Pde6b Cas9-CKO Strategy

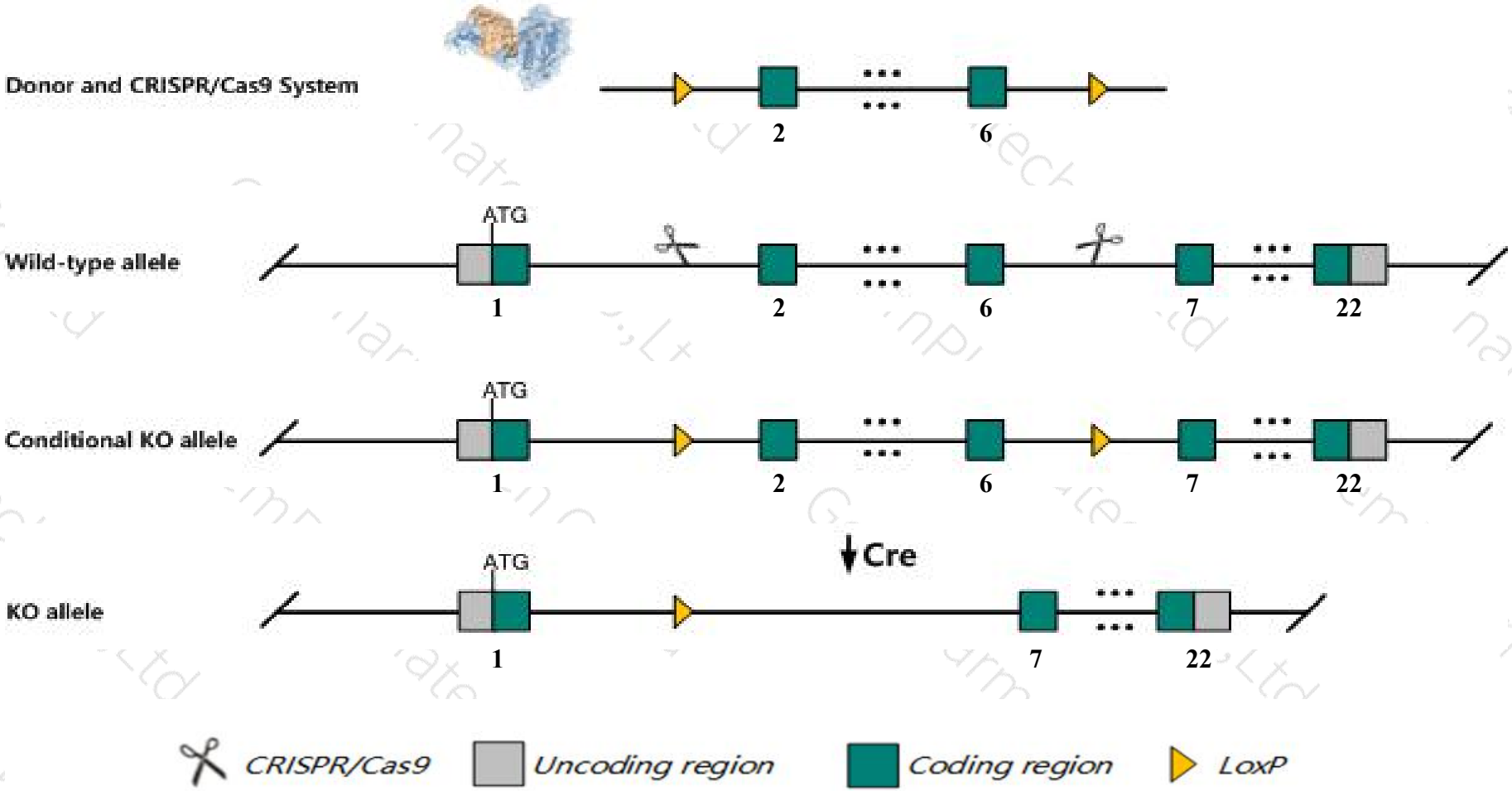
Designer: Xueting Zhang
Design Date: 2019-7-22

