Robo1 Cas9-CKO Strategy

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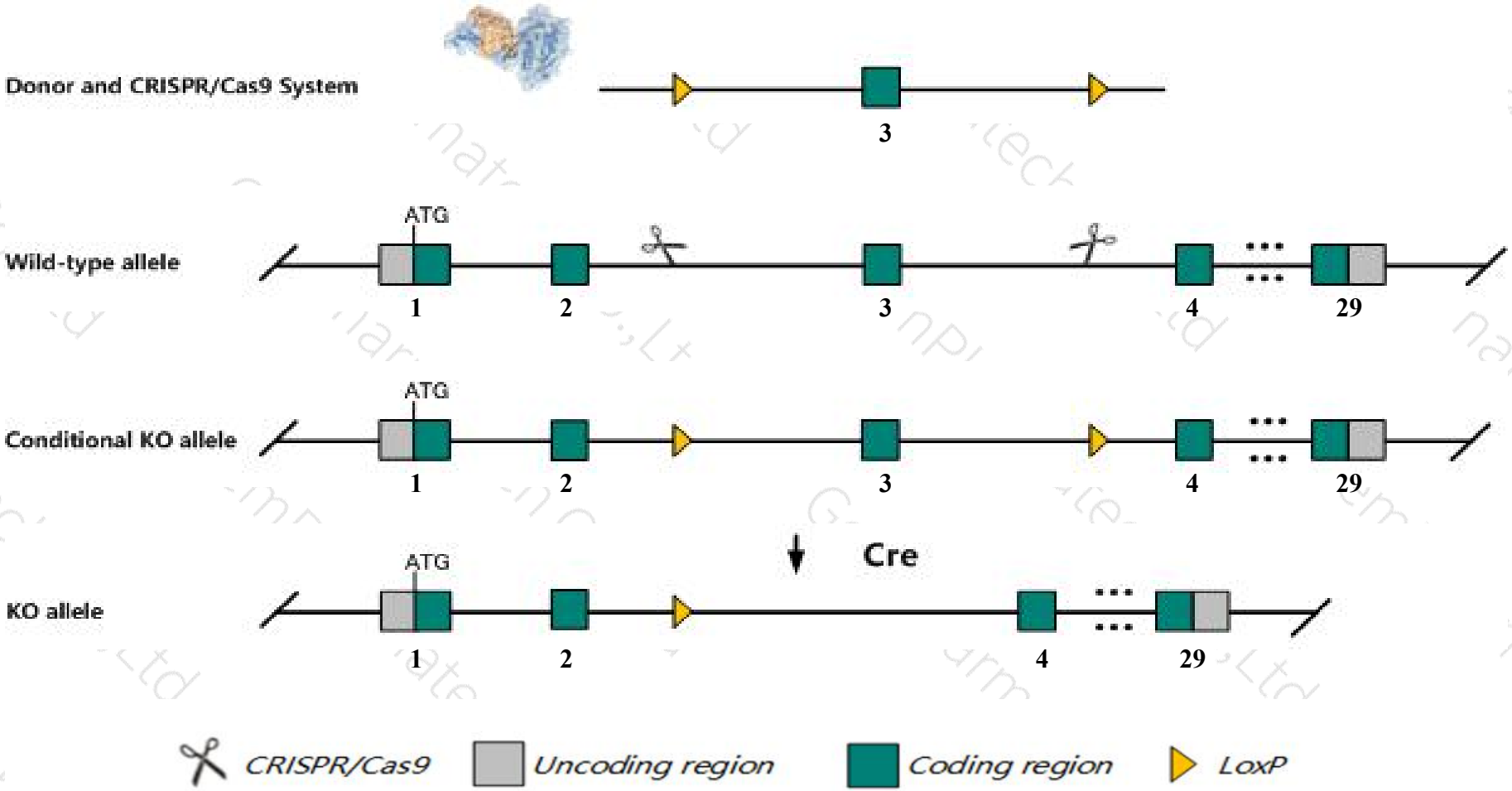
Project Overview

Project Name	***Robo1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Robo1* gene. The schematic diagram is as follows:



Technical routes

- The *Robo1* gene has 9 transcripts. According to the structure of *Robo1* gene, exon3 of *Robo1-201* (ENSMUST00000023600.7) transcript is recommended as the knockout region.The region contains 158bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Robo1* 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 reporter allele show altered axon guidance. Mice homozygous for a null allele die at birth showing aberrant axon pathfinding and cortical interneuron migration. Homozygotes for another null allele show neonatal death, aphagia, delayed lung maturation and bronchial hyperplasia.

