Crlf1 Cas9-CKO Strategy

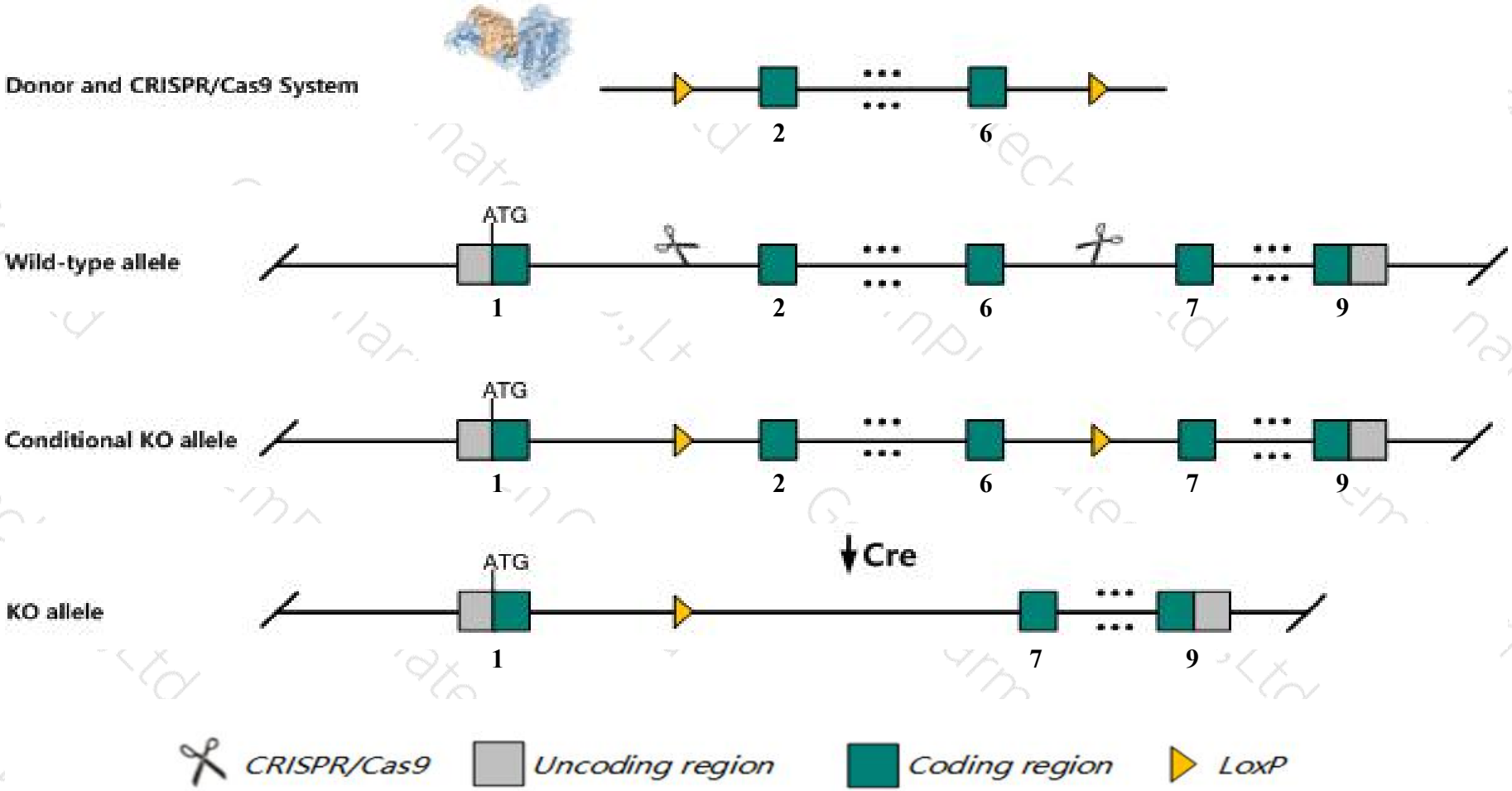
Designer: Xueting Zhang
Design Date: 2019-7-29

Project Overview

Project Name	***Crlf1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Crlf1* gene. The schematic diagram is as follows:



Technical routes

- The *Crlf1* gene has 3 transcripts. According to the structure of *Crlf1* gene, exon2-exon6 of *Crlf1-201* (ENSMUST00000008032.13) 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 *Crlf1* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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.

