Ripk2 Cas9-KO Strategy

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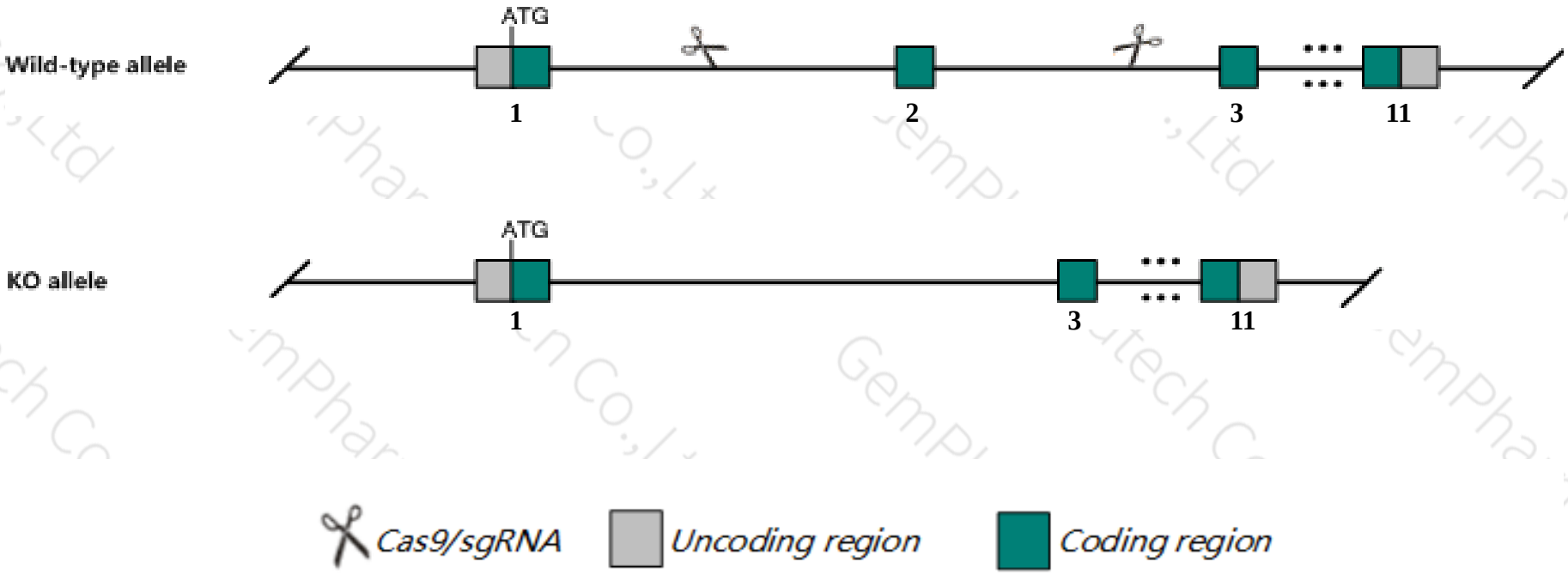
Project Overview

Project Name	***Ripk2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ripk2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ripk2* gene has 2 transcripts. According to the structure of *Ripk2* gene, exon2 of *Ripk2-201* (ENSMUST00000037035.11) transcript is recommended as the knockout region.The region contains 154bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ripk2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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,Homozygous inactivation of this gene leads to impaired cytokine production in response to LPS treatment, and may result in resistance to LPS-induced septic shock and defects in Toll-like receptor and T-cell receptor signaling. Macrophages homozygous for a knock-in allele show normal LPS signaling.

