Limk2 Cas9-CKO Strategy

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Project Overview

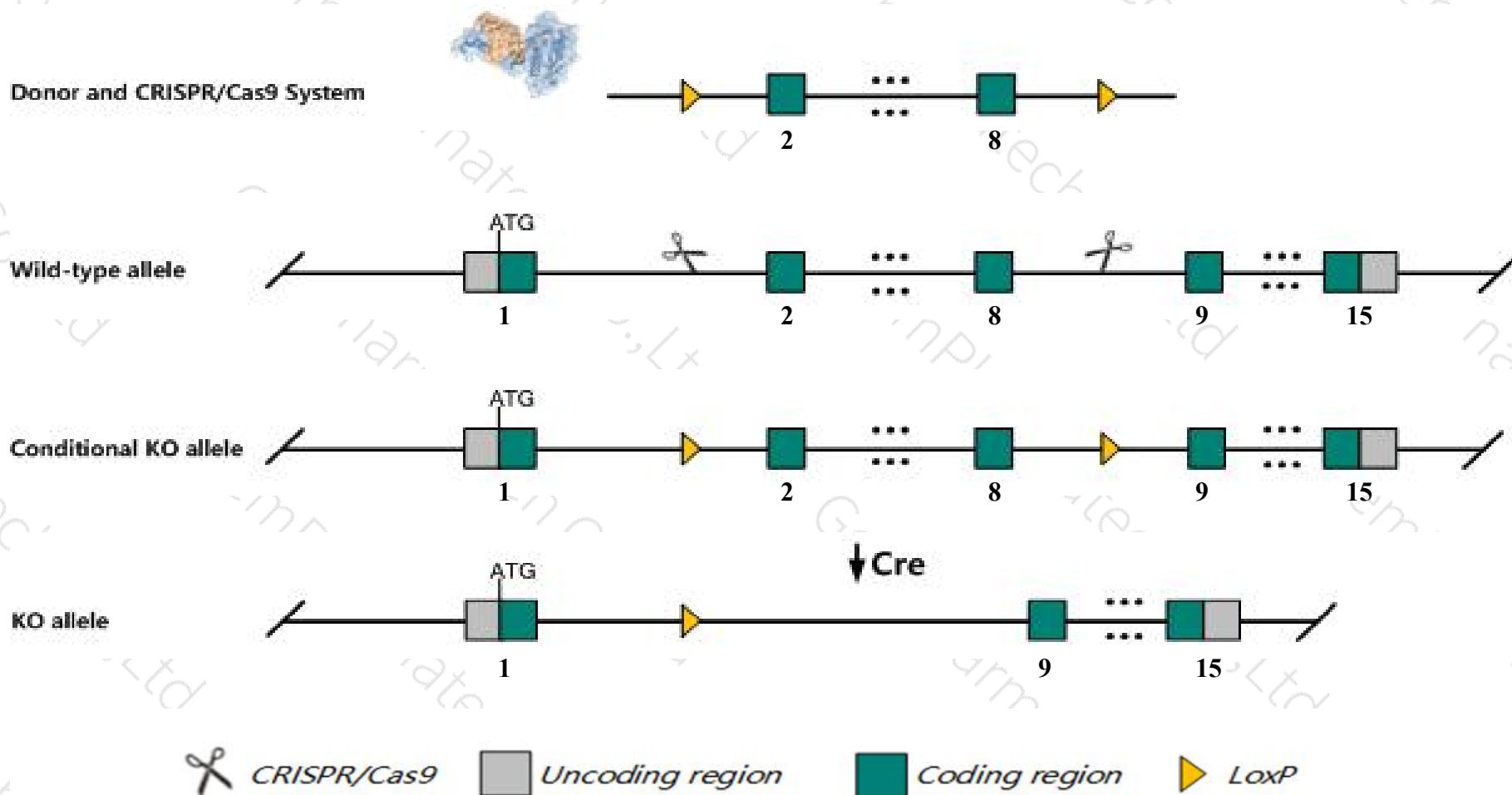|	
Project Name	*Limk2*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Limk2* gene. The schematic diagram is as follows:

Technical routes

➤ The *Limk2* gene has 13 transcripts. According to the structure of *Limk2* gene, exon2-exon8 of *Limk2-203* (ENSMUST00000101642.9) transcript is recommended as the knockout region.The region contains Ens 1012bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Limk2* 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,Male homozygotes for targeted null mutations exhibit small testes but are fertile. Mutant kidneys have fewer glomeruli and dilated renal tubules, but function normally. Mice homozygous for a gene trap allele or spontaneous mutation have open eyelids at birth, corneal abnormalities and inflammation.

➤ The *Limk2* gene is located on the Chr11. 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）

Limk2 LIM motif-containing protein kinase 2 [Mus musculus (house mouse)]

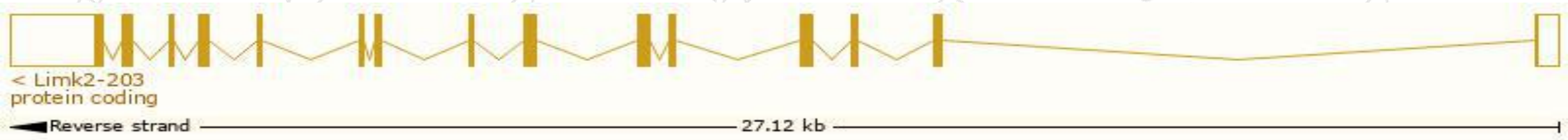Gene ID: 16886, updated on 31-Jan-2019

▲Summary

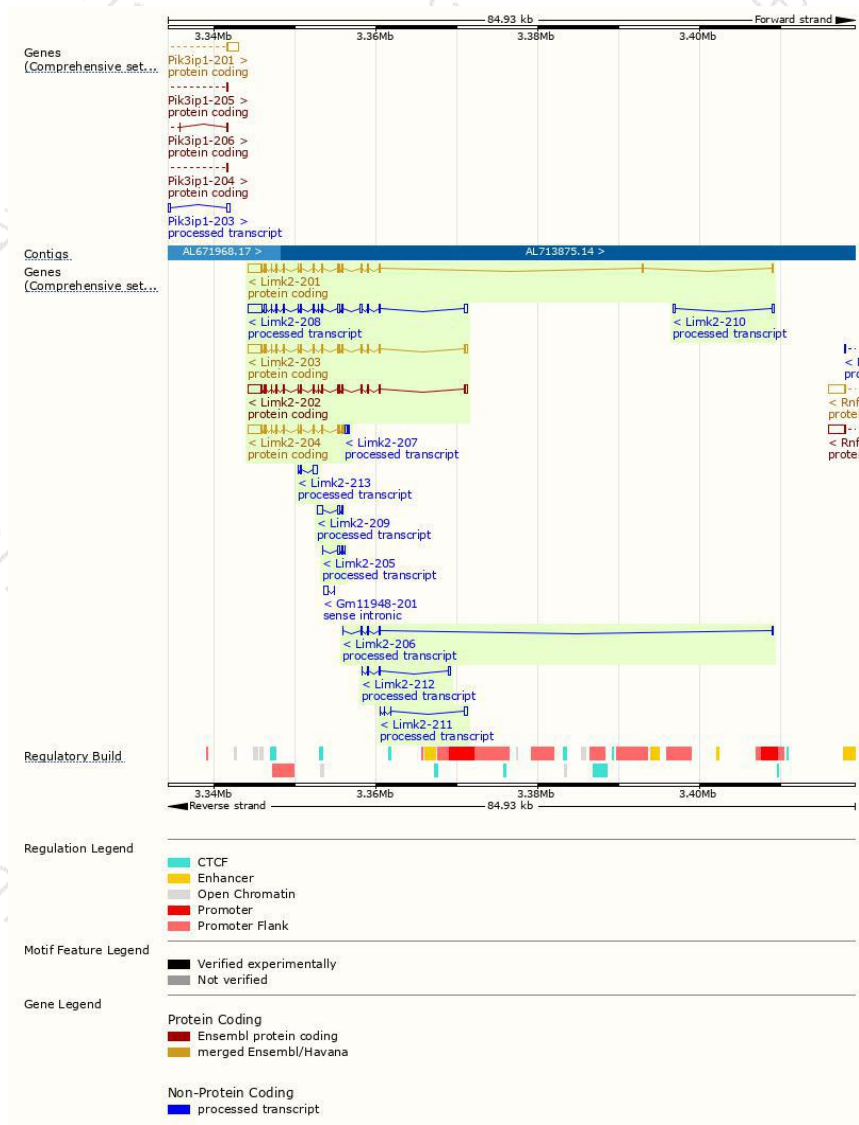|	
Official Symbol	Limk2 provided by MGI
Official Full Name	LIM motif-containing protein kinase 2 provided byMGI
Primary source	MGI:MGI:1197517
See related	Ensembl:ENSMUSG00000020451
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Limk-2, whe
Expression	Ubiquitous expression in placenta adult (RPKM 23.2), colon adult (RPKM 19.9) and 27 other tissuesSee 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
Limk2-203	ENSMUST00000101642.9	3693	617aa	Protein coding	CCDS24359	O54785	TSL:1 GENCODE basic APPRIS ALT1
Limk2-201	ENSMUST00000101638.3	3514	638aa	Protein coding	CCDS24358	O54785	TSL:1 GENCODE basic APPRIS P4
Limk2-204	ENSMUST00000110029.8	2939	451aa	Protein coding	CCDS24360	O54785	TSL:1 GENCODE basic
Limk2-202	ENSMUST00000101640.9	3711	623aa	Protein coding	-	Q5NC03	TSL:5 GENCODE basic
Limk2-208	ENSMUST00000134576.7	3849	No protein	Processed transcript	-	-	TSL:5
Limk2-209	ENSMUST00000142926.7	949	No protein	Processed transcript	-	-	TSL:3
Limk2-213	ENSMUST00000148771.1	716	No protein	Processed transcript	-	-	TSL:5
Limk2-206	ENSMUST00000125832.7	613	No protein	Processed transcript	-	-	TSL:3
Limk2-211	ENSMUST00000147344.1	550	No protein	Processed transcript	-	-	TSL:5
Limk2-212	ENSMUST00000148091.1	473	No protein	Processed transcript	-	-	TSL:5
Limk2-207	ENSMUST00000132479.1	465	No protein	Processed transcript	-	-	TSL:5
Limk2-210	ENSMUST00000145223.1	400	No protein	Processed transcript	-	-	TSL:2
Limk2-205	ENSMUST00000123689.1	382	No protein	Processed transcript	-	-	TSL:3

The strategy is based on the design of *Limk2-203* transcript,The transcription is shown below
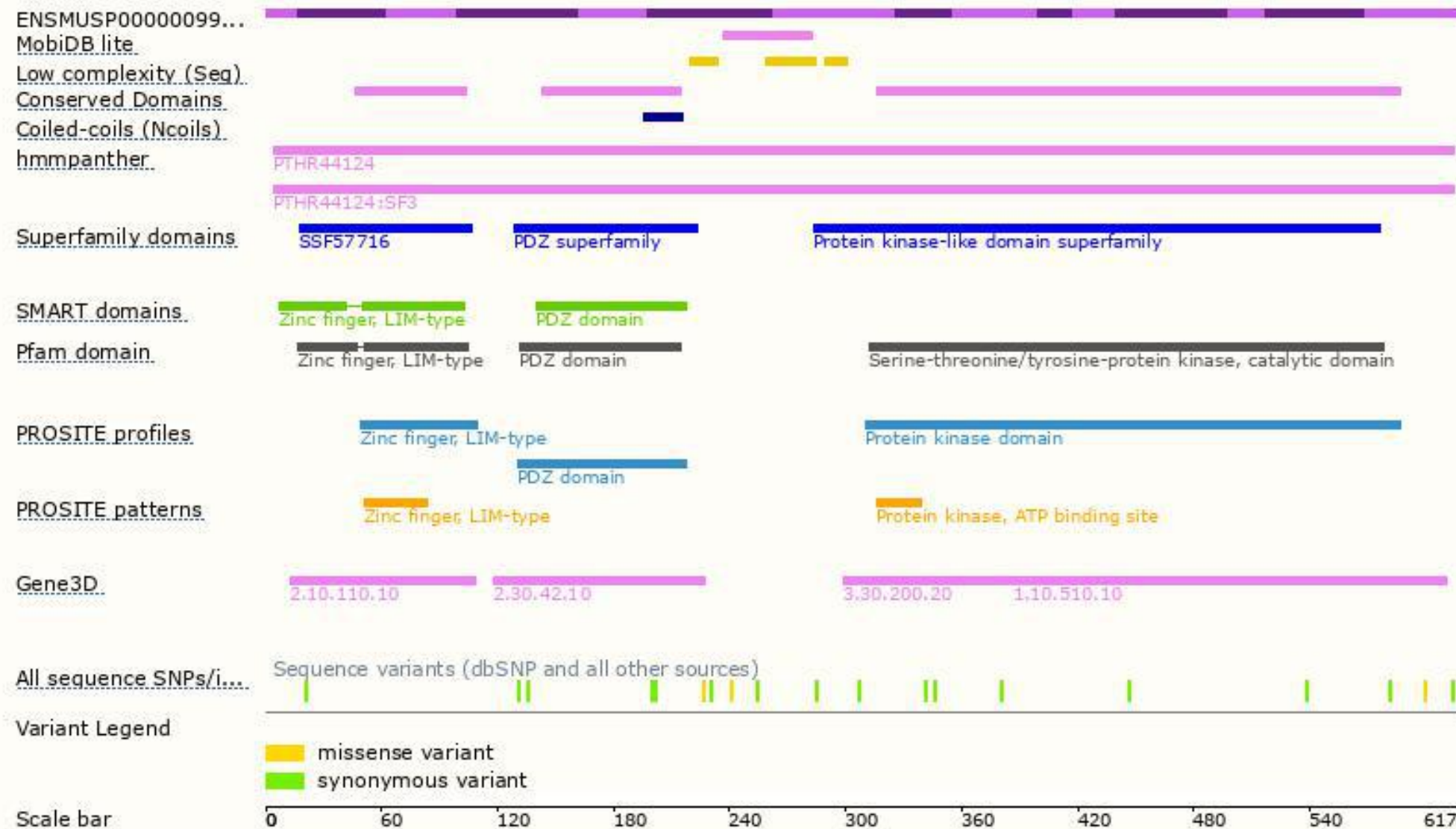
< Limk2-203
protein coding

◄ Reverse strand

27.12 kb

Genomic location distribution

Protein domain

ENSMUSP00000099...
MobiDB lite
Low complexity (Seg)
Conserved Domains
Coiled-coils (Ncoils)
hmmpanther
PTHR44124
PTHR44124:SF3
Superfamily domains
SSF57716   PDZ superfamily   Protein kinase-like domain superfamily
SMART domains
Zinc finger, LIM-type   PDZ domain
Pfam domain
Zinc finger, LIM-type   PDZ domain   Serine-threonine/tyrosine-protein kinase, catalytic domain
PROSITE profiles
Zinc finger, LIM-type   Protein kinase domain
PDZ domain
PROSITE patterns
Zinc finger, LIM-type   Protein kinase, ATP binding site
Gene3D
2.10.110.10   2.30.42.10   3.30.200.20   1.10.510.10
All sequence SNPs/i....
Sequence variants (dbSNP and all other sources)
Variant Legend
█ missense variant
█ synonymous variant
Scale bar
0   60   120   180   240   300   360   420   480   540   617

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,Male homozygotes for targeted null mutations exhibit small testes but are fertile. Mutant kidneys have fewer glomeruli and dilated renal tubules, but function normally. Mice homozygous for a gene trap allele or spontaneous mutation have open eyelids at birth, corneal abnormalities and inflammation.

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