- The *Robo1* gene is located on the Chr16. 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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Robo1 roundabout guidance receptor 1 [Mus musculus (house mouse)]

Gene ID: 19876, updated on 9-Apr-2019

Summary

Official Symbol	Robo1 provided by MGI
Official Full Name	roundabout guidance receptor 1 provided by MGI
Primary source	MGI:MGI:1274781
See related	Ensembl:ENSMUSG00000022883
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	AW494633, AW742721, DUTT1, Gm310
Expression	Biased expression in adrenal adult (RPKM 19.0), whole brain E14.5 (RPKM 14.9) and 14 other tissues See more
Orthologs	human all

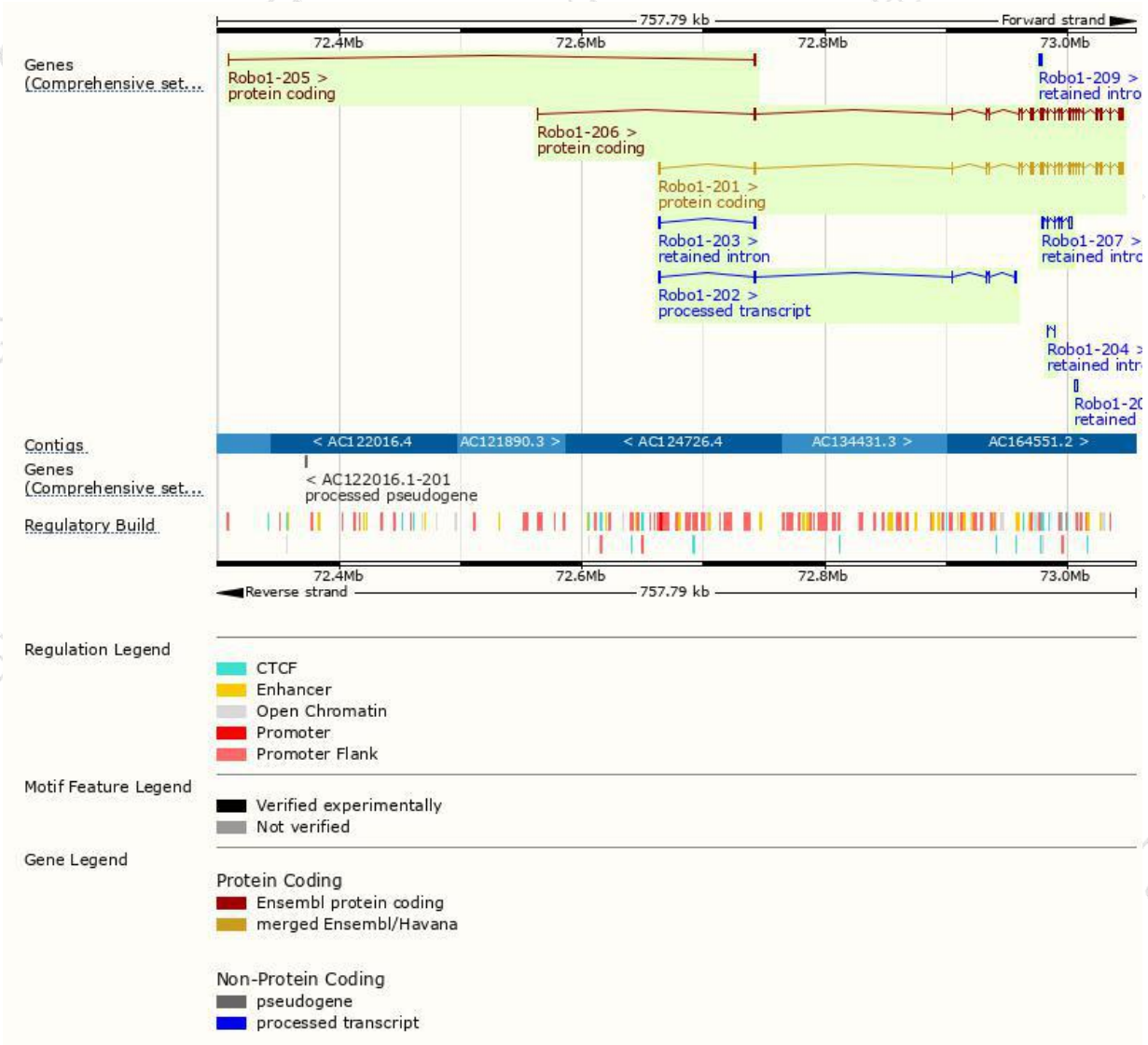
Transcript information（Ensembl）

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

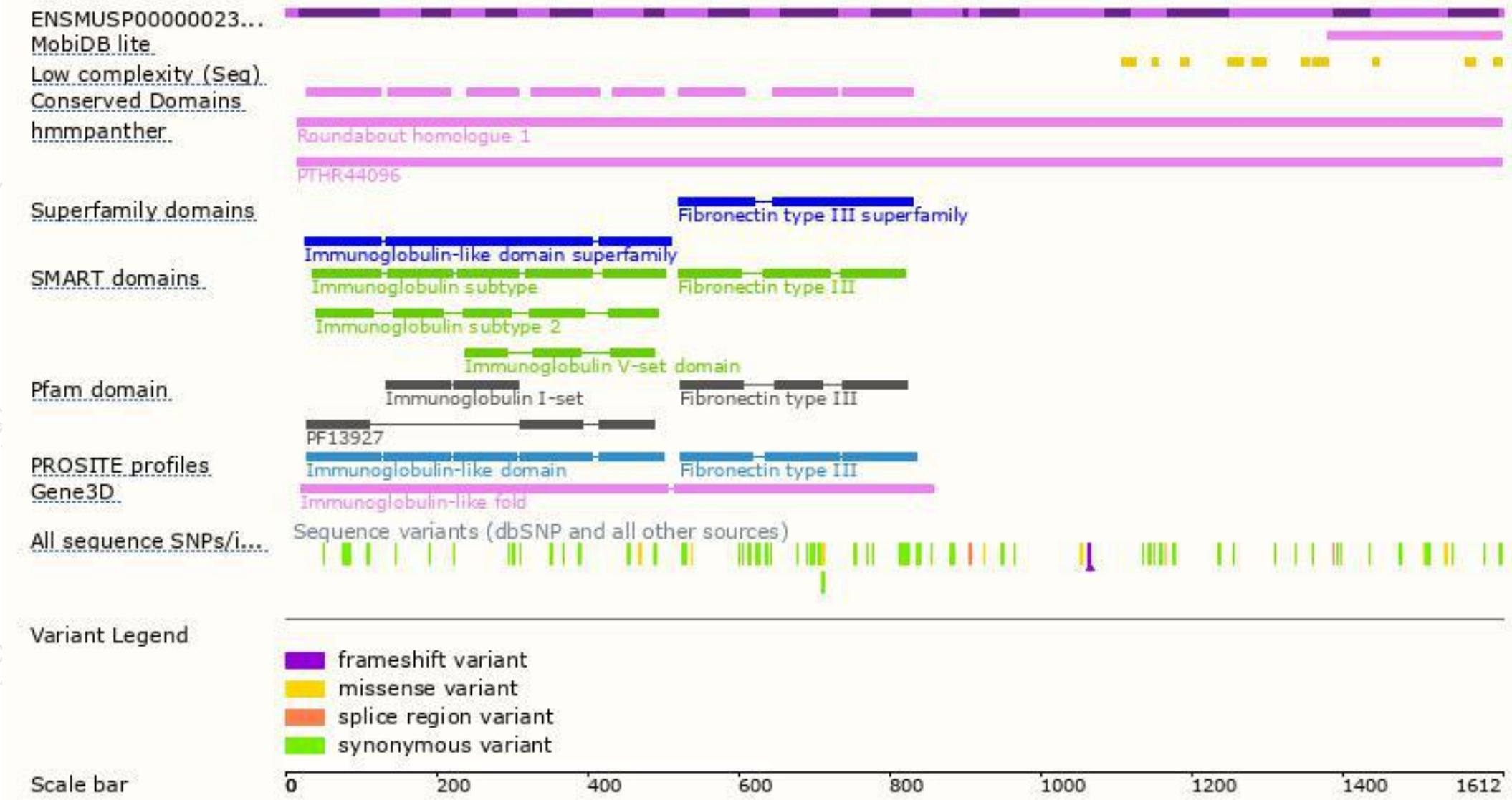
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Robo1-201	ENSMUST00000023600.7	7563	1612aa	Protein coding	CCDS37376	G5E843	TSL:1 GENCODE basic APPRIS P1
Robo1-206	ENSMUST00000232205.1	6601	1622aa	Protein coding	-	A0A338P6F7	CDS 5' incomplete
Robo1-205	ENSMUST00000231580.1	546	133aa	Protein coding	-	A0A338P6U2	CDS 3' incomplete
Robo1-202	ENSMUST00000231265.1	3834	No protein	Processed transcript	-	-	
Robo1-207	ENSMUST00000232264.1	2416	No protein	Retained intron	-	-	
Robo1-208	ENSMUST00000232432.1	2326	No protein	Retained intron	-	-	
Robo1-203	ENSMUST00000231474.1	2277	No protein	Retained intron	-	-	
Robo1-209	ENSMUST00000232545.1	2118	No protein	Retained intron	-	-	
Robo1-204	ENSMUST00000231566.1	406	No protein	Retained intron	-	-	

