Krit1 Cas9-KO Strategy

Designer: Xiaojing Li
Design Date: 2019-9-11
Reviewer: JiaYu

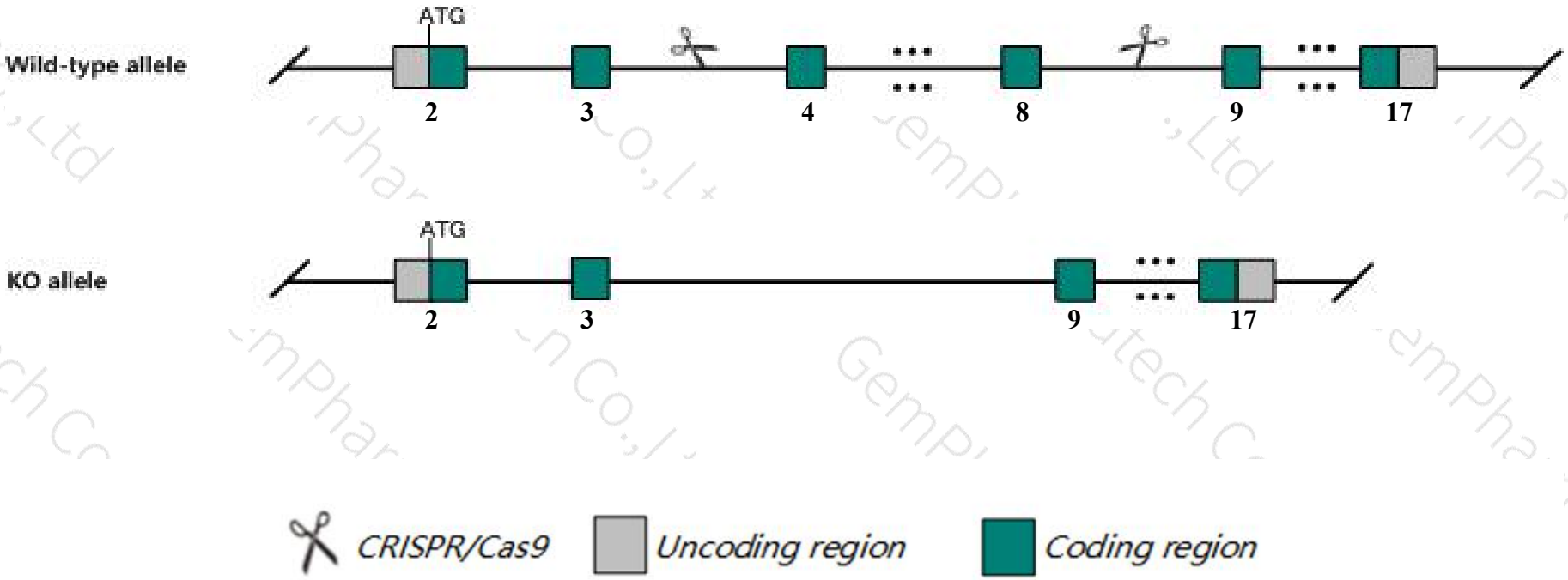
集萃药康 GemPharmatech
GemPharmatech Co.,Ltd

Project Overview

Project Name	***Krit1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Krit1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Krit1* gene has 12 transcripts. According to the structure of *Krit1* gene, exon4-exon8 of *Krit1-201* (ENSMUST00000080085.8) transcript is recommended as the knockout region.The region contains 727bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Krit1* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

➢ According to the existing MGI data,Targeted disruption of this gene results in embryonic lethality by E11. Embryos display prominent vascular defects that disrupt arterial modeling and phenocopy the human disorder of cerebral cavernous malformations.

➢ The *Krit1* gene is located on the Chr5. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

集萃药康 GemPharmatech

Krit1 KRIT1, ankyrin repeat containing [Mus musculus (house mouse)]

Gene ID: 79264, updated on 12-Mar-2019

Summary

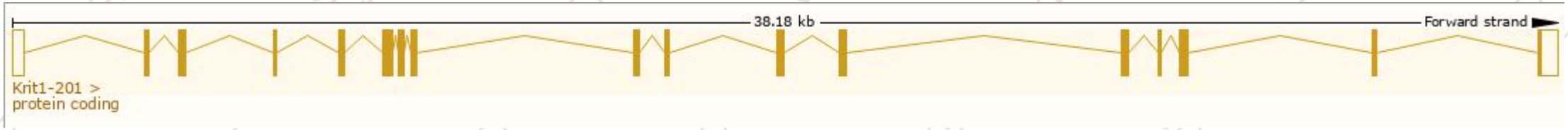
Official Symbol	Krit1 provided by MGI
Official Full Name	KRIT1, ankyrin repeat containing provided by MGI
Primary source	MGI:MGI:1930618
See related	Ensembl:ENSMUSG00000000600
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2010007K12Rik, A630036P20Rik, AA432855, AI450393, AI643869, BB155247, BB235701, Ccm1
Expression	Broad expression in CNS E14 (RPKM 7.1), CNS E11.5 (RPKM 6.7) and 24 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

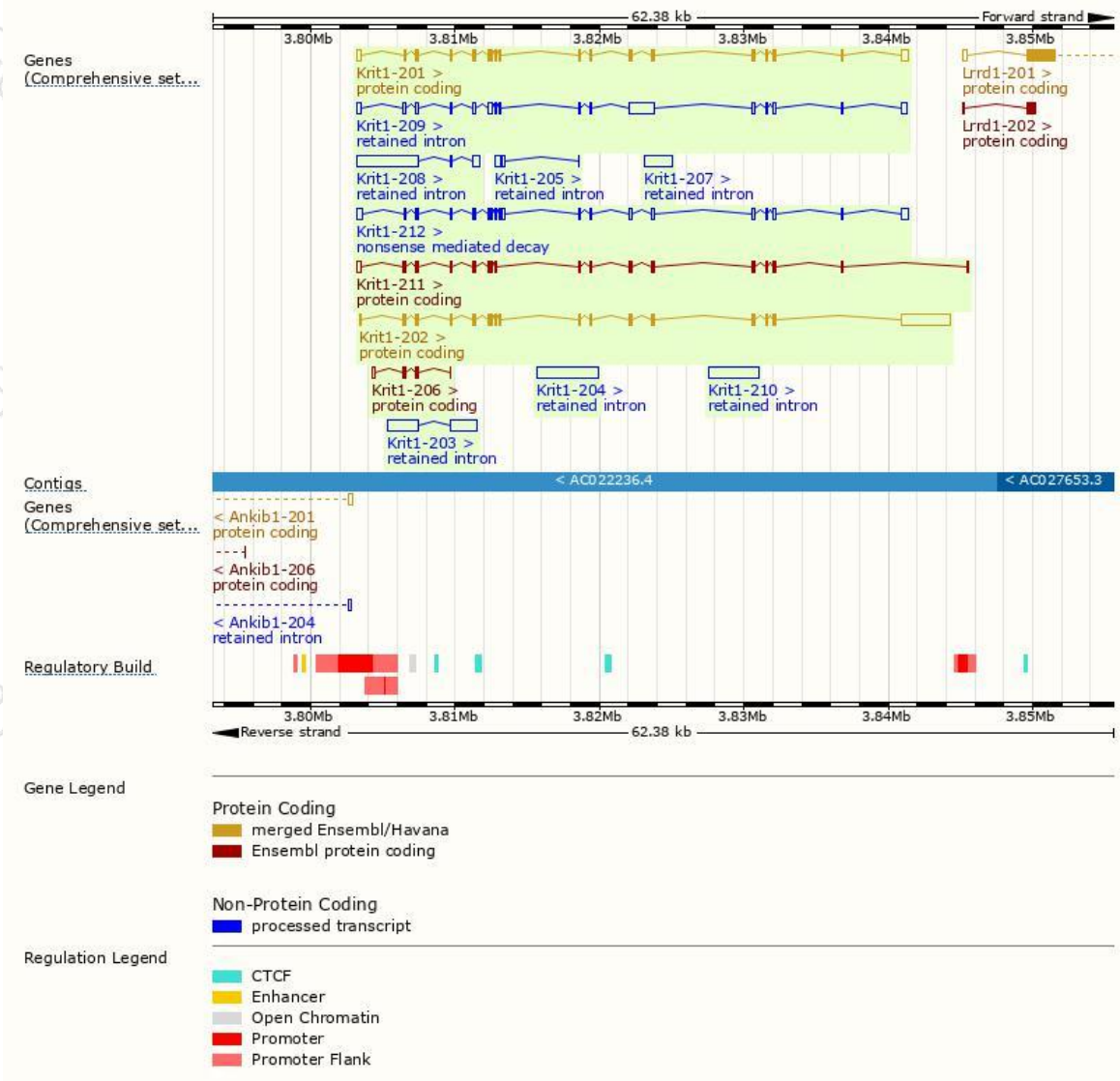
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Krit1-202	ENSMUST00000171023.7	5582	697aa	Protein coding	CCDS51409	Q6S5J6	TSL:1 GENCODE basic
Krit1-201	ENSMUST00000080085.8	2930	736aa	Protein coding	CCDS39002	B2RUA8 Q6S5J6	TSL:1 GENCODE basic APPRIS P1
Krit1-211	ENSMUST00000200386.4	2343	647aa	Protein coding	-	A0A0G2JGG7	TSL:5 GENCODE basic
Krit1-206	ENSMUST00000198079.1	571	132aa	Protein coding	-	A0A0G2JE71	CDS 3' incomplete TSL:3
Krit1-212	ENSMUST00000200577.4	3188	329aa	Nonsense mediated decay	-	Q6S5J6	TSL:1
Krit1-208	ENSMUST00000199475.1	4828	No protein	Retained intron	-	-	TSL:2
Krit1-209	ENSMUST00000199845.4	4293	No protein	Retained intron	-	-	TSL:5
Krit1-204	ENSMUST00000197611.1	4262	No protein	Retained intron	-	-	TSL:NA
Krit1-203	ENSMUST00000196098.1	4040	No protein	Retained intron	-	-	TSL:2
Krit1-210	ENSMUST00000200004.1	3481	No protein	Retained intron	-	-	TSL:NA
Krit1-207	ENSMUST00000199234.1	1914	No protein	Retained intron	-	-	TSL:NA
Krit1-205	ENSMUST00000197742.1	608	No protein	Retained intron	-	-	TSL:3

