

Tnf Cas9-KO Strategy

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Project Overview

Project Name

Tnf

Project type

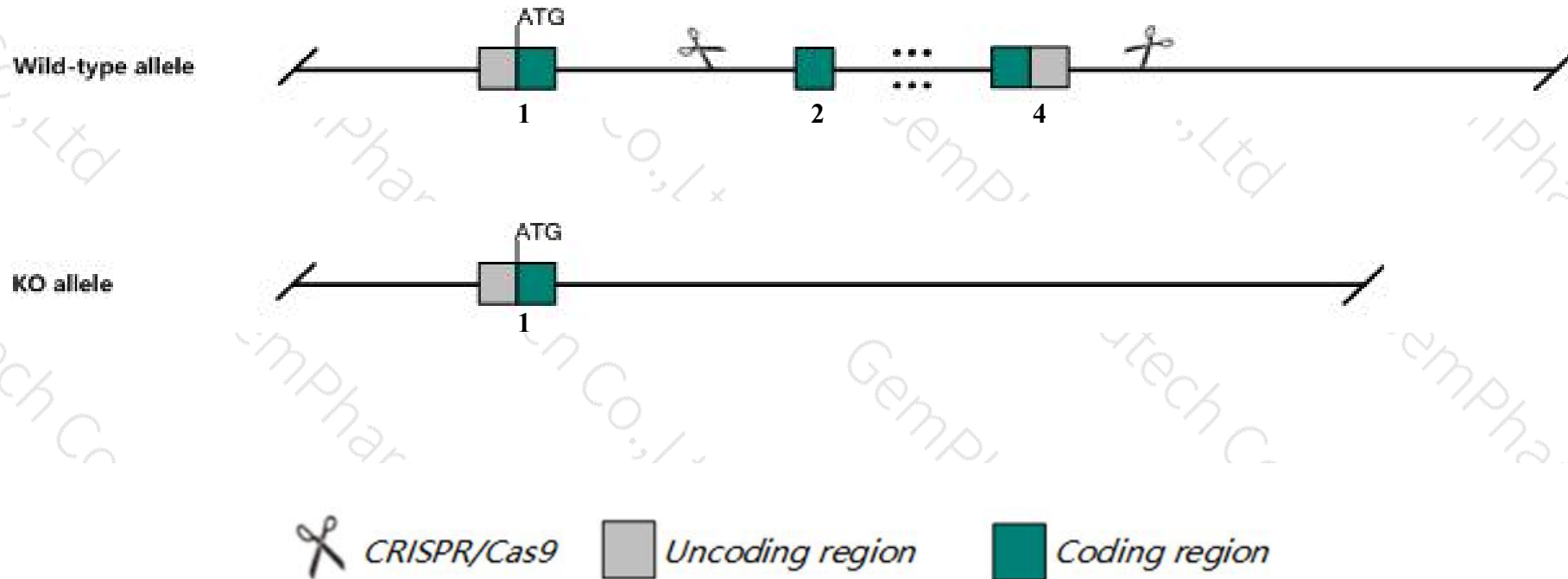
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

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



- The floxed region is near to the C-terminal of *Lta* and *Mir6974* gene, this strategy may influence the regulatory function of the N-terminal of these gene.
- The *Tnf* gene has 2 transcripts. According to the structure of *Tnf* gene, exon2-exon4 of *Tnf-201* (ENSMUST00000025263.14) 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 *Tnf* 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.

- According to the existing MGI data, Mutations at this locus primarily affect the immune system, causing increased susceptibility to infection, failure to form splenic B-cell follicles, increased inflammation and impaired contact hypersensitivity. Homozygotes also may show metabolic defects.
- The *Tnf* gene is located on the Chr17. 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)

Tnf tumor necrosis factor [Mus musculus (house mouse)]

Gene ID: 21926, updated on 9-Apr-2019

Summary



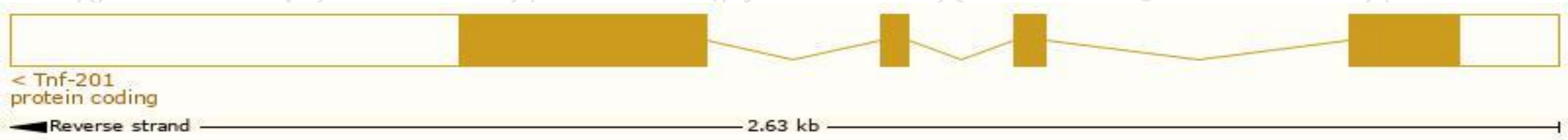
Official Symbol	Tnf provided by MGI
Official Full Name	tumor necrosis factor provided by MGI
Primary source	MGI:MGI:104798
See related	Ensembl:ENSMUSG00000024401
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	DIF, TNF-a, TNF-alpha, TNFSF2, TNFalpha, Tnfa, Tnfsf1a, Tnlg1f
Summary	This gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. Members of this family are classified based on primary sequence, function, and structure. This protein is synthesized as a type-II transmembrane protein and is reported to be cleaved into products that exert distinct biological functions. It plays an important role in the innate immune response as well as regulating homeostasis but is also implicated in diseases of chronic inflammation. In mouse deficiency of this gene is associated with defects in response to bacterial infection, with defects in forming organized follicular dendritic cell networks and germinal centers, and with a lack of primary B cell follicles. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jun 2013]
Expression	Broad expression in spleen adult (RPKM 4.1), thymus adult (RPKM 3.0) and 16 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

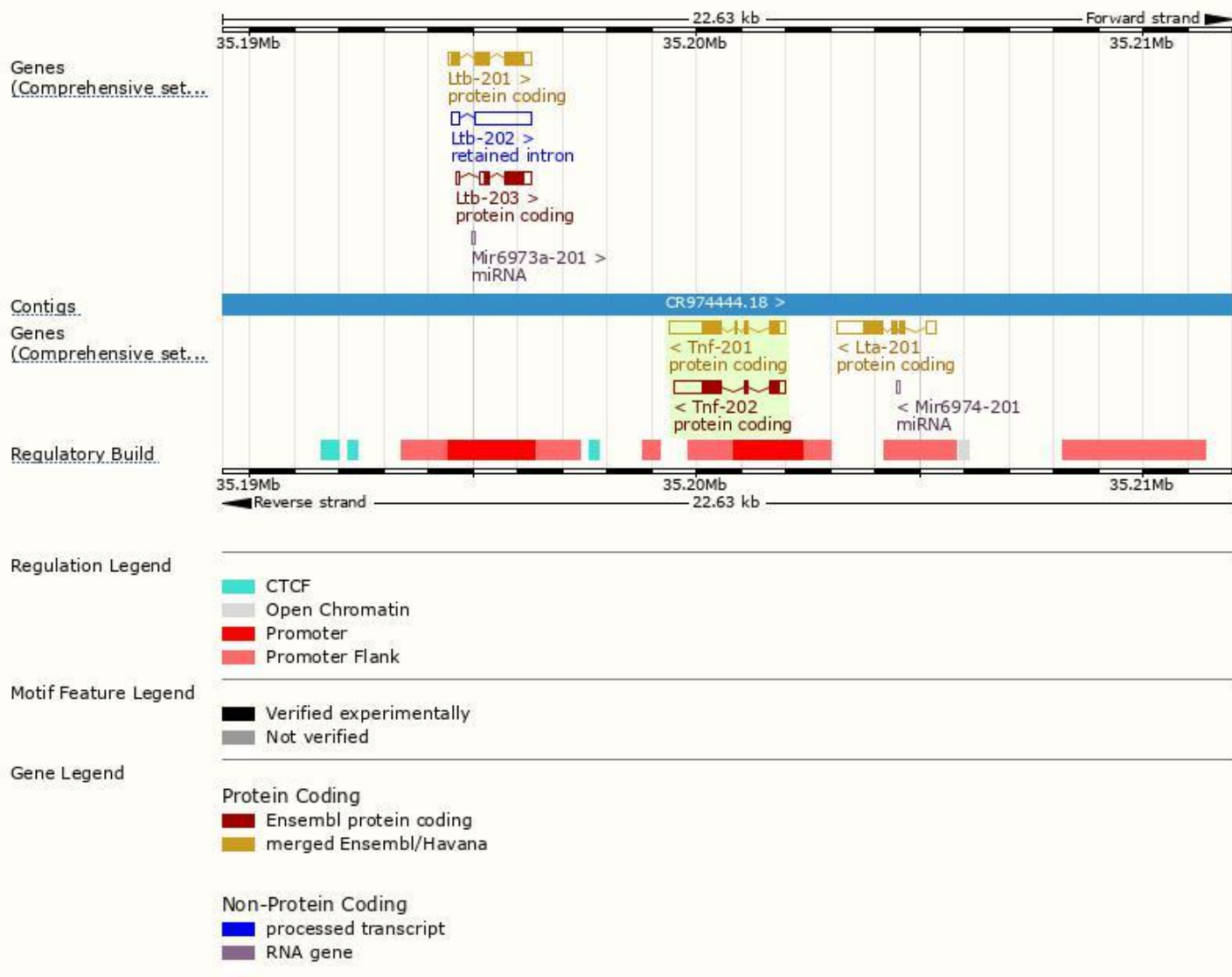
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tnf-201	ENSMUST00000025263.14	1639	235aa	Protein coding	CCDS28691	P06804 Q3U593	TSL:1 GENCODE basic APPRIS P1
Tnf-202	ENSMUST00000167924.1	1462	219aa	Protein coding	CCDS70801	A0A0R4J210	TSL:1 GENCODE basic

