

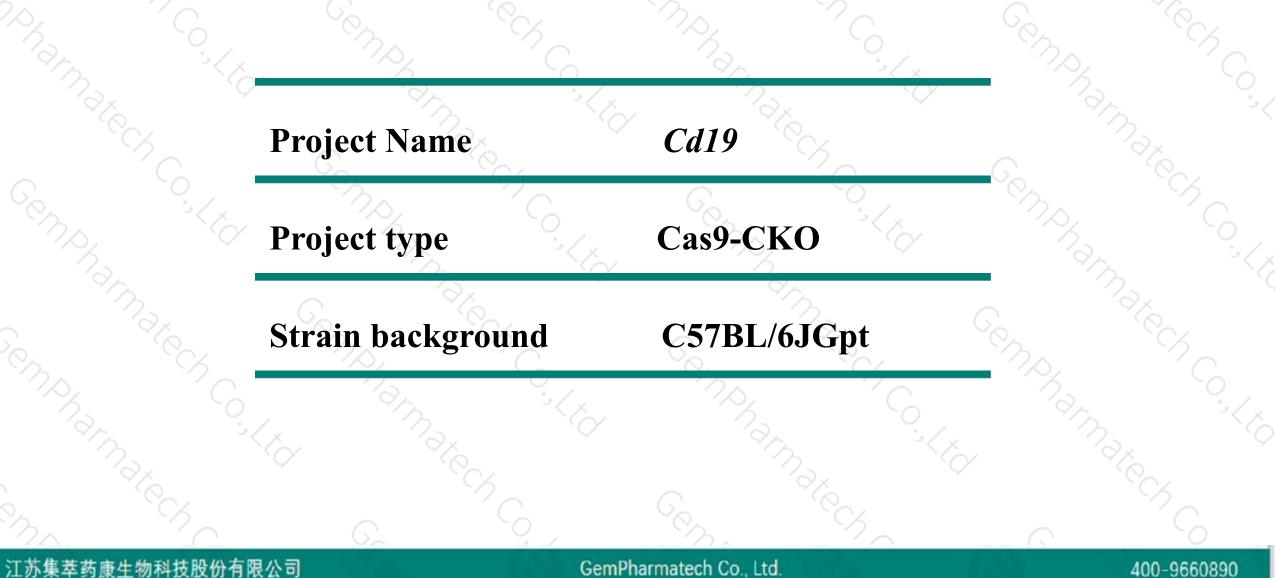
# anphamatech Co. ND AMAKA CHO-14 Cd19 Cas9-CKO Strategy Romphamater Control

Cemphamatech Co.

Emphamater Co. Lity Designer: Lixin Lv

# **Project Overview**





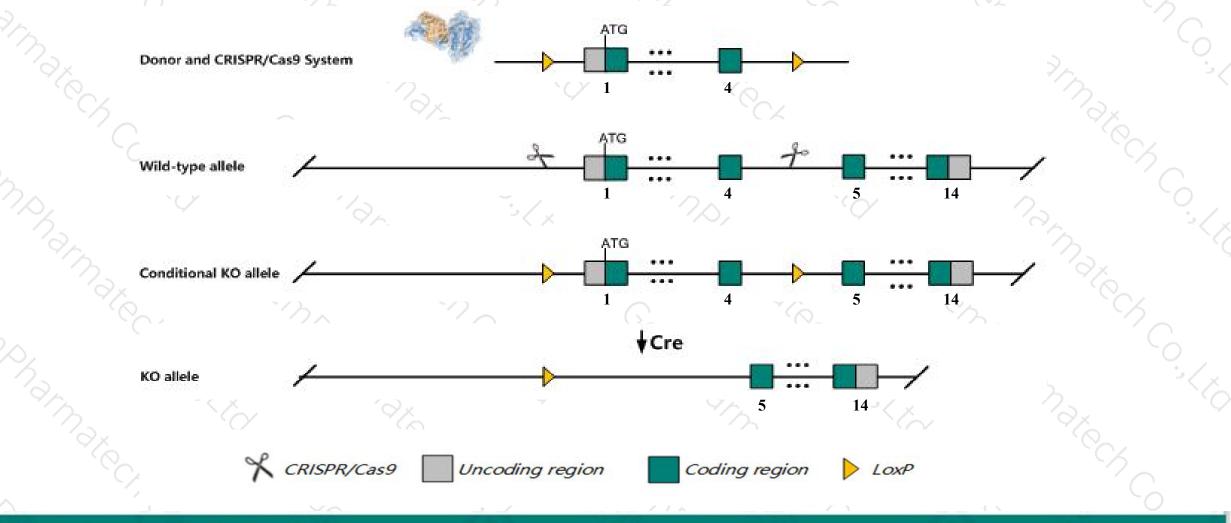
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## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Cd19 gene. The schematic diagram is as follows:



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The Cd19 gene has 5 transcripts. According to the structure of Cd19 gene, exon1-exon4 of Cd19-204 (ENSMUST00000206325.1) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Cd19* 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 abnormal B lymphocyte development, activation and differentiation, altered mast cell activation in a model for acute septic peritonitis, inhibition of bleomycin-induced fibrosis and autoantibody production, and increased susceptibility to EAE.
- > The *Cd19* gene is located on the Chr7. 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)



☆ ?

### Cd19 CD19 antigen [Mus musculus (house mouse)]

Gene ID: 12478, updated on 9-Apr-2019

#### Summary

Official SymbolCd19 provided by MGIOfficial Full NameCD19 antigen provided by MGIPrimary sourceMGI:MGI:88319See relatedEnsembl:ENSMUSG0000030724Gene typeprotein codingvolten CodingVALIDATEDOrganianMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;<br/>Muroidea; Murinae; Mus; MusAlso knowaneAW495831Bised expression in spleen adult (RPKM 117.1), mammary gland adult (RPKM 26.5) and 2 other tissuesSee more<br/>human all

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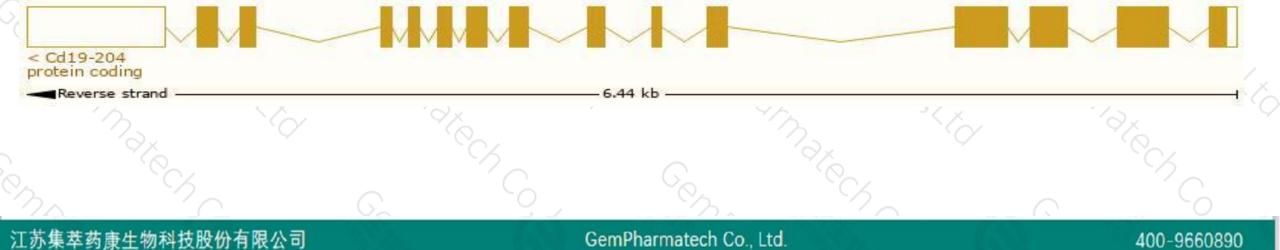
# **Transcript information (Ensembl)**



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

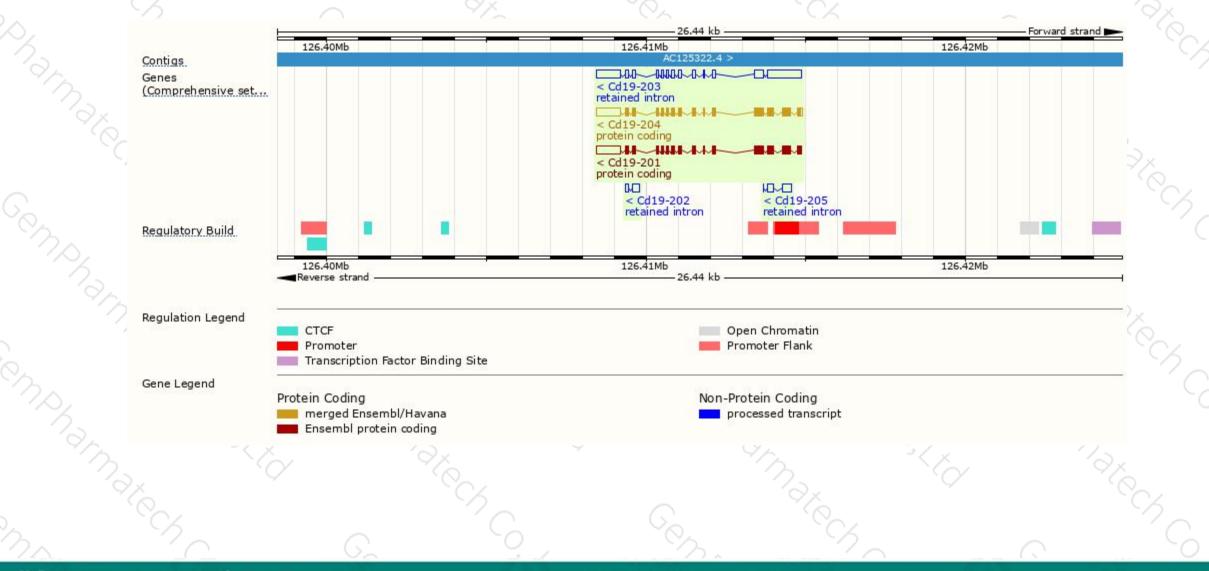
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cd19-204	ENSMUST00000206325.1	2452	<u>547aa</u>	Protein coding	CCDS21828	P25918	TSL:1 GENCODE basic APPRIS P2
Cd19-201	ENSMUST0000032968.6	2432	<u>546aa</u>	Protein coding	-	A0A0X1KG58	TSL:1 GENCODE basic APPRIS ALT2
Cd19-203	ENSMUST00000205997.1	2928	No protein	Retained intron	42	-	TSL:2
Cd19-205	ENSMUST00000206871.1	493	No protein	Retained intron	20 20	-	TSL:3
Cd19-202	ENSMUST00000205848.1	315	No protein	Retained intron	50		TSL:3

The strategy is based on the design of Cd19-204 transcript, The transcription is shown below



### **Genomic location distribution**





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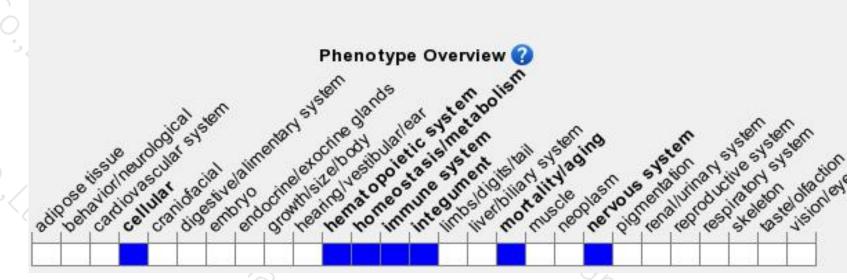
### **Protein domain**





## 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, Mice homozygous for a knock-out allele exhibit abnormal B lymphocyte development, activation and differentiation, altered mast cell activation in a model for acute septic peritonitis, inhibition of bleomycin-induced fibrosis and autoantibody production, and increased susceptibility to EAE.

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