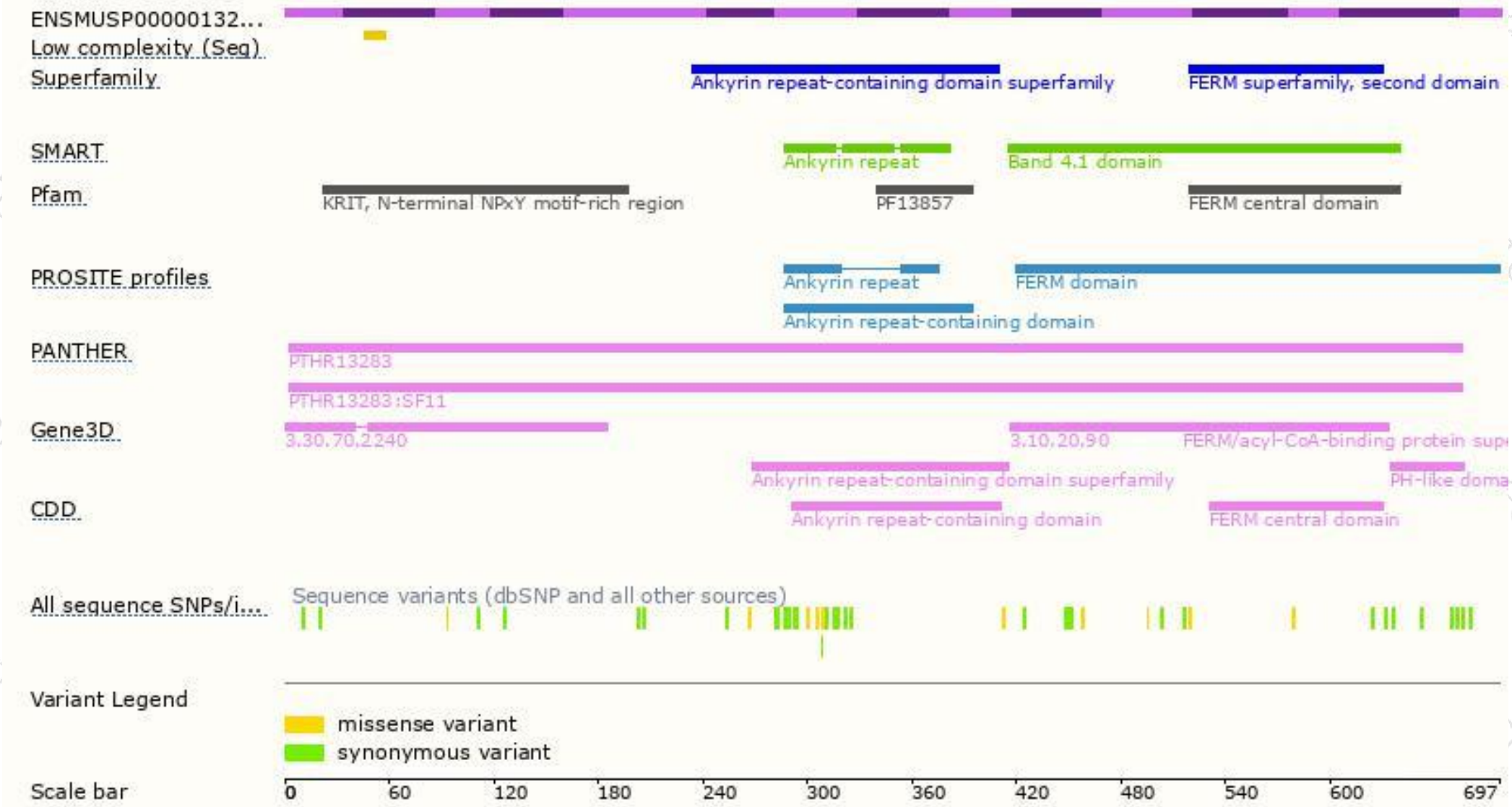
The strategy is based on the design of *Krit1-201* transcript,The transcription is shown below



