E2f4 Cas9-CKO Strategy

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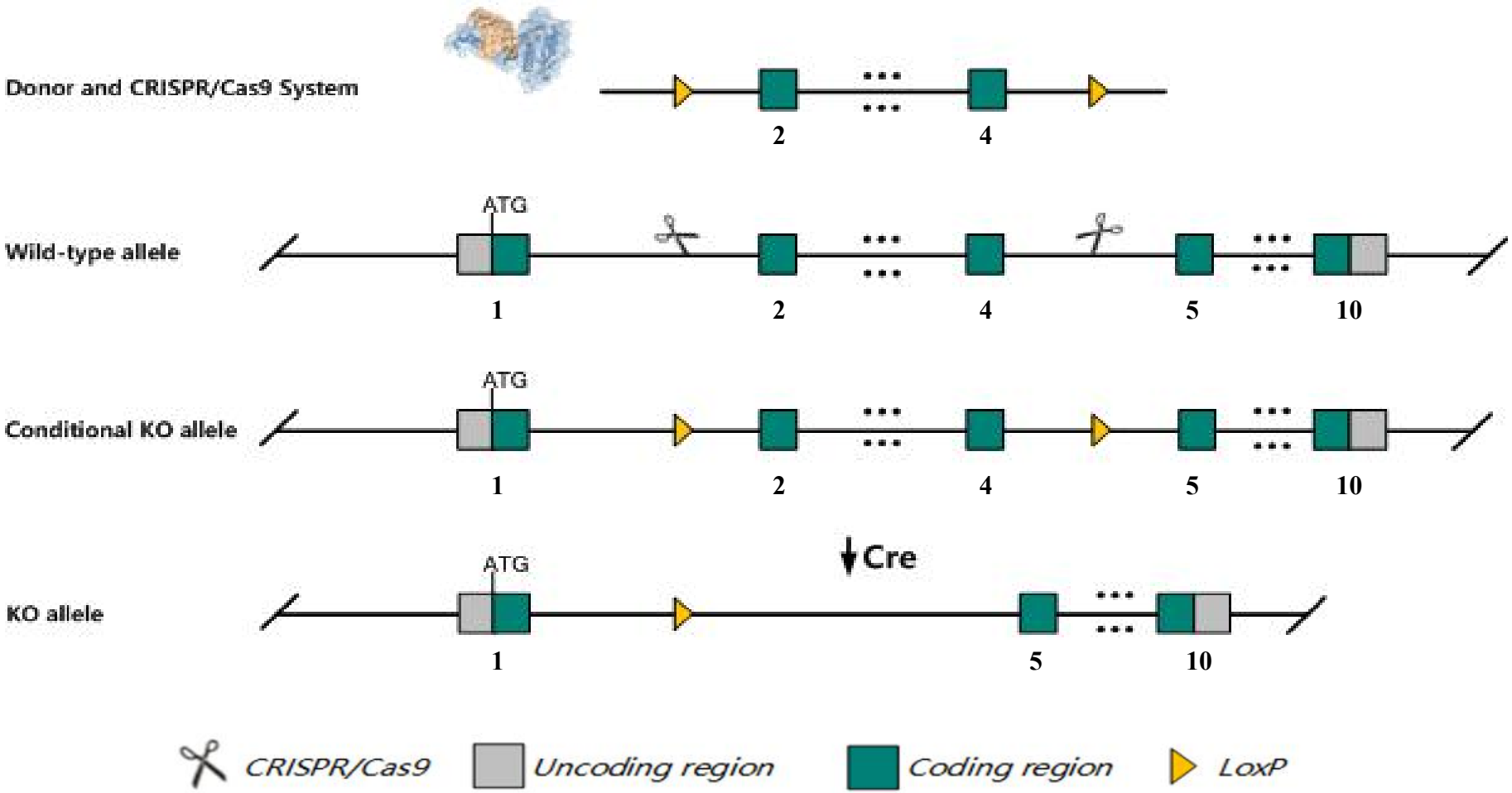
Design Date: 2020-8-5

Project Overview

Project Name	***E2f4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *E2f4* gene. The schematic diagram is as follows:



Technical routes

The *E2f4* gene has 3 transcripts. According to the structure of *E2f4* gene, exon2-exon4 of *E2f4-201*(ENSMUST00000015003.9) transcript is recommended as the knockout region.The region contains 316bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *E2f4* 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.