Notice

➢ The influence on *Crlf1*-202&203 is unknown.

➢ According to the existing MGI data,Mice homozygous for a targeted mutation fail to suckle effectively and do not survive beyond 24 hrs after birth. Newborns exhibit reduced numbers of hematopoietic progenitor cells as well as a significant reduction in the number of motoneurons in the lumbar spinal cord and facial nucleus.

➢ The *Crlf1* gene is located on the Chr8. 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）

集萃药康 GemPharmatech

Crlf1 cytokine receptor-like factor 1 [Mus musculus (house mouse)]

Gene ID: 12931, updated on 31-Jan-2019

Summary

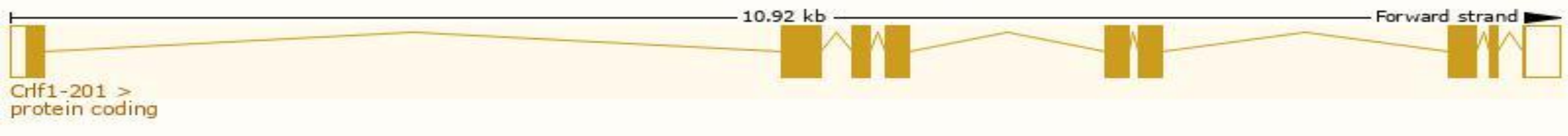
Official Symbol	Crlf1 provided by MGI
Official Full Name	cytokine receptor-like factor 1 provided by MGI
Primary source	MGI:MGI:1340030
See related	Ensembl:ENSMUSG00000007888
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CLF-1, CRLM-3, CRLM3, NR6, NR6.1
Summary	This gene encodes a member of the cytokine type I receptor family. The encoded protein functions as a cytokine receptor subunit and may be involved in immune system regulation and fetal development. [provided by RefSeq, Dec 2015]
Expression	Biased expression in limb E14.5 (RPKM 28.5), testis adult (RPKM 18.6) and 7 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

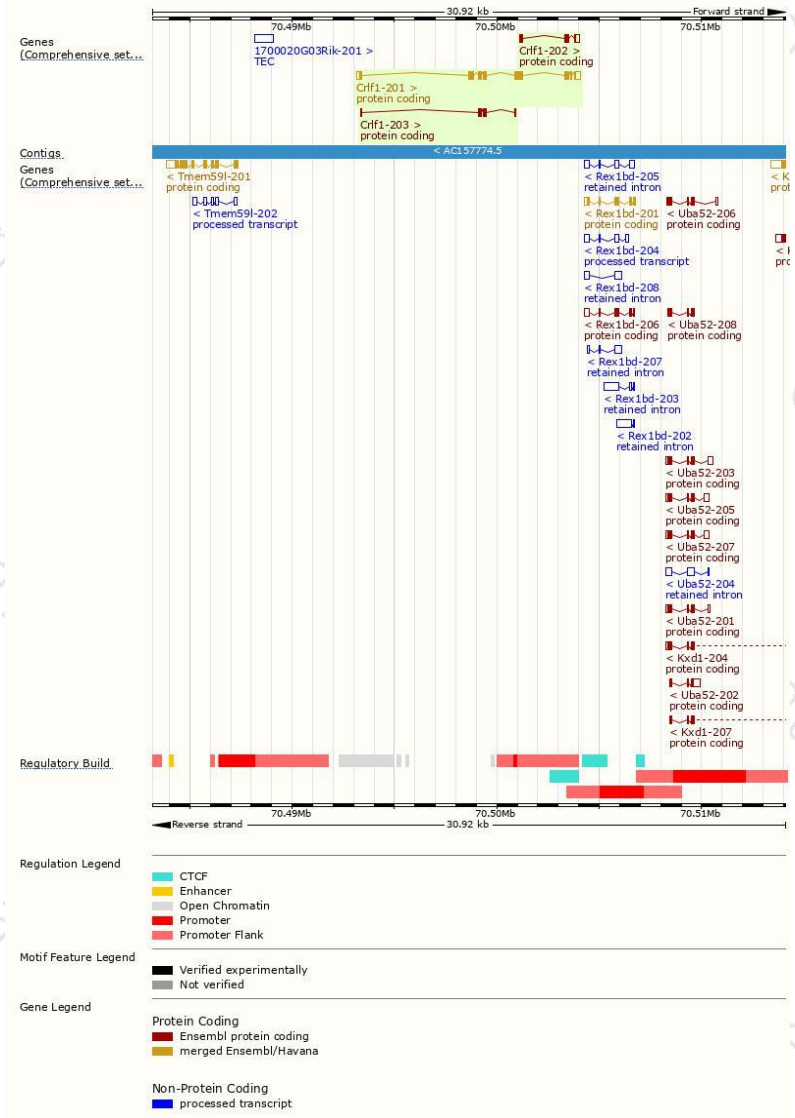
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Crlf1-201	ENSMUST00000008032.13	1644	425aa	Protein coding	CCDS22369	Q9JM58	TSL:1 GENCODE basic APPRIS P1
Crlf1-202	ENSMUST00000127983.1	544	118aa	Protein coding	-	F6T1W4	CDS 5' incomplete TSL:2
Crlf1-203	ENSMUST00000132648.1	387	129aa	Protein coding	-	F6RP70	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3

