Sqstm1 Cas9-CKO Strategy

Designer: Huan Fan
Reviewer: Jiayuan Yao
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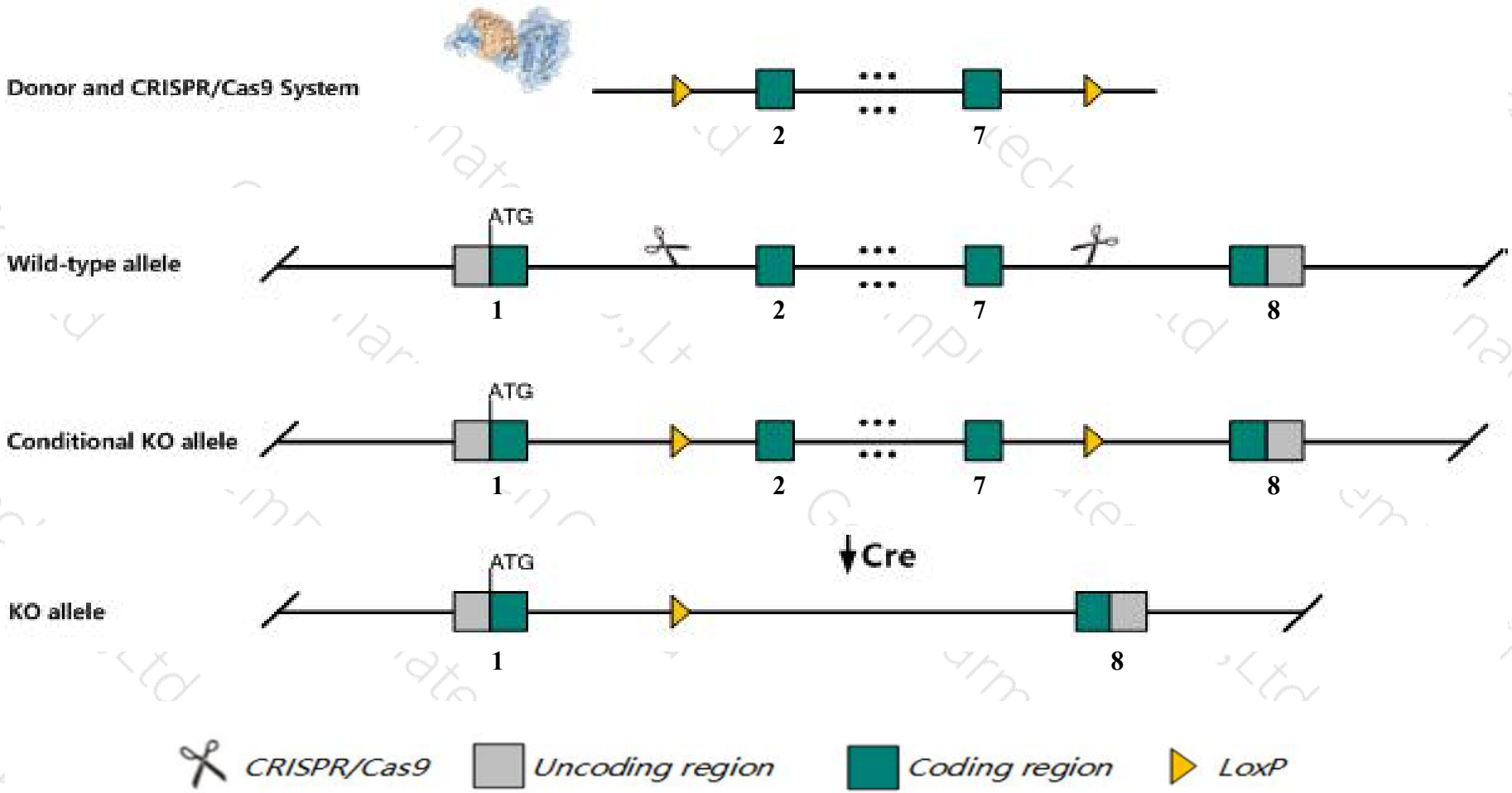
GemPharmatech Co.,Ltd

Project Overview

Project Name	***Sqstm1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sqstm1* gene. The schematic diagram is as follows:



