

***Ext2* Cas9-CKO Strategy**

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Project Overview

Project Name

Ext2

Project type

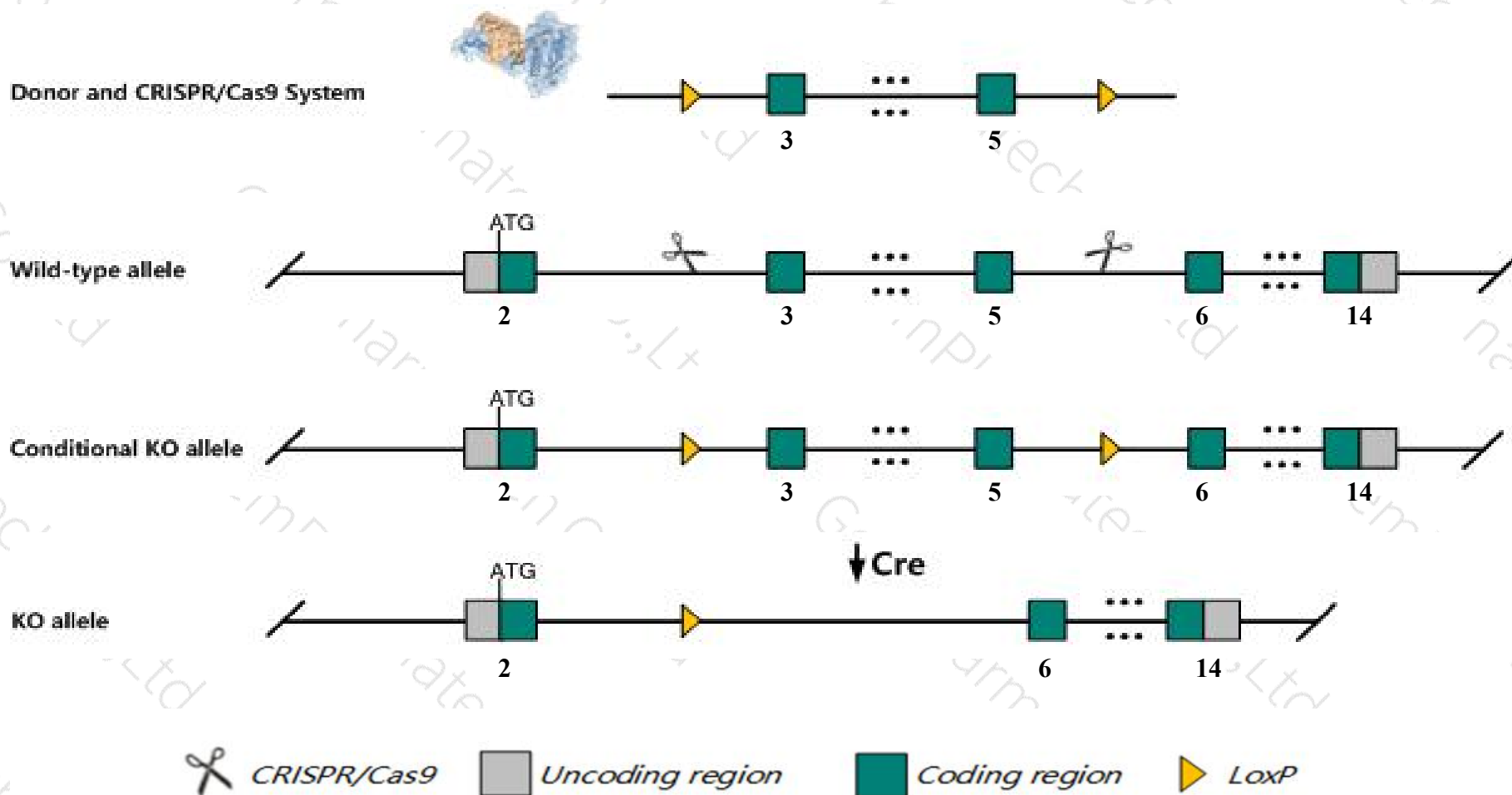
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ext2* gene. The schematic diagram is as follows:



- The *Ext2* gene has 12 transcripts. According to the structure of *Ext2* gene, exon3-exon5 of *Ext2-201* (ENSMUST00000028623.12) transcript is recommended as the knockout region. The region contains 403bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ext2* 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.

