

***Grk2* Cas9-CKO Strategy**

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Project Overview

Project Name

Grk2

Project type

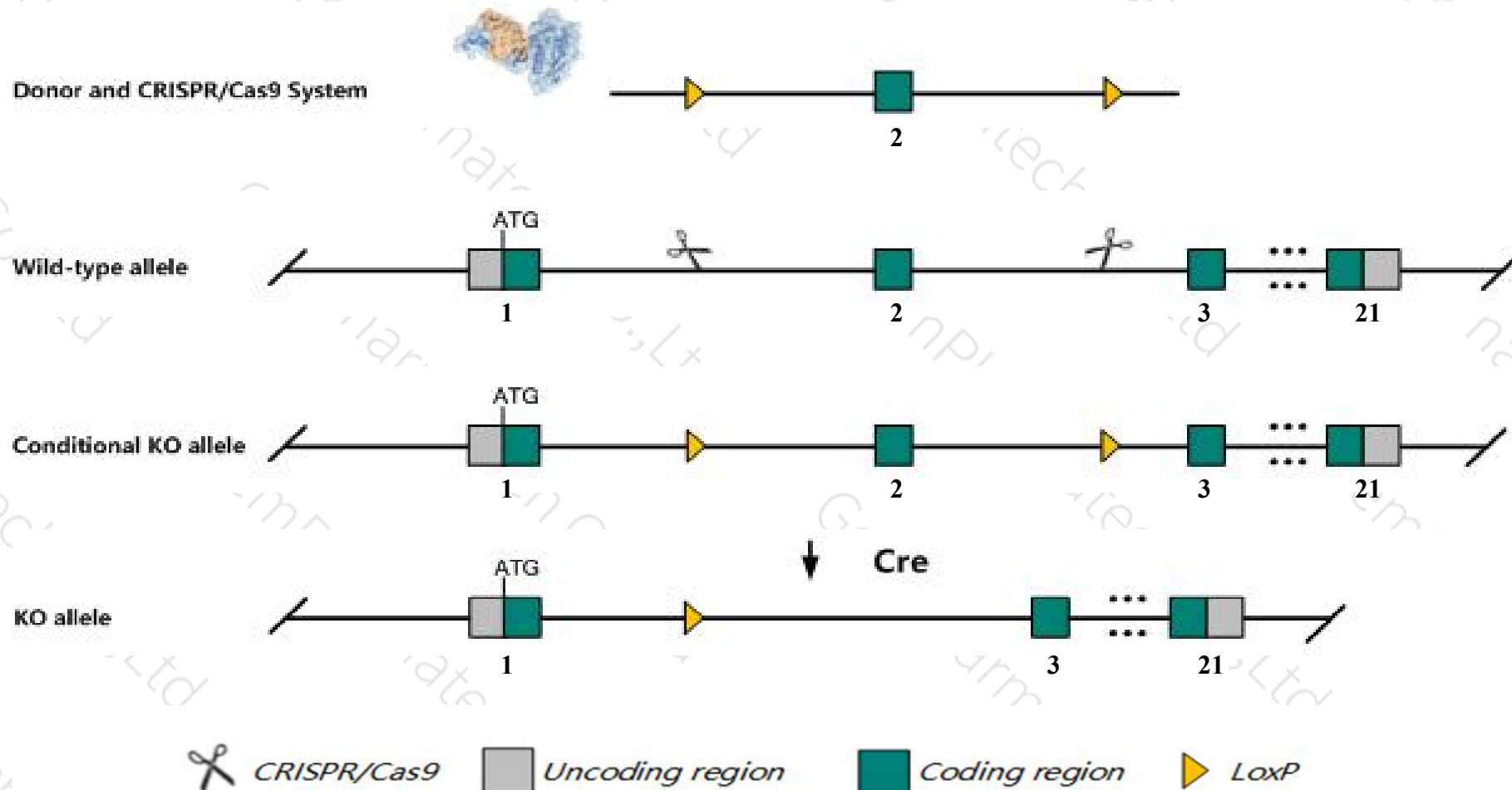
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Grk2* gene. The schematic diagram is as follows:



Technical routes

- The *Grk2* gene has 9 transcripts. According to the structure of *Grk2* gene, exon2 of *Grk2*-202 (ENSMUST00000088737.10) transcript is recommended as the knockout region. The region contains 77bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Grk2* 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 mutation of this gene results in embryonic lethality likely due to heart failure. Homozygous mutant embryos are pale in appearance and exhibit ventricular hypoplasia.
- The *Grk2* gene is located on the Chr19. 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)