Technical routes

- The *Sqstm1* gene has 7 transcripts. According to the structure of *Sqstm1* gene, exon2-exon7 of *Sqstm1-201* (ENSMUST00000015981.11) 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 *Sqstm1* 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 one knock-out allele exhibit impaired osteoclastogenesis in response to osteoclastogenic factors. Mice homozygous and heterozygous for a knock-in allele exhibit osteolytic lesion with increased bone formation, mineral apposition rate,and osteoclast numbers.
- The KO region deletes most of the coding sequence, but does not result in frameshift.
- The floxed region is 2.5kb away from *Mgat4b*，*Mgat4b* may be affected.
- The *Sqstm1* gene is located on the Chr11. 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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Sqstm1 sequestosome 1 [Mus musculus (house mouse)]

Gene ID: 18412, updated on 9-Apr-2019

Summary

Official Symbol	Sqstm1 provided by MGI
Official Full Name	sequestosome 1 provided by MGI
Primary source	MGI:MGI:107931
See related	Ensembl:ENSMUSG00000015837
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	A170, OSF-6, Osi, STAP, STONE14, p62
Expression	Ubiquitous expression in adrenal adult (RPKM 275.7), placenta adult (RPKM 170.1) and 28 other tissues See more
Orthologs	human all

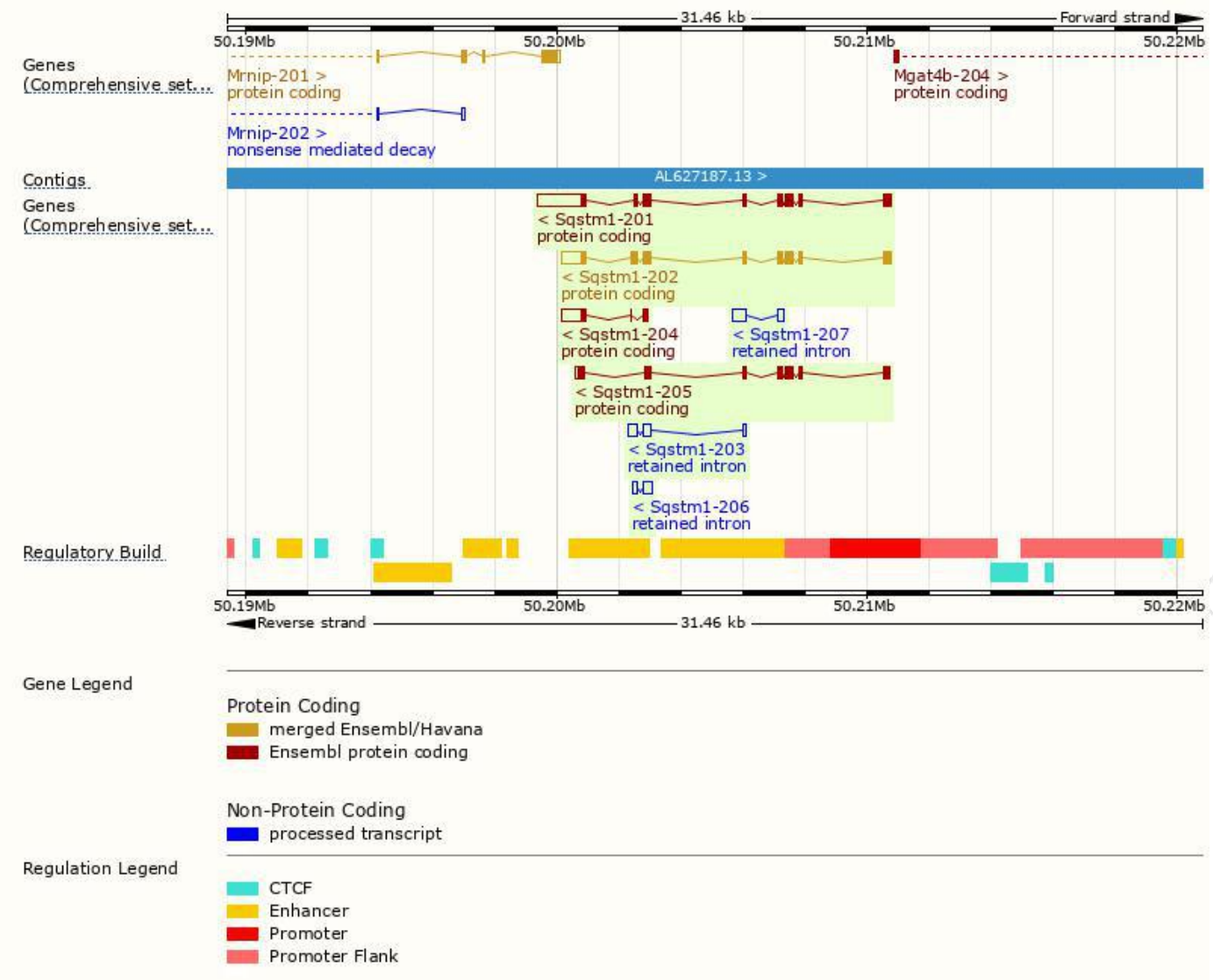
Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