- According to the existing MGI data, Homozygous null embryos lack heparan sulfate, initiate primitive streak formation but fail to form mesoderm, become growth arrested and die around gastrulation. Heterozygotes show various abnormalities in cartilage differentiation; about one-third form one or more exostoses on the ribs.
- Some amino acids will remain at the N-terminus and some functions may be retained.
- Transcripts 204,207,210,211 may not be affected. The effect of transcript 202,206,208 is unknown.
- The *Ext2* gene is located on the Chr2. 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)

Ext2 exostosin glycosyltransferase 2 [*Mus musculus* (house mouse)]

Gene ID: 14043, updated on 11-Feb-2020

Summary

Official Symbol	Ext2 provided by MGI
Official Full Name	exostosin glycosyltransferase 2 provided by MGI
Primary source	MGI:MGI:108050
See related	Ensembl:ENSMUSG00000027198
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	AI893565
Expression	Ubiquitous expression in ovary adult (RPKM 29.4), limb E14.5 (RPKM 23.3) and 28 other tissues See more
Orthologs	human all

Genomic context

Location: 2 E1; 2 51.62 cM

Exon count: 17

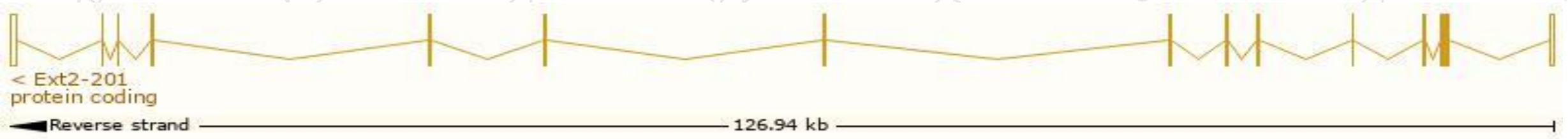
See Ext2 in [Genome Data Viewer](#)

Transcript information (Ensembl)

