Mcoln2 Cas9-CKO Strategy

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Project Overview

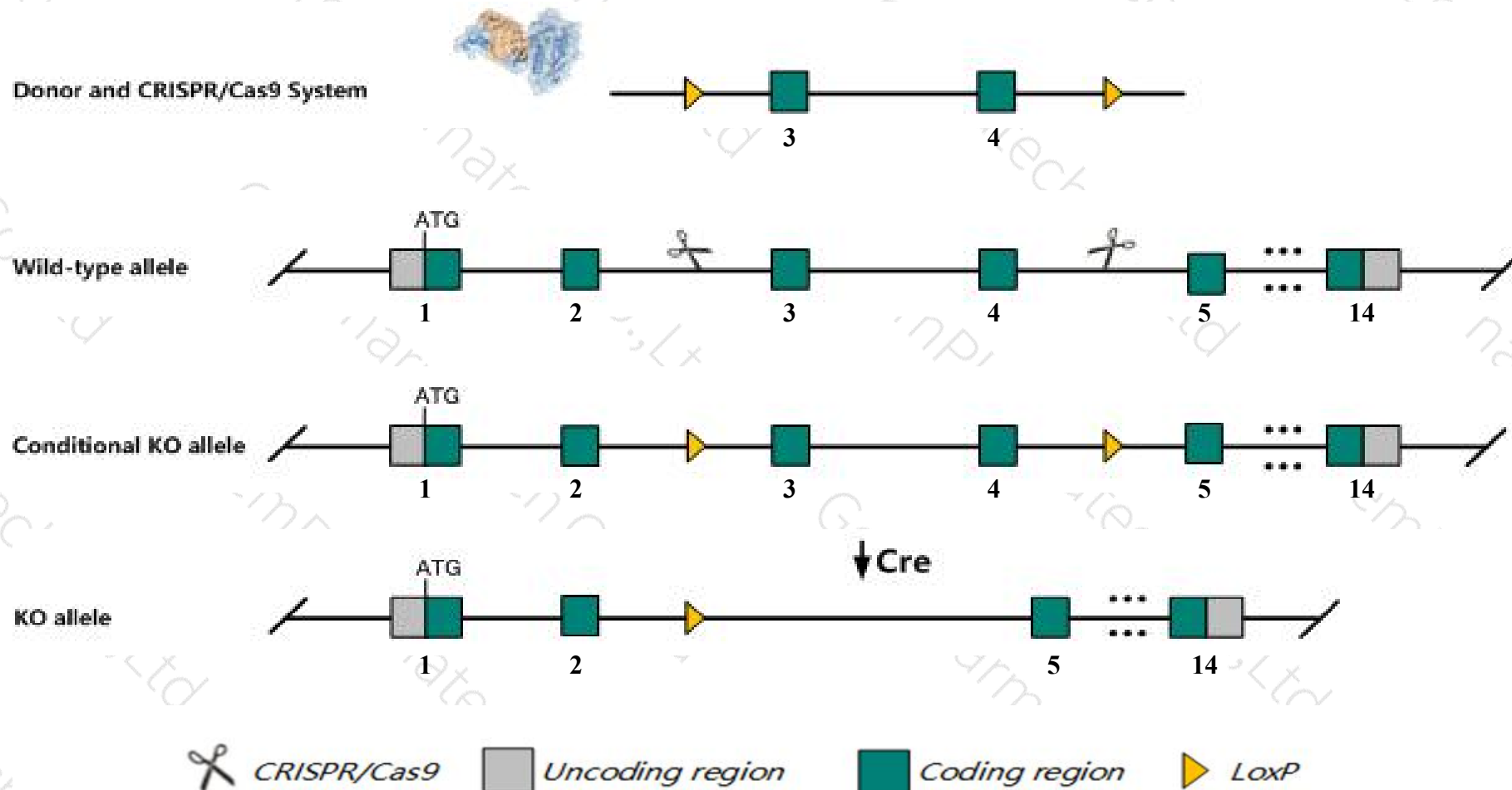|	
Project Name	*Mcoln2*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mcoln2* gene. The schematic diagram is as follows:

Technical routes

➤ The *Mcoln2* gene has 5 transcripts. According to the structure of *Mcoln2* gene, exon3-exon4 of *Mcoln2-201* (ENSMUST00000011152.13) transcript is recommended as the knockout region.The region contains 328bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Mcoln2* 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,Mice homozygous for a knock-out allele exhibit reduced chemokine production in bone marrow-derived macrophages and impaired recruitment of peripheral macrophages in response to i.p. injections of LPS or live bacteria.

➤ The 5'region of transcript Mcoln2-205 is incomplete, so the effect on it is unknown.

➤ The *Mcoln2* gene is located on the Chr3. 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）

Mcoln2 mucolipin 2 [Mus musculus (house mouse)]
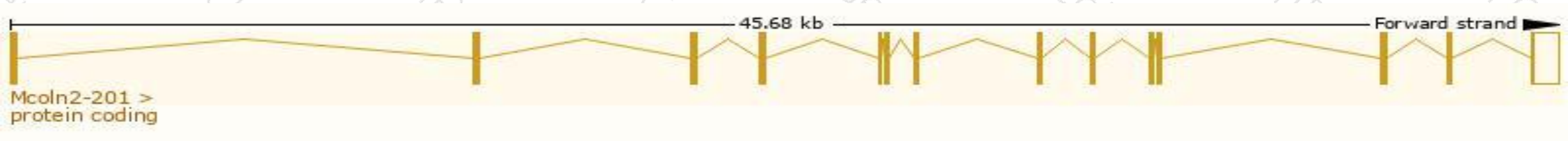
Gene ID: 68279, updated on 19-Mar-2019

▲ Summary

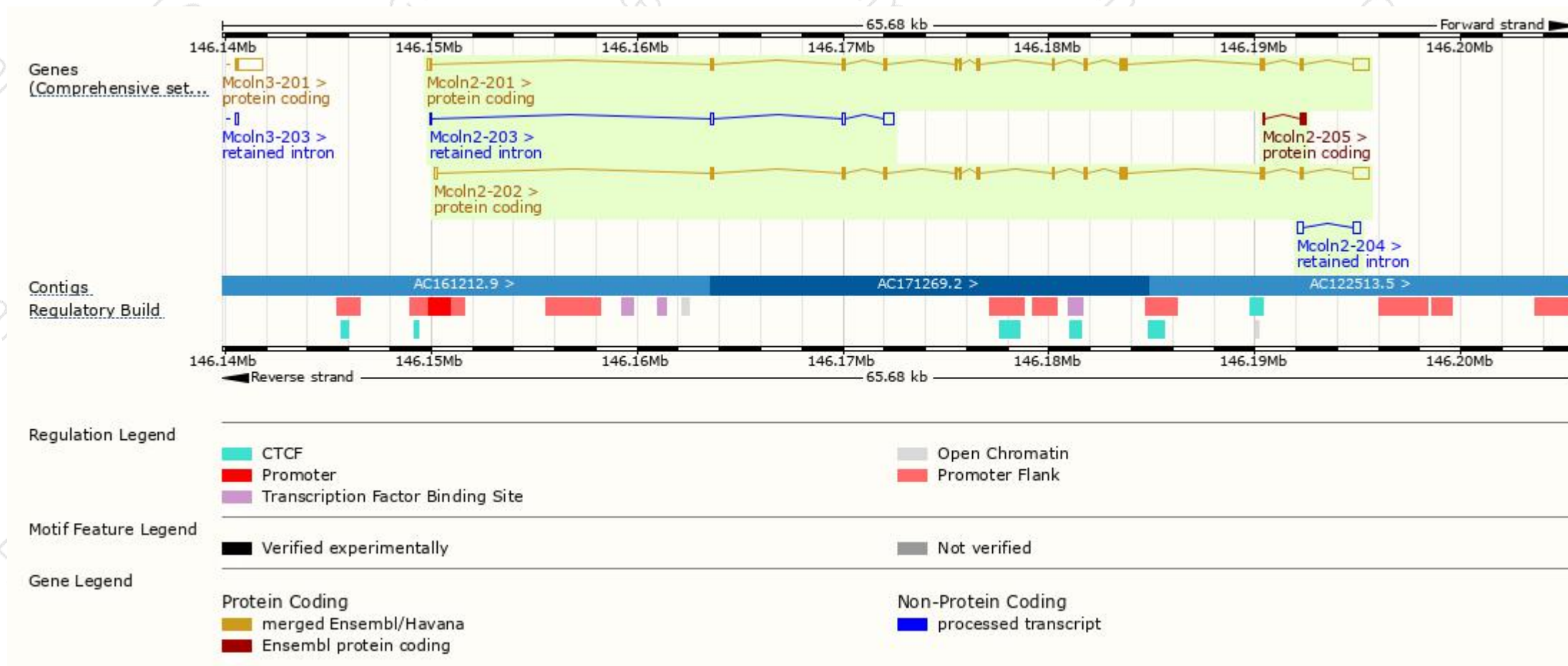|	
Official Symbol	Mcoln2 provided by MGI
Official Full Name	mucolipin 2 provided byMGI
Primary source	MGI:MGI:1915529
See related	Ensembl:ENSMUSG00000011008
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	3300002C04Rik, AI549968, C86638, TRPML2
Expression	Biased expression in placenta adult (RPKM 10.1), large intestine adult (RPKM 2.8) and 11 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 5 transcripts,all transcripts are shown below:

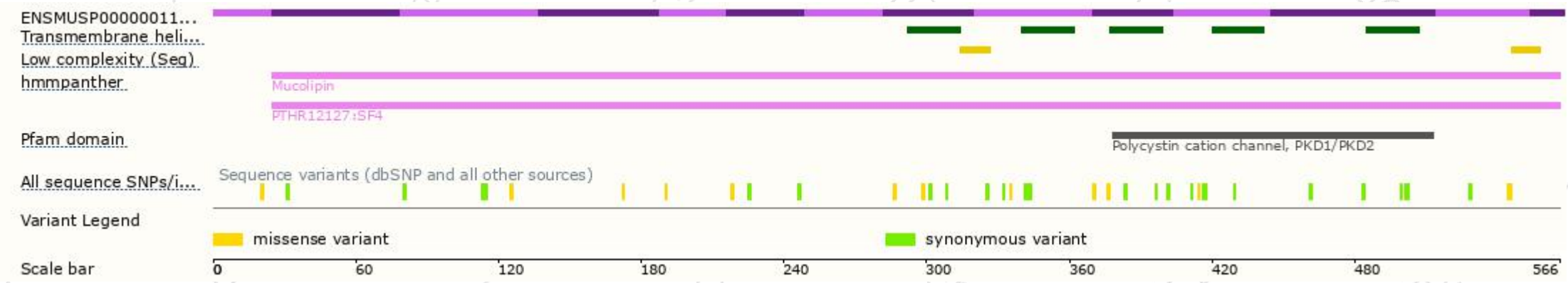| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mcoln2-201	ENSMUST00000011152.13	2520	566aa	Protein coding	CCDS38663	Q8K595	TSL:1 GENCODE basic
Mcoln2-202	ENSMUST00000098524.4	2492	538aa	Protein coding	CCDS38664	Q8K595	TSL:1 GENCODE basic APPRIS P1
Mcoln2-205	ENSMUST00000170972.1	361	113aa	Protein coding	-	F7B2J7	CDS 5' incomplete TSL:3
Mcoln2-203	ENSMUST00000169533.1	855	No protein	Retained intron	-	-	TSL:2
Mcoln2-204	ENSMUST00000169984.1	653	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Mcoln2-201* transcript,The transcription is shown below
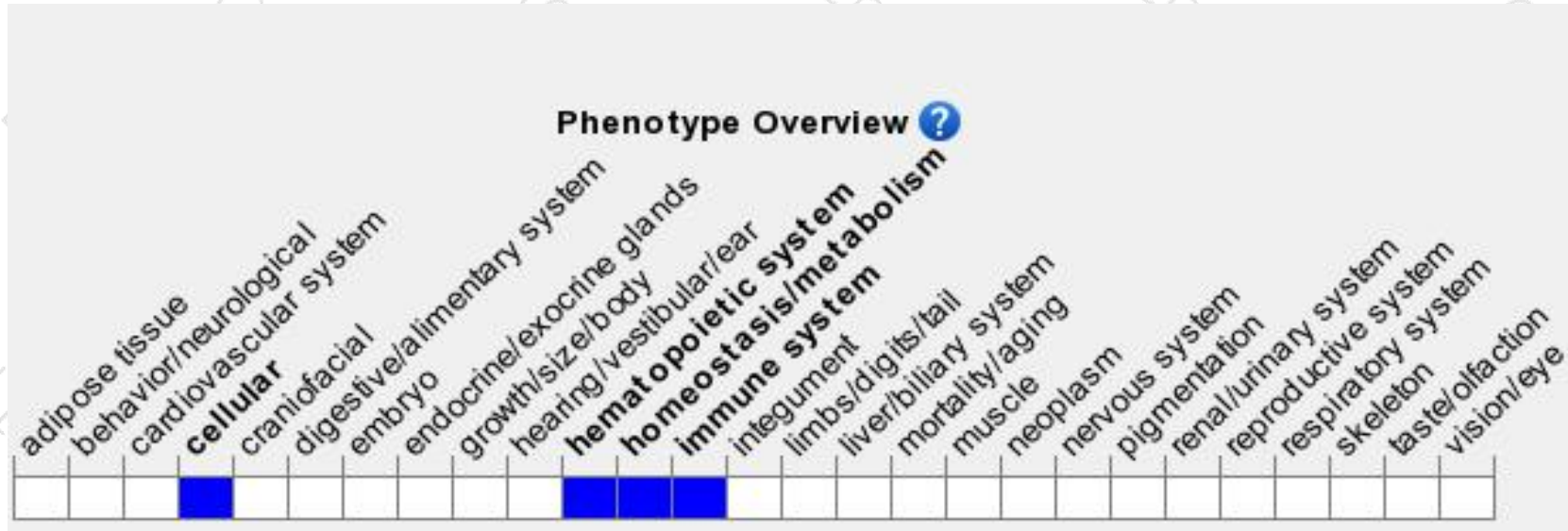

Mcoln2-201 >
protein coding

Genomic location distribution

Protein domain

ENSMUSP00000011...		
Transmembrane heli...		
Low complexity (Seg)		
hmmpanther	Mucolipin	
	PTHR12127:SF4	
Pfam domain		Polycystin cation channel, PKD1/PKD2
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   566

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 a knock-out allele exhibit reduced chemokine production in bone marrow-derived macrophages and impaired recruitment of peripheral macrophages in response to i.p. injections of LPS or live bacteria.

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