Wasf3 Cas9-CKO Strategy

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Project Overview

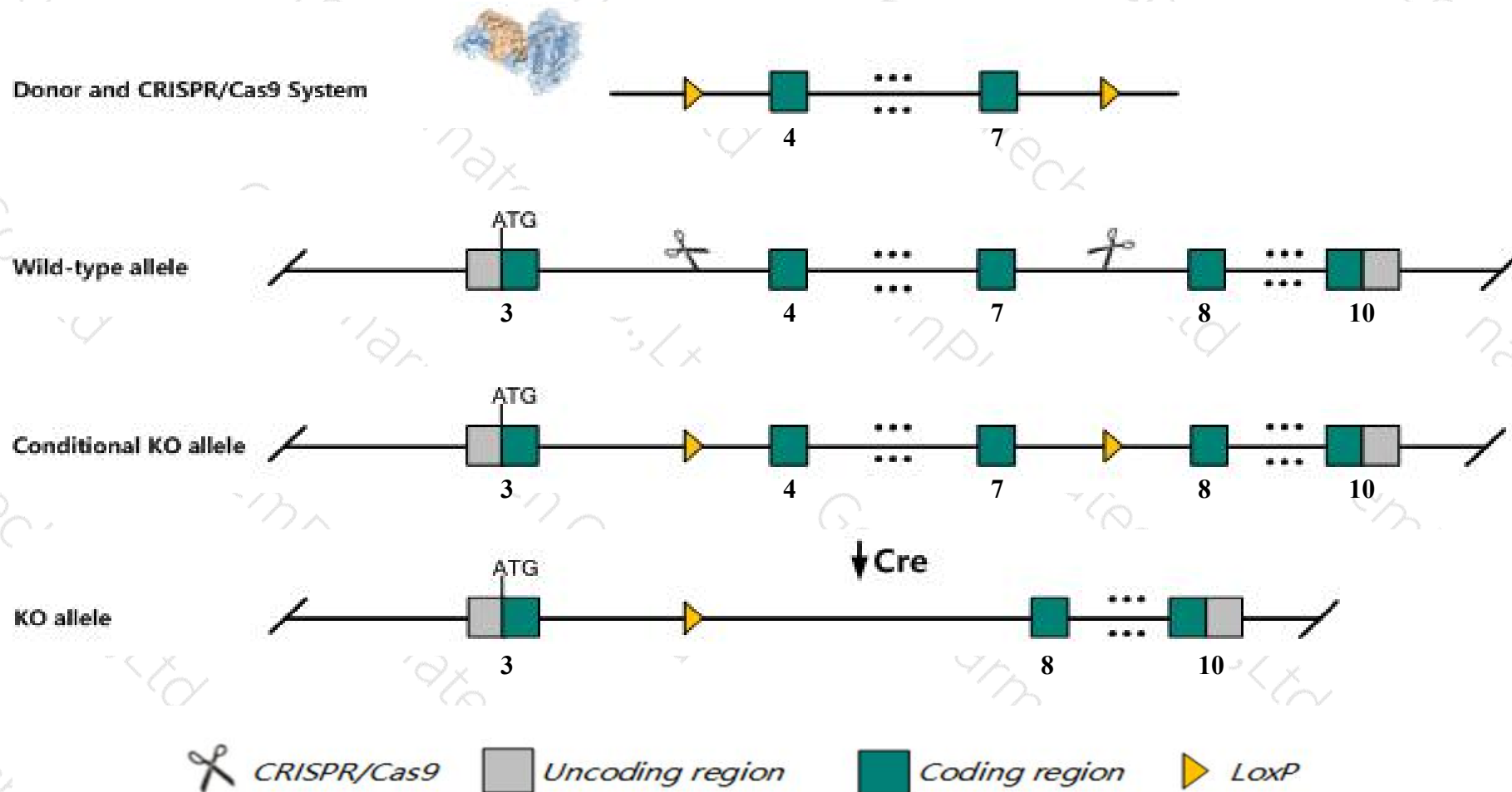|	
Project Name	*Wasf3*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Wasf3* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele

Cre

CRISPR/Cas9   Uncoding region   Coding region   LoxP

Technical routes

➤ The *Wasf3* gene has 2 transcripts. According to the structure of *Wasf3* gene, exon4-exon7 of *Wasf3-201* (ENSMUST00000016143.8) transcript is recommended as the knockout region.The region contains Ens 583bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Wasf3* 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 knock-out allele exhibit normal platelet physiology.

➢The effect on transcript *Wasf3*-202 is unknown.

➢ The *Wasf3* gene is located on the Chr5. 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）

Wasf3   WAS protein family, member 3 [_Mus musculus_ (house mouse)]

Gene ID: 245880, updated on 8-Oct-2019

▲ Summary

Official Symbol	Wasf3 provided by MGI
Official Full Name	WAS protein family, member 3 provided by MGI
Primary source	MGI:MGI:2658986
See related	Ensembl:ENSMUSG00000029636
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	Scar3; Wave3
Expression	Broad expression in cerebellum adult (RPKM 17.1), frontal lobe adult (RPKM 16.1) and 15 other tissues See more
Orthologs	human all

▲ Genomic context

Location: 5; 5 G3

See Wasf3 in Genome Data Viewer

Exon count: 12

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	5	NC_000071.6 (146385006..146473615)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	5	NC_000071.5 (147196582..147282701)

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Transcript information（Ensembl）

The gene has 2 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wasf3-201	ENSMUST00000016143.8	5196	501aa	Protein coding	CCDS19870	Q8VHI6	TSL:1 GENCODE basic APPRIS P1
Wasf3-202	ENSMUST00000238592.1	532	177aa	Protein coding	-	-	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete

The strategy is based on the design of *Wasf3-201* transcript,The transcription is shown below
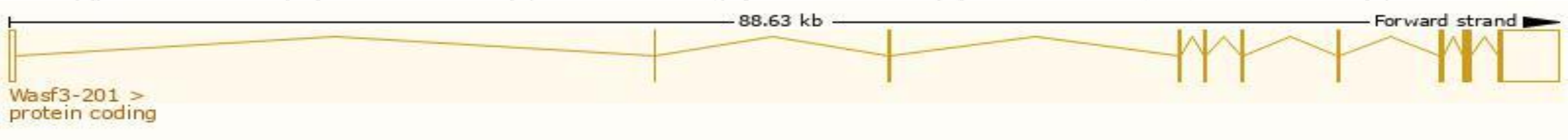
Genomic location distribution
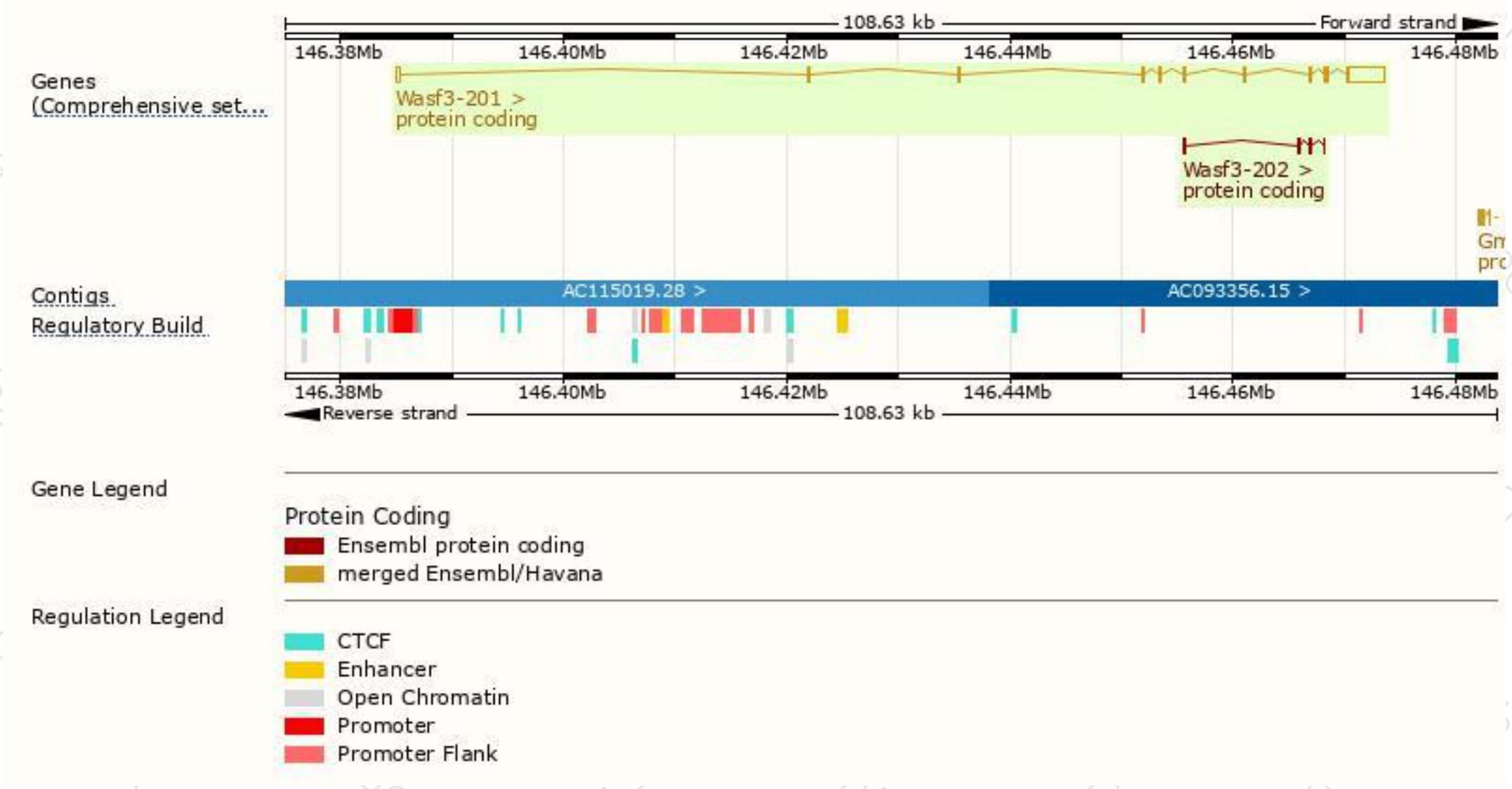
Protein domain
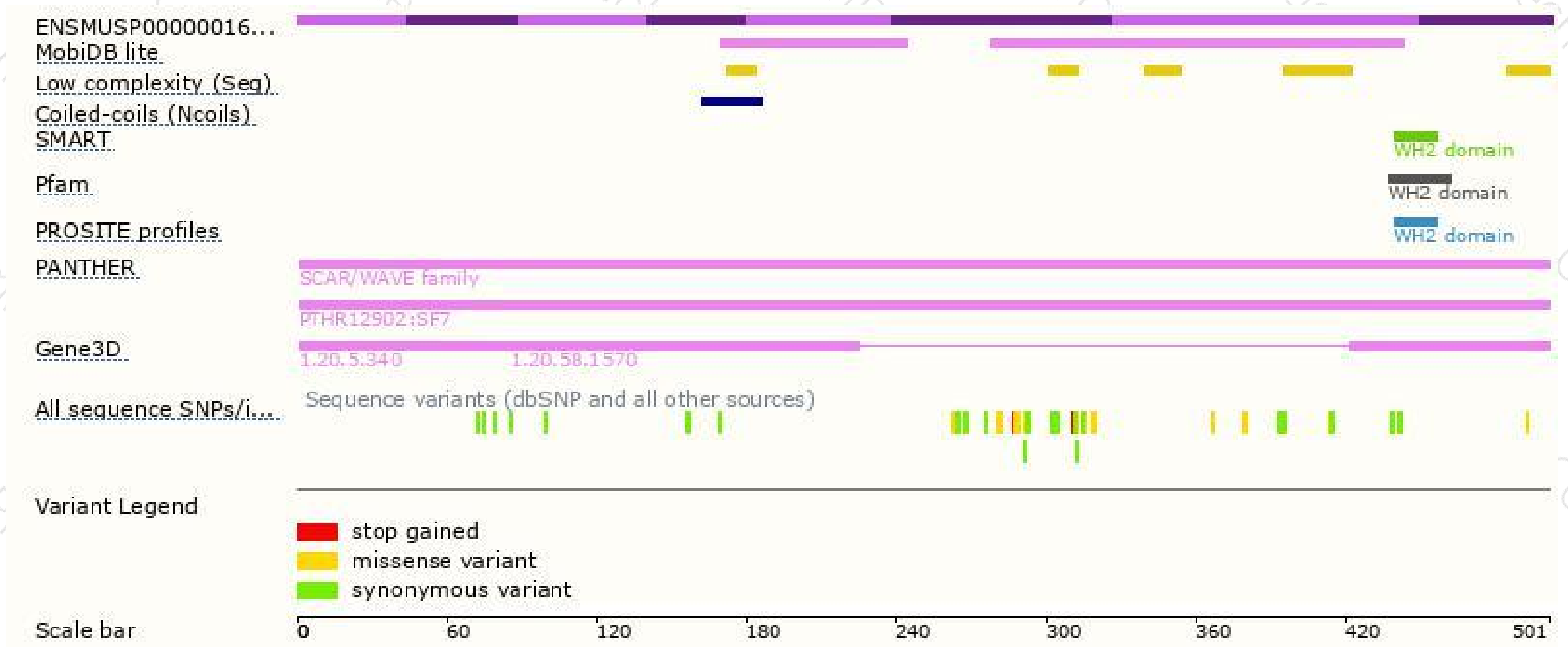
If you have any questions, you are welcome to inquire.
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