

Pnpla8 Cas9-CKO Strategy

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Project Overview

Project Name

Pnpla8

Project type

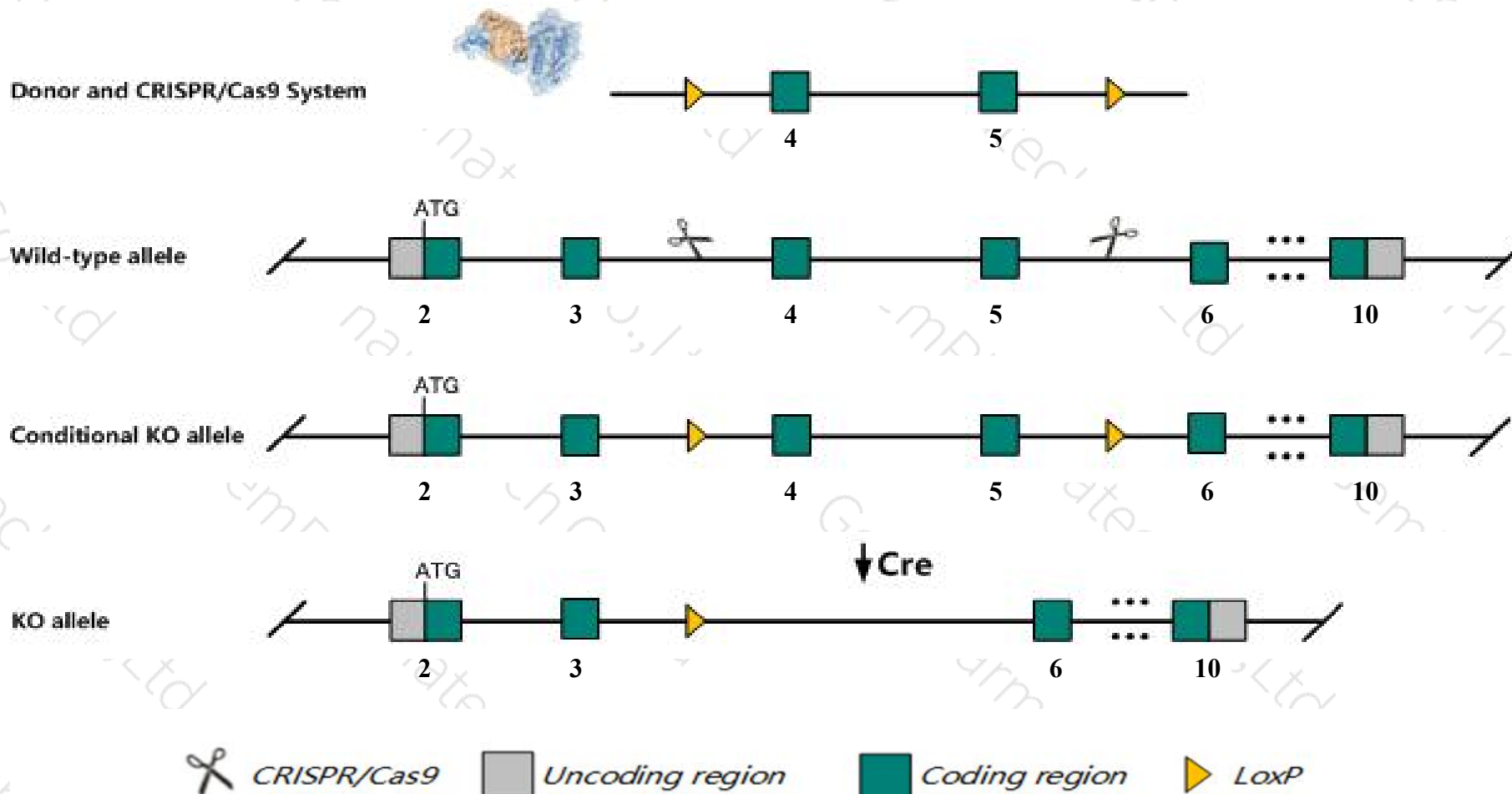
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pnpla8* gene. The schematic diagram is as follows:



- The *Pnpla8* gene has 7 transcripts. According to the structure of *Pnpla8* gene, exon4-exon5 of *Pnpla8*-201(ENSMUST00000043082.15) transcript is recommended as the knockout region. The region contains 247bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pnpla8* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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 null allele exhibit some female-specific embryonic lethality, reduced body weight and temperature, cold intolerance, decreased exercise tolerance and decreased mitochondria function.
- Transcript *Pnpla8*-203, *Pnpla8*-205 and *Pnpla8*-207 may not be affected.
- The *Pnpla8* gene is located on the Chr12. 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)

Pnpla8 patatin-like phospholipase domain containing 8 [Mus musculus (house mouse)]

Gene ID: 67452, updated on 13-Mar-2020

Summary

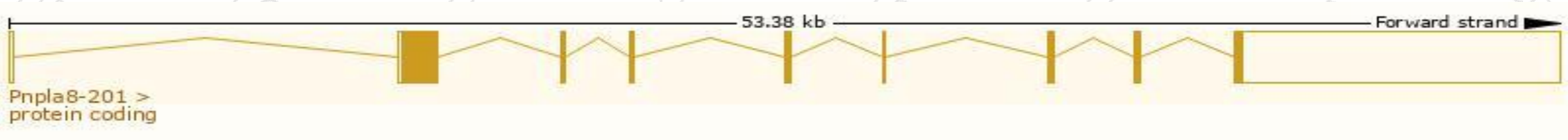
Official Symbol	Pnpla8 provided by MGI
Official Full Name	patatin-like phospholipase domain containing 8 provided by MGI
Primary source	MGI:MGI:1914702
See related	Ensembl:ENSMUSG00000036257
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1200006O19Rik, AI467579, Ipla2(gamma)
Expression	Ubiquitous expression in cortex adult (RPKM 9.4), frontal lobe adult (RPKM 9.1) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

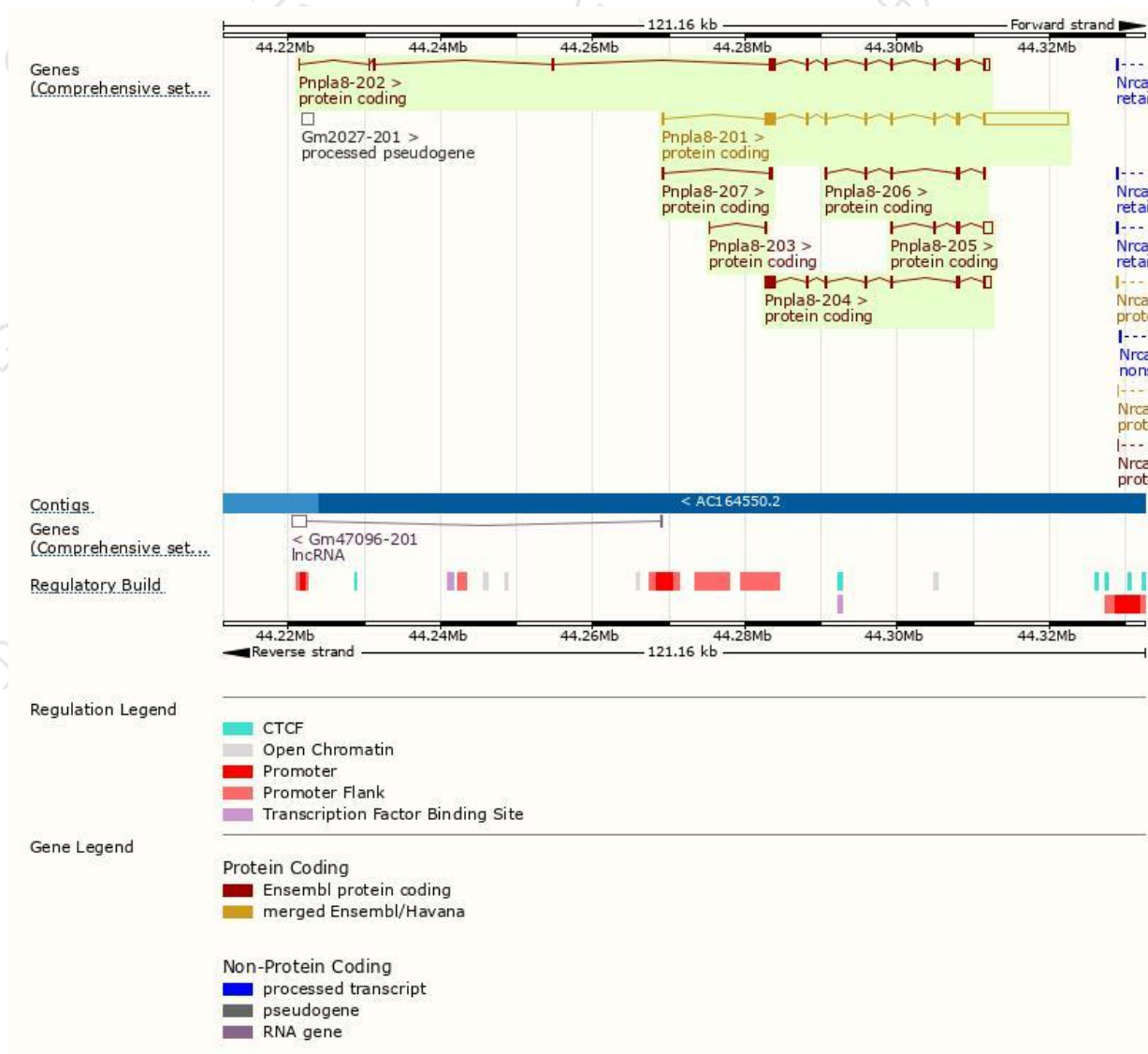
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pnpla8-201	ENSMUST00000043082.15	13507	776aa	Protein coding	CCDS36436	Q8K1N1	TSL:1 GENCODE basic APPRIS P2
Pnpla8-204	ENSMUST00000143771.1	2974	711aa	Protein coding	-	E9QAC9	TSL:5 GENCODE basic
Pnpla8-202	ENSMUST00000122902.7	2728	558aa	Protein coding	-	E9PXB0	TSL:1 GENCODE basic APPRIS ALT2
Pnpla8-205	ENSMUST00000156082.2	1611	137aa	Protein coding	-	F7B2N8	CDS 5' incomplete TSL:1
Pnpla8-206	ENSMUST00000218803.1	811	160aa	Protein coding	-	A0A1W2P6P7	CDS 5' incomplete TSL:3
Pnpla8-203	ENSMUST00000125757.1	394	74aa	Protein coding	-	D3Z5W9	CDS 3' incomplete TSL:2
Pnpla8-207	ENSMUST00000218954.1	342	65aa	Protein coding	-	A0A1W2P7I1	CDS 3' incomplete TSL:2

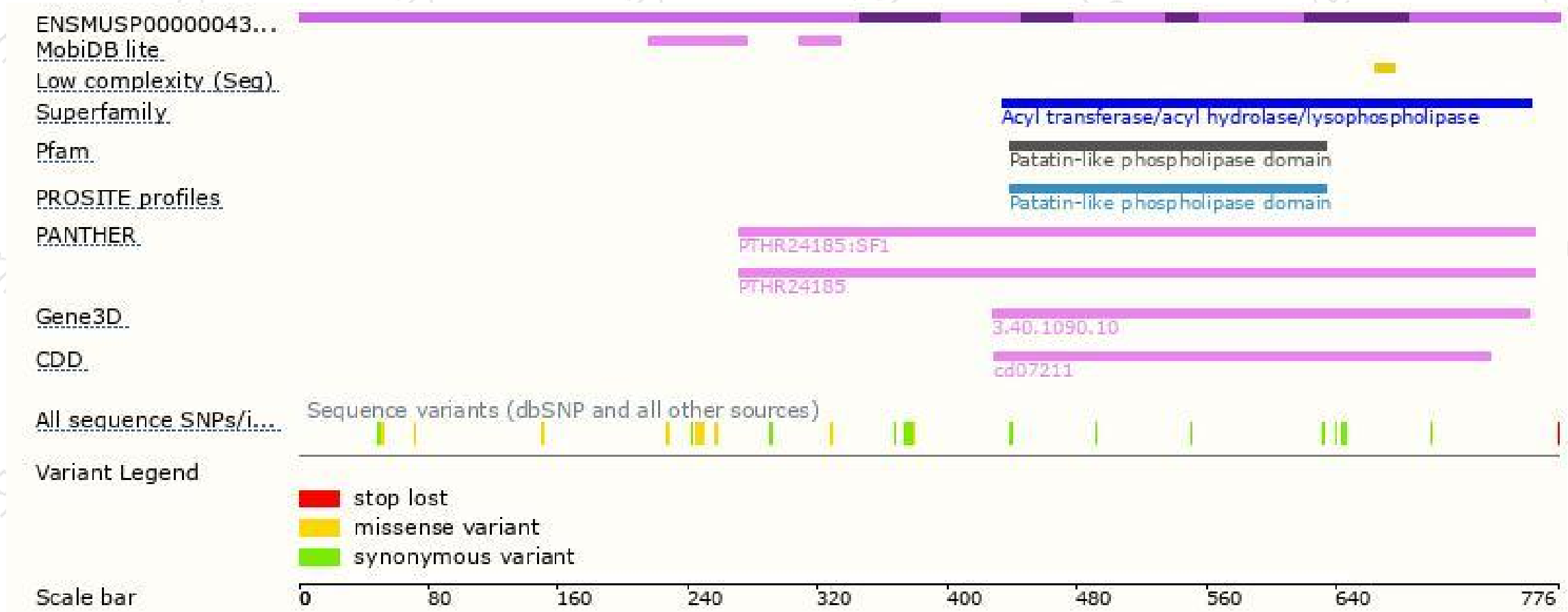
The strategy is based on the design of *Pnpla8-201* transcript,the transcription is shown below:



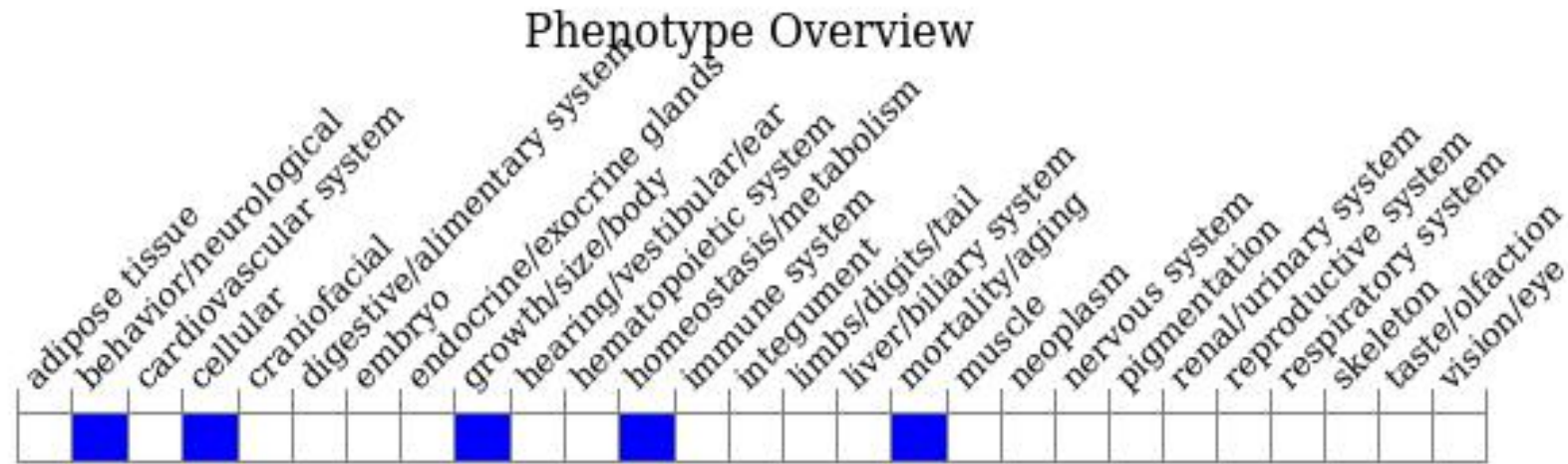
Genomic location distribution



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, mice homozygous for a null allele exhibit some female-specific embryonic lethality, reduced body weight and temperature, cold intolerance, decreased exercise tolerance and decreased mitochondria function.

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

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