Notice

According to the existing MGI data,homozygous null mice die postnatally of an increased susceptibility to bacterial infection and exhibit craniofacial defects, erythroid abnormalities, and growth retardation.

The KO region contains functional region of the *E2f4* gene.Knockout the region may affect the function of *Exoc3l* gene.

The Intron1 is only 405bp ,loxp insertion may affect mRNA splicing.

The *E2f4* gene is located on the Chr8. 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

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E2f4 E2F transcription factor 4 [Mus musculus (house mouse)]

Gene ID: 104394, updated on 13-Mar-2020

Summary

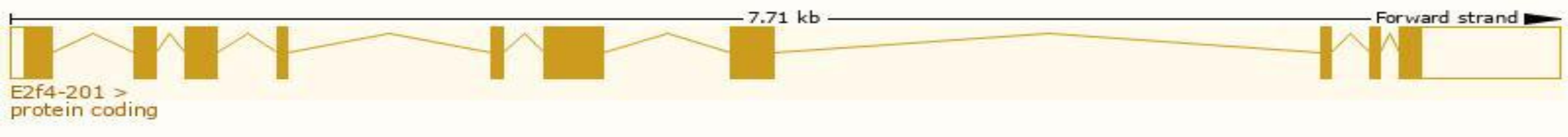
Official Symbol	E2f4 provided by MGI
Official Full Name	E2F transcription factor 4 provided by MGI
Primary source	MGI:MGI:103012
See related	Ensembl:ENSMUSG00000014859
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2010111M04Rik, AI427446
Expression	Ubiquitous expression in liver E14.5 (RPKM 188.0), liver E14 (RPKM 147.2) and 28 other tissues See more
Orthologs	human all

Transcript information Ensembl

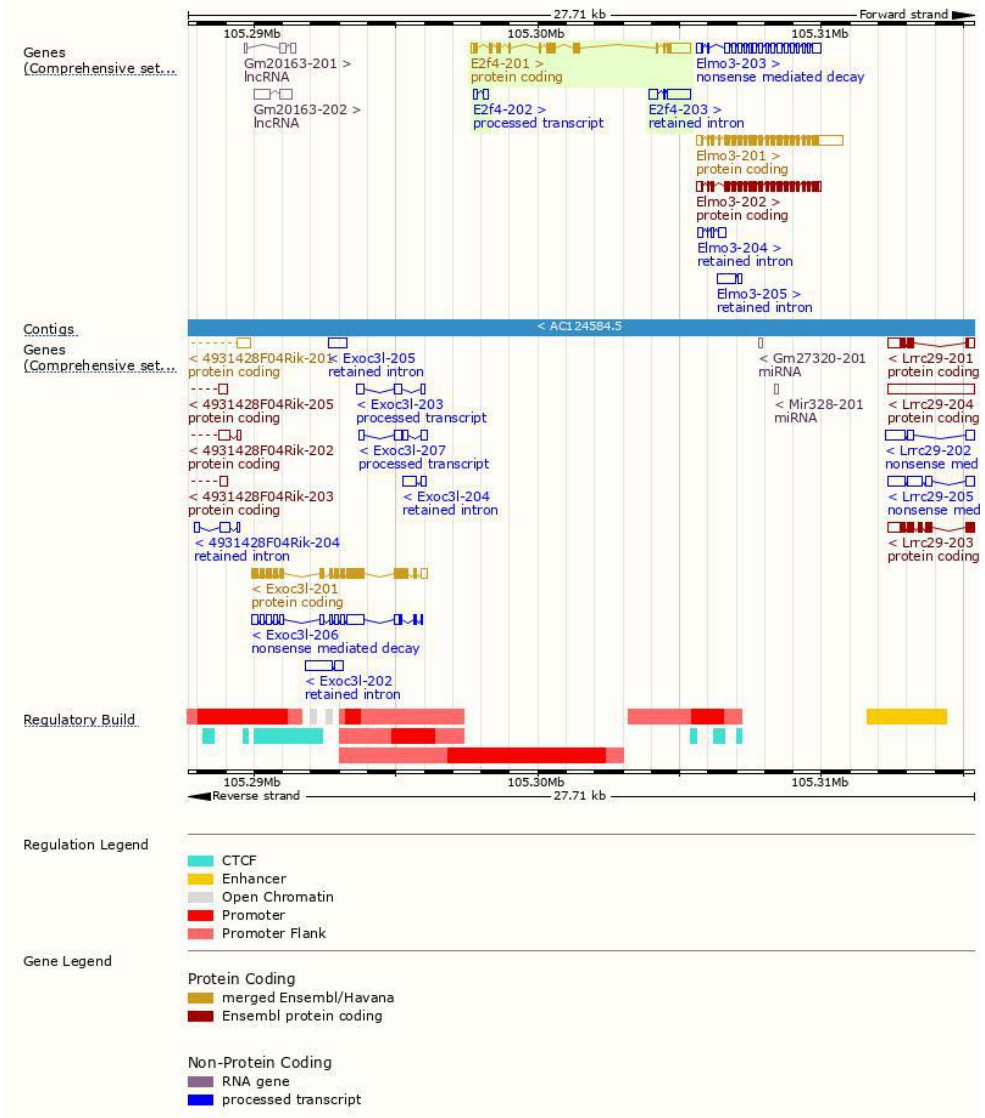
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
E2f4-201	ENSMUST00000015003.9	1993	410aa	Protein coding	CCDS40456	Q8R0K9	TSL:1 GENCODE basic APPRIS P1
E2f4-202	ENSMUST00000212037.1	358	No protein	Processed transcript	-	-	TSL:3
E2f4-203	ENSMUST00000212572.1	1151	No protein	Retained intron	-	-	TSL:1

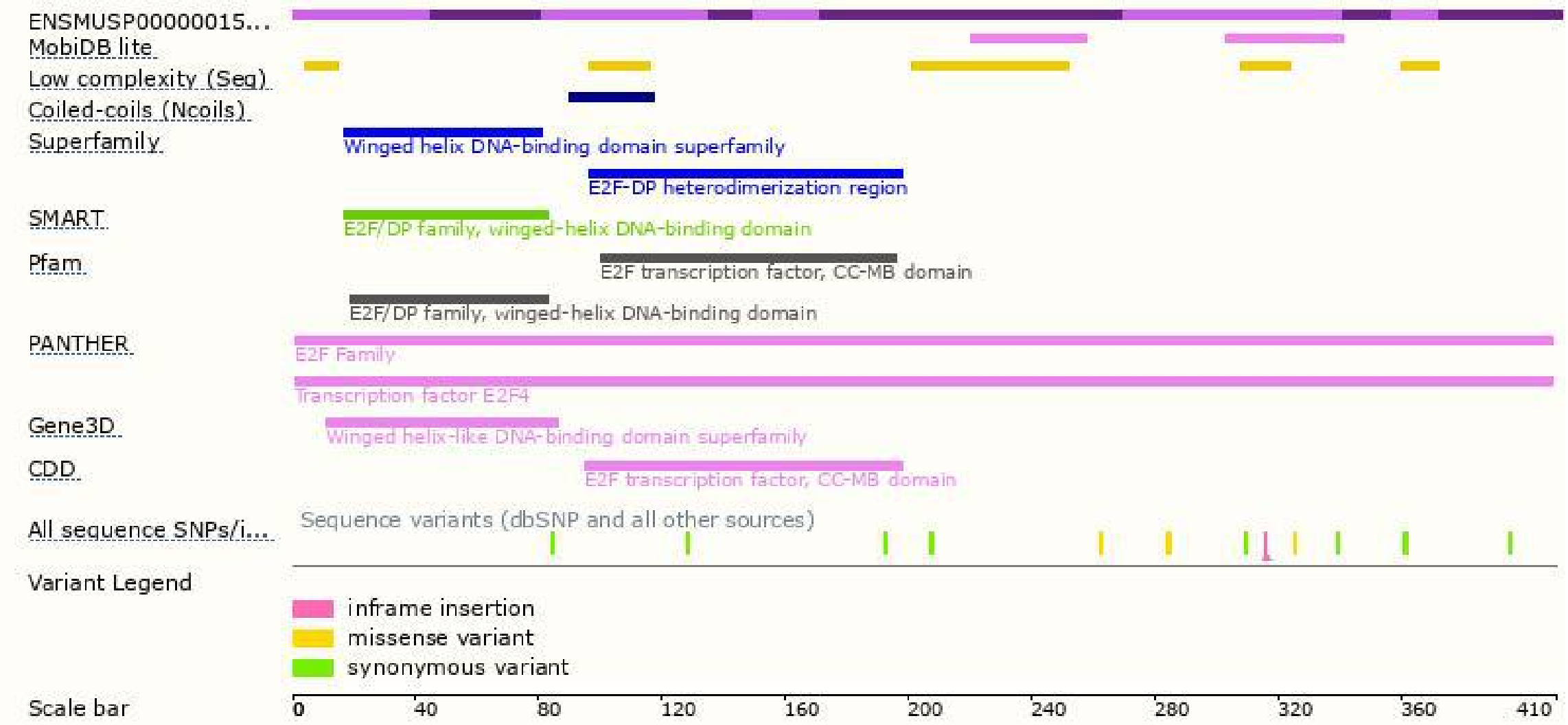
The strategy is based on the design of *E2f4-201* transcript,the transcription is shown below:



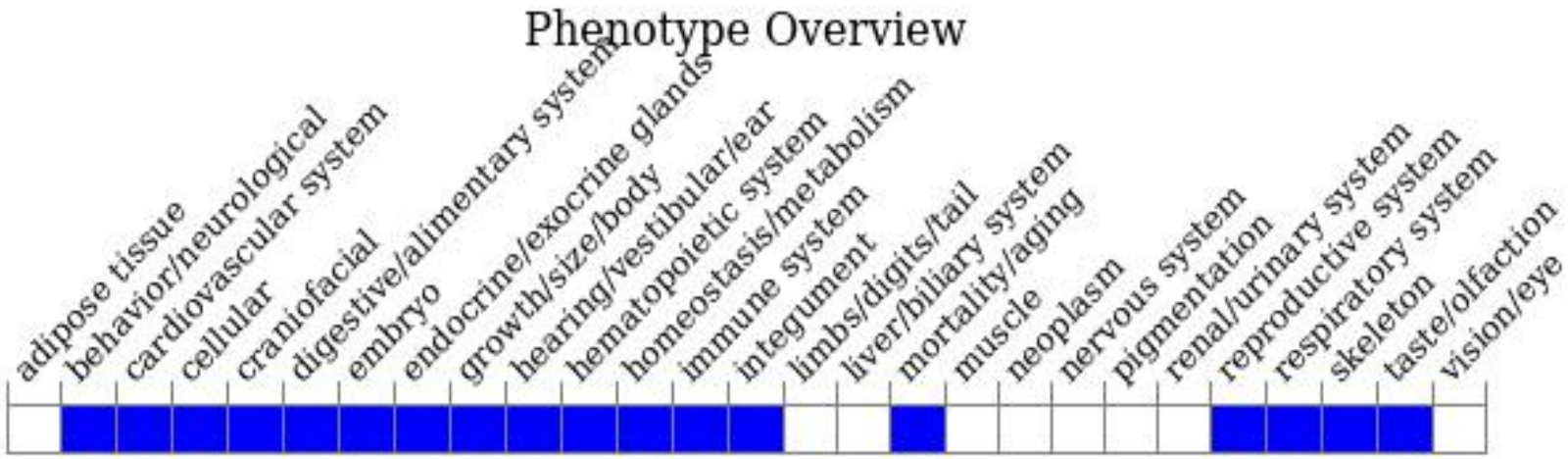
Genomic location distribution



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,homozygous null mice die postnatally of an increased susceptibility to bacterial infection and exhibit craniofacial defects, erythroid abnormalities, and growth retardation.

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