➢ The *Ripk2* gene is located on the Chr4. 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）

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Ripk2 receptor (TNFRSF)-interacting serine-threonine kinase 2 [*Mus musculus* (house mouse)]

Gene ID: 192656, updated on 26-Nov-2019

Summary

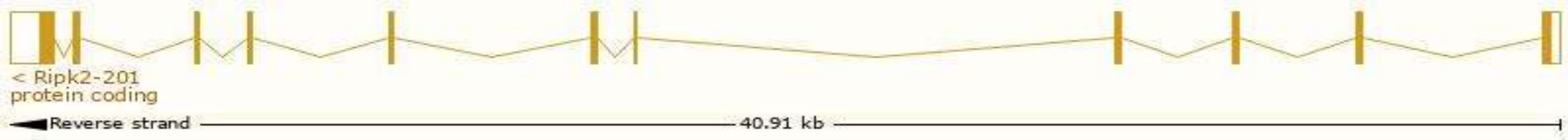
Official Symbol	Ripk2 provided by MGI
Official Full Name	receptor (TNFRSF)-interacting serine-threonine kinase 2 provided by MGI
Primary source	MGI:MGI:1891456
See related	Ensembl:ENSMUSG00000041135
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CCK; RICK; RIP2; CARD3; CARDIAK; D4Bwg0615e; 2210420D18Rik
Summary	This gene encodes a member of the receptor-interacting protein family of serine/threonine protein kinases. The encoded protein contains a C-terminal caspase activation and recruitment domain, and is a component of signaling complexes in both the innate and adaptive immune pathways. It is a potent activator of nuclear factor kappa B and inducer of apoptosis in response to various stimuli. Alternate splicing results in multiple transcript variants. [provided by RefSeq, Jul 2016]
Expression	Ubiquitous expression in ovary adult (RPKM 9.1), adrenal adult (RPKM 8.0) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

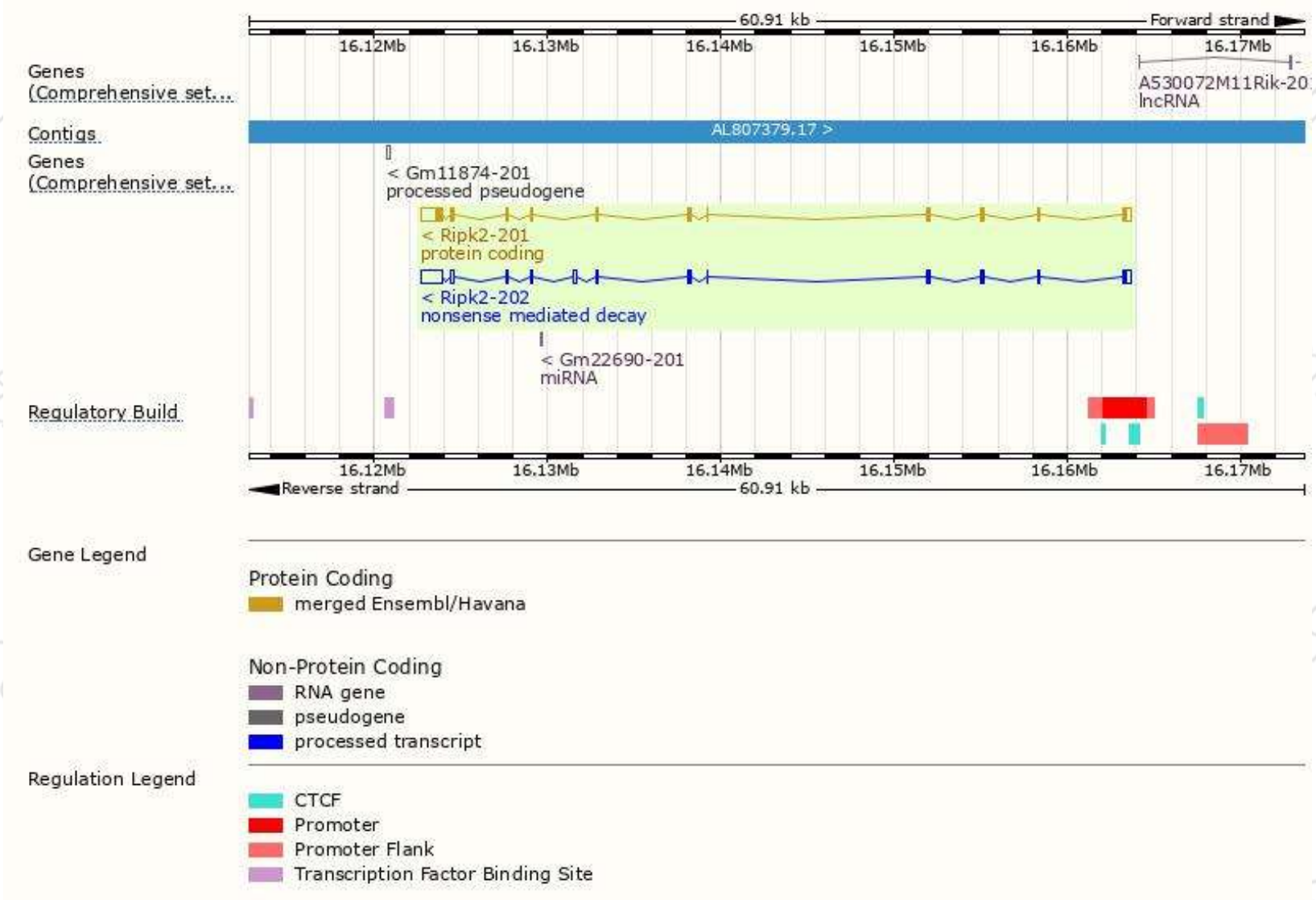
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ripk2-201	ENSMUST00000037035.11	2692	539aa	Protein coding	CCDS17988	P58801 Q547H1	TSL:1 GENCODE basic APPRIS P1
Ripk2-202	ENSMUST00000183871.1	2804	315aa	Nonsense mediated decay	-	V9GXY4	TSL:1