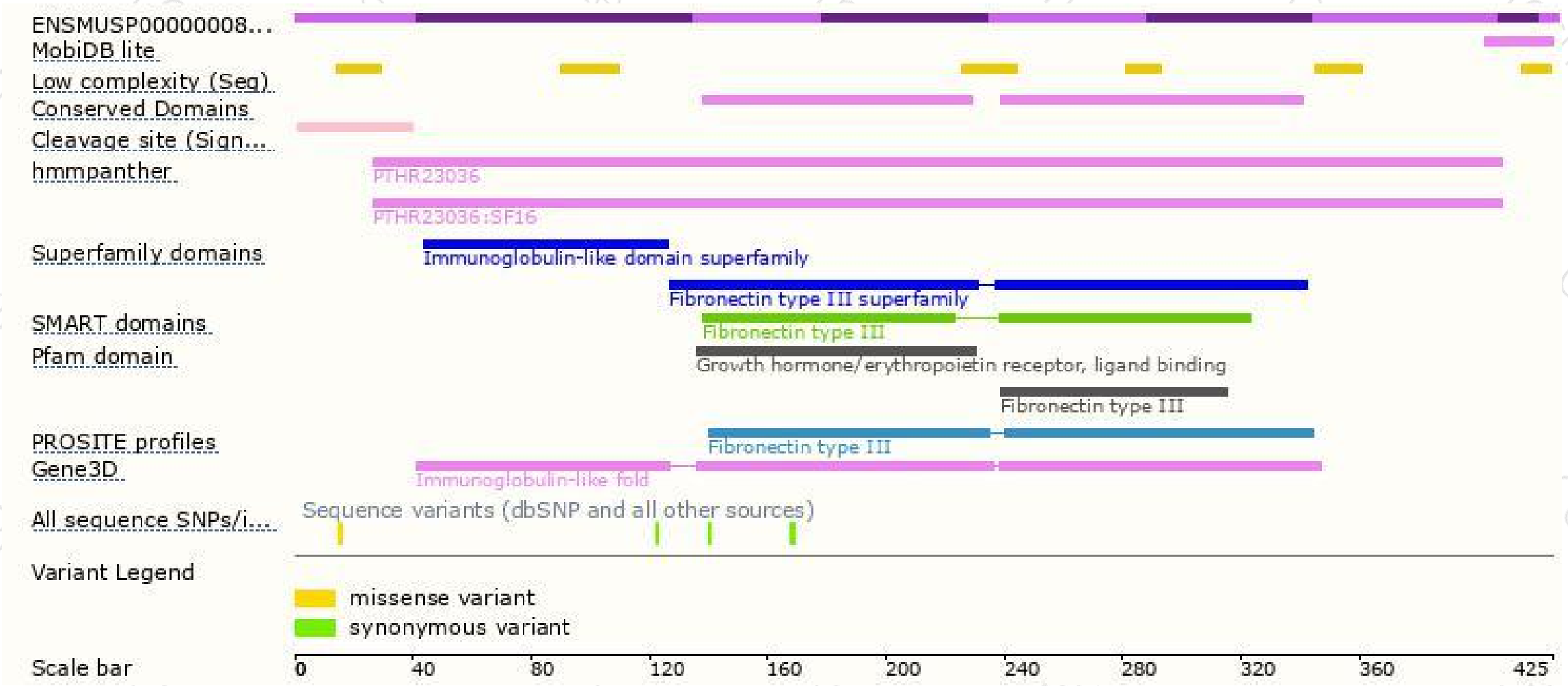
The strategy is based on the design of *Crlf1-201* transcript,The transcription is shown below