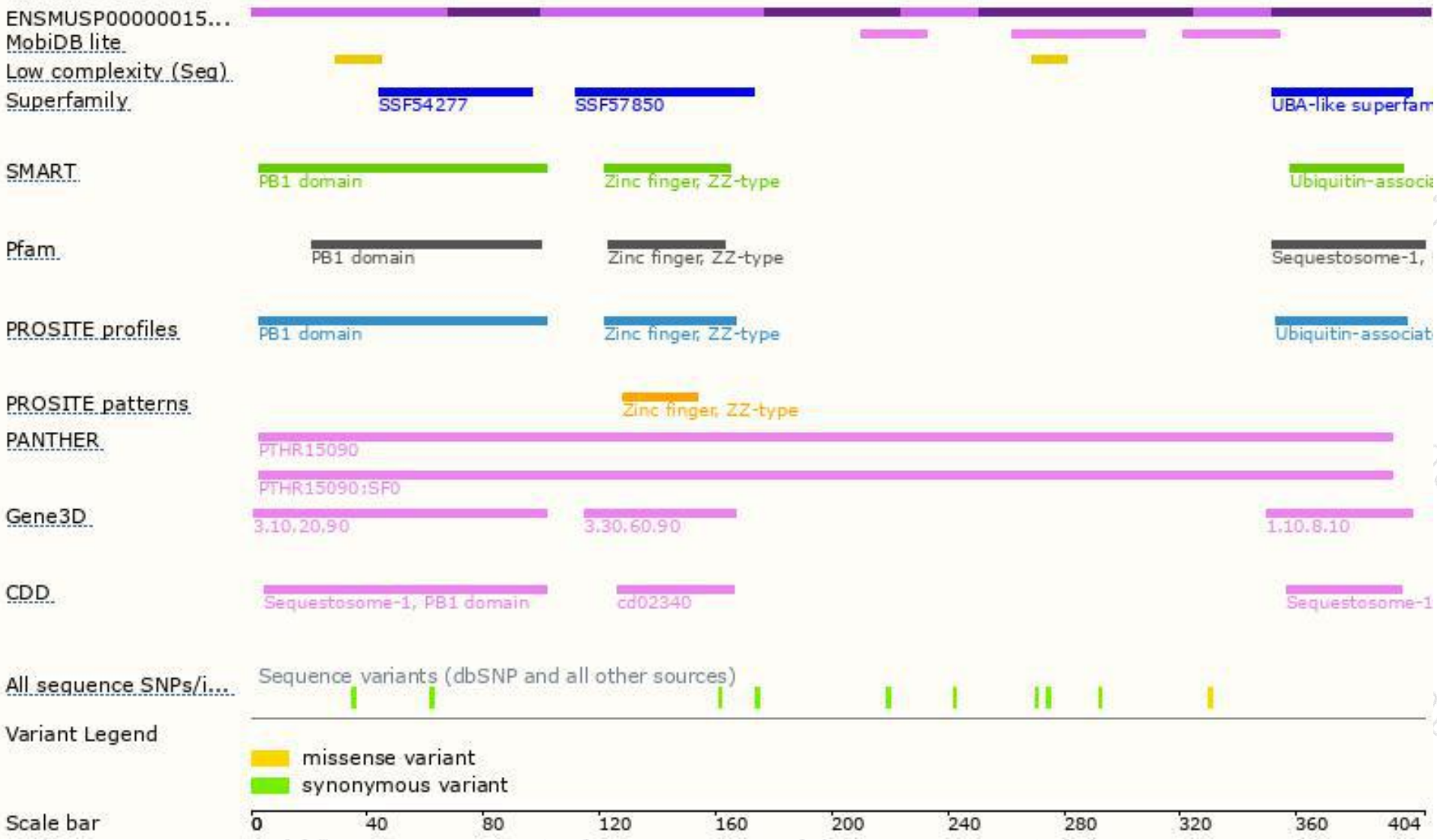
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sqstm1-201	ENSMUST00000015981.11	2673	404aa	Protein coding	CCDS70176	Q64337	TSL:1 GENCODE basic APPRIS ALT2
Sqstm1-202	ENSMUST00000102774.10	2037	442aa	Protein coding	CCDS24629	Q64337	TSL:1 GENCODE basic APPRIS P3
Sqstm1-205	ENSMUST00000143379.1	1266	382aa	Protein coding	-	D3YZJ1	TSL:5 GENCODE basic
Sqstm1-204	ENSMUST00000136936.1	953	109aa	Protein coding	-	F6VD69	CDS 5' incomplete TSL:3
Sqstm1-207	ENSMUST00000154805.1	585	No protein	Retained intron	-	-	TSL:2
Sqstm1-203	ENSMUST00000131214.1	582	No protein	Retained intron	-	-	TSL:3
Sqstm1-206	ENSMUST00000147846.1	457	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Sqstm1-201* transcript,The transcription is shown below

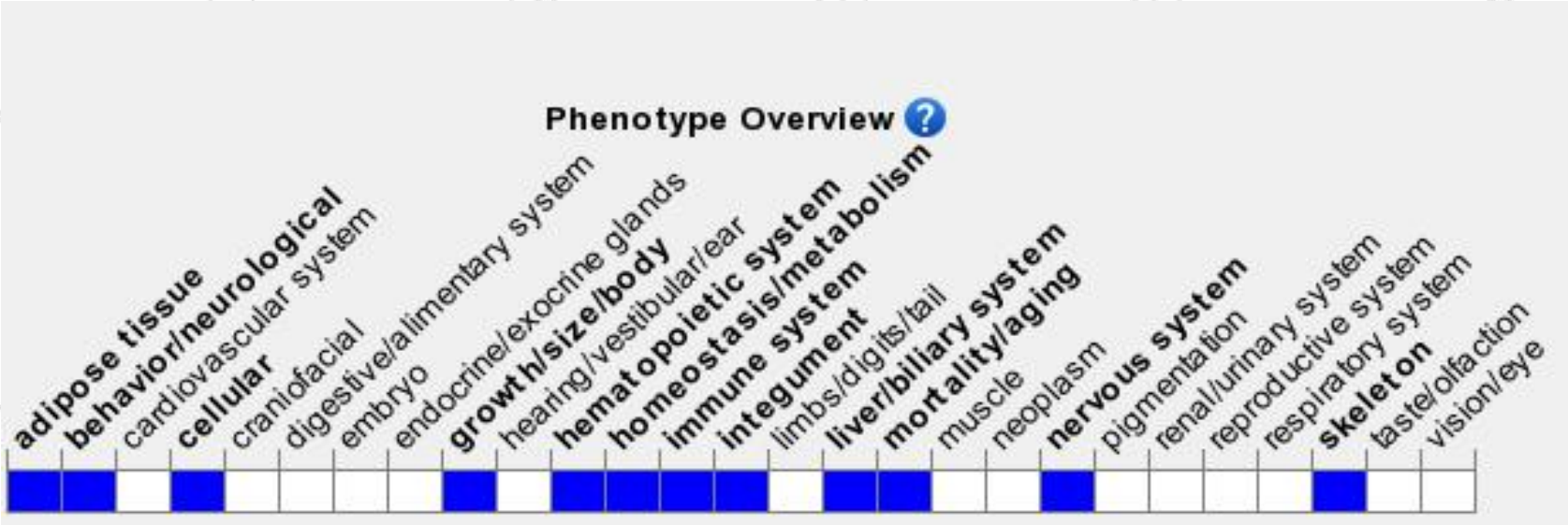
Genomic location distribution



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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GemPharmatech

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

Tel: 400-9660890

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