

Lhfpl2 Cas9-CKO Strategy

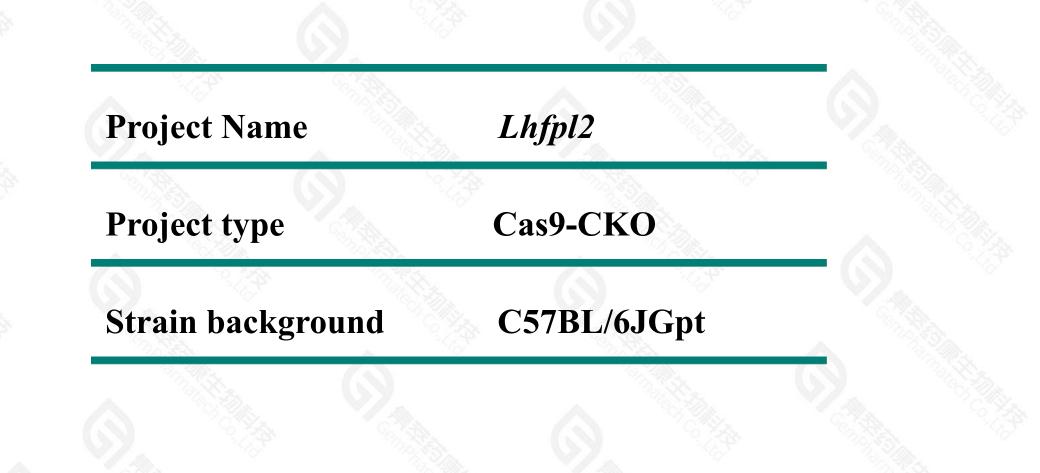
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Reviewer: Miaomiao Cui

Design Date: 2021-3-1

Project Overview



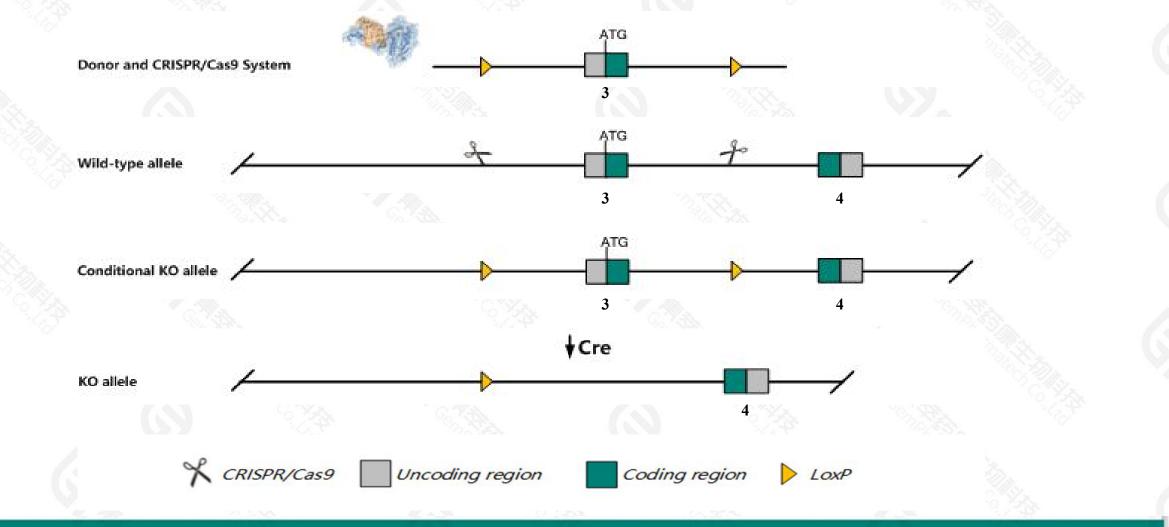


Conditional Knockout strategy

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This model will use CRISPR/Cas9 technology to edit the *Lhfpl2* gene. The schematic diagram is as follows:



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Technical routes



The Lhfpl2 gene has 9 transcripts. According to the structure of Lhfpl2 gene, exon3 of Lhfpl2-201(ENSMUST00000054274.7) transcript is recommended as the knockout region. The region contains start codon ATG.Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Lhfpl2* 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.



- > According to the existing MGI data, females homozygous for a spontaneous point mutation have a completely closed vagina, soft swelling of the perineum and buildup of viscous fluid in the uteri.
- The *Lhfpl2* gene is located on the Chr13. 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.

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Gene information (NCBI)

Lhfpl2 lipoma HMGIC fusion partner-like 2 [Mus musculus (house mouse)]

Gene ID: 218454, updated on 13-Mar-2020

Summary

Official Symbol	Lhfpl2 provided by MGI
Official Full Name	lipoma HMGIC fusion partner-like 2 provided by MGI
Primary source	MGI:MGI:2145236
See related	Ensembl:ENSMUSG0000045312
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6030465B15, Al447312, AW050335, mKIAA0206, vgim
Expression	Broad expression in subcutaneous fat pad adult (RPKM 19.7), limb E14.5 (RPKM 15.8) and 23 other tissues See more
Orthologs	human all



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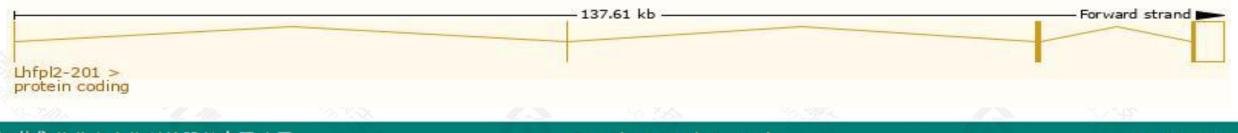
Transcript information (Ensembl)

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

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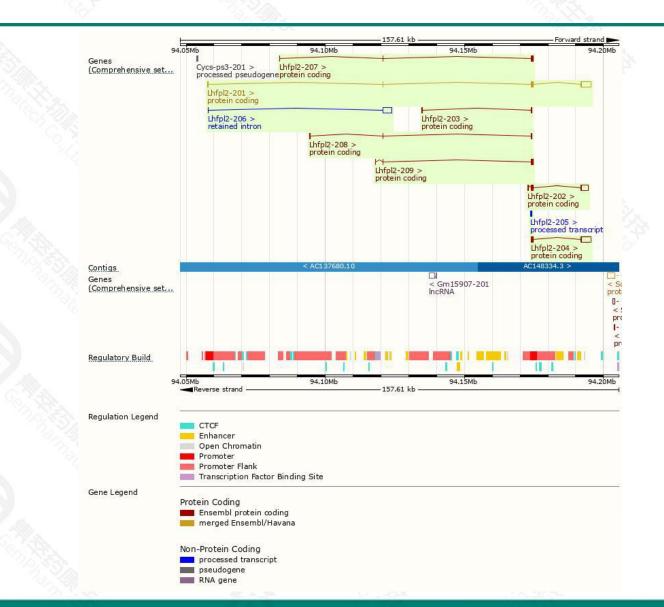
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lhfpl2-201	ENSMUST0000054274.7	4348	<u>222aa</u>	Protein coding	CCD526692	Q8BGA2	TSL:1 GENCODE basic APPRIS P1
Lhfpl2-204	ENSMUST00000121618.1	4209	<u>222aa</u>	Protein coding	CCDS26692	Q8BGA2	TSL:1 GENCODE basic APPRIS P1
Lhfpl2-202	ENSMUST00000118195.1	3611	<u>222aa</u>	Protein coding	CCDS26692	Q8BGA2	TSL:1 GENCODE basic APPRIS P1
Lhfpl2-209	ENSMUST00000223423.1	771	<u>141aa</u>	Protein coding		A0A1Y7VJ20	CDS 3' incomplete TSL:3
Lhfpl2-207	ENSMUST00000156071.1	768	<u>139aa</u>	Protein coding	-	D3Z698	CDS 3' incomplete TSL:3
.hfpl2-203	ENSMUST00000120051.1	488	<u>34aa</u>	Protein coding	2	<u>D3Z4C4</u>	CDS 3' incomplete TSL:5
hfpl2-208	ENSMUST00000221096.1	362	<u>19aa</u>	Protein coding	-	A0A1Y7VNJ3	CDS 3' incomplete TSL:3
hfpl2-205	ENSMUST00000131595.1	347	No protein	Processed transcript	2	-	TSL:2
Lhfpl2-206	ENSMUST00000144838.1	3273	No protein	Retained intron		-	TSL:1

The strategy is based on the design of *Lhfpl2-201* transcript, the transcription is shown below:



Genomic location distribution





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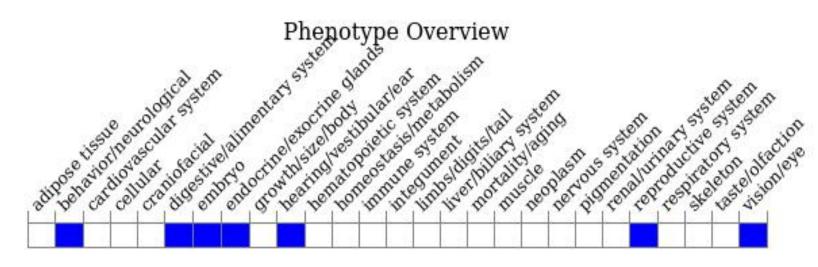
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Protein domain



NSMUSP0000062												
ransmembrane heli .ow.complexity (Seg)												
<u>fam</u>	Lipo	ma HMGIO	fusion par	tner-like pro	otein						1	
ANTHER	Lipoma H	MGIC fusi	on partner-	like protein								
	PTHR124	89:SF19										
Gene3D	1.20.	140,150										- 1
All sequence SNPs/i	Sequen	ce variant	s (dbSNP	and all oth	er sources)				10	D	E.	
/ariant Legend		ssense va nonymous										
scale bar	0	20	40	60	80	100	120	140	160	180	200	222

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, females homozygous for a spontaneous point mutation have a completely closed vagina, soft swelling of the perineum and buildup of viscous fluid in the uteri.



If you have any questions, you are welcome to inquire. Tel: 400-9660890