Grk2 G protein-coupled receptor kinase 2 [Mus musculus (house mouse)]

Gene ID: 110355, updated on 2-Apr-2019

Summary



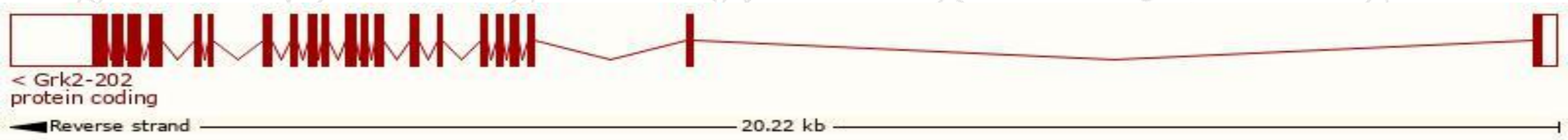
Official Symbol	Grk2 provided by MGI
Official Full Name	G protein-coupled receptor kinase 2 provided by MGI
Primary source	MGI:MGI:87940
See related	Ensembl:ENSMUSG00000024858
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Adrbk-1, Adrbk1, Bark-1, beta ARK, betaARK1
Expression	Ubiquitous expression in spleen adult (RPKM 143.0), thymus adult (RPKM 142.5) and 27 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

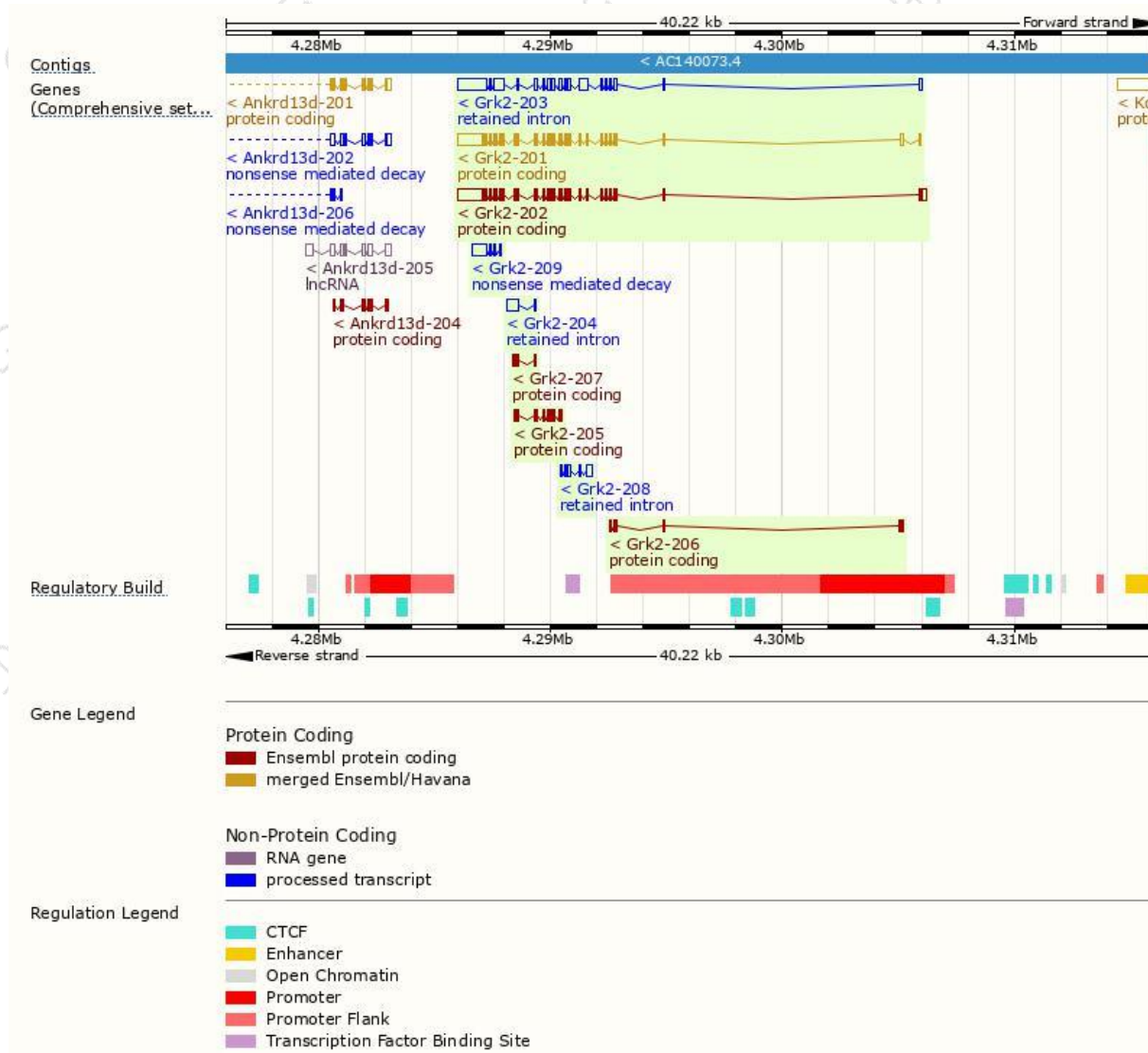
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Grk2-202	ENSMUST00000088737.10	3376	689aa	Protein coding	CCDS70915	Q3U1V3 Q99MK8	TSL:1 GENCODE basic APPRIS P1
Grk2-201	ENSMUST00000025791.11	3182	647aa	Protein coding	CCDS29426	Q7TS64	TSL:1 GENCODE basic
Grk2-205	ENSMUST00000165954.1	624	208aa	Protein coding	-	F6QY34	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Grk2-206	ENSMUST00000167511.2	403	110aa	Protein coding	-	F6Y9P3	CDS 3' incomplete TSL:3
Grk2-207	ENSMUST00000168594.7	261	87aa	Protein coding	-	F7AEX1	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Grk2-209	ENSMUST00000235681.1	853	26aa	Nonsense mediated decay	-	-	CDS 5' incomplete
Grk2-203	ENSMUST00000113837.8	3476	No protein	Retained intron	-	-	TSL:2
Grk2-204	ENSMUST00000164427.1	608	No protein	Retained intron	-	-	TSL:3
Grk2-208	ENSMUST00000169991.1	557	No protein	Retained intron	-	-	TSL:3