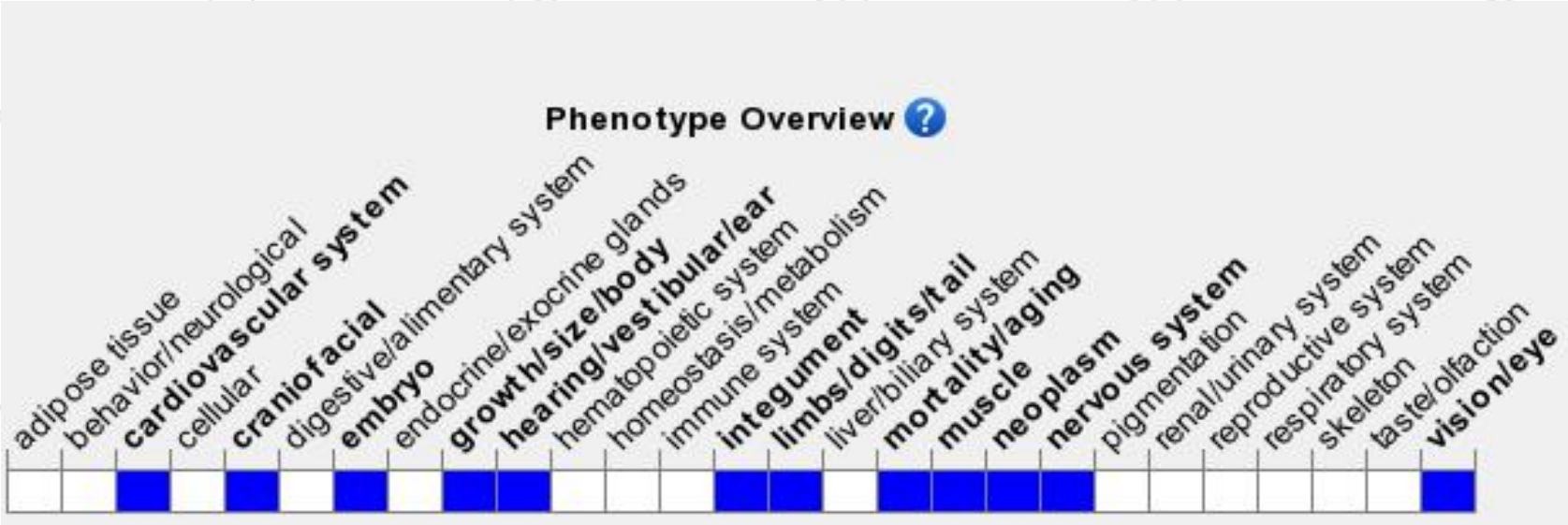
Genomic location distribution



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,Targeted disruption of this gene results in embryonic lethality by E11. Embryos display prominent vascular defects that disrupt arterial modeling and phenocopy the human disorder of cerebral cavernous malformations.

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

GemPharmatech Co.,Ltd