Fbxw4 Cas9-CKO Strategy

Designer: Ruirui Zhang
Reviewer: Huimin Su
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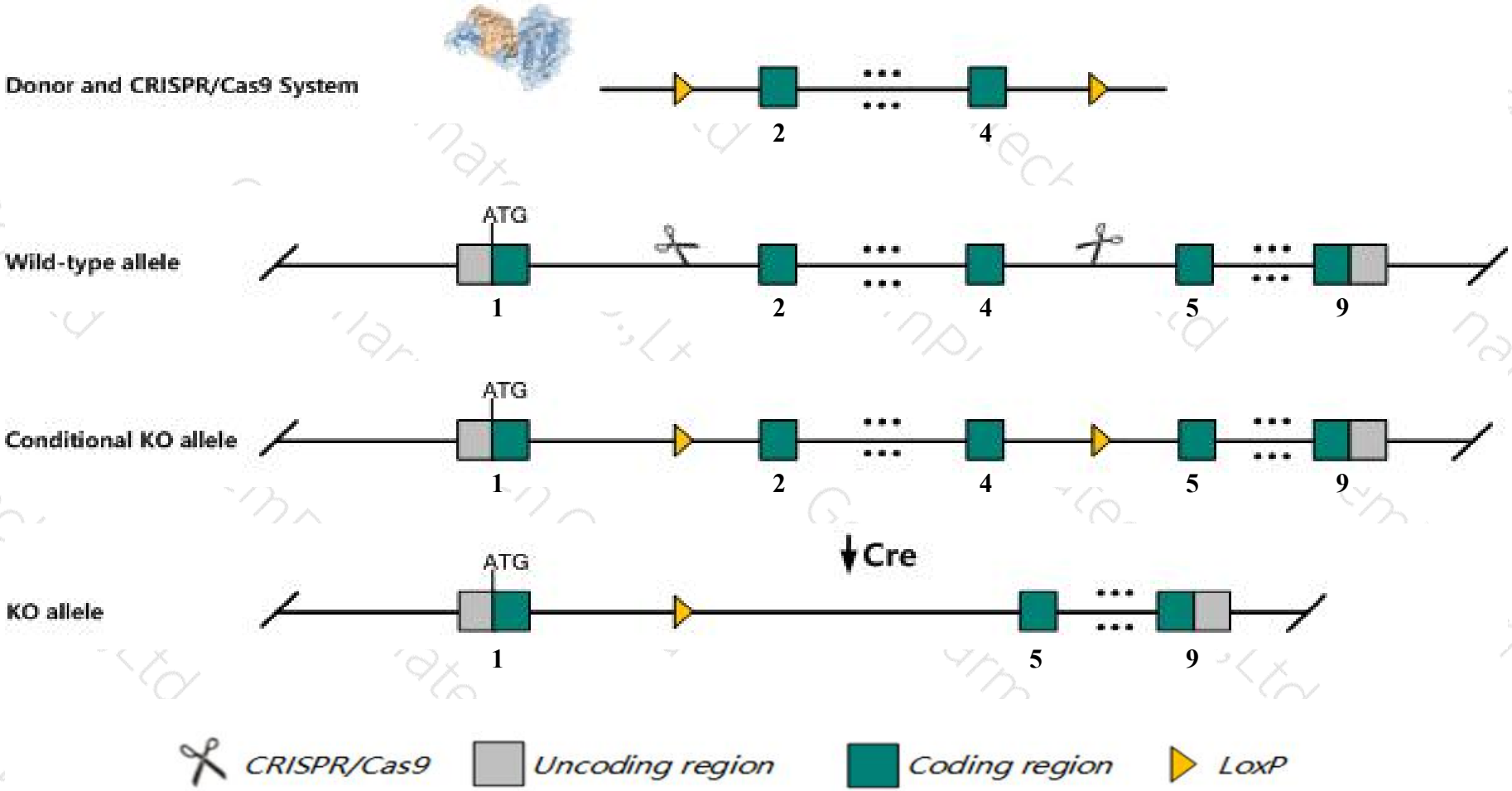
Project Overview

Project Name	***Fbxw4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Fbxw4* gene. The schematic diagram is as follows:



Technical routes

- The *Fbxw4* gene has 10 transcripts. According to the structure of *Fbxw4* gene, exon2-exon4 of *Fbxw4-201* (ENSMUST00000046869.10) transcript is recommended as the knockout region.The region contains 415bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Fbxw4* 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,Homozygotes for a spontaneous null mutation lack feet except for a single fused digit and die prenatally. Heterozygotes, in the presence of a recessive modifying allele, show loss of digits, frequently with fused metatarsal and metacarpal bones.