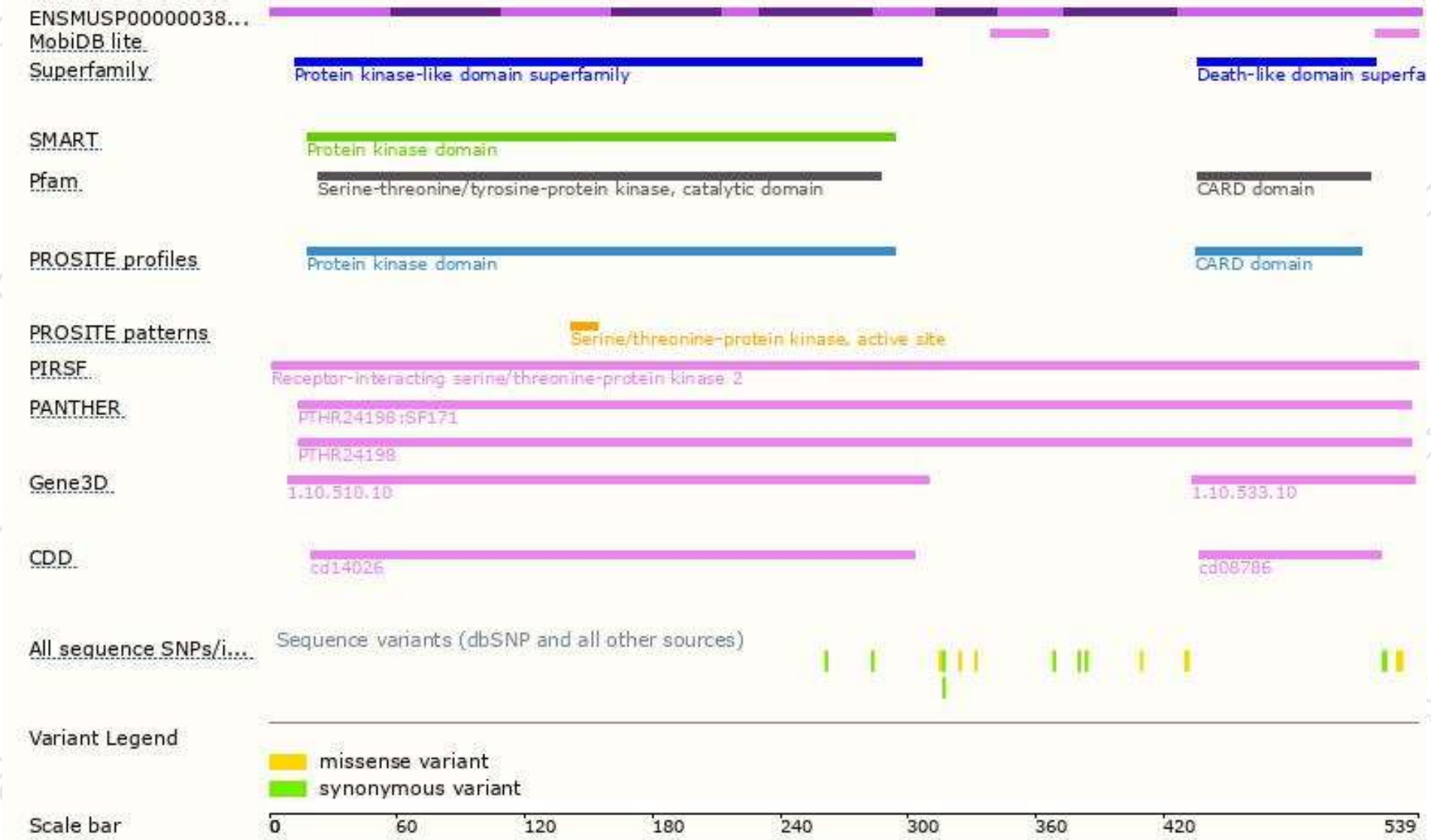
The strategy is based on the design of *Ripk2-201* transcript,The transcription is shown below



