

Pparg Cas9-KO Strategy

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Project Overview



Project Name Pparg

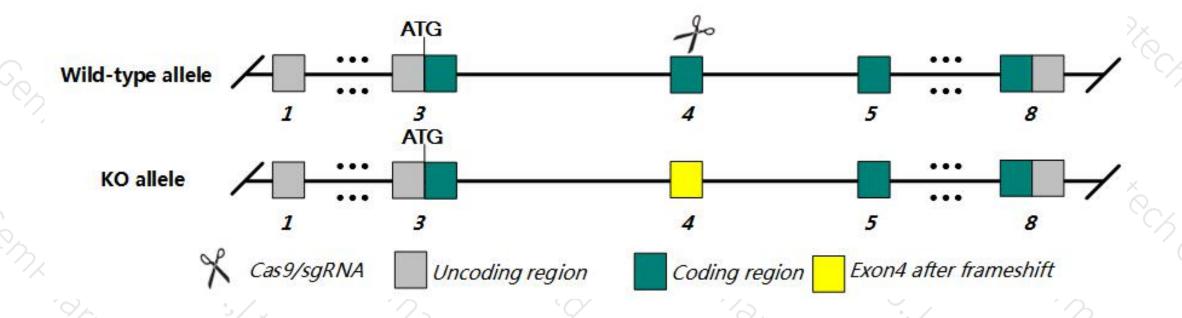
Project type Cas9-KO

Strain background C57BL/6J

Knockout strategy



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



Technical routes



- ➤ The *Pparg