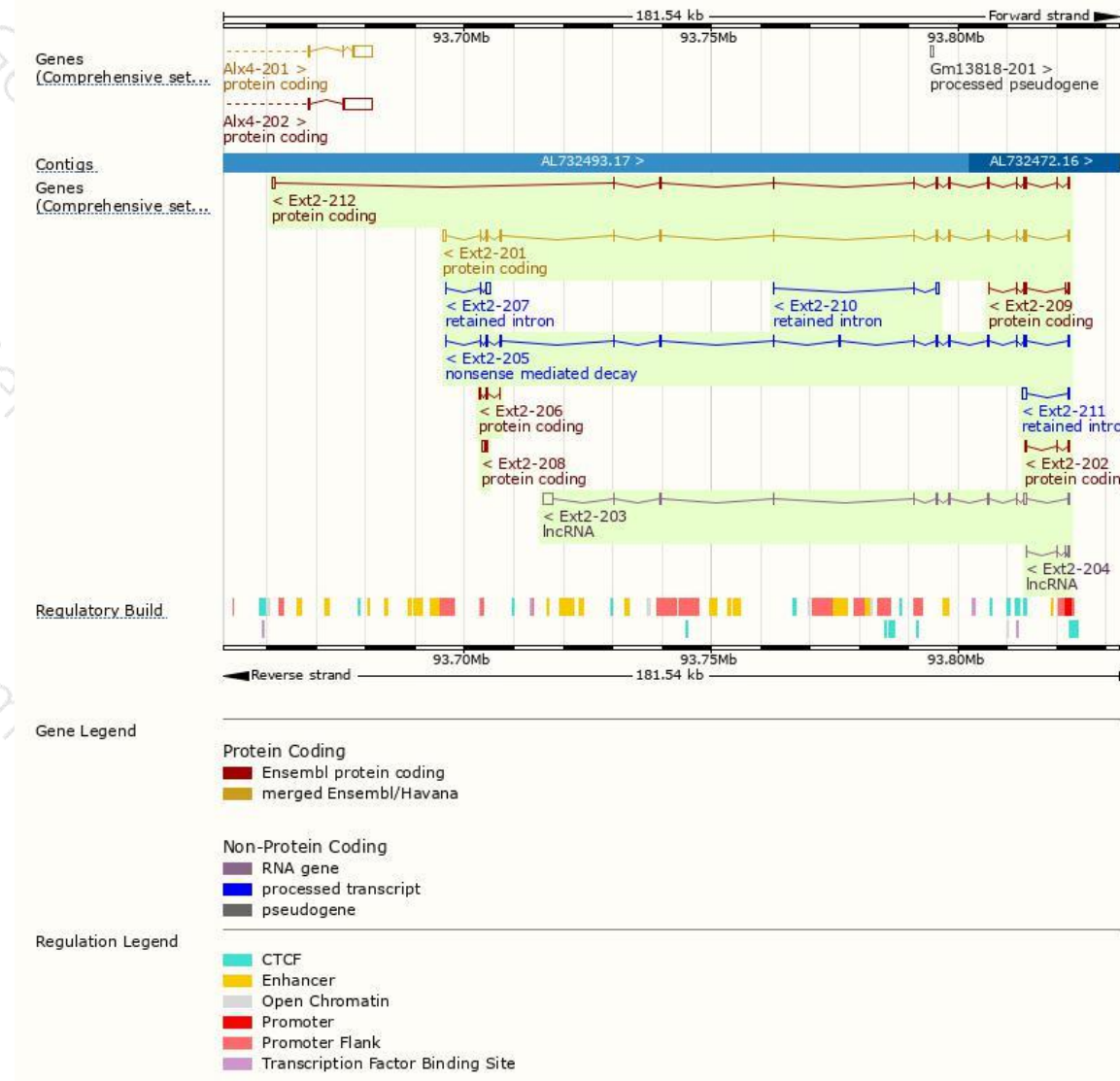
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ext2-201	ENSMUST00000028623.12	2871	718aa	Protein coding	CCDS16456	P70428	TSL:1 GENCODE basic APPRIS P1
Ext2-212	ENSMUST00000184931.7	2629	586aa	Protein coding	-	V9GX32	TSL:1 GENCODE basic
Ext2-209	ENSMUST00000145838.7	921	213aa	Protein coding	-	A2AIG3	CDS 3' incomplete TSL:5
Ext2-202	ENSMUST00000111248.7	679	122aa	Protein coding	-	A2AIG6	CDS 3' incomplete TSL:2
Ext2-206	ENSMUST00000132429.1	535	100aa	Protein coding	-	F6WHC9	CDS 5' incomplete TSL:2
Ext2-208	ENSMUST00000144780.1	478	20aa	Protein coding	-	B0QZZ7	CDS 5' incomplete TSL:3
Ext2-205	ENSMUST00000125407.7	2376	407aa	Nonsense mediated decay	-	E9Q1M5	TSL:5
Ext2-211	ENSMUST00000157046.1	981	No protein	Retained intron	-	-	TSL:1
Ext2-207	ENSMUST00000139321.7	869	No protein	Retained intron	-	-	TSL:2
Ext2-210	ENSMUST00000152344.1	573	No protein	Retained intron	-	-	TSL:2
Ext2-203	ENSMUST00000123649.7	3721	No protein	lncRNA	-	-	TSL:1
Ext2-204	ENSMUST00000125027.1	371	No protein	lncRNA	-	-	TSL:5