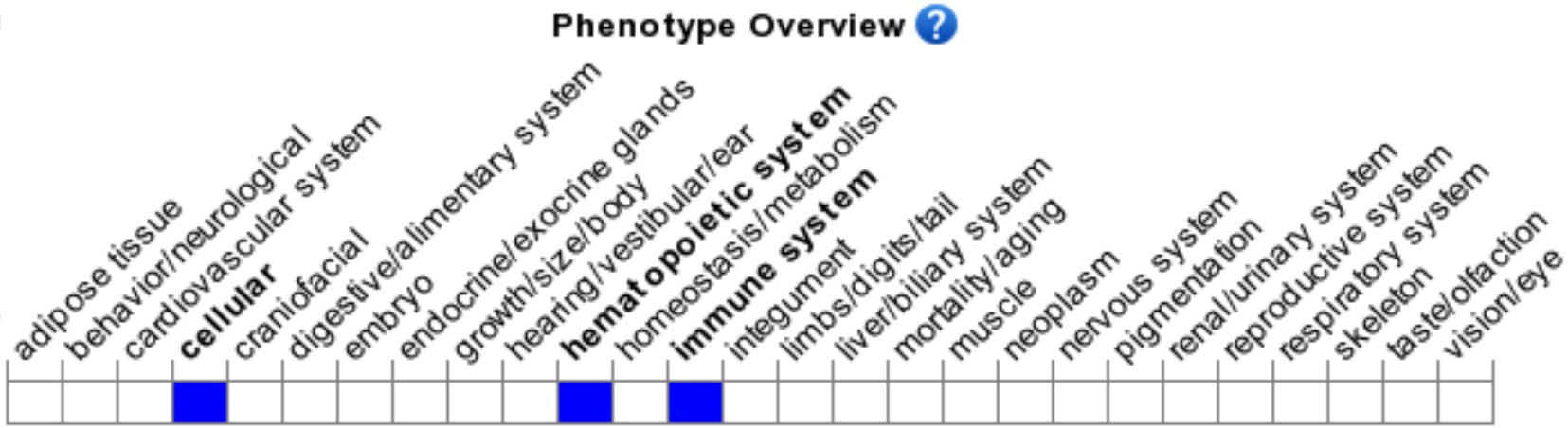
Genomic location distribution



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,Homozygous inactivation of this gene leads to impaired cytokine production in response to LPS treatment, and may result in resistance to LPS-induced septic shock and defects in Toll-like receptor and T-cell receptor signaling. Macrophages homozygous for a knock-in allele show normal LPS signaling.

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

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