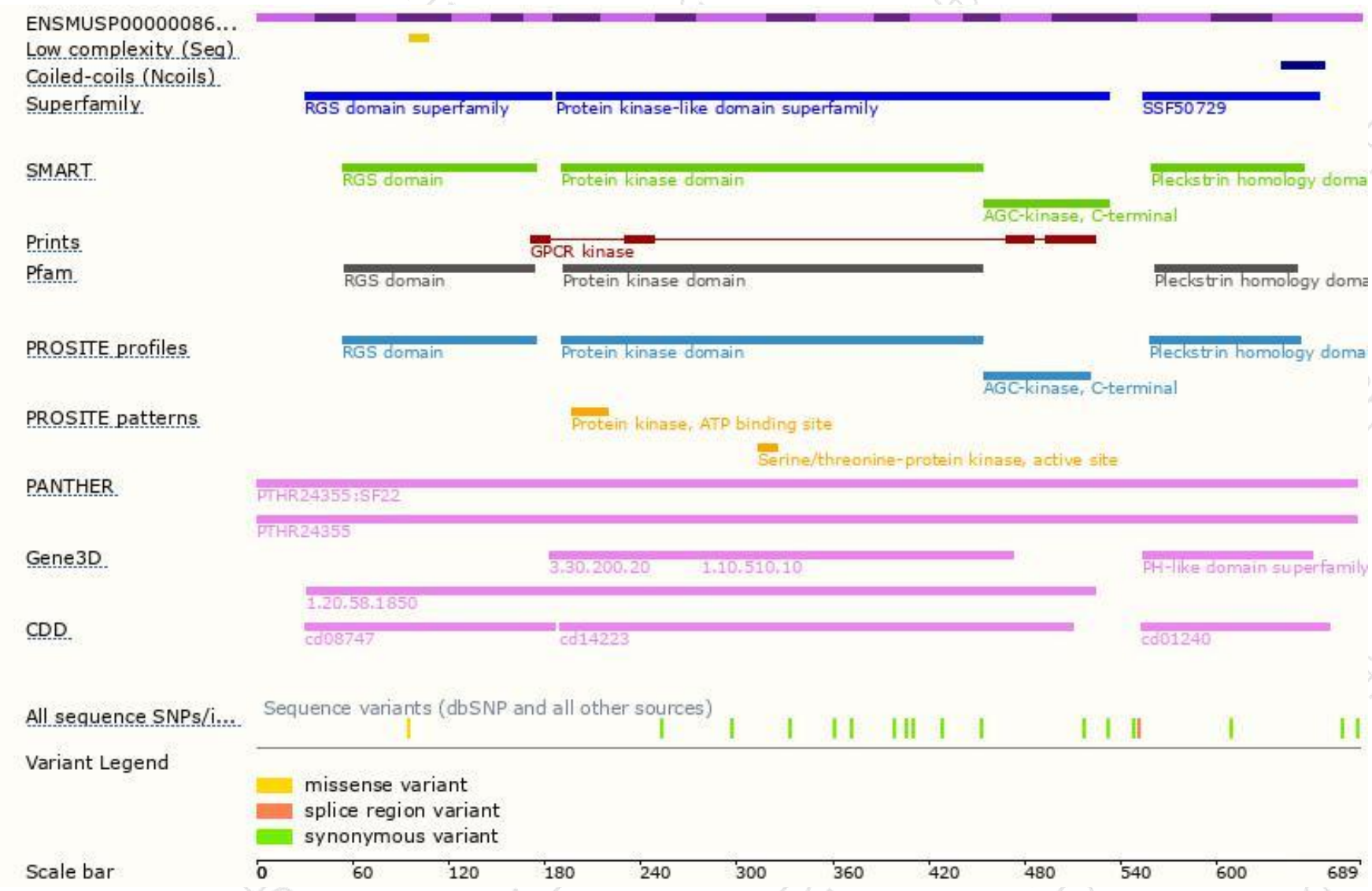
The strategy is based on the design of *Grk2-202* transcript,The transcription is shown below



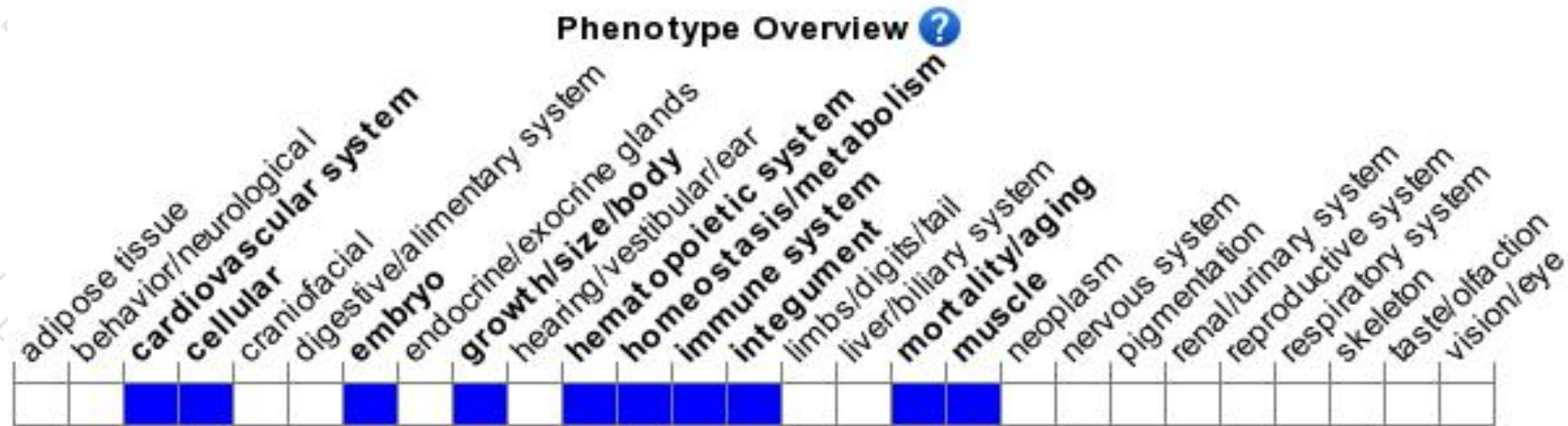
Genomic location distribution



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 mutation of this gene results in embryonic lethality likely due to heart failure. Homozygous mutant embryos are pale in appearance and exhibit ventricular hypoplasia.

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

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