Rcn3 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Daohua Shen

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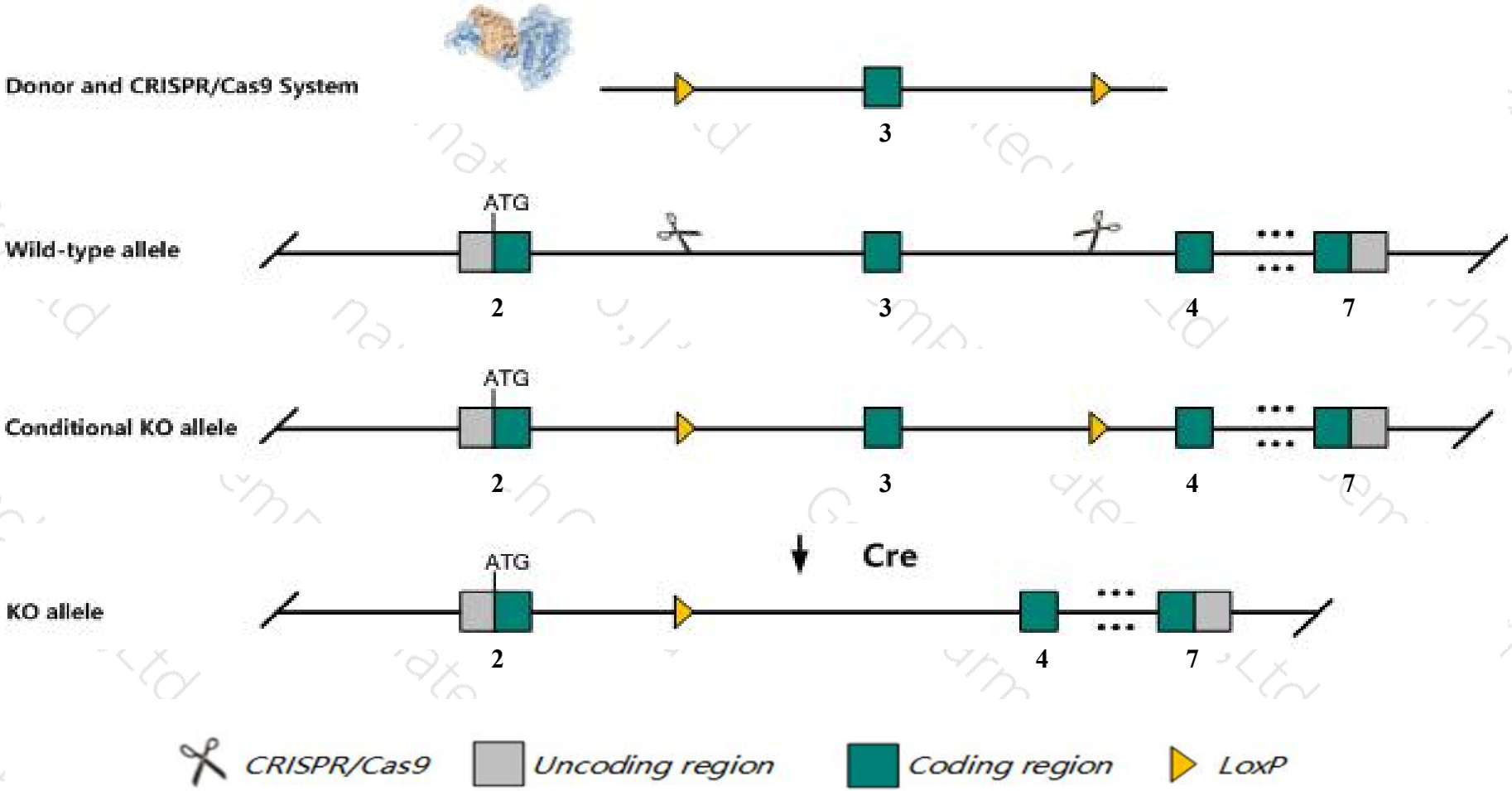
Project Overview

Project Name	***Rcn3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rcn3* gene. The schematic diagram is as follows:



Technical routes

- The *Rcn3* gene has 9 transcripts. According to the structure of *Rcn3* gene, exon3 of *Rcn3-201* (ENSMUST00000019683.10) transcript is recommended as the knockout region.The region contains 203bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rcn3* 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.