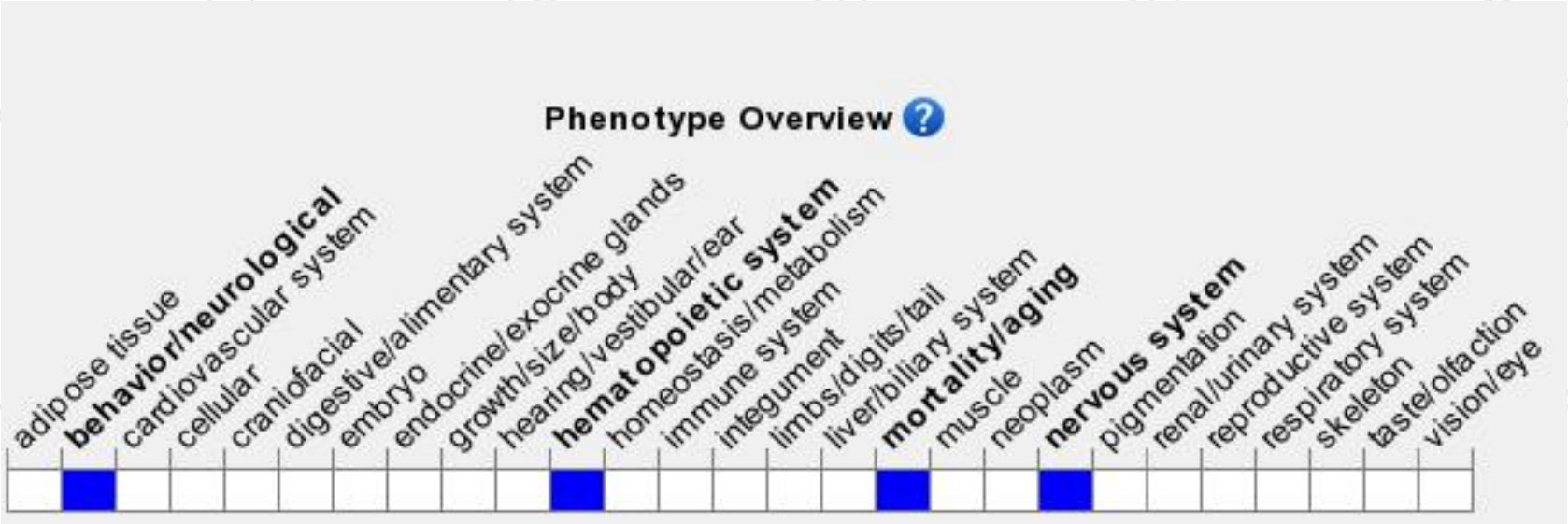
Genomic location distribution



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 targeted mutation fail to suckle effectively and do not survive beyond 24 hrs after birth. Newborns exhibit reduced numbers of hematopoietic progenitor cells as well as a significant reduction in the number of motoneurons in the lumbar spinal cord and facial nucleus.

集萃药康
GemPharmatech

If you have any questions, you are welcome to inquire.

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd