Mcoln1 Cas9-CKO Strategy

Designer: Daohua Xu
Reviewer: Huimin Su

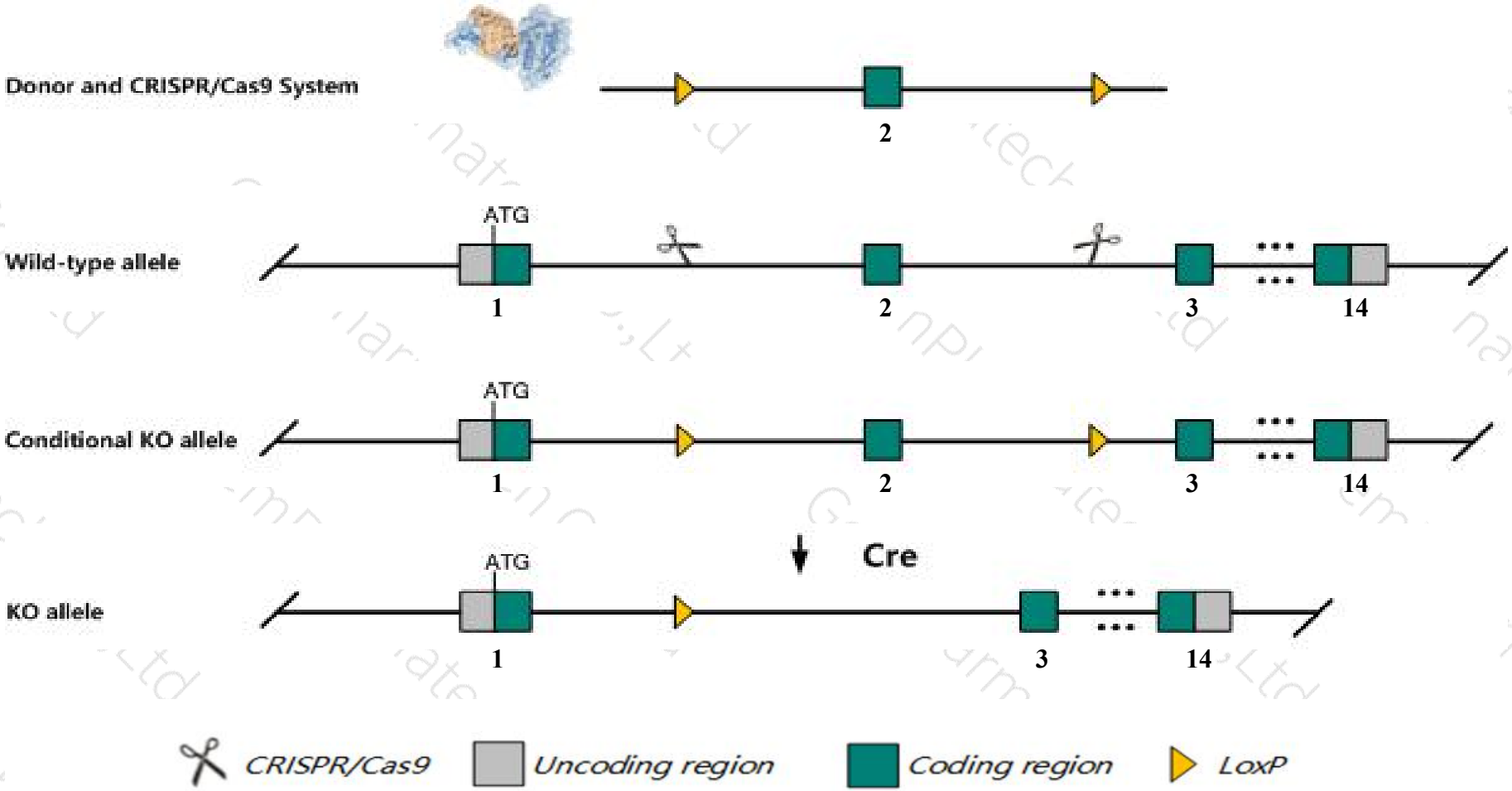
Design Date: 2019-11-22

Project Overview

Project Name	***Mcoln1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mcoln1* gene. The schematic diagram is as follows:



Technical routes

集萃药康 GemPharmatech

- The *Mcoln1* gene has 11 transcripts. According to the structure of *Mcoln1* gene, exon2 of *Mcoln1-201* (ENSMUST00000004683.12) transcript is recommended as the knockout region.The region contains 206bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Mcoln1* 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 null allele exhibit premature death around 8 months of age preceeded by weight loss, weakness, lethargy, bladder and stomach distension, and retinal degradation.

➢Transcript *Mcoln1-206, Mcoln1-209 and Mcoln1-211* may not be affected.

➢ The *Mcoln1* gene is located on the Chr8. 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）

集萃药康 GemPharmatech

Mcoln1 mucolipin 1 [Mus musculus (house mouse)]

Gene ID: 94178, updated on 19-Mar-2019

Summary

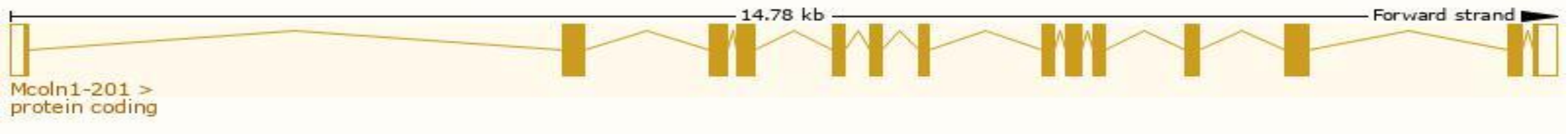
Official Symbol	Mcoln1 provided by MGI
Official Full Name	mucolipin 1 provided by MGI
Primary source	MGI:MGI:1890498
See related	Ensembl:ENSMUSG00000004567
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2210015I05Rik, TRPML1, mucolipidin
Expression	Ubiquitous expression in adrenal adult (RPKM 109.5), ovary adult (RPKM 65.5) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

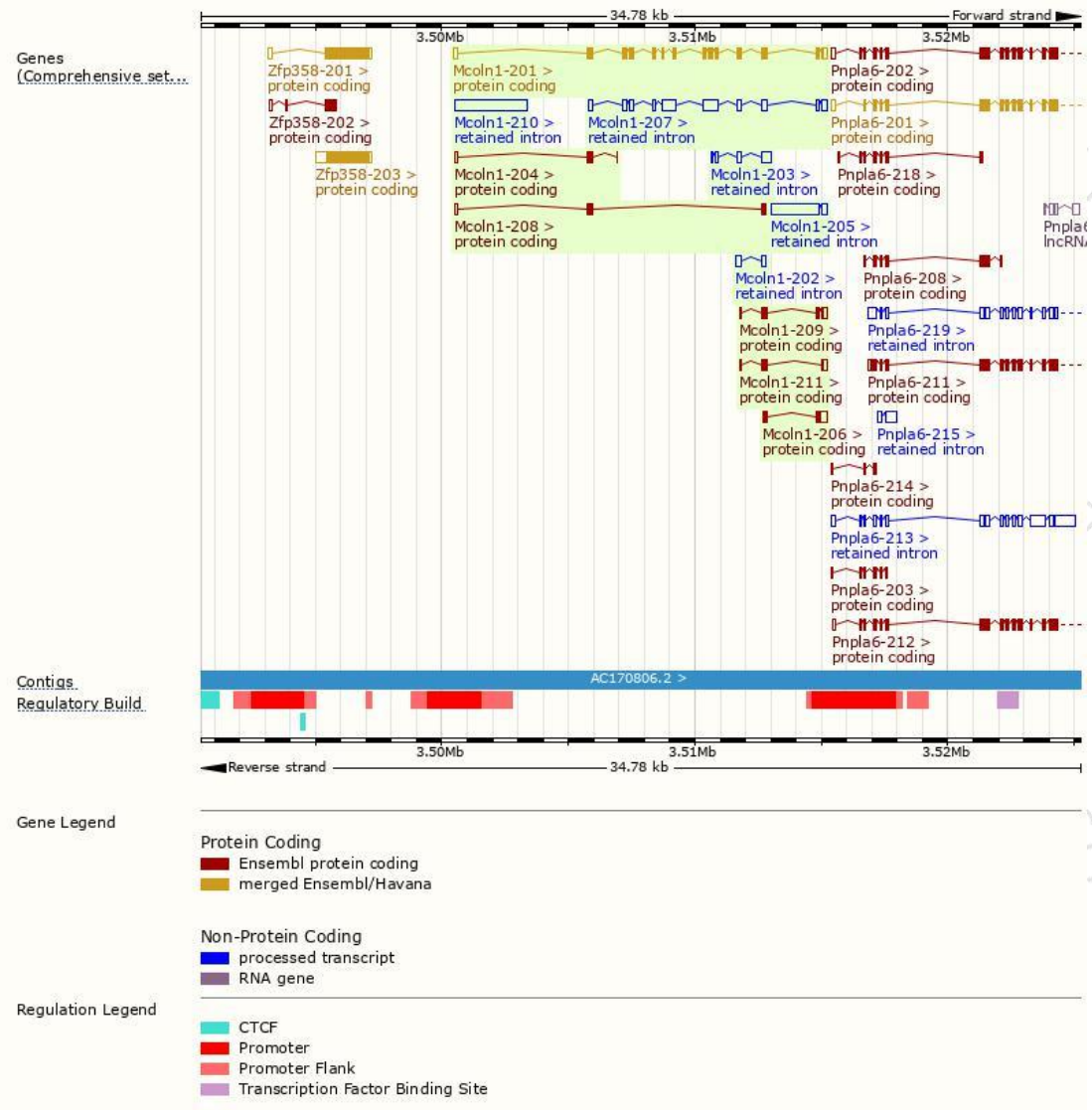
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mcoln1-201	ENSMUST00000004683.12	2065	580aa	Protein coding	CCDS22063	Q99J21	TSL:1 GENCODE basic APPRIS P1
Mcoln1-206	ENSMUST00000161842.1	626	115aa	Protein coding	-	A0A140LHY3	CDS 5' incomplete TSL:1
Mcoln1-209	ENSMUST00000208359.1	577	130aa	Protein coding	-	A0A140LI94	CDS 5' incomplete TSL:5
Mcoln1-211	ENSMUST00000208943.1	445	94aa	Protein coding	-	A0A140LHK5	CDS 5' incomplete TSL:5
Mcoln1-208	ENSMUST00000208306.1	435	124aa	Protein coding	-	A0A140LHW6	CDS 3' incomplete TSL:2
Mcoln1-204	ENSMUST00000160338.1	363	90aa	Protein coding	-	E0CZ07	CDS 3' incomplete TSL:5
Mcoln1-210	ENSMUST00000208739.1	2889	No protein	Retained intron	-	-	TSL:NA
Mcoln1-207	ENSMUST00000162797.7	2445	No protein	Retained intron	-	-	TSL:2
Mcoln1-205	ENSMUST00000161705.2	2110	No protein	Retained intron	-	-	TSL:2
Mcoln1-203	ENSMUST00000159808.1	621	No protein	Retained intron	-	-	TSL:2
Mcoln1-202	ENSMUST00000159538.1	373	No protein	Retained intron	-	-	TSL:1

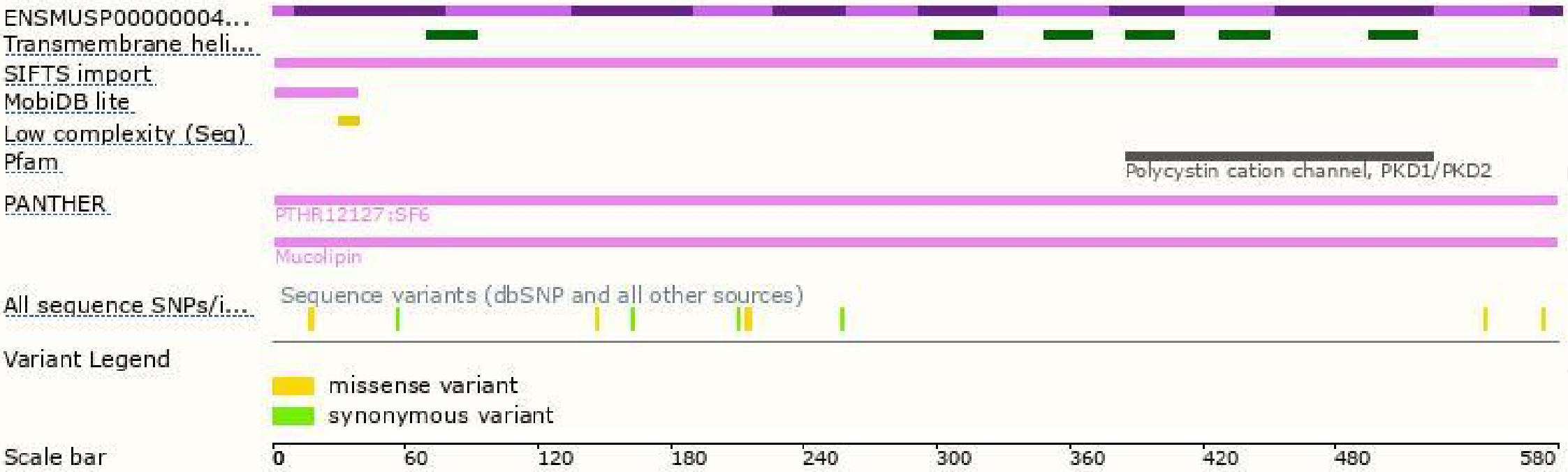
The strategy is based on the design of *Mcoln1-201* transcript,The transcription is shown below



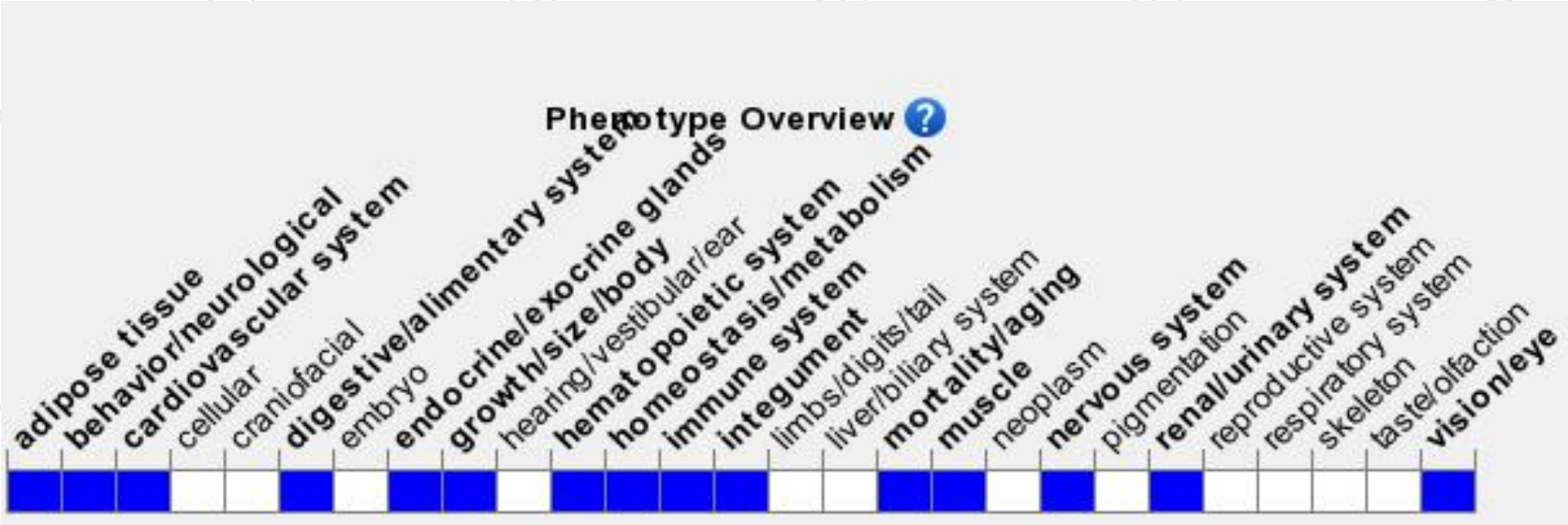
Genomic location distribution



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 a null allele exhibit premature death around 8 months of age preceeded by weight loss, weakness, lethargy, bladder and stomach distension, and retinal degradation.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd