr1 gene, exon2-exon14 of Cnksr1-201 (ENSMUST00000030645.8) transcript is recommended as the knockout region. The region contains 1229bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Cnksr1* 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.



- The Cnksr1 gene is located on the Chr4. 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)



\$?

Cnksr1 connector enhancer of kinase suppressor of Ras 1 [Mus musculus (house mouse)]

Gene ID: 194231, updated on 31-Jan-2019

Summary

Official Symbol	Cnksr1 provided by MGI
Official Full Name	connector enhancer of kinase suppressor of Ras 1 provided by MGI
Primary source	MGI:MGI:2670958
See related	Ensembl:ENSMUSG0000028841
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BC037758
Expression	Biased expression in large intestine adult (RPKM 14.7), colon adult (RPKM 13.4) and 14 other tissuesSee more
Orthologs	human all

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The gene has 5 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cnksr1-201	ENSMUST0000030645.8	2468	<u>700aa</u>	Protein coding	CCDS38912	<u>A2A9K7</u>	TSL:1 GENCODE basic APPRIS P1
Cnksr1-203	ENSMUST00000144504.7	2395	No protein	Processed transcript	87	070	TSL:5
Cnksr1-202	ENSMUST00000124181.1	956	No protein	Processed transcript	8 <u>4</u>	120	TSL:5
Cnksr1-204	ENSMUST00000145998.7	450	No protein	Processed transcript	62	121	TSL:5
Cnksr1-205	ENSMUST00000146283.1	396	No protein	Processed transcript	117	120	TSL:3

The strategy is based on the design of Cnksr1-201 transcript, The transcription is shown below



Reverse strand -

– 10.36 kb –

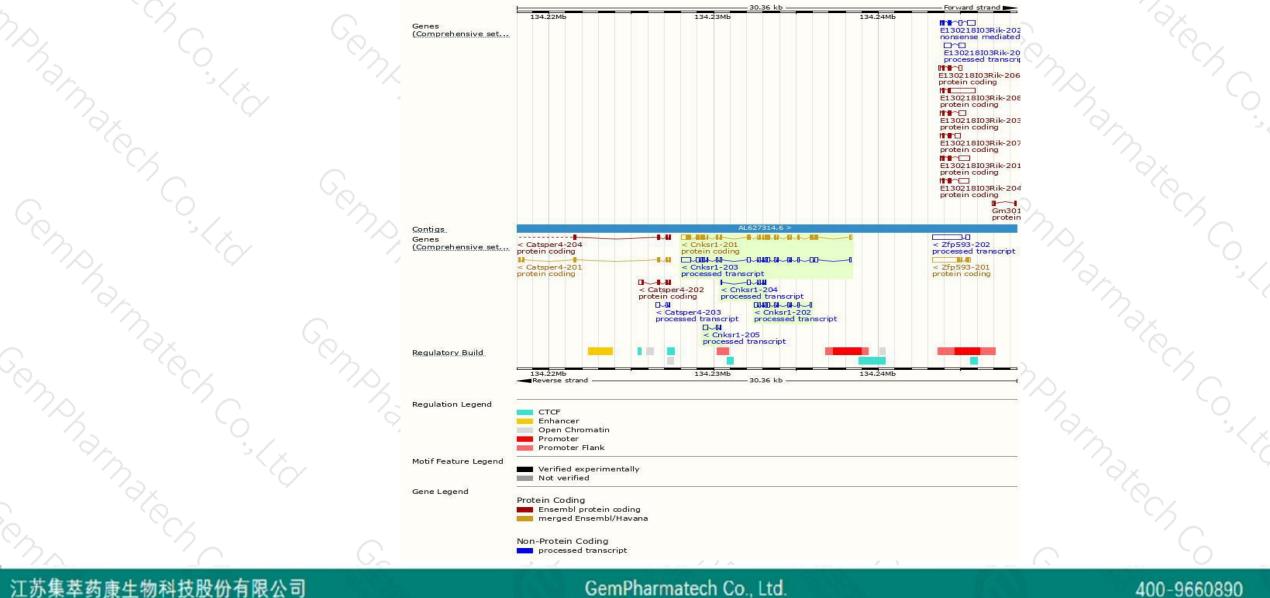
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Genomic location distribution

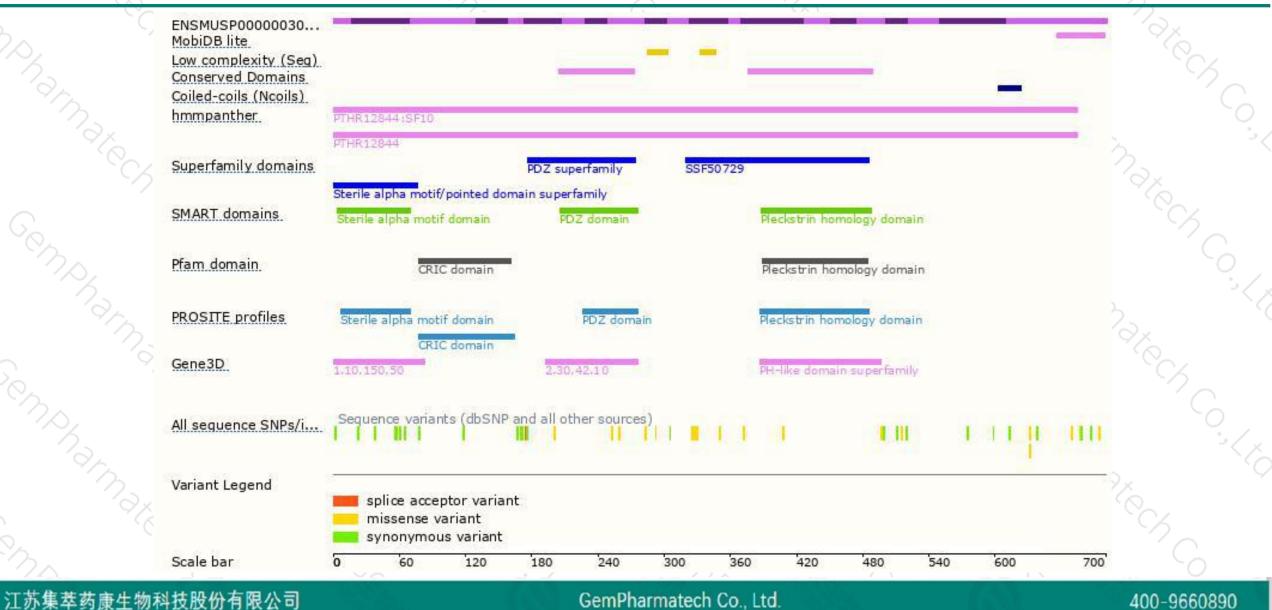




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Protein domain







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