Notice

- According to the existing MGI data,mice homozygous for a knock-out allele exhibit neonatal lethality due to atelectasis-induced respiratory distress associated with failure of type ii pneumocyte maturation, decreased surfactant protein secretion, altered surfactant phospholipid homeostasis, and abnormal lamellar body formation.
- Transcript *Rcn3*-203&206 may not be affected.
- The effect on transcript *Rcn3*-205&209 is unknown.
- The floxed region is near to the N-terminal of *Gm45669* gene,this strategy may influence the regulatory function of the N-terminal of *Gm45669* gene.
- The *Rcn3* 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）

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Rcn3 reticulocalbin 3, EF-hand calcium binding domain [Mus musculus (house mouse)]

Gene ID: 52377, updated on 13-Mar-2020

Summary

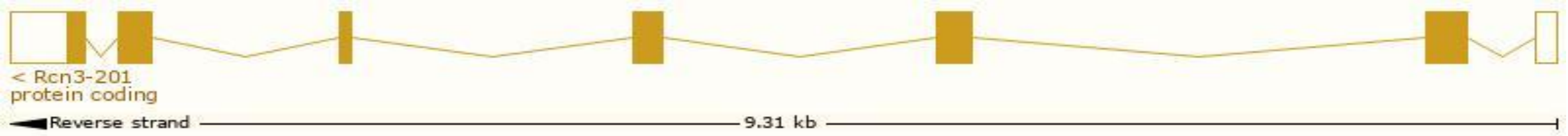
Official Symbol	Rcn3 provided by MGI
Official Full Name	reticulocalbin 3, EF-hand calcium binding domain provided by MGI
Primary source	MGI:MGI:1277122
See related	Ensembl:ENSMUSG00000019539
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6030455P07Rik, D530026G20Rik, D7Ertd671e, RLP49
Expression	Broad expression in limb E14.5 (RPKM 194.2), ovary adult (RPKM 178.1) and 21 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

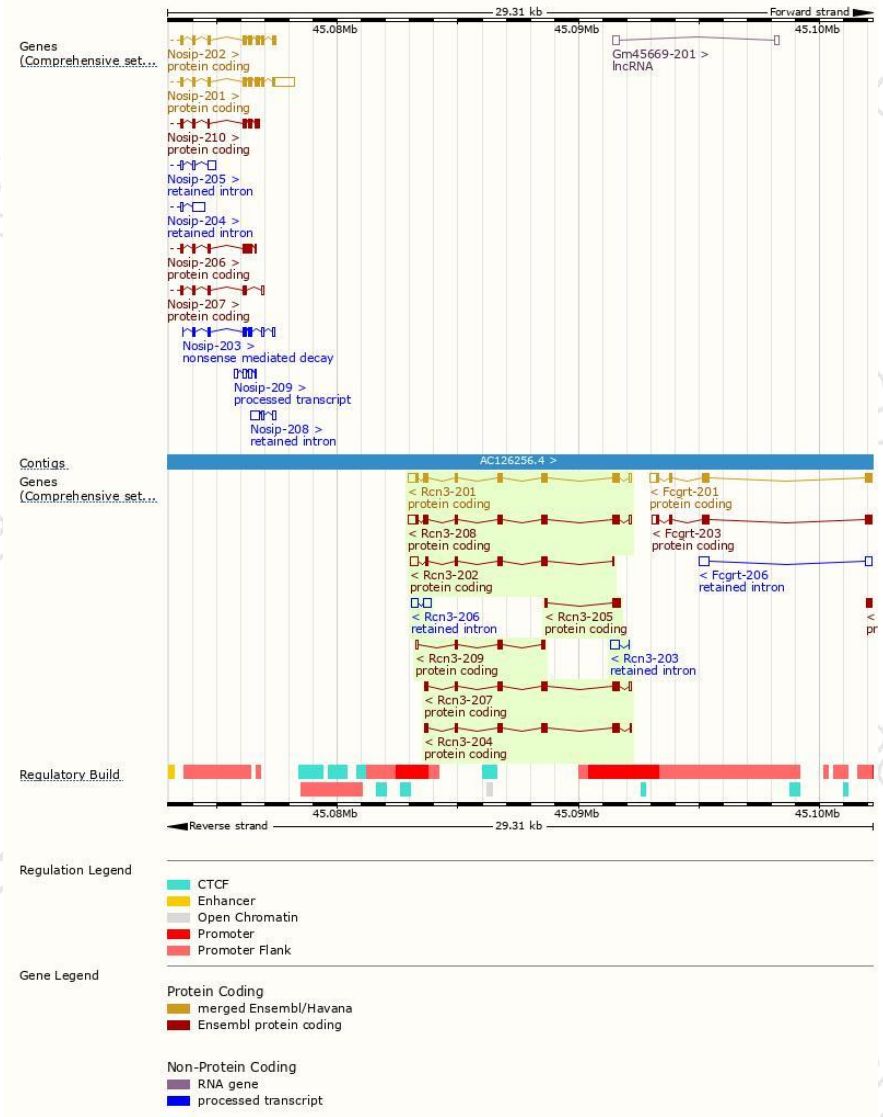
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rcn3-201	ENSMUST00000019683.10	1462	328aa	Protein coding	CCDS21229	Q8BH97	TSL:1 GENCODE basic APPRIS P1
Rcn3-208	ENSMUST00000211352.1	1453	328aa	Protein coding	CCDS21229	Q8BH97	TSL:1 GENCODE basic APPRIS P1
Rcn3-207	ENSMUST00000210734.1	905	280aa	Protein coding	-	A0A1B0GR86	CDS 3' incomplete TSL:5
Rcn3-202	ENSMUST00000209761.1	892	186aa	Protein coding	-	A0A1B0GSK5	CDS 5' incomplete TSL:5
Rcn3-204	ENSMUST00000210469.1	884	274aa	Protein coding	-	A0A1B0GR19	CDS 3' incomplete TSL:3
Rcn3-209	ENSMUST00000211760.1	471	122aa	Protein coding	-	A0A1B0GS22	CDS 5' incomplete TSL:5
Rcn3-205	ENSMUST00000210527.1	389	115aa	Protein coding	-	A0A1B0GRI0	CDS 3' incomplete TSL:2
Rcn3-206	ENSMUST00000210615.1	589	No protein	Retained intron	-	-	TSL:2
Rcn3-203	ENSMUST00000210355.1	381	No protein	Retained intron	-	-	TSL:2

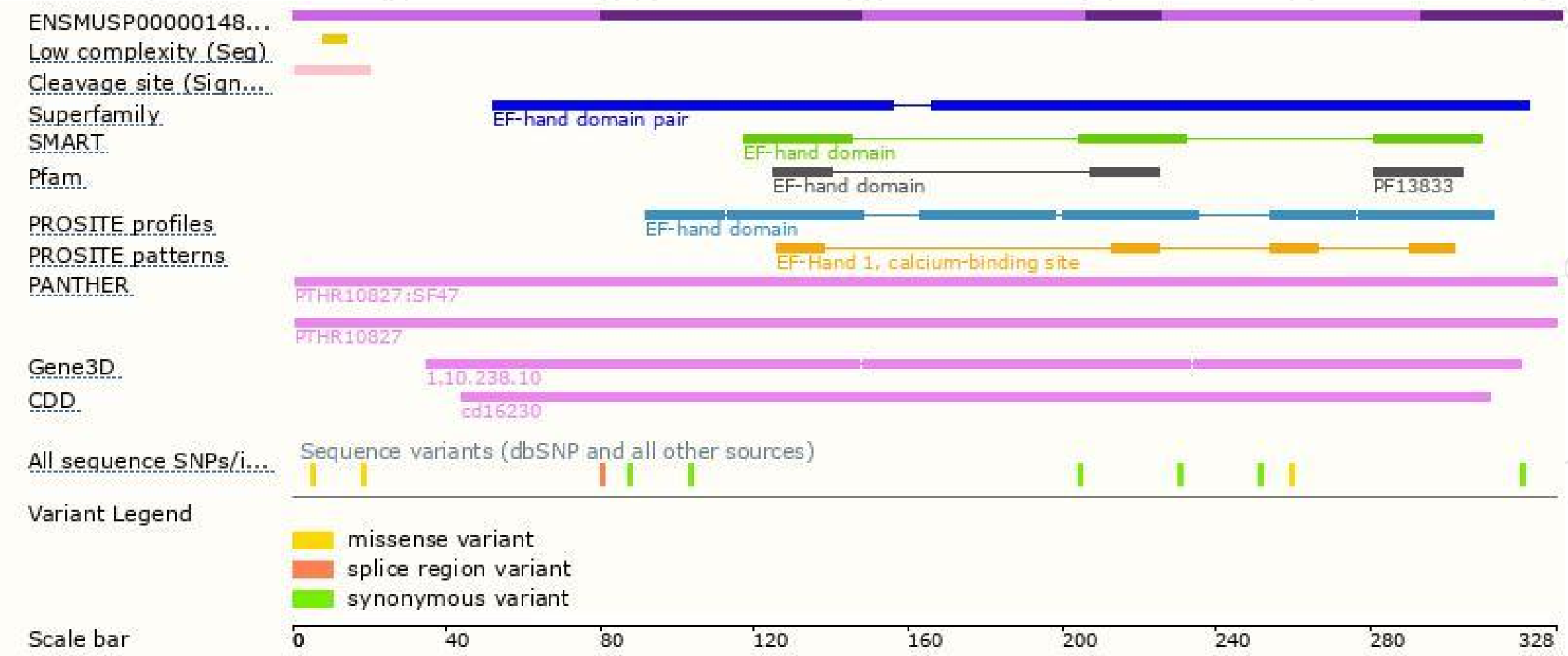
The strategy is based on the design of *Rcn3-201* transcript,the transcription is shown below:



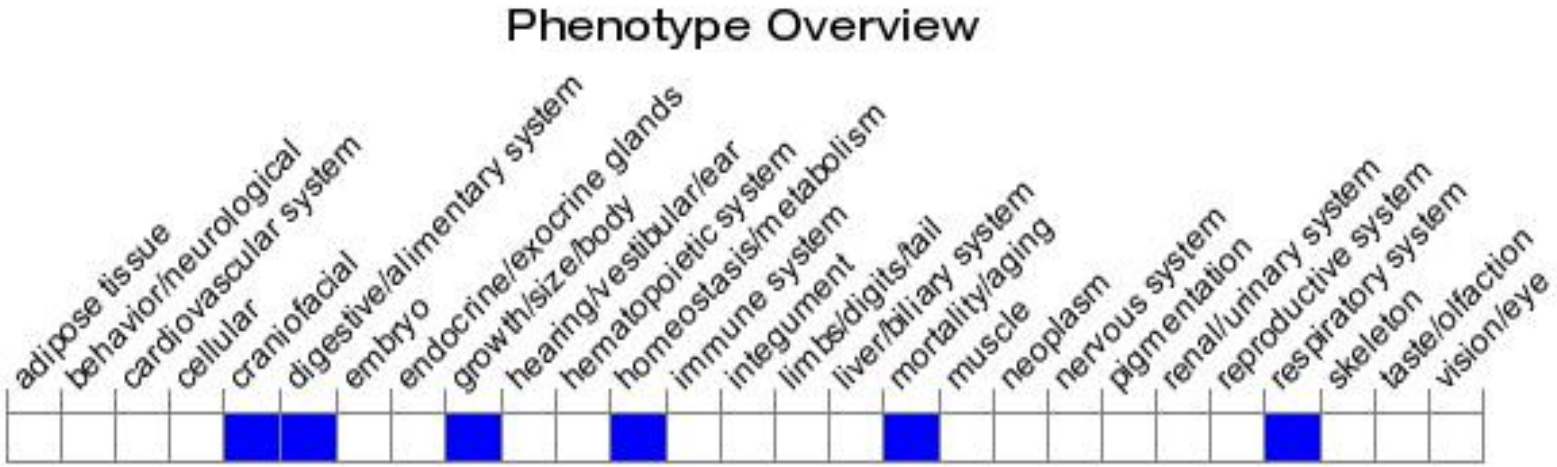
Genomic location distribution



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/).

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集萃药康
GemPharmatech

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

Tel: 400-9660890

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GemPharmatech Co.,Ltd