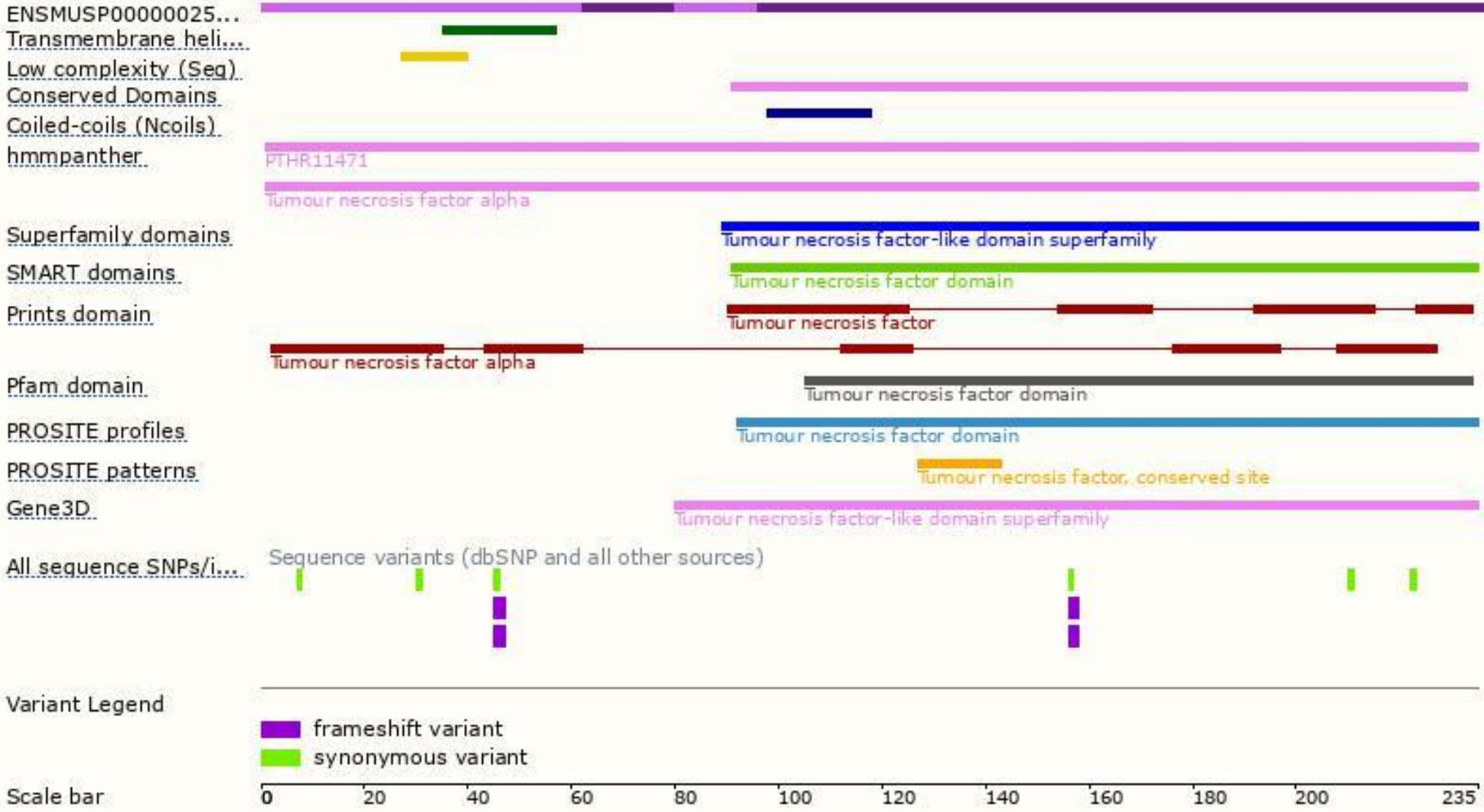
The strategy is based on the design of *Tnf-201* transcript,The transcription is shown below



