Sharpin Cas9-KO Strategy

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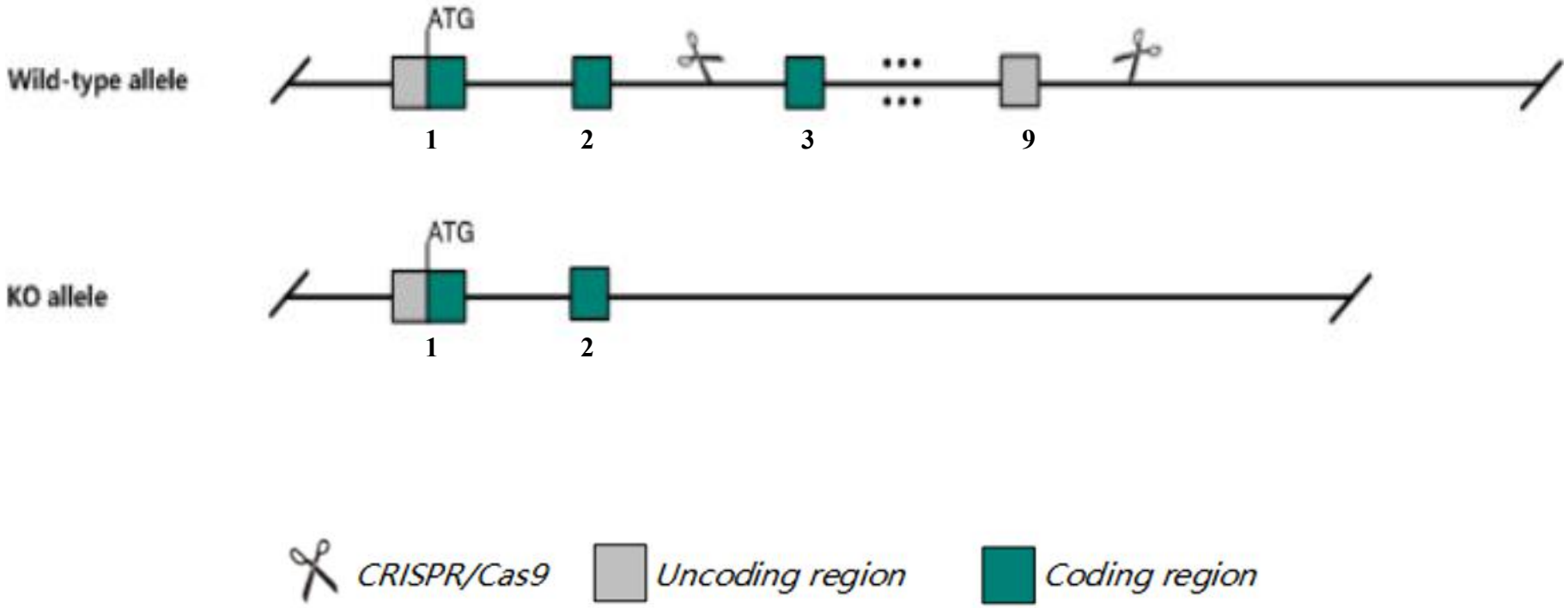
Project Overview

Project Name	***Sharpin***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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



Technical routes

The *Sharpin* gene has 13 transcripts. According to the structure of *Sharpin* gene, exon3-exon9 of *Sharpin-201*(ENSMUST00000023211.15) transcript is recommended as the knockout region.The region contains 770bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Sharpin* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

According to the existing MGI data,mutations in this gene produces chronic skin lesions.

The *Sharpin* gene is located on the Chr15. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

The KO region is less than 5kb from the adjacent gene *Maf1* .

Gene information NCBI

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Sharpin SHANK-associated RH domain interacting protein [Mus musculus (house mouse)]

Gene ID: 106025, updated on 20-Mar-2020

Summary

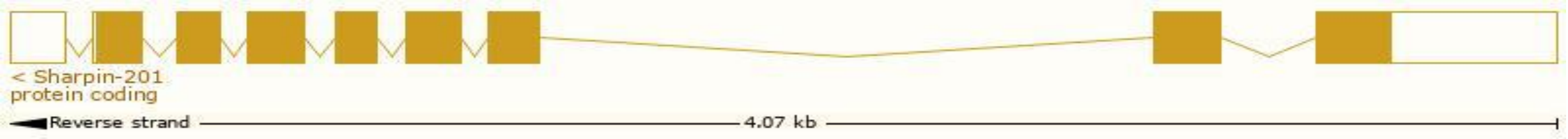
Official Symbol	Sharpin provided by MGI
Official Full Name	SHANK-associated RH domain interacting protein provided by MGI
Primary source	MGI:MGI:1913331
See related	Ensembl:ENSMUSG00000022552
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	0610041B22Rik, AW121341, RBCKL1, SIPL1, cpdm
Expression	Ubiquitous expression in spleen adult (RPKM 39.1), thymus adult (RPKM 38.9) and 28 other tissues See more
Orthologs	human all

Transcript information Ensembl

The gene has 13 transcripts,all transcripts are shown below:

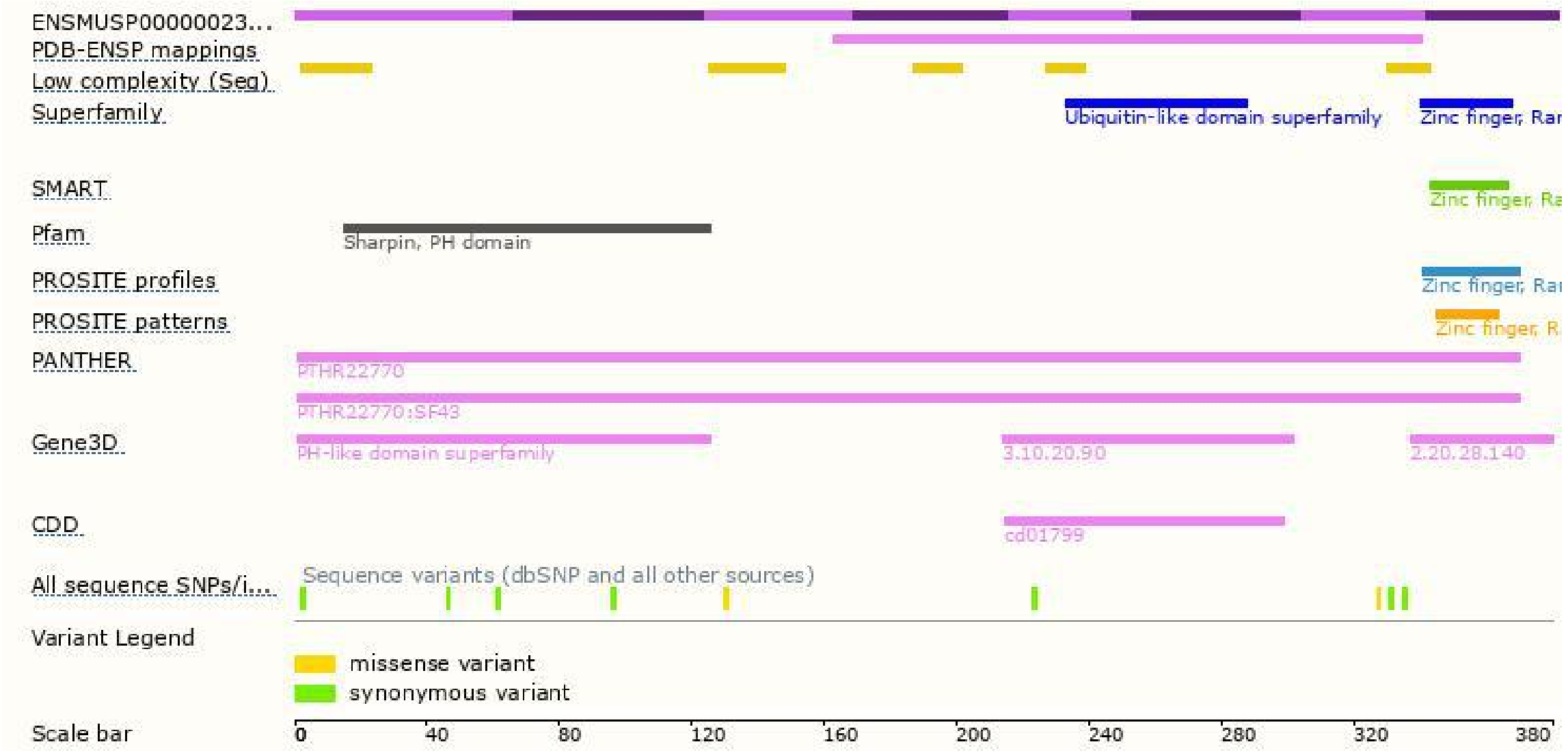
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sharpin-201	ENSMUST00000023211.15	1734	380aa	Protein coding	CCDS37121	Q91WA6	TSL:1 GENCODE basic APPRIS P1
Sharpin-209	ENSMUST00000159429.1	418	83aa	Protein coding	-	F6UHH8	CDS 5' incomplete TSL:3
Sharpin-213	ENSMUST00000230314.1	1271	157aa	Nonsense mediated decay	-	E0CZH2	
Sharpin-210	ENSMUST00000160560.1	835	157aa	Nonsense mediated decay	-	E0CZH2	TSL:3
Sharpin-212	ENSMUST00000230016.1	478	No protein	Processed transcript	-	-	
Sharpin-211	ENSMUST00000229536.1	395	No protein	Processed transcript	-	-	
Sharpin-208	ENSMUST00000154477.7	1905	No protein	Retained intron	-	-	TSL:1
Sharpin-202	ENSMUST00000123112.7	1896	No protein	Retained intron	-	-	TSL:2
Sharpin-203	ENSMUST00000133993.1	1884	No protein	Retained intron	-	-	TSL:1
Sharpin-205	ENSMUST00000144816.8	1669	No protein	Retained intron	-	-	TSL:1
Sharpin-206	ENSMUST00000147001.9	854	No protein	Retained intron	-	-	TSL:5
Sharpin-204	ENSMUST00000137106.1	835	No protein	Retained intron	-	-	TSL:5
Sharpin-207	ENSMUST00000152151.8	819	No protein	Retained intron	-	-	TSL:2

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

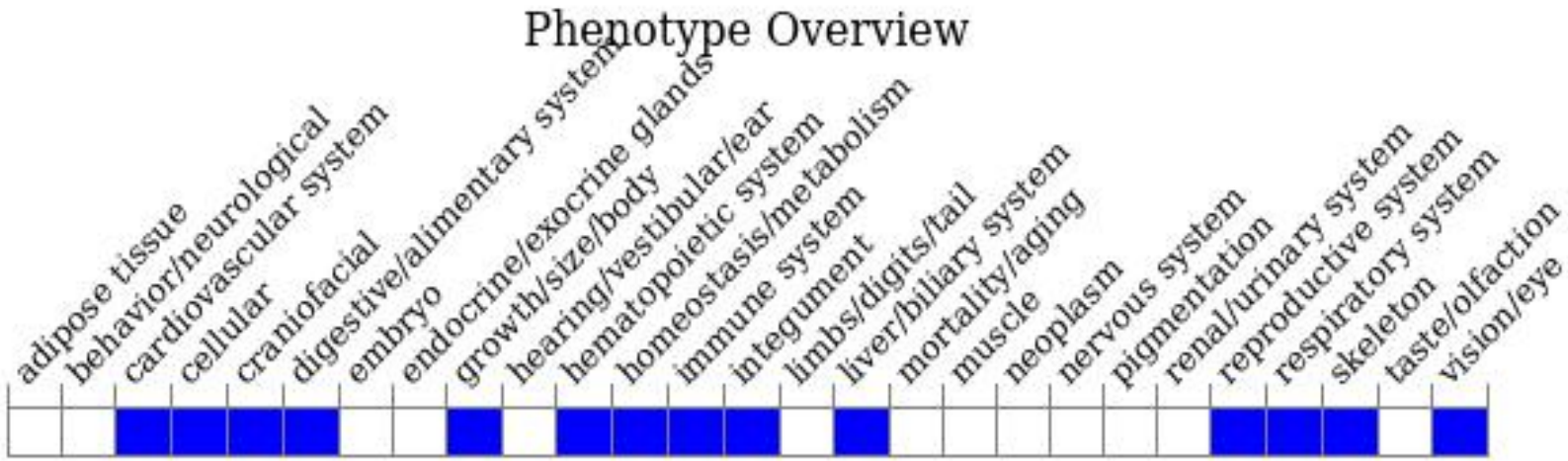


Genomic location distribution

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,mutations in this gene produces chronic skin lesions.

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