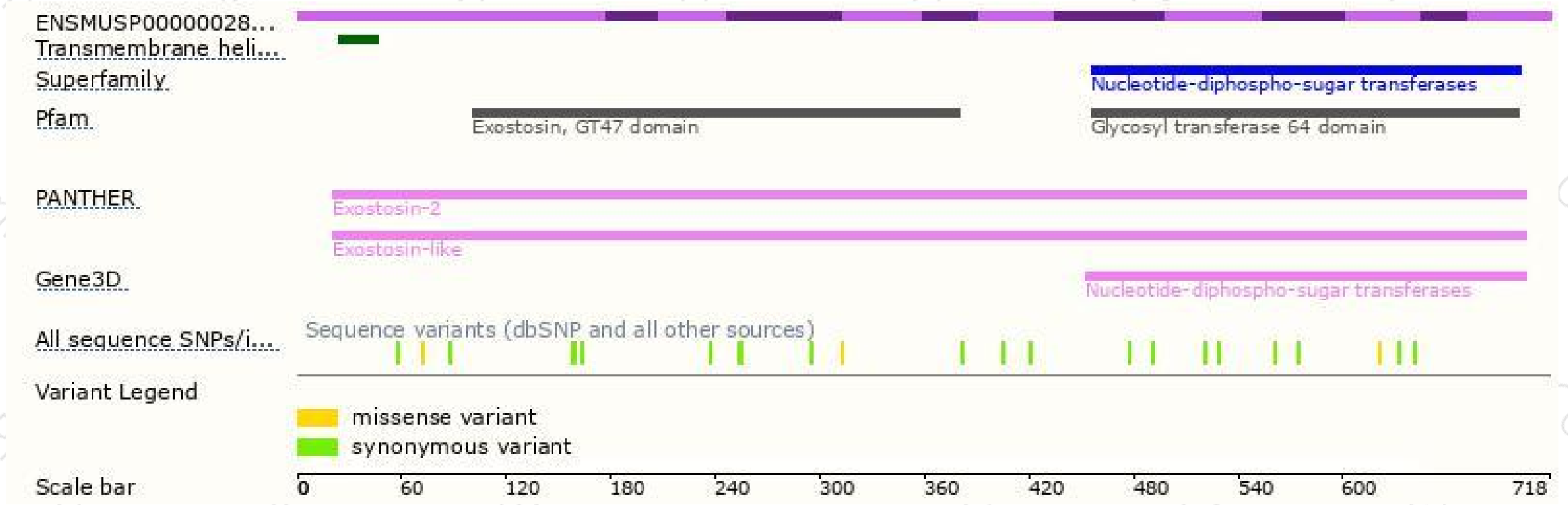
The strategy is based on the design of *Ext2-201* transcript,The transcription is shown below



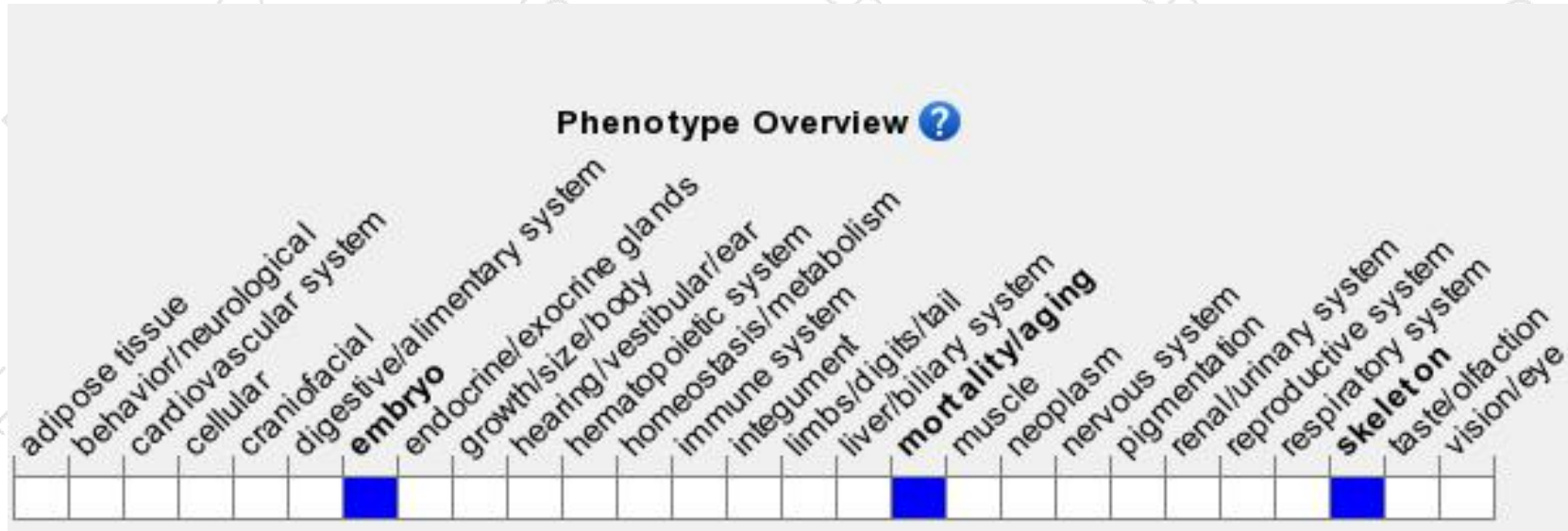
Genomic location distribution



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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