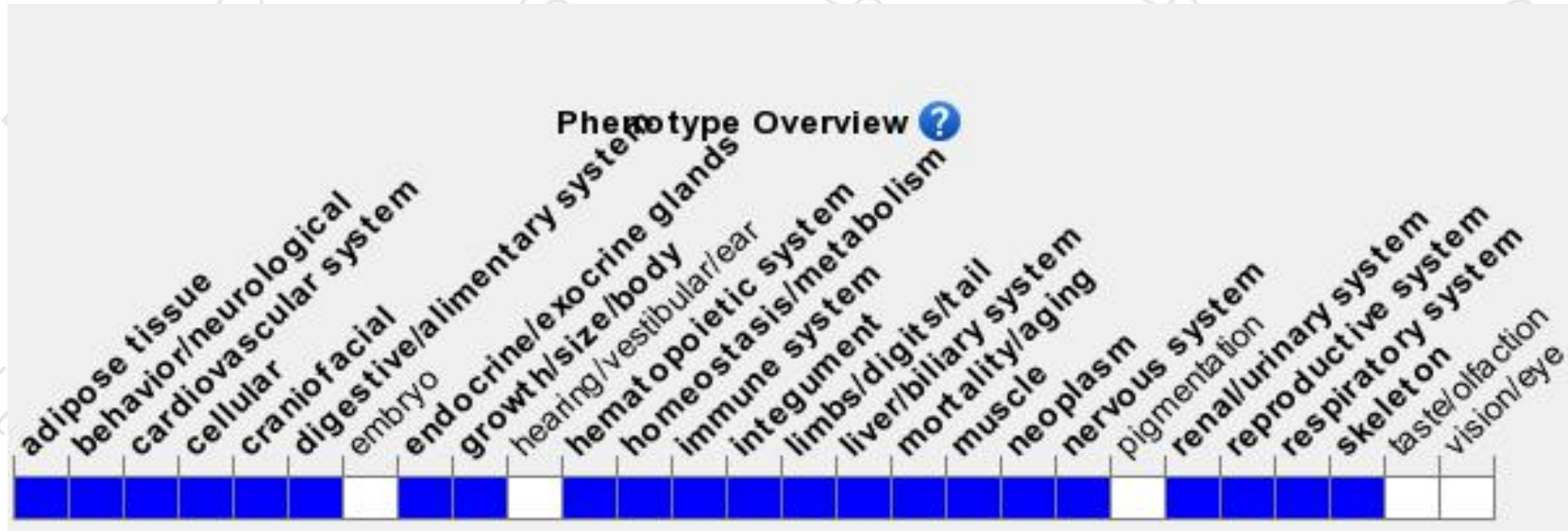
Genomic location distribution



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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