Ift172 Cas9-CKO Strategy

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Project Overview

Project Name	***Ift172***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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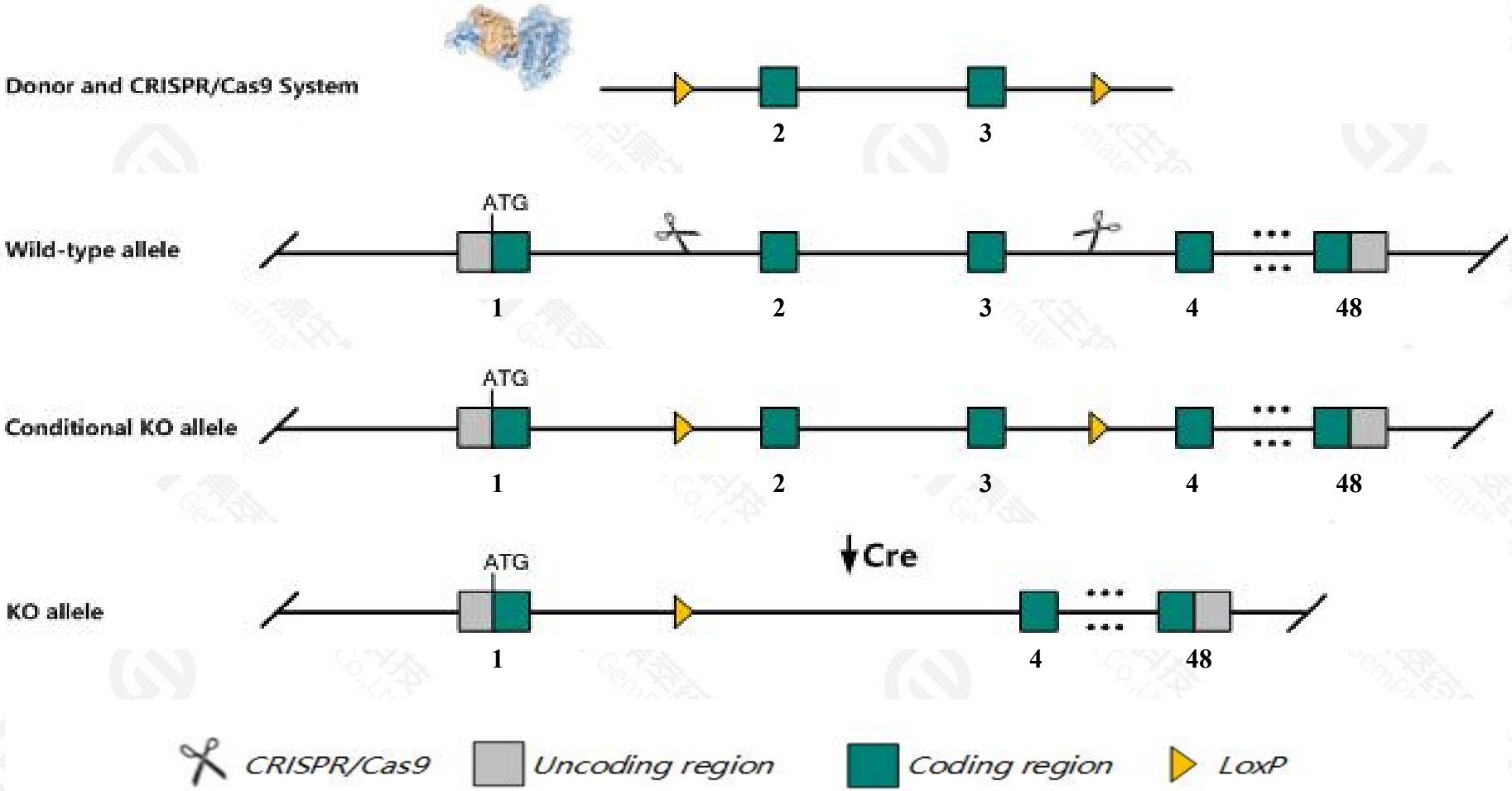
This model will use CRISPR/Cas9 technology to edit the *Ift172* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ift172* gene has 11 transcripts. According to the structure of *Ift172* gene, exon2-exon3 of *Ift172-201*(ENSMUST00000041565.11) transcript is recommended as the knockout region.The region contains 257bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ift172* 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 was 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,mice homozygous for disruptions in this gene display embryonic lethality during organogenesis, neural tube defects, and developmental patterning abnormalities. Mice homozygous for a conditional allele activated in the early limb bud exhibit polydactyly and short limbs.

➢Transcript *Ift172-210* and *Ift172-211* may not be affected.

➢ The Intron3 is only 459bp,loxp insertion may affect mRNA splicing.

➢ The *Ift172* 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）

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Ift172 intraflagellar transport 172 [Mus musculus (house mouse)]

Gene ID: 67661, updated on 17-Feb-2019

Summary

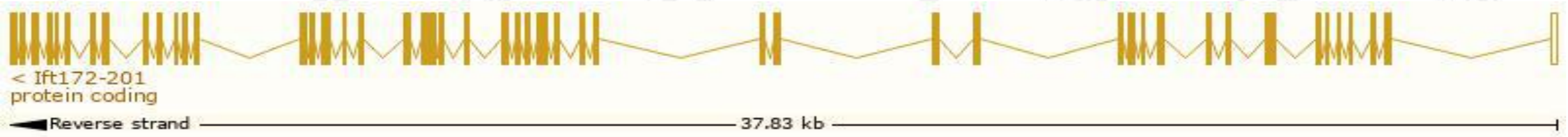
Official Symbol	Ift172 provided by MGI
Official Full Name	intraflagellar transport 172 provided by MGI
Primary source	MGI:MGI:2682064
See related	Ensembl:ENSMUSG00000038564
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4930553F24Rik, Slb, avc1, wim
Expression	Broad expression in testis adult (RPKM 50.1), cerebellum adult (RPKM 7.6) and 15 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

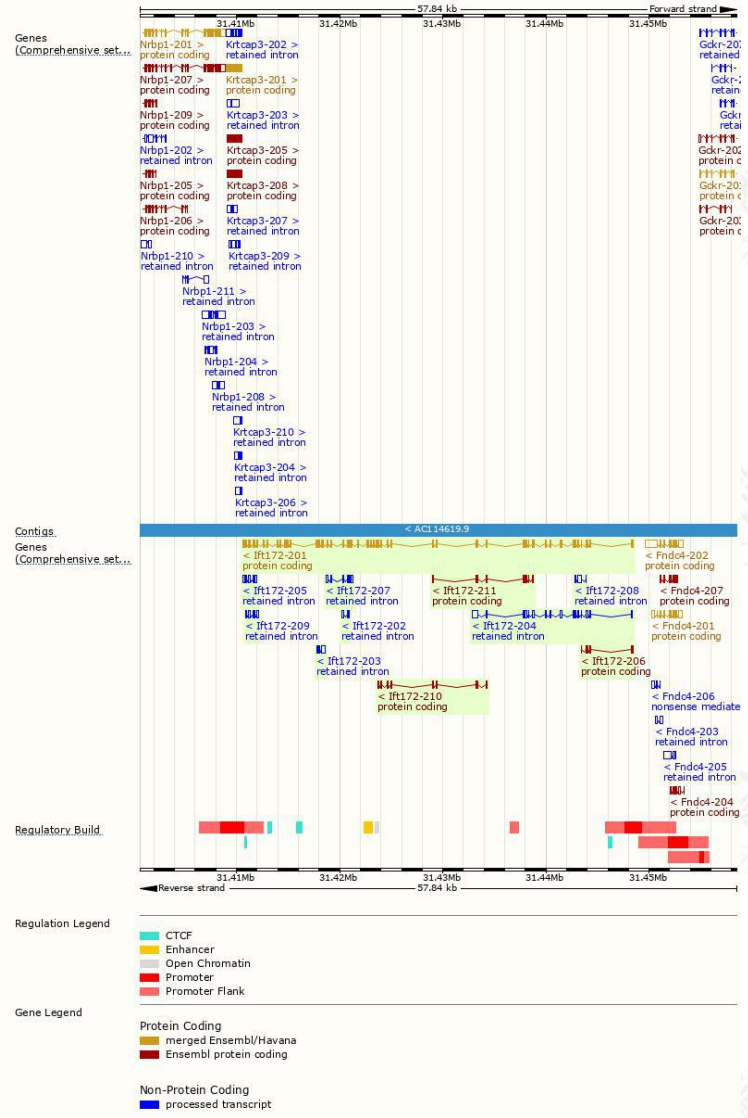
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ift172-201	ENSMUST00000041565.10	5403	1749aa	Protein coding	CCDS39054	Q6VH22	TSL:1 GENCODE basic APPRIS P1
Ift172-210	ENSMUST00000202585.4	774	258aa	Protein coding	-	A0A0J9YUJ7	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
Ift172-211	ENSMUST00000202589.1	727	242aa	Protein coding	-	A0A0J9YU41	CDS 5' incomplete TSL:3
Ift172-206	ENSMUST00000201809.1	405	83aa	Protein coding	-	A0A0J9YV04	TSL:5 GENCODE basic
Ift172-204	ENSMUST00000201274.1	2063	No protein	Retained intron	-	-	TSL:1
Ift172-207	ENSMUST00000201953.3	785	No protein	Retained intron	-	-	TSL:5
Ift172-205	ENSMUST00000201426.3	742	No protein	Retained intron	-	-	TSL:1
Ift172-209	ENSMUST00000202560.1	563	No protein	Retained intron	-	-	TSL:3
Ift172-203	ENSMUST00000201057.1	473	No protein	Retained intron	-	-	TSL:3
Ift172-208	ENSMUST00000202410.2	435	No protein	Retained intron	-	-	TSL:3
Ift172-202	ENSMUST00000200936.1	409	No protein	Retained intron	-	-	TSL:3

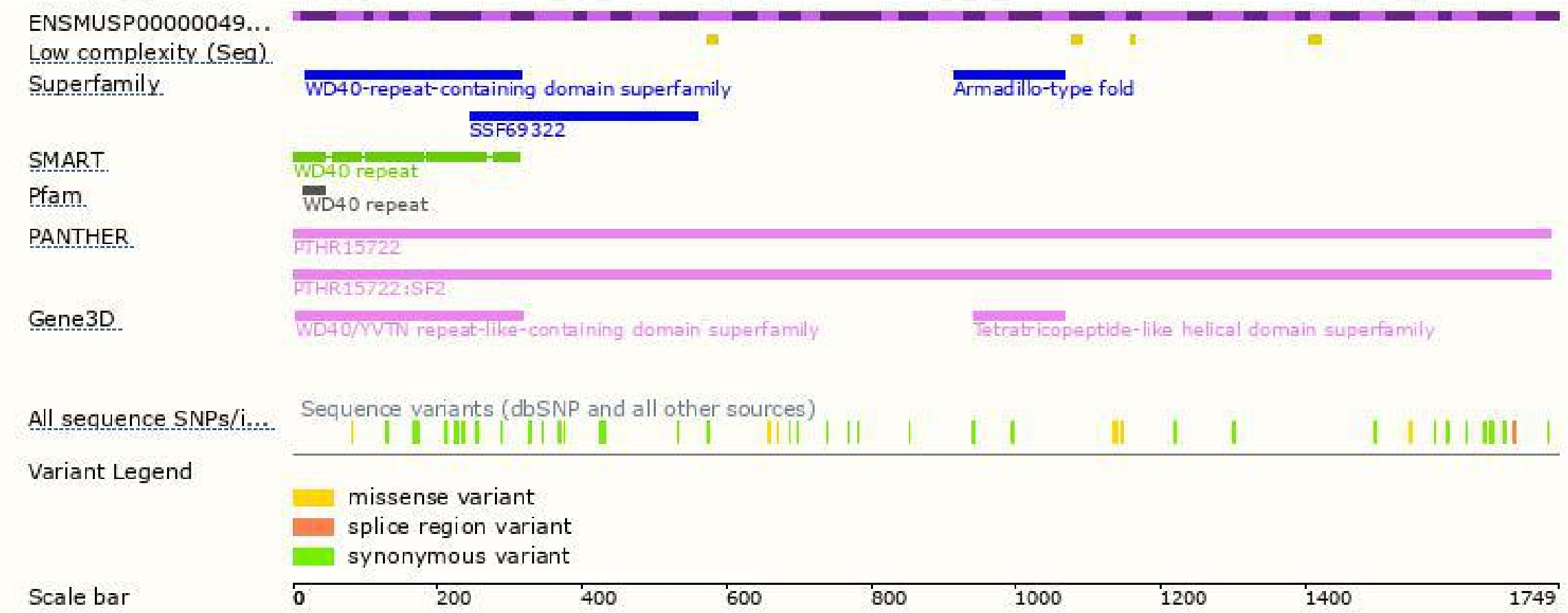
The strategy is based on the design of *Ift172-201* transcript,the transcription is shown below:



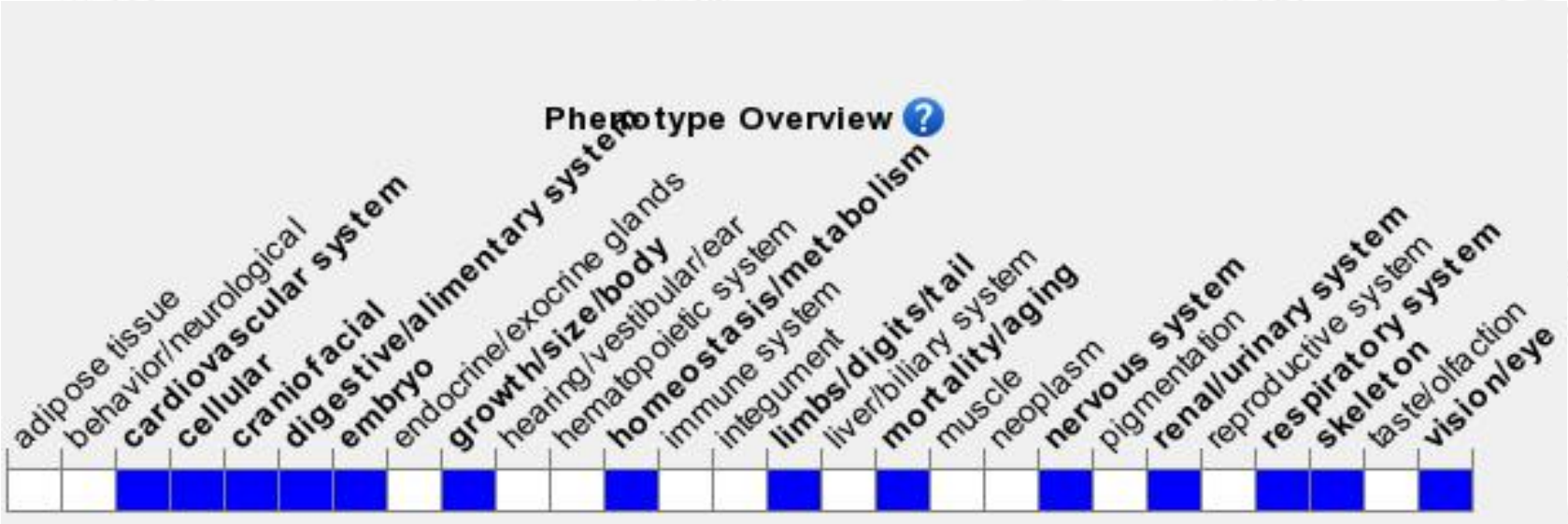
Genomic location distribution



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,mice homozygous for disruptions in this gene display embryonic lethality during organogenesis, neural tube defects, and developmental patterning abnormalities. Mice homozygous for a conditional allele activated in the early limb bud exhibit polydactyly and short limbs.

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