Project Overview

Project Name	***Pde6b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pde6b* gene. The schematic diagram is as follows:



Technical routes

- The *Pde6b* gene has 3 transcripts. According to the structure of *Pde6b* gene, exon2-exon6 of *Pde6b-201* (ENSMUST00000031456.7) transcript is recommended as the knockout region.The region contains 524bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Pde6b* 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,Homozygotes for the rd1 mutation have severe retinal degeneration and vision loss. Rod cells are lost by 35 days of age; cone cells degenerate slower and some light sensitivity persists. Other allelic mutations produce similar or milder phenotypes.
- The N-terminal of *Pde6b* gene will remain 168aa,it may remain the partial function of *Pde6b* gene.
- Transcript *Pde6b*-202 may not be affected .
- The *Pde6b* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Pde6b phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide [Mus musculus (house mouse)]

Gene ID: 18587, updated on 2-Apr-2019

Summary

Official Symbol	Pde6b provided by MGI
Official Full Name	phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide provided by MGI
Primary source	MGI:MGI:97525
See related	Ensembl:ENSMUSG00000029491
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	Pdeb, r, rd, rd-1, rd1, rd10
Expression	Low expression observed in reference dataset See more
Orthologs	human all

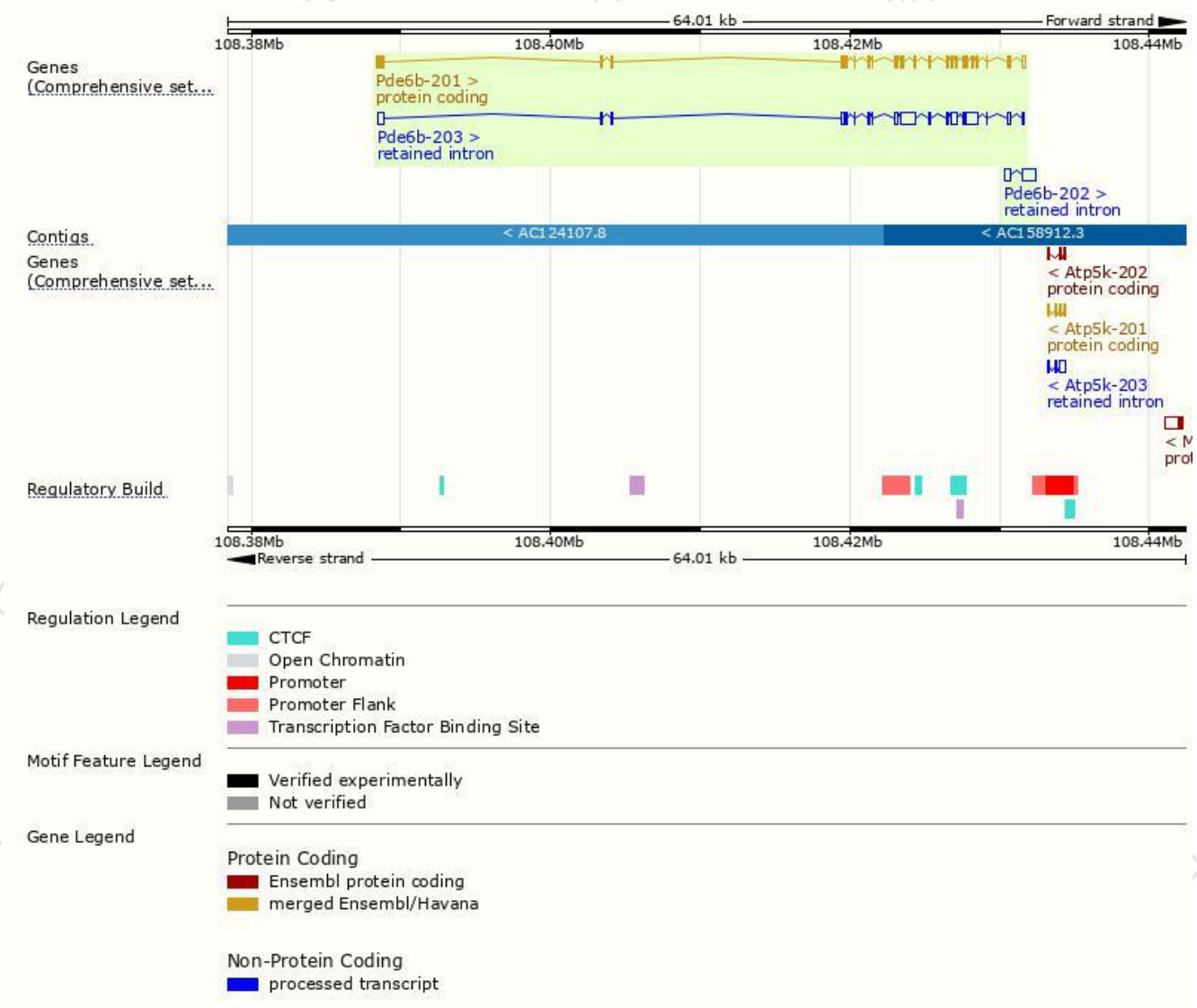
Transcript information（Ensembl）

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

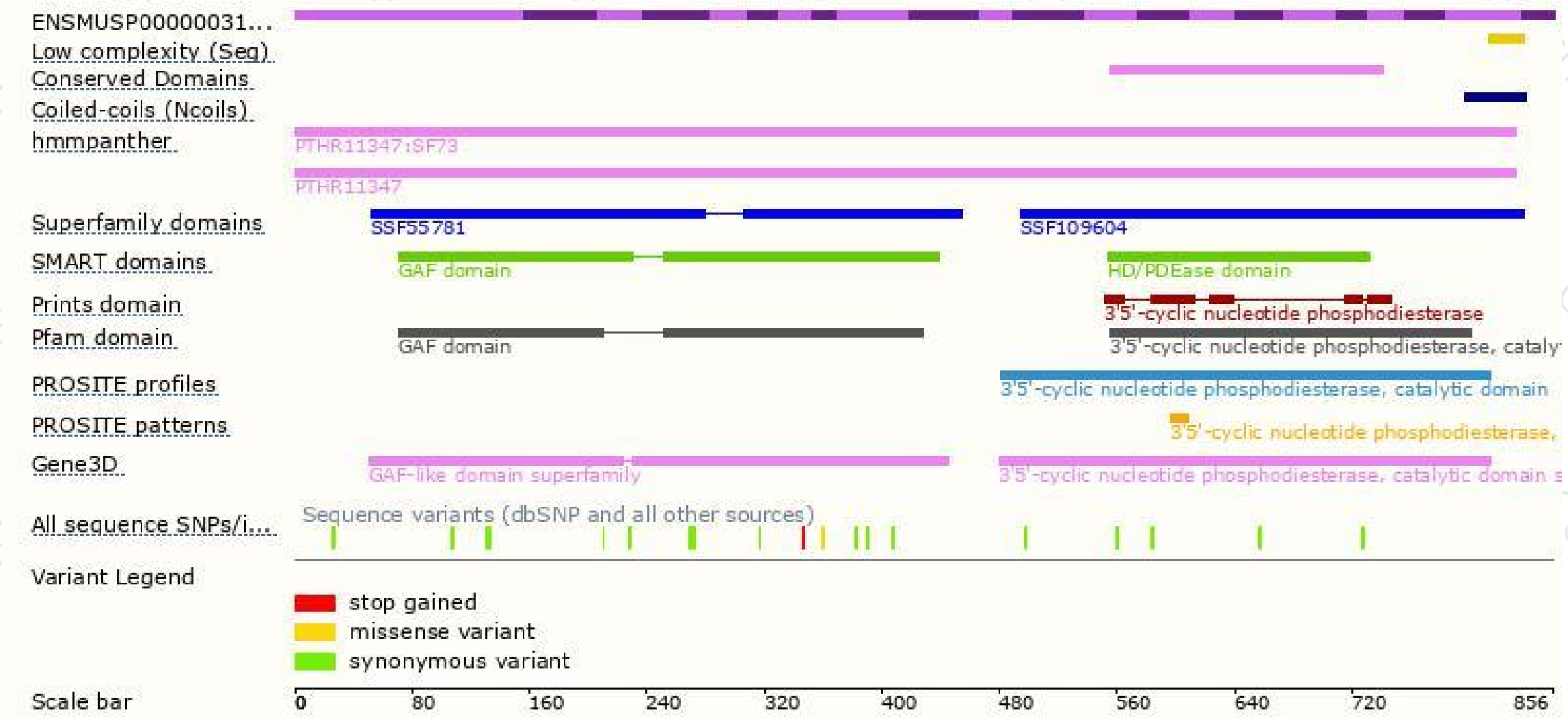
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pde6b-201	ENSMUST00000031456.7	2799	856aa	Protein coding	CCDS19510	P23440	TSL:1 GENCODE basic APPRIS P1
Pde6b-203	ENSMUST00000134865.1	4088	No protein	Retained intron	-	-	TSL:2
Pde6b-202	ENSMUST00000130448.1	1225	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Pde6b-201* transcript,The transcription is shown below

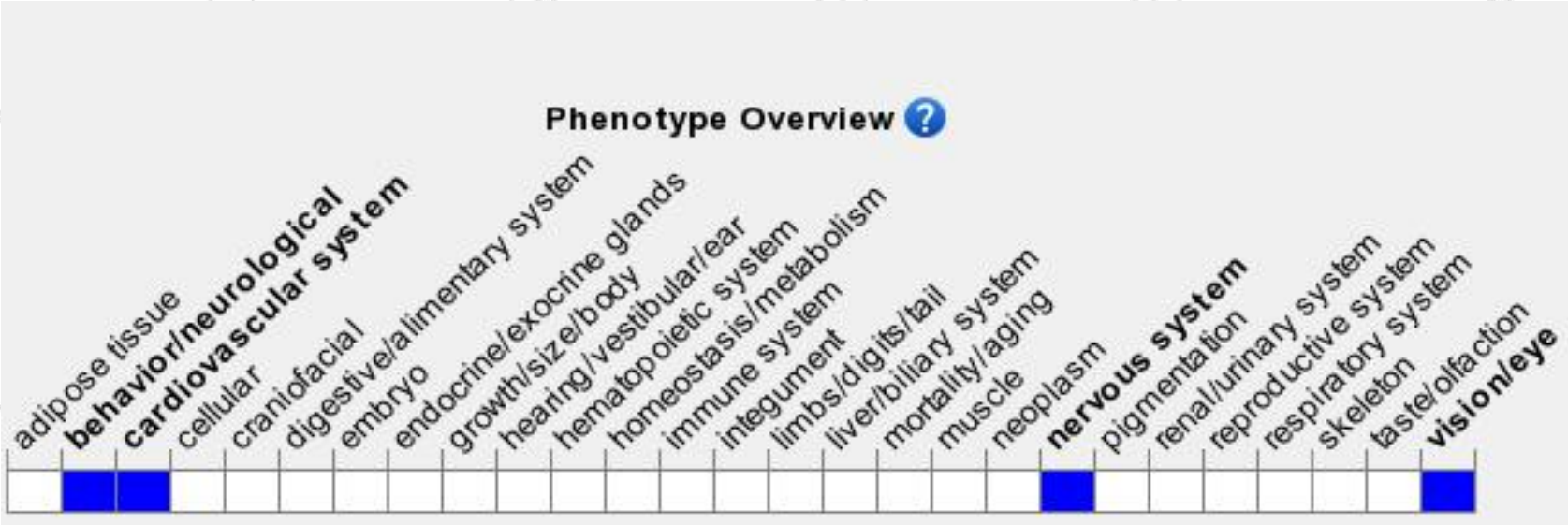
Genomic location distribution



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

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