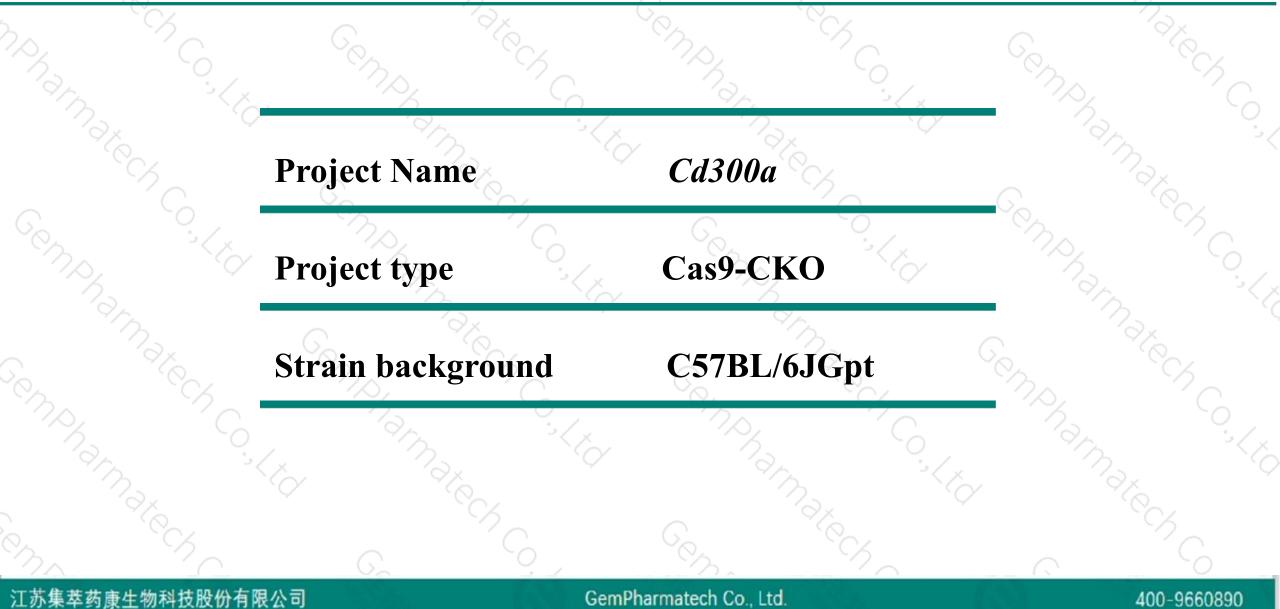


Cd300a Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2020-2-13

Project Overview

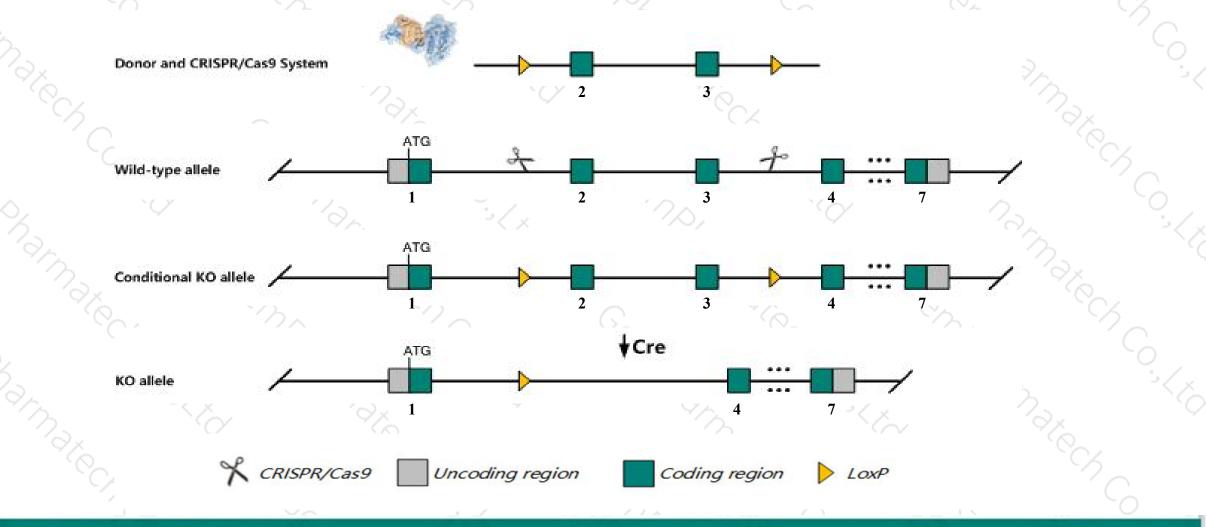




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Cd300a gene. The schematic diagram is as follows:



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The Cd300a gene has 3 transcripts. According to the structure of Cd300a gene, exon2-exon3 of Cd300a-202 (ENSMUST00000106582.8) transcript is recommended as the knockout region. The region contains 478bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Cd300a* 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, Mice homozygous for a knock-out allele exhibit decreased susceptibility to cecal ligation and puncture with increased production of chemoattractants by mast cells.
 - The Cd300a 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)



☆ ?

Cd300a CD300A molecule [Mus musculus (house mouse)]

Gene ID: 217303, updated on 31-Jan-2019

Summary

Official SymbolCd300a provided by MGIOfficial Full NameCD300A molecule provided byMGIPrimary soureMci:Mci:2443411See relatedEnsembl:ENSMUSG0000034652Gene typeprotein codingvaliDATEDValiDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Rolentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso knowaneB230315M08Rik, Clm8, LMIR1, MAIR-1, MAIR-1a, MMAC8, Pigr4, mcpir1ExpressionBroad expression in spleen adult (RPKM 4.3), liver E18 (RPKM 1.5) and 21 other tissues
See more

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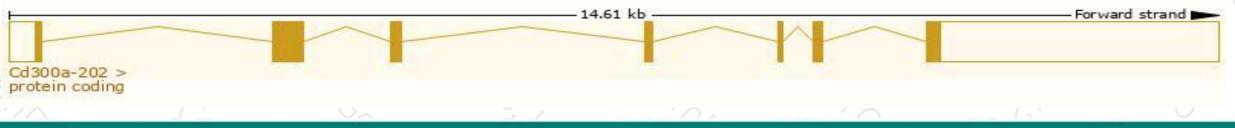
Transcript information (Ensembl)



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

Name 🖕	Transcript ID	bp 🍦	Protein 🖕	Biotype	CCDS 🖕	UniProt 🖕	Flags 🔶		
Cd300a-202	ENSMUST00000106582.8	4645	<u>318aa</u>	Protein coding	<u>CCDS25613</u> 교	<u>Q6SJQ0</u> &	TSL:1	GENCODE basic	APPRIS P3
Cd300a-201	ENSMUST0000045151.5	4633	<u>314aa</u>	Protein coding	<u>CCDS83923</u> &	<u>Q6SJQ0</u> &	TSL:1	GENCODE basic	APPRIS ALT2
Cd300a-203	ENSMUST00000153245.1	467	No protein	Processed transcript	1 2	11 <u>2</u> 2		TSL:3	

The strategy is based on the design of Cd300a-202 transcript, The transcription is shown below



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Genomic location distribution



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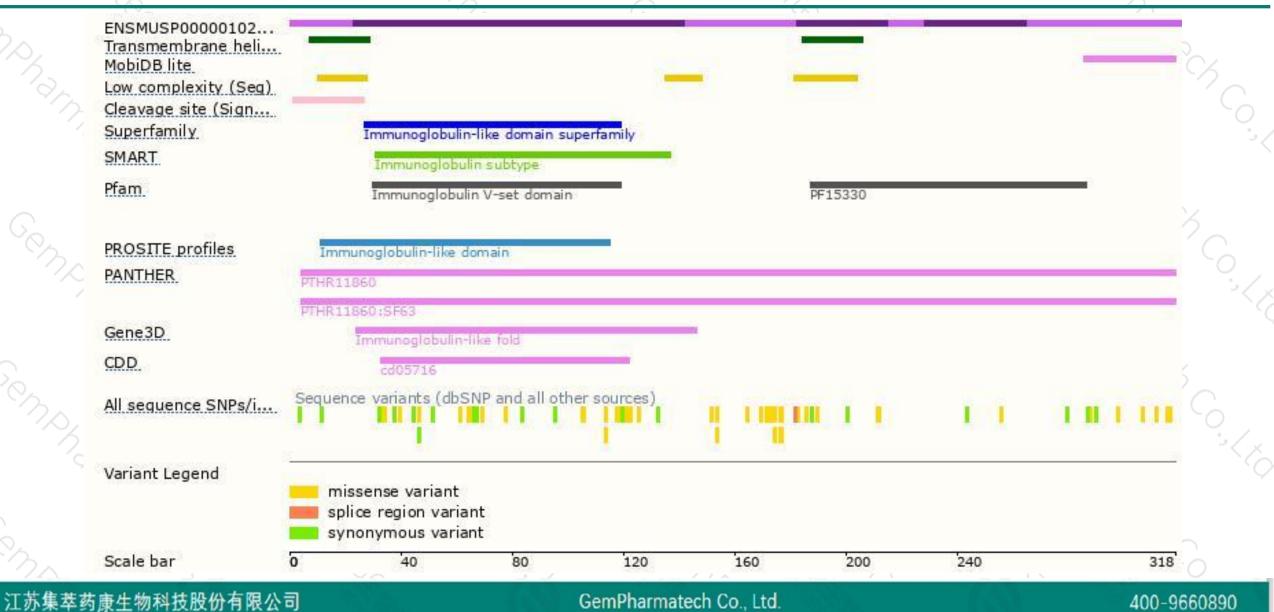
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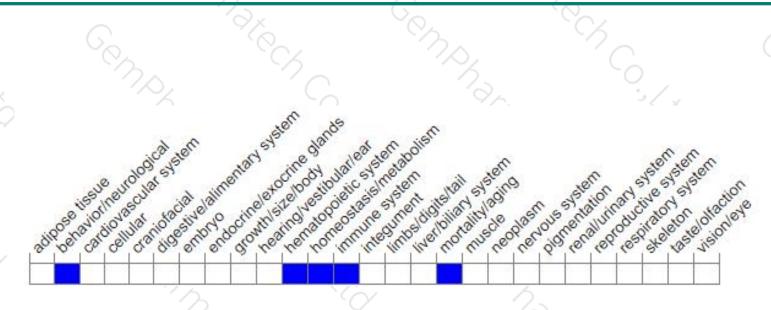
Protein domain





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, Mice homozygous for a knock-out allele exhibit decreased susceptibility to cecal ligation and puncture with increased production of chemoattractants by mast cells.



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



