

***Dio1* Cas9-CKO Strategy**

Designer:

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Project Overview

Project Name

Dio1

Project type

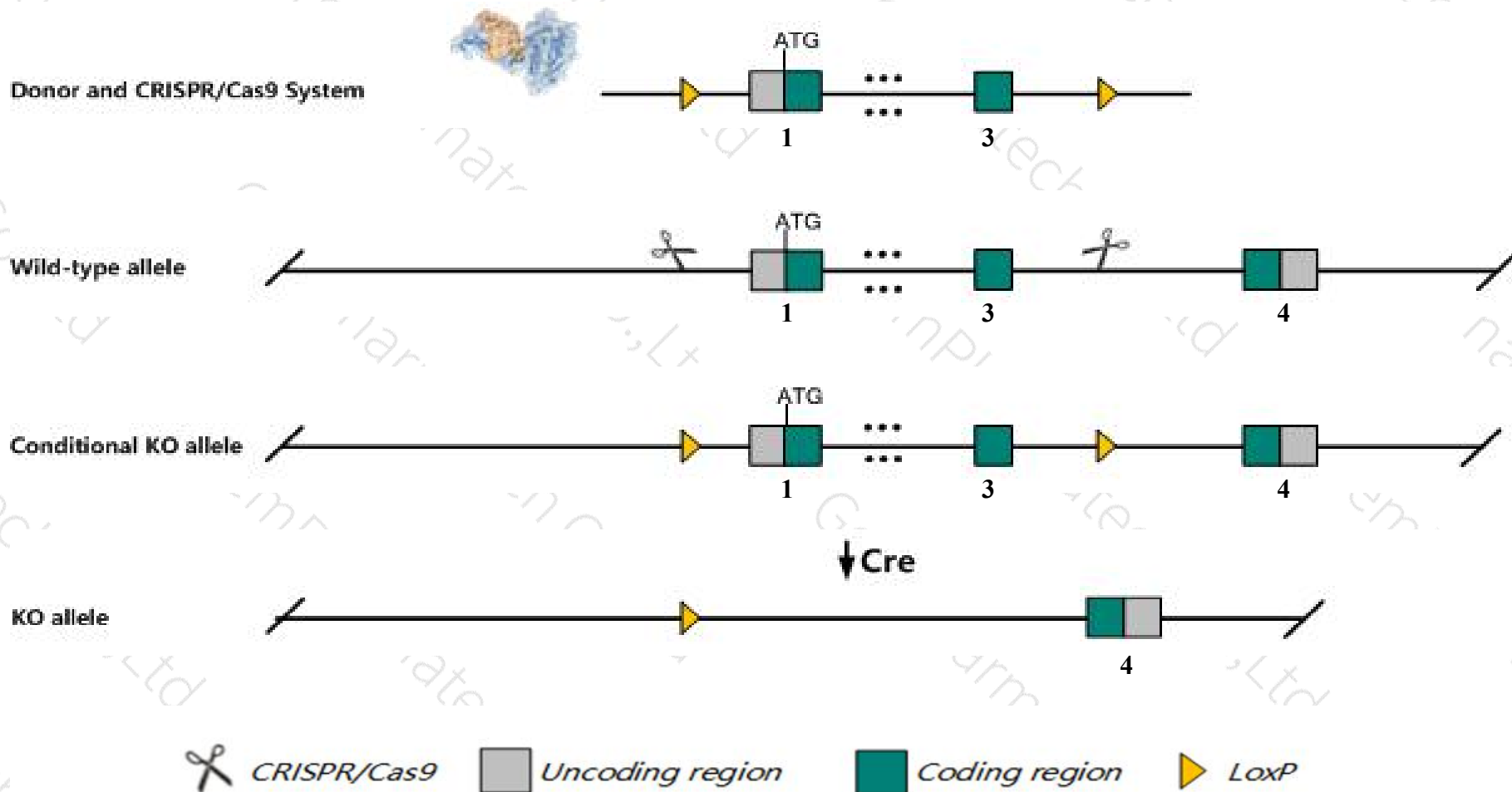
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dio1* gene. The schematic diagram is as follows:



- The *Dio1* gene has 8 transcripts. According to the structure of *Dio1* gene, exon1-exon3 of *Dio1-201* (ENSMUST00000082426.9) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Dio1* 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, Mice homozygous for a disruption in this gene display elevated thyroxine (T4) and reverse triiodothyronine (rT3) levels and changes in the metabolism and excretion of iodothyronines.
- The *Dio1* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Dio1 deiodinase, iodothyronine, type I [Mus musculus (house mouse)]

Gene ID: 13370, updated on 19-Feb-2019

Summary

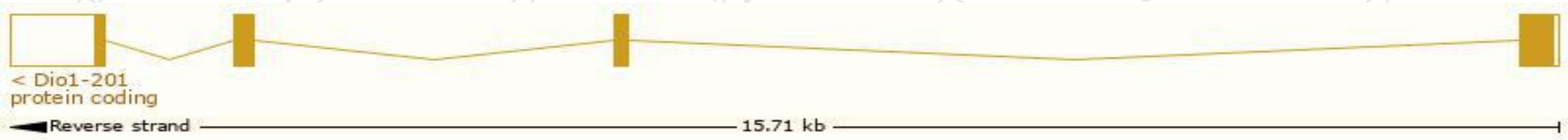
Official Symbol	Dio1 provided by MGI
Official Full Name	deiodinase, iodothyronine, type I provided by MGI
Primary source	MGI:MGI:94896
See related	Ensembl:ENSMUSG00000034785
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5DI, D1, ITDI1, TXDI1
Summary	The protein encoded by this gene belongs to the iodothyronine deiodinase family. It catalyzes the activation, as well as the inactivation of thyroid hormone by outer and inner ring deiodination, respectively. The activation reaction involves the conversion of the prohormone thyroxine (3,5,3',5'-tetraiodothyronine, T4), secreted by the thyroid gland, to the bioactive thyroid hormone (3,5,3'-triiodothyronine, T3) by 5'-deiodination. This protein is expressed predominantly in the liver and kidney and provides most of the circulating T3, which is essential for growth, differentiation and basal metabolism in vertebrates. This protein is a selenoprotein, containing the rare amino acid selenocysteine (Sec) at its active site. Sec is encoded by the UGA codon, which normally signals translation termination. The 3' UTRs of selenoprotein mRNAs contain a conserved stem-loop structure, designated the Sec insertion sequence (SECIS) element, that is necessary for the recognition of UGA as a Sec codon, rather than as a stop signal. [provided by RefSeq, Apr 2016]
Expression	Biased expression in liver adult (RPKM 54.1), kidney adult (RPKM 25.9) and 5 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

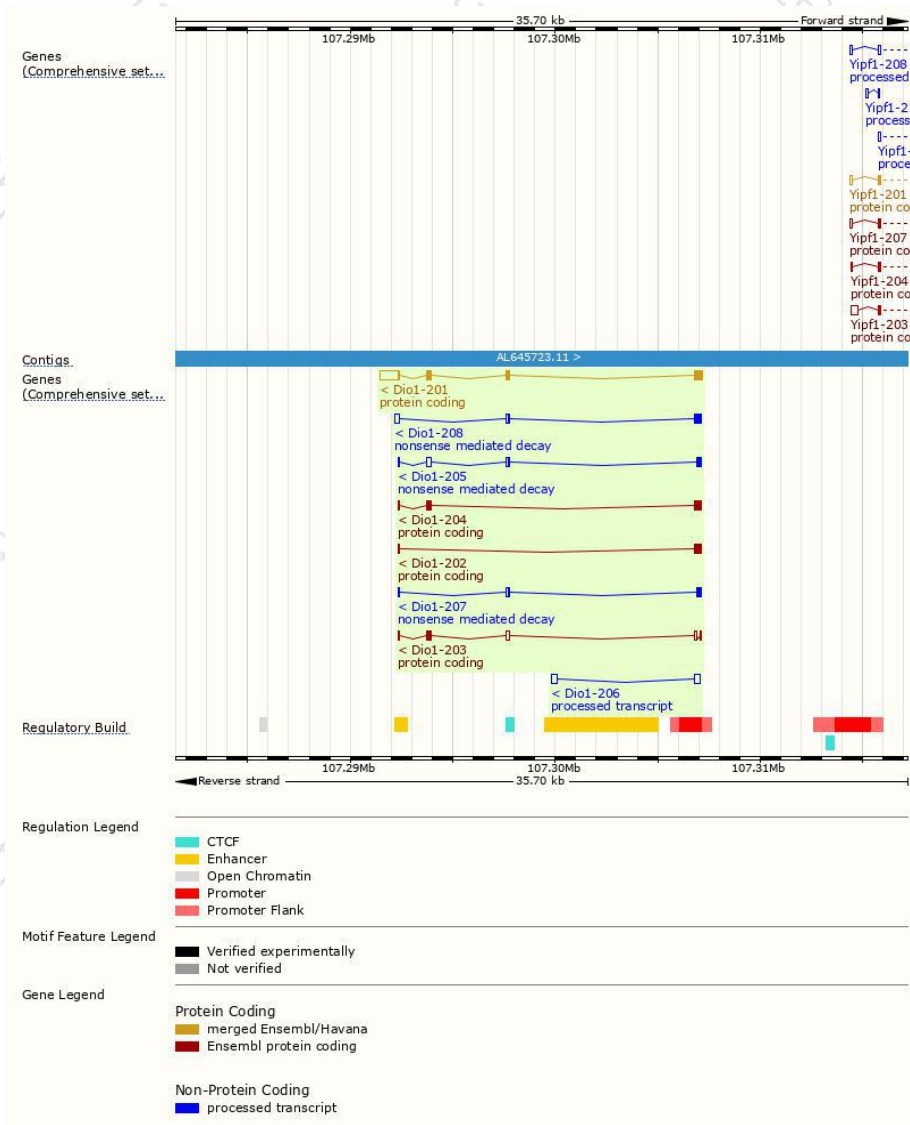
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dio1-201	ENSMUST00000082426.9	1690	257aa	Protein coding	CCDS18436	A0A0R4J110	TSL:1 GENCODE basic APPRIS P1
Dio1-204	ENSMUST00000129138.1	632	208aa	Protein coding	-	D3YX15	CDS 3' incomplete TSL:5
Dio1-203	ENSMUST00000126291.1	616	61aa	Protein coding	-	D6RDQ6	CDS 3' incomplete TSL:5
Dio1-202	ENSMUST00000106748.1	396	113aa	Protein coding	-	D3YVM7	TSL:5 GENCODE basic
Dio1-208	ENSMUST00000150974.1	761	125aa	Nonsense mediated decay	-	A0A0R4J1U2	TSL:5
Dio1-205	ENSMUST00000134366.7	671	82aa	Nonsense mediated decay	-	D6RCH6	TSL:5
Dio1-207	ENSMUST00000147709.1	431	82aa	Nonsense mediated decay	-	D6RCH6	TSL:5
Dio1-206	ENSMUST00000145332.1	565	No protein	Processed transcript	-	-	TSL:5

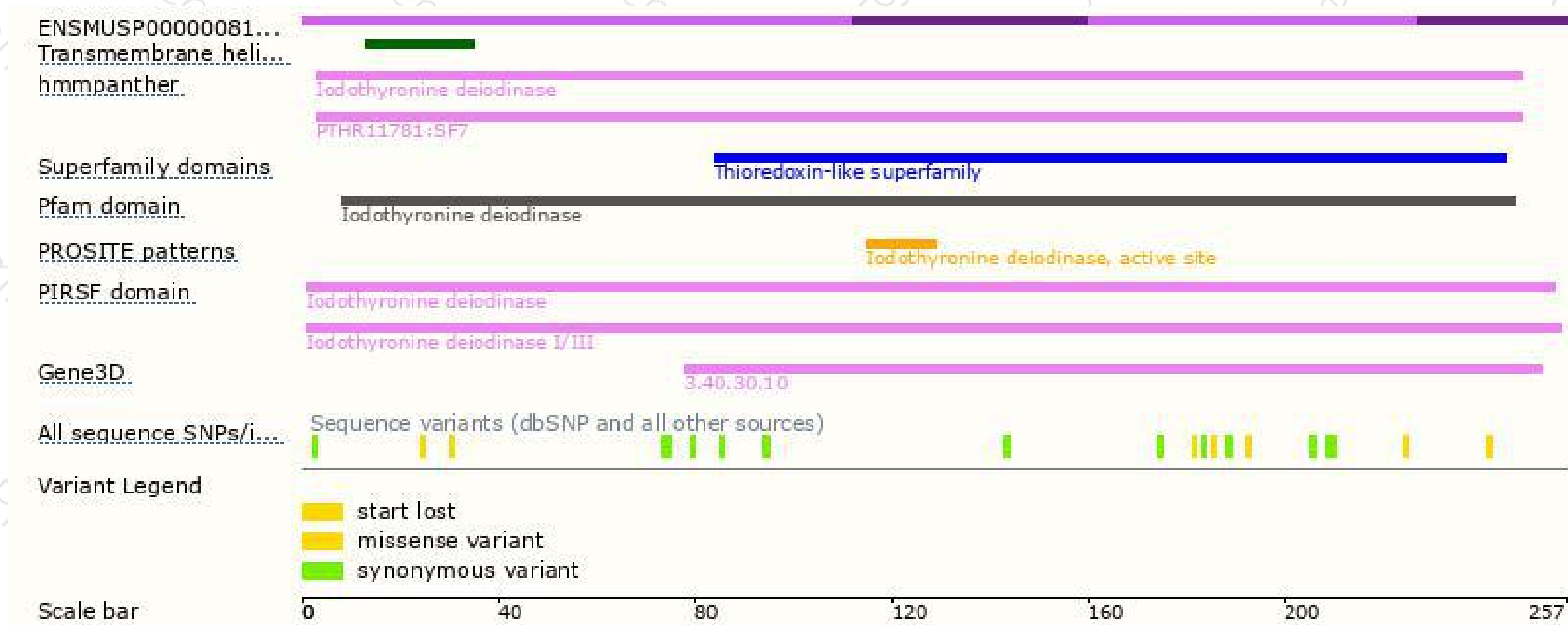
The strategy is based on the design of *Dio1-201* transcript,The transcription is shown below



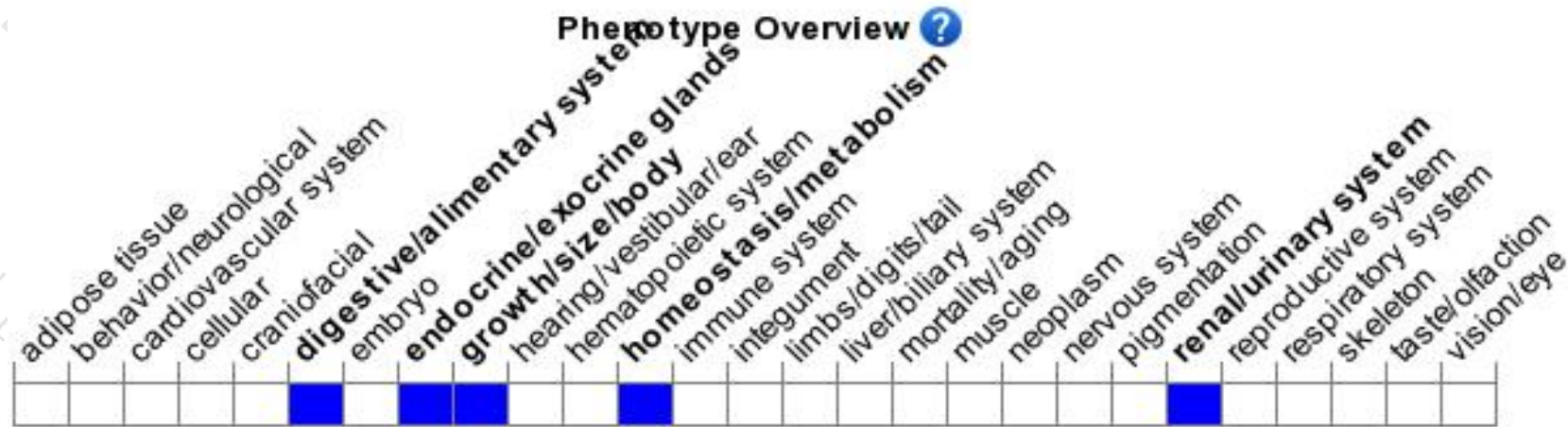
Genomic location distribution



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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