

Cd80 Cas9-CKO Strategy

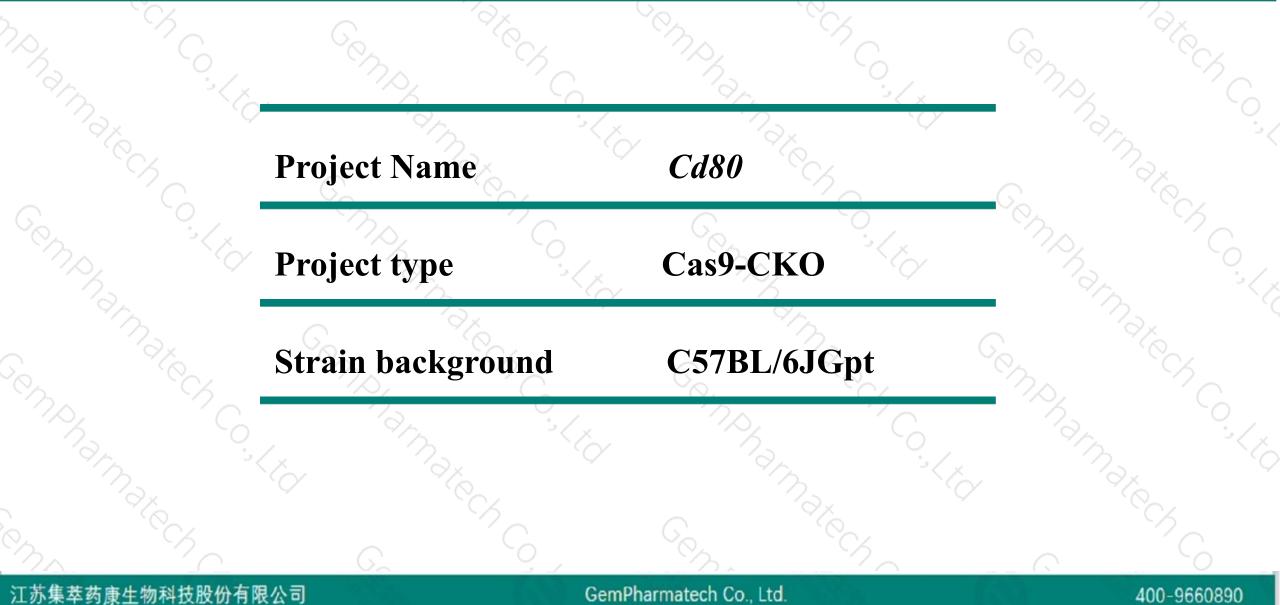
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2019-11-14

Project Overview

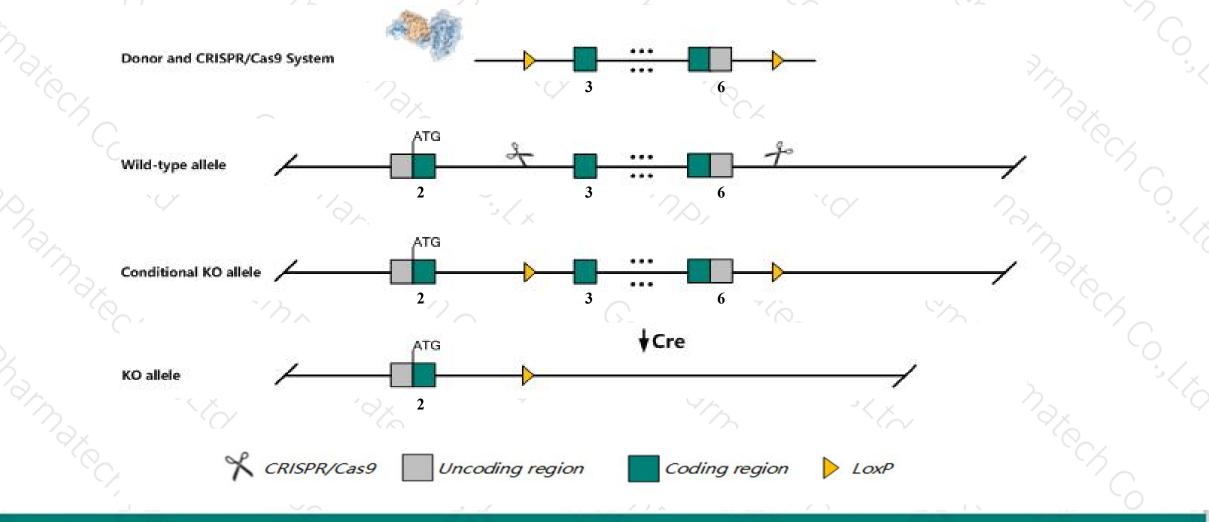




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Cd80 gene. The schematic diagram is as follows:



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The Cd80 gene has 3 transcripts. According to the structure of Cd80 gene, exon3-exon6 of Cd80-202 (ENSMUST00000231716.1) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify Cd80 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, Homozygous mutation of this gene results in a 70% reduction in the mixed lymphocyte response in LPS- and dextran sulfate-stimulated B cells.
 - The Cd80 gene is located on the Chr16. 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)



☆ ?

Cd80 CD80 antigen [Mus musculus (house mouse)]

Gene ID: 12519, updated on 12-Feb-2019

Summary

Official SymbolCd80 provided by MGIOfficial Full NameCD80 antigen provided by MGIPrimary sourceMGI:MGI:101775See relatedEnsembl:ENSMUSG0000075122Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso knownasB71, Cd281, Ly-53, Ly53, MIC17, TSA1

Expression Low expression observed in reference datasetSee more

Orthologs human all

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Transcript information (Ensembl)



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

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Cd80-202	ENSMUST00000231716.1	3173	<u>306aa</u>	Protein coding	CCDS28168	Q00609 Q549R2	GENCODE basic APPRIS P1	
Cd80-201	ENSMUST0000099816.2	1701	<u>306aa</u>	Protein coding	CCDS28168	Q00609 Q549R2	TSL:1 GENCODE basic APPRIS P1	
Cd80-203	ENSMUST00000232409.1	748	<u>212aa</u>	Protein coding	(12)	<u>Q00609</u>	GENCODE basic	

The strategy is based on the design of Cd80-202 transcript, The transcription is shown below

	40.77 kb	- Forward strand
Cd80-202 >		
protein coding		

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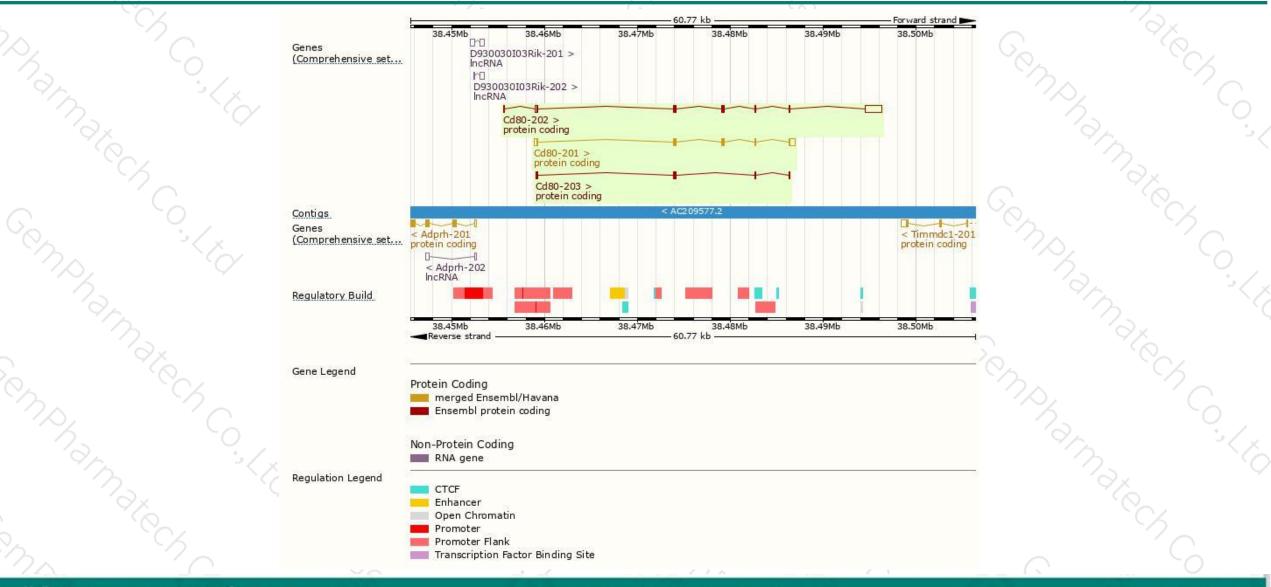
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Genomic location distribution



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Protein domain

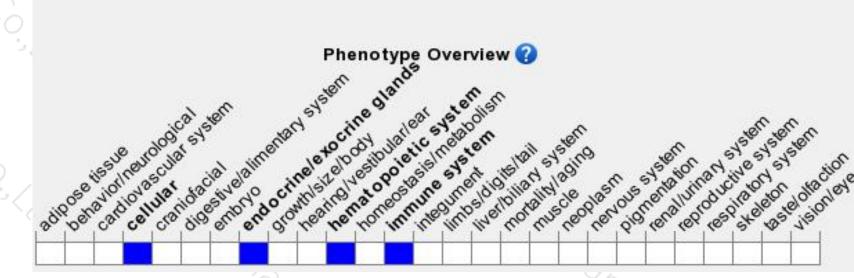
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	Scale bar	0	40	80	120	160	200	240	306
- 1	Variant Legend	— missen	e insertion se variant mous variant						
×.	All sequence SNPs/i	Sequence v	ariants (dbSNF 	and all othe	er sources)	<u>e</u>	<u>.</u>	1 11	1 6.
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	Pfam.		No. of Concession, Name of Concession, Name	ulin subtype oulin V-set dor	main	CD80-like, imm	unoglobulin C2-se	t	
5	ENSMUSP00000156 Transmembrane heli SIFTS import Low complexity (Seg) Cleavage site (Sign Superfamily SMART		Statement of the local division of the local	ALC: NO REAL PROPERTY.	nain superfamily				

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, Homozygous mutation of this gene results in a 70% reduction in the mixed lymphocyte response in LPS- and dextran sulfate-stimulated B cells.



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