The strategy is based on the design of *Robo1-201* transcript,The transcription is shown below

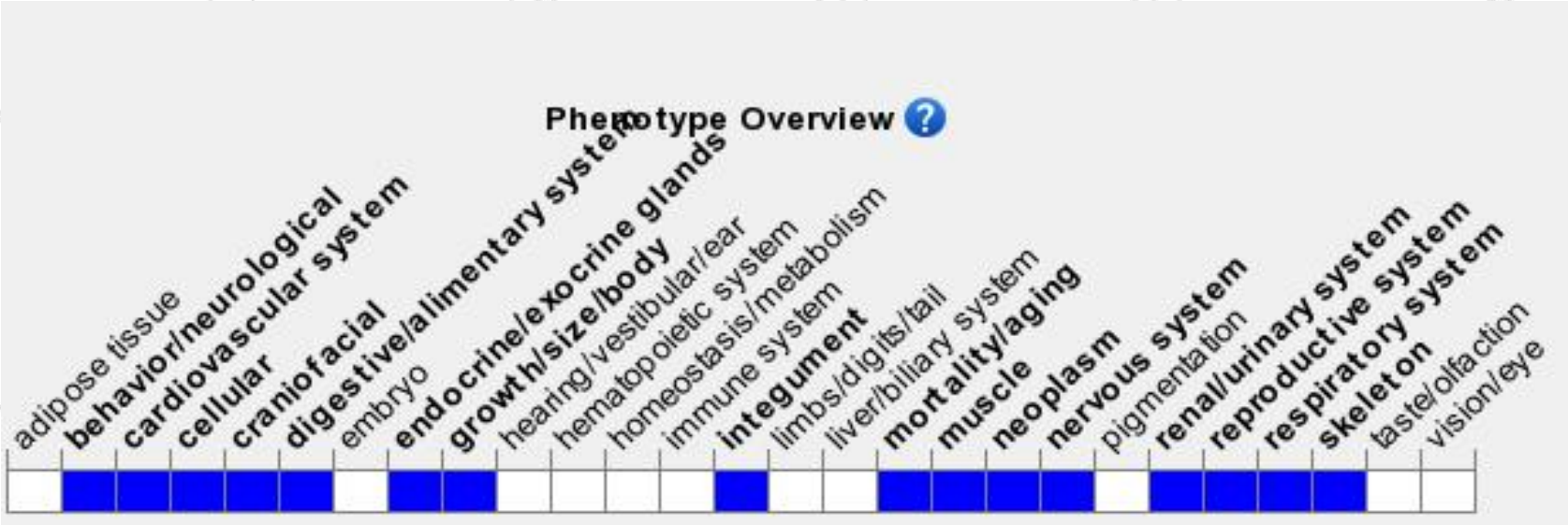
Genomic location distribution



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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