Plagl1 Cas9-KO Strategy

Designer: Xueting Zhang

Reviewer: Daohua Xu

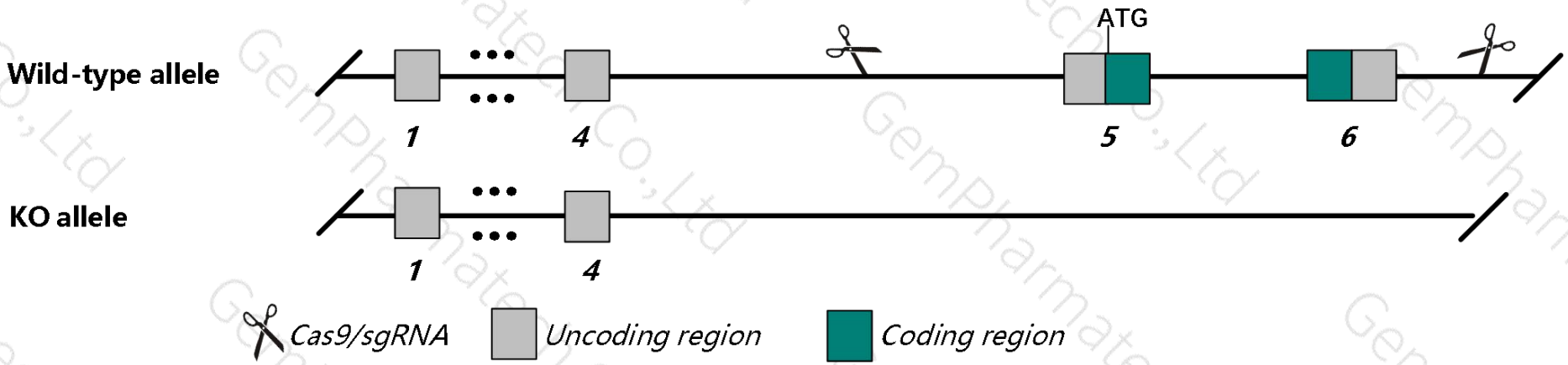
Design Date: 2020-7-20

Project Overview

Project Name	***Plagl1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Plagl1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Plagl1* gene has 25 transcripts. According to the structure of *Plagl1* gene, exon5-exon6 of *Plagl1-202*(ENSMUST00000121646.7) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Plagl1* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,homozygous null mice exhibit significantly reduced birth weights. Heterozygous mice with a paternal copy of the null allele show reduced fetal and birth weights, altered ossification, dyspnea and background-dependent neonatal lethality, as well as wrinkled skin and curly tails with 30% penetrance.

➢ The effect on transcript *Plagl1*-208&220&&222&225 is unknown.

➢ The *Plagl1* gene is located on the Chr10. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Plagl1 pleiomorphic adenoma gene-like 1 [Mus musculus (house mouse)]

Gene ID: 22634, updated on 13-Mar-2020

Summary

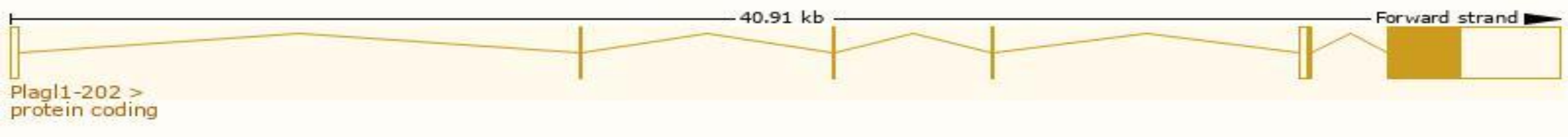
Official Symbol	Plagl1 provided by MGI
Official Full Name	pleiomorphic adenoma gene-like 1 provided by MGI
Primary source	MGI:MGI:1100874
See related	Ensembl:ENSMUSG00000019817
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2610311E24Rik, Lot1, Zac1
Expression	Biased expression in limb E14.5 (RPKM 270.7), CNS E11.5 (RPKM 64.6) and 8 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

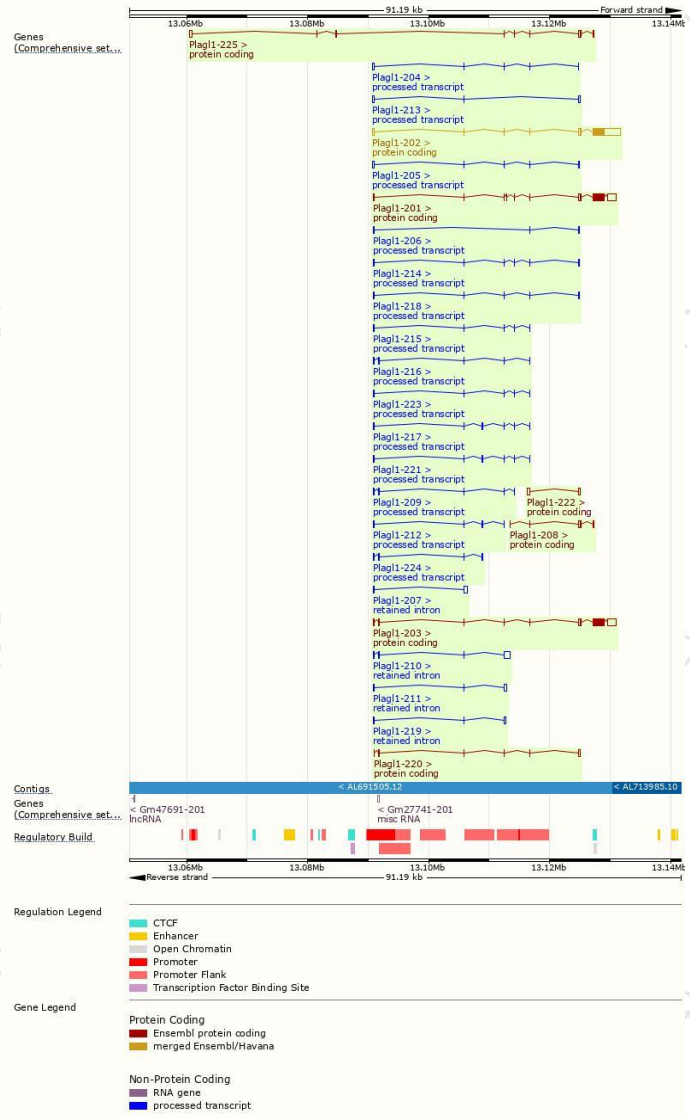
The gene has 25 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Plagl1-202	ENSMUST00000121646.7	5260	704aa	Protein coding	CCDS48501	Q9JLQ4	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 P2
Plagl1-201	ENSMUST00000121325.7	3946	678aa	Protein coding	-	Q3UQW2	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 ALT2
Plagl1-203	ENSMUST00000121766.7	3897	678aa	Protein coding	-	Q3UQW2	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 ALT2
Plagl1-225	ENSMUST00000193426.5	1237	121aa	Protein coding	-	A0A0A6YWE3	CDS 3' incomplete TSL:1
Plagl1-222	ENSMUST00000145103.1	630	17aa	Protein coding	-	B0QZX5	CDS 3' incomplete TSL:2
Plagl1-208	ENSMUST00000130313.1	624	117aa	Protein coding	-	B0QZX4	CDS 3' incomplete TSL:3
Plagl1-220	ENSMUST00000143582.7	364	4aa	Protein coding	-	-	CDS 3' incomplete TSL:5
Plagl1-213	ENSMUST00000135095.7	512	No protein	Processed transcript	-	-	TSL:2
Plagl1-204	ENSMUST00000123135.7	478	No protein	Processed transcript	-	-	TSL:3
Plagl1-214	ENSMUST00000135261.7	475	No protein	Processed transcript	-	-	TSL:3
Plagl1-205	ENSMUST00000124252.7	424	No protein	Processed transcript	-	-	TSL:2
Plagl1-217	ENSMUST00000139262.7	424	No protein	Processed transcript	-	-	TSL:5
Plagl1-221	ENSMUST00000144437.7	422	No protein	Processed transcript	-	-	TSL:5
Plagl1-215	ENSMUST00000135826.7	399	No protein	Processed transcript	-	-	TSL:3
Plagl1-218	ENSMUST00000141068.7	390	No protein	Processed transcript	-	-	TSL:2
Plagl1-206	ENSMUST00000126880.7	386	No protein	Processed transcript	-	-	TSL:2
Plagl1-216	ENSMUST00000138433.7	357	No protein	Processed transcript	-	-	TSL:3
Plagl1-209	ENSMUST00000130449.7	352	No protein	Processed transcript	-	-	TSL:5
Plagl1-224	ENSMUST00000150773.7	345	No protein	Processed transcript	-	-	TSL:3
Plagl1-212	ENSMUST00000130894.7	344	No protein	Processed transcript	-	-	TSL:5
Plagl1-223	ENSMUST00000149881.7	336	No protein	Processed transcript	-	-	TSL:5
Plagl1-210	ENSMUST00000130638.7	1187	No protein	Retained intron	-	-	TSL:3
Plagl1-207	ENSMUST00000129989.7	712	No protein	Retained intron	-	-	TSL:2
Plagl1-211	ENSMUST00000130800.7	552	No protein	Retained intron	-	-	TSL:2
Plagl1-219	ENSMUST00000141944.7	452	No protein	Retained intron	-	-	TSL:2

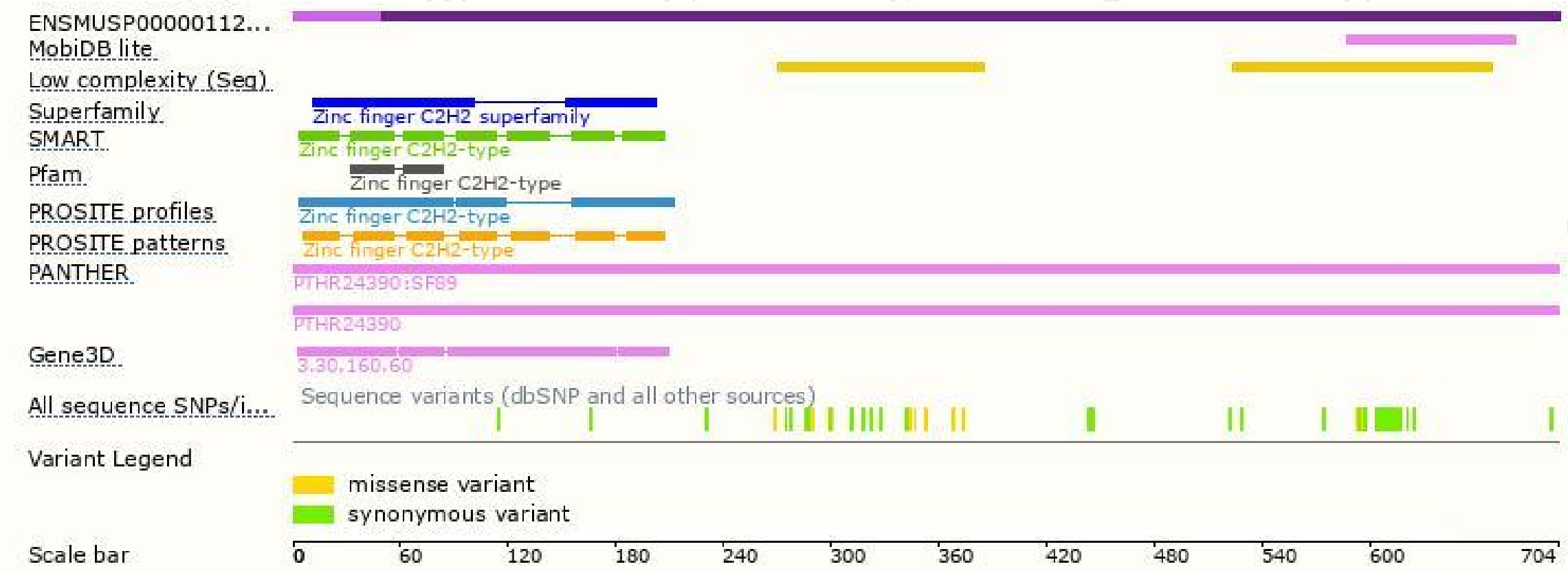
The strategy is based on the design of *Plagl1-202* transcript,the transcription is shown below:



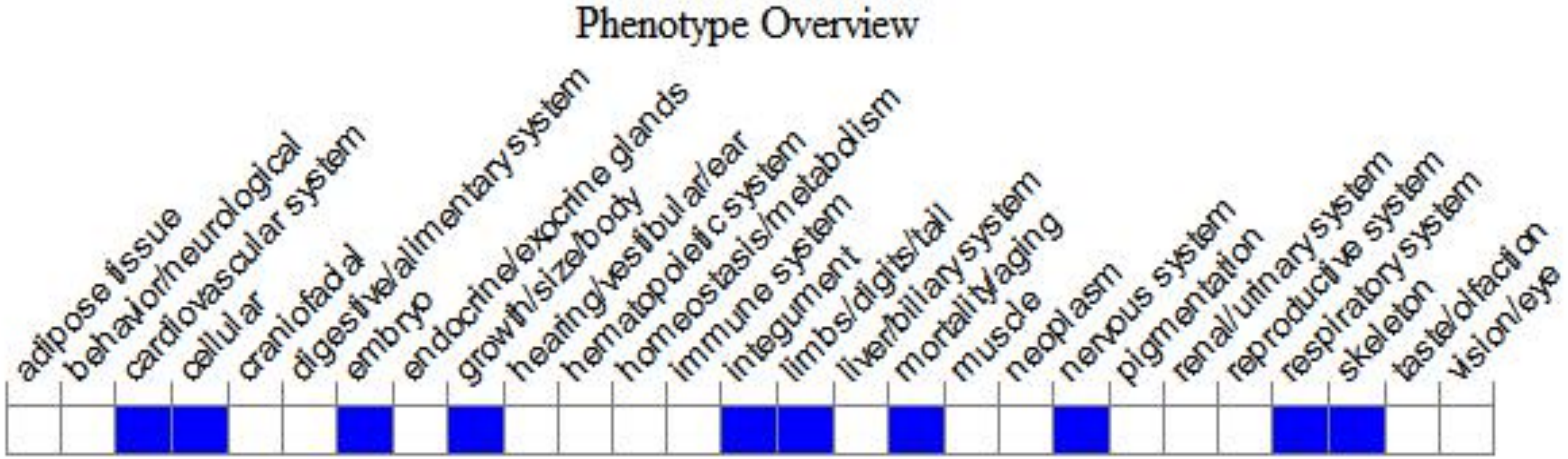
Genomic location distribution



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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