- Transcript *Fbxw4-203,204,205,209,210* may not be affected.

- The *Fbxw4* 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）

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Fbxw4 F-box and WD-40 domain protein 4 [*Mus musculus* (house mouse)]

Gene ID: 30838, updated on 12-Aug-2019

Summary

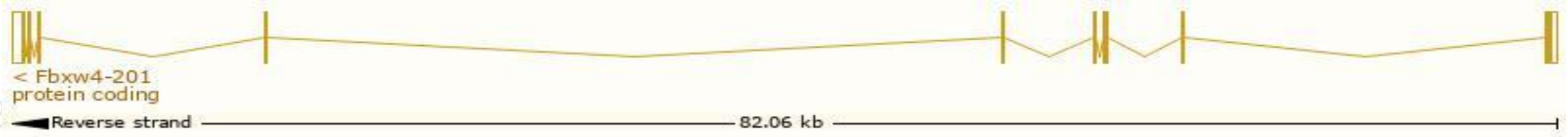
Official Symbol	Fbxw4 provided by MGI
Official Full Name	F-box and WD-40 domain protein 4 provided by MGI
Primary source	MGI:MGI:1354698
See related	Ensembl:ENSMUSG00000040913
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Dac; Fbw4; SHFM3; SHSF3; dactylin; dactylyn
Expression	Ubiquitous expression in adrenal adult (RPKM 14.9), ovary adult (RPKM 14.4) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

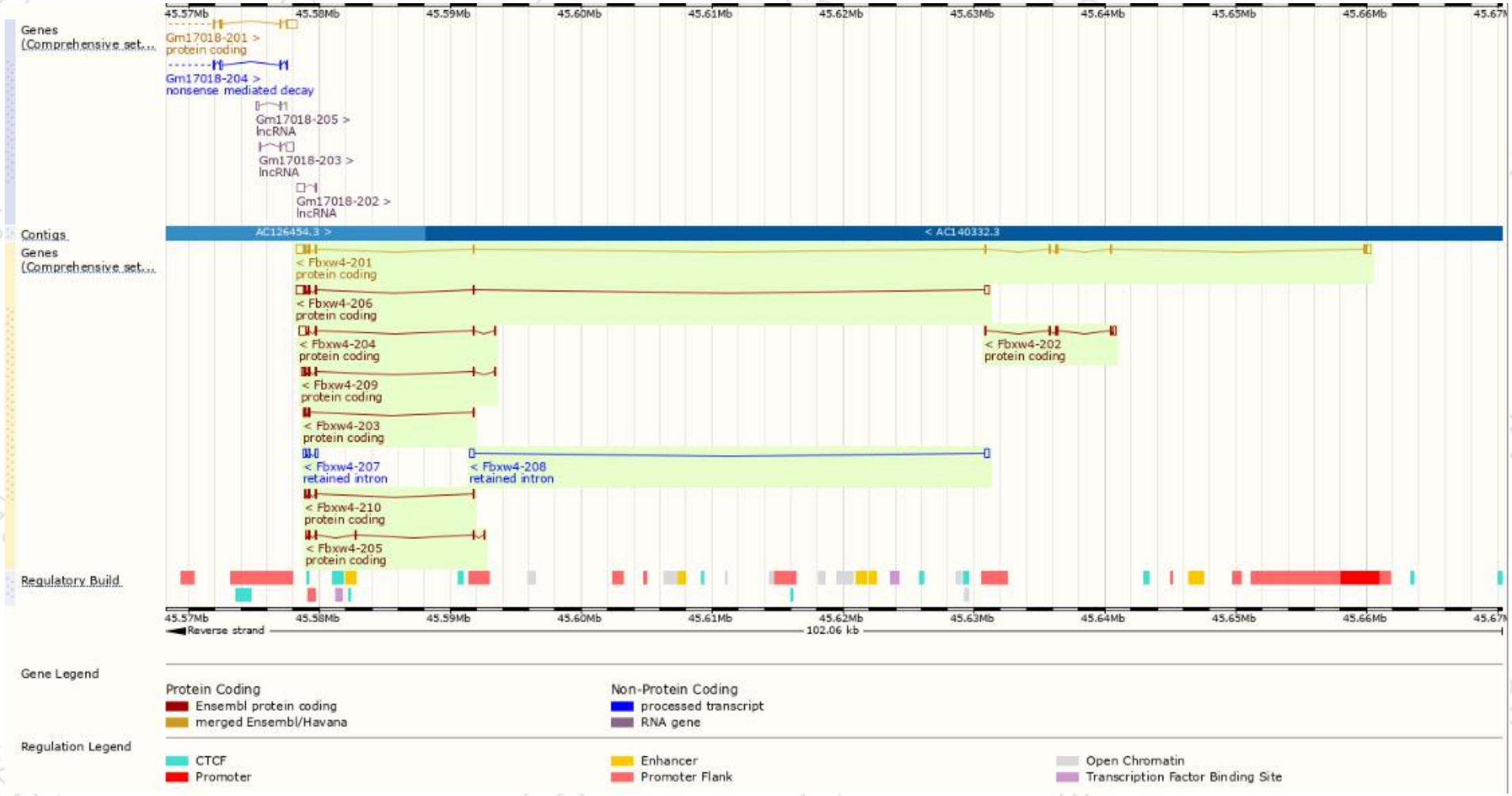
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fbxw4-201	ENSMUST00000046869.10	2068	410aa	Protein coding	CCDS29863	Q9JMJ2	TSL:1 GENCODE basic APPRIS P1
Fbxw4-206	ENSMUST00000160718.8	1295	113aa	Protein coding	-	-	TSL:2 GENCODE basic
Fbxw4-204	ENSMUST00000160018.7	931	59aa	Protein coding	-	E0CZ74	TSL:3 GENCODE basic
Fbxw4-209	ENSMUST00000162433.7	789	113aa	Protein coding	-	E0CX21	TSL:3 GENCODE basic
Fbxw4-202	ENSMUST00000159590.1	630	166aa	Protein coding	-	E0CYL5	CDS 3' incomplete TSL:3
Fbxw4-205	ENSMUST00000160438.2	570	78aa	Protein coding	-	E0CXP5	CDS 3' incomplete TSL:5
Fbxw4-210	ENSMUST00000162879.7	482	102aa	Protein coding	-	E0CXV9	CDS 3' incomplete TSL:2
Fbxw4-203	ENSMUST00000160003.7	387	83aa	Protein coding	-	E0CXZ3	TSL:3 GENCODE basic
Fbxw4-208	ENSMUST00000161556.1	609	No protein	Retained intron	-	-	TSL:3
Fbxw4-207	ENSMUST00000161039.1	509	No protein	Retained intron	-	-	TSL:2

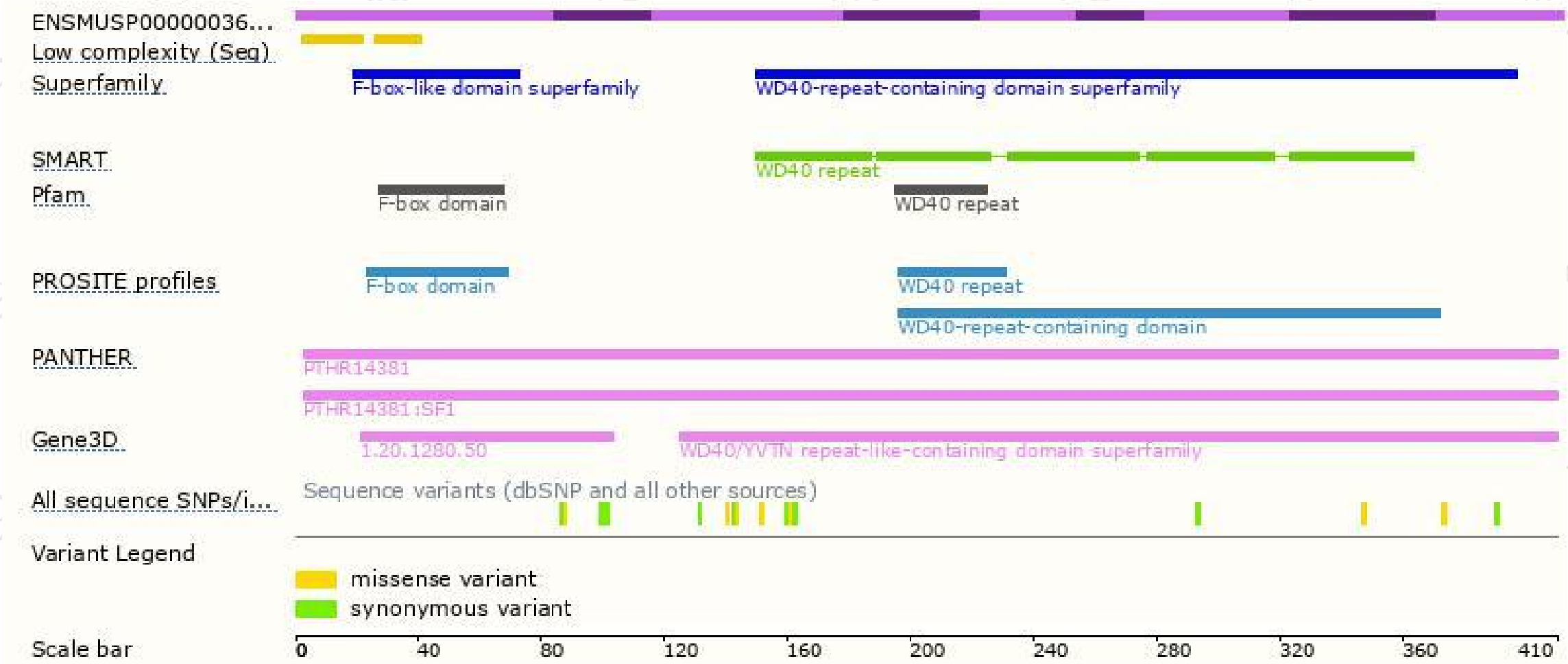
The strategy is based on the design of *Fbxw4-201* transcript,The transcription is shown below



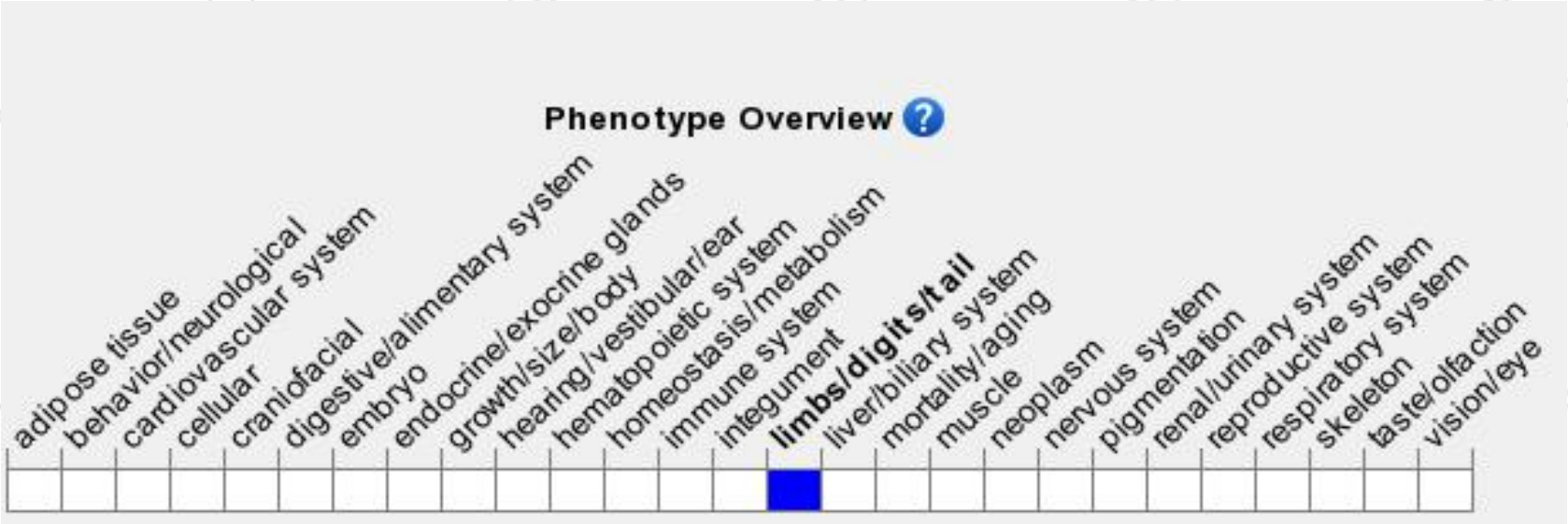
Genomic location distribution



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.

Tel: 400-9660890

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GemPharmatech Co.,Ltd