Kcnj8 Cas9-CKO Strategy

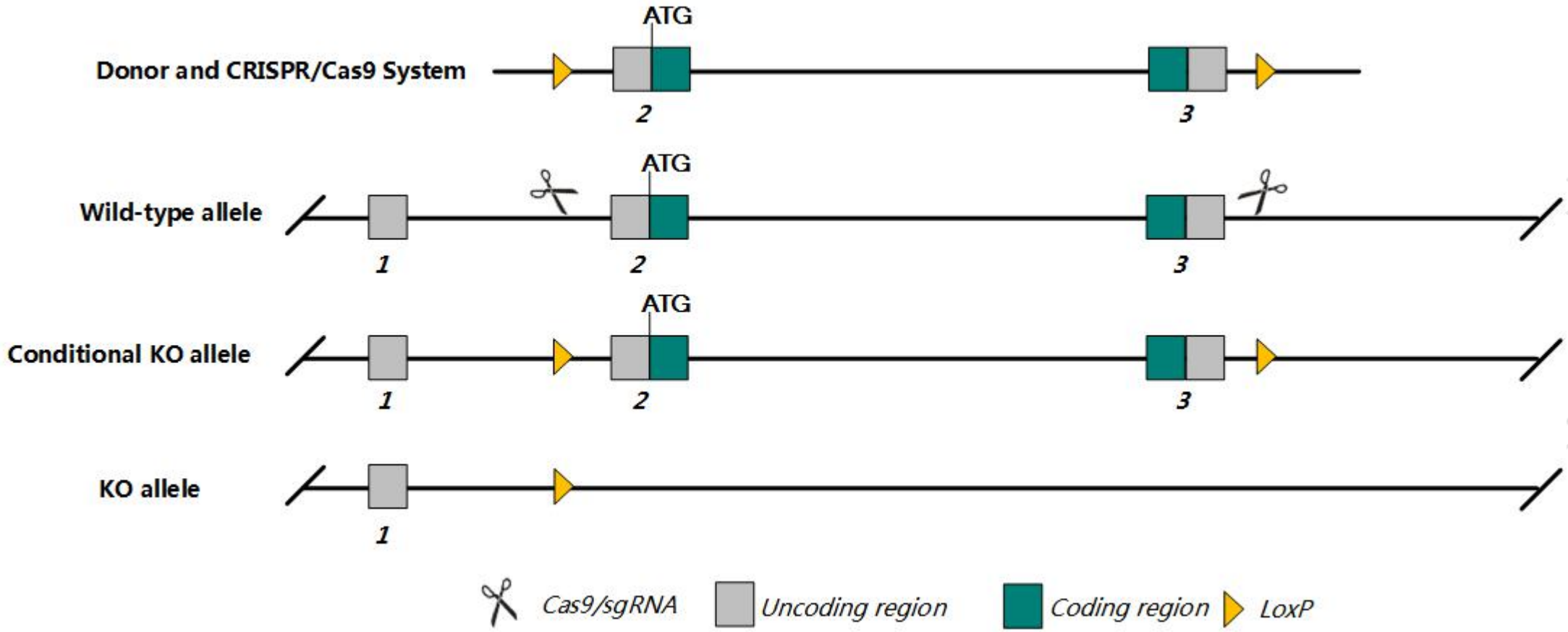
Designer: Xueting Zhang
Design Date: 2019-7-25

Project Overview

Project Name	***Kcnj8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Kcnj8* gene. The schematic diagram is as follows:



Technical routes

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- The *Kcnj8* gene has 2 transcripts. According to the structure of *Kcnj8* gene, exon2-exon3 of *Kcnj8-202* (ENSMUST00000203945.2) transcript is recommended as the knockout region.The region contains all the coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Kcnj8* 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

- According to the existing MGI data, Mice homozygous for a targeted null mutation exhibit sudden cardiac death due to dysregulation of the vascular tonus in the coronary arteries, and exhibit a phenotype resembling Prinzmetal (or variant) angina in humans.
- The *Kcnj8* gene is located on the Chr6. 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）

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Kcnj8 potassium inwardly-rectifying channel, subfamily J, member 8 [*Mus musculus* (house mouse)]

Gene ID: 16523, updated on 13-Apr-2019

Summary

Official Symbol	Kcnj8 provided by MGI
Official Full Name	potassium inwardly-rectifying channel, subfamily J, member 8 provided by MGI
Primary source	MGI:MGI:1100508
See related	Ensembl:ENSMUSG00000030247
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	sltr; gnite; slmbr; Kir6.1; uKATP-1; AI448900
Expression	Broad expression in subcutaneous fat pad adult (RPKM 13.7), heart adult (RPKM 13.0) and 25 other tissues See more
Orthologs	human all

Genomic context

Location: 6 G2; 6 74.31 cM

See Kcnj8 in Genome Data Viewer

Exon count: 3

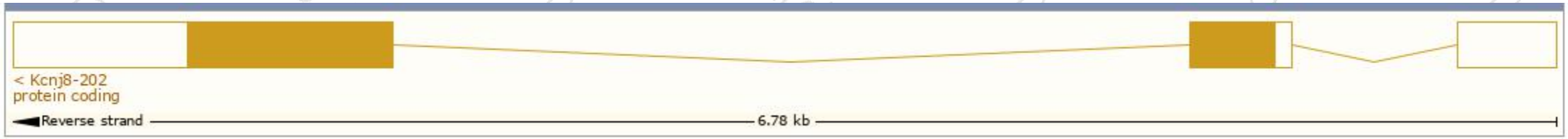
Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	6	NC_000072.6 (142564837..142571647, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	6	NC_000072.5 (142513459..142519876, complement)

Transcript information（Ensembl）

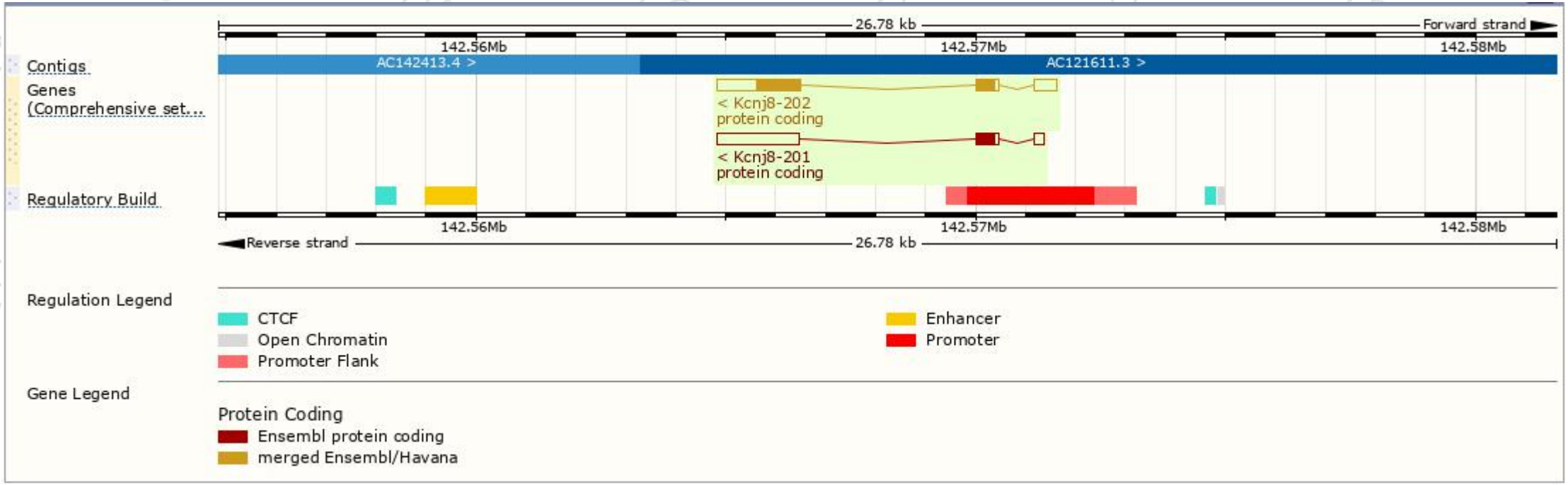
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID ▲	bp	Protein	Biotype	CCDS	UniProt	Flags
Kcnj8-201	ENSMUST00000032374.8	2269	127aa	Protein coding	-	A0A0J9YMM3	TSL:1 GENCODE basic
Kcnj8-202	ENSMUST00000203945.2	2548	424aa	Protein coding	CCDS20685	P97794 Q3U118	TSL:1 GENCODE basic APPRIS P1

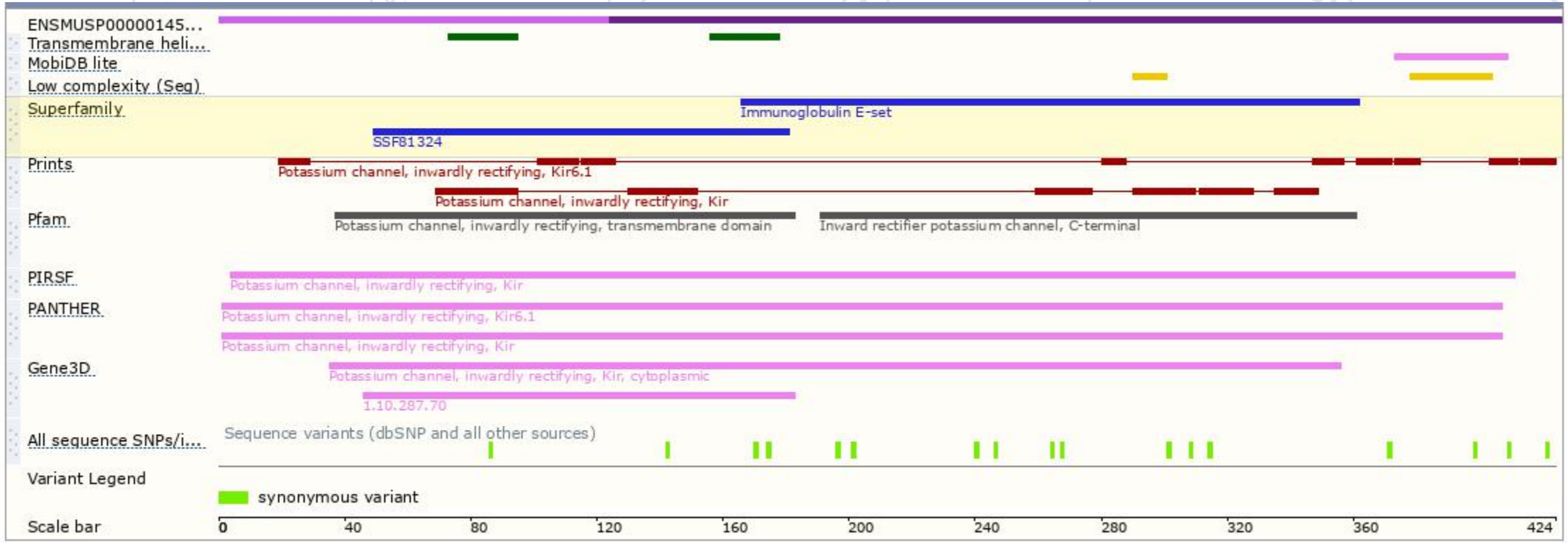
The strategy is based on the design of *Kcnj8-202* transcript,The transcription is shown below



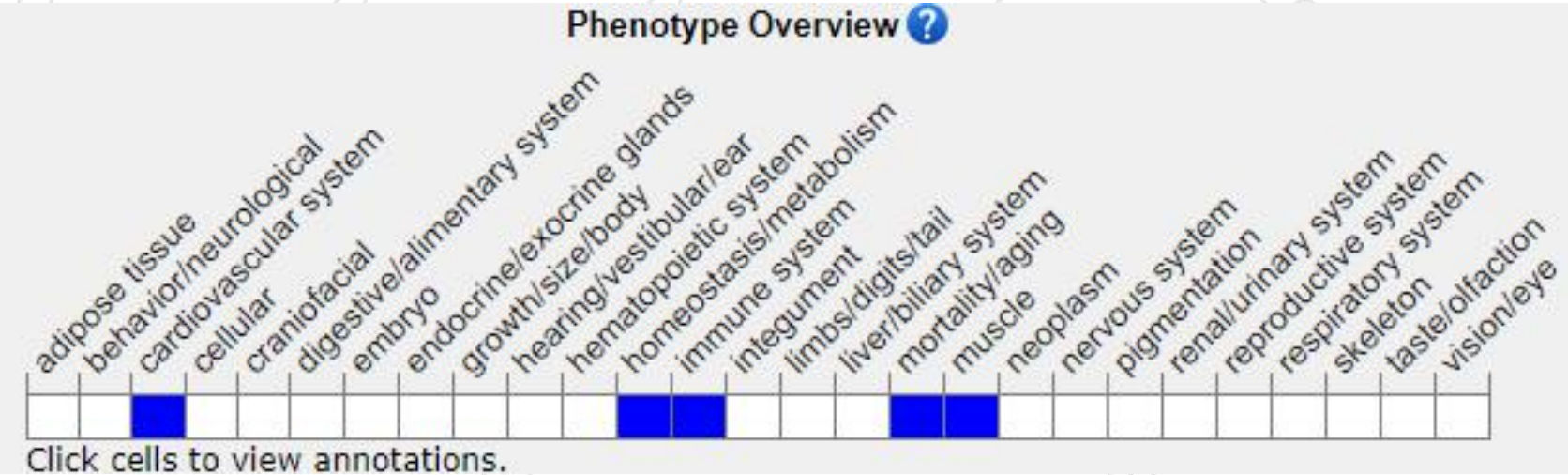
Genomic location distribution



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/).

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd