Mlh1 Cas9-CKO Strategy

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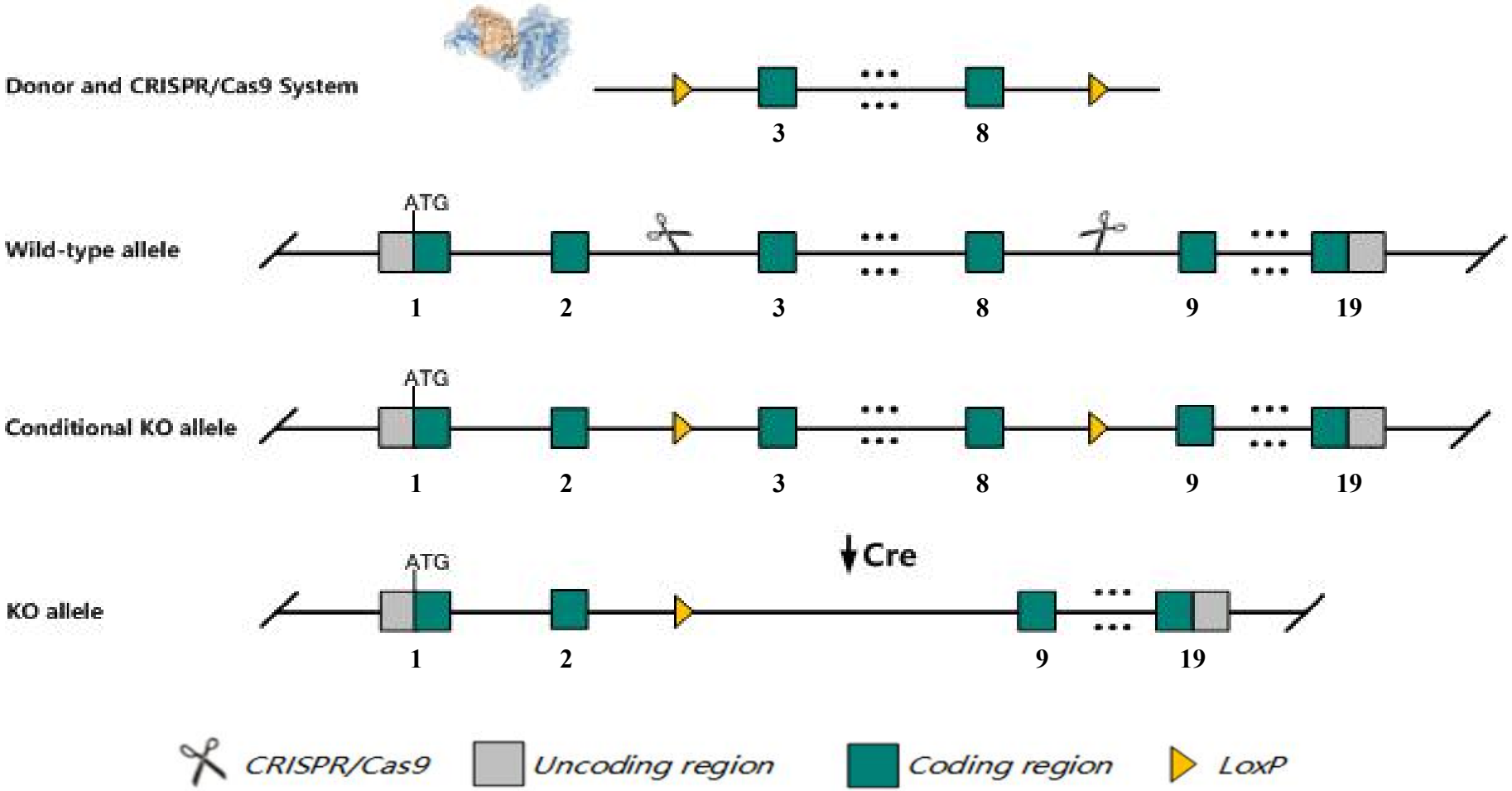
Project Overview

Project Name	***Mlh1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mlh1* gene. The schematic diagram is as follows:



Technical routes

The *Mlh1* gene has 9 transcripts. According to the structure of *Mlh1* gene, exon3-exon8 of *Mlh1-201*(ENSMUST00000035079.9) transcript is recommended as the knockout region.The region contains 470bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Mlh1* 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,homozygotes for targeted null mutations exhibit reduced pairing in meiotic prophase I and produce no mature germ cells. Mutants also display increased microsatellite instability and a predisposition for developing intestinal and other tumors.

The *Mlh1* gene is located on the Chr9. 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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Mlh1 mutL homolog 1 [Mus musculus (house mouse)]

Gene ID: 17350, updated on 13-Mar-2020

Summary

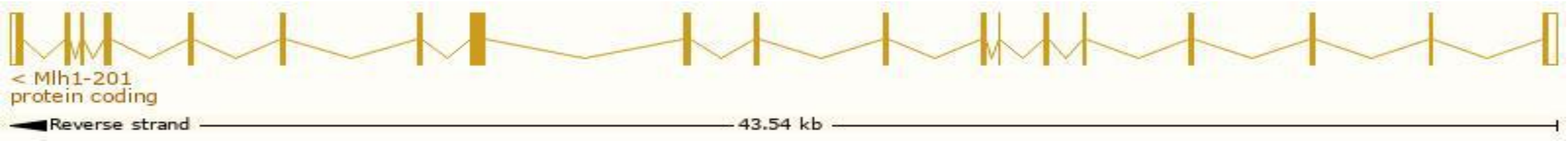
Official Symbol	Mlh1 provided by MGI
Official Full Name	mutL homolog 1 provided by MGI
Primary source	MGI:MGI:101938
See related	Ensembl:ENSMUSG00000032498
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110035C23Rik, AI317206, AI325952, AI561766
Expression	Ubiquitous expression in CNS E11.5 (RPKM 5.9), limb E14.5 (RPKM 4.8) and 28 other tissues See more
Orthologs	human all

Transcript information Ensembl

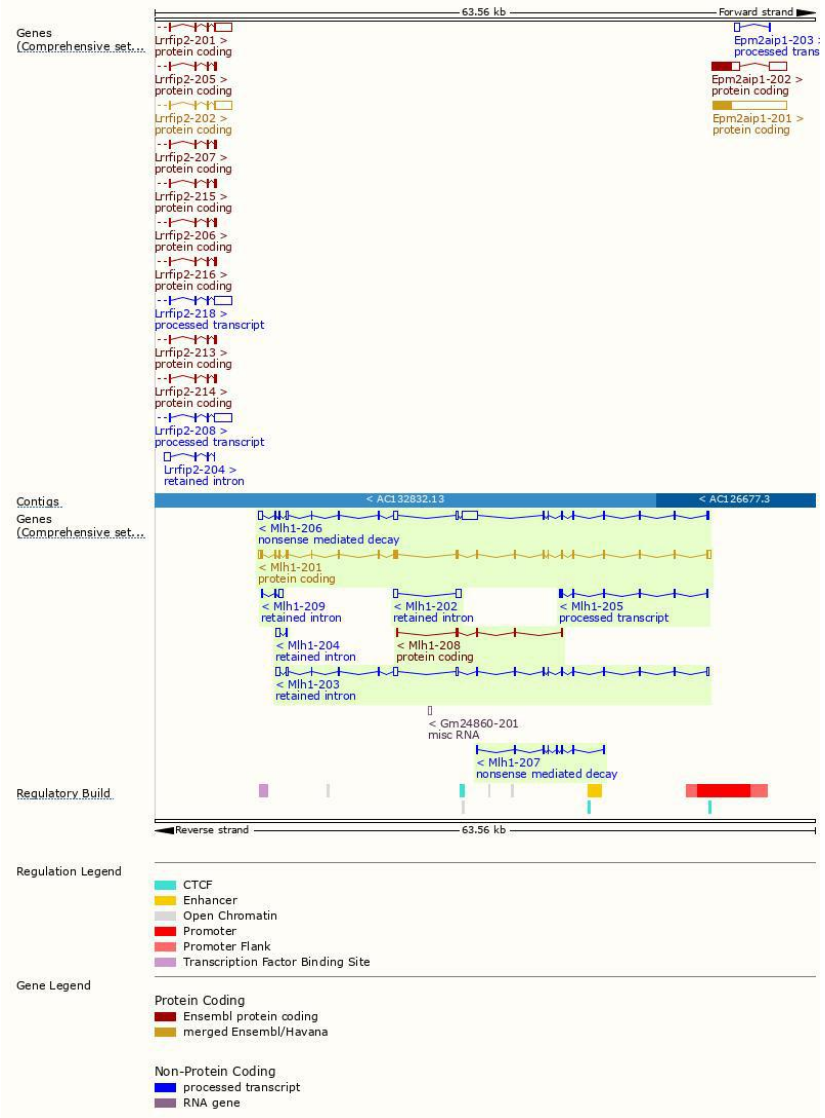
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mlh1-201	ENSMUST00000035079.9	2754	760aa	Protein coding	CCDS40784	Q9JK91	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Mlh1-208	ENSMUST00000199404.1	465	155aa	Protein coding	-	A0A0G2JDY9	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Mlh1-206	ENSMUST00000135218.7	3762	38aa	Nonsense mediated decay	-	A0A0G2JH14	TSL:1
Mlh1-207	ENSMUST00000135695.1	697	94aa	Nonsense mediated decay	-	F6R1G0	CDS 5' incomplete TSL:3
Mlh1-205	ENSMUST00000134316.1	667	No protein	Processed transcript	-	-	TSL:3
Mlh1-203	ENSMUST00000123869.7	2394	No protein	Retained intron	-	-	TSL:1
Mlh1-202	ENSMUST00000123713.1	825	No protein	Retained intron	-	-	TSL:2
Mlh1-209	ENSMUST00000200145.1	603	No protein	Retained intron	-	-	TSL:2
Mlh1-204	ENSMUST00000128795.1	514	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Mlh1-201* transcript,the transcription is shown below:

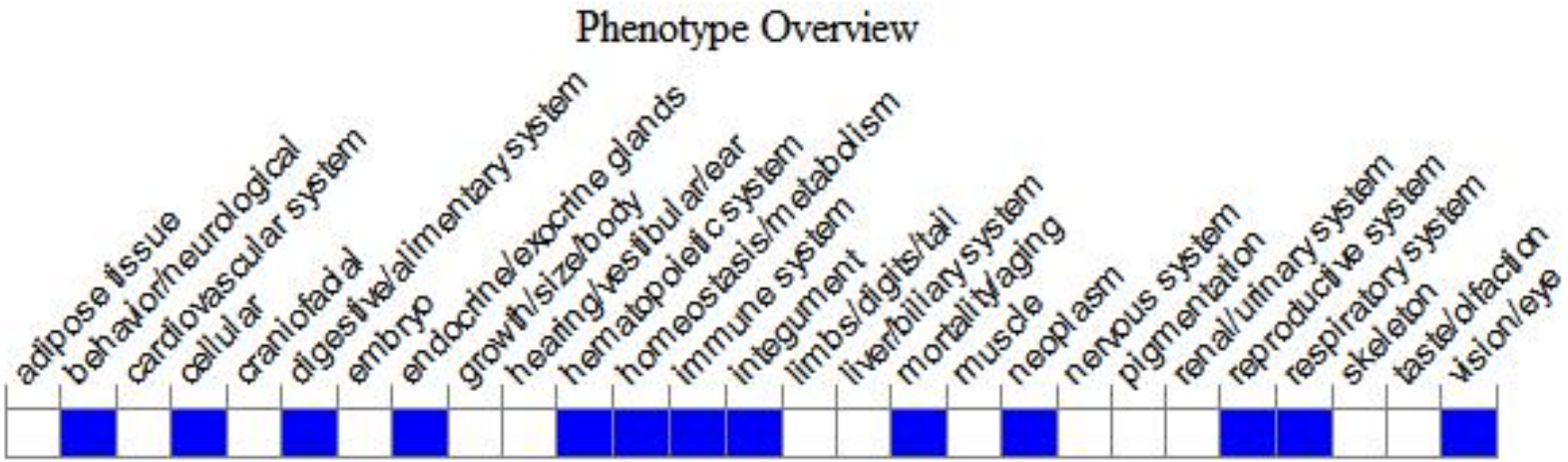


Genomic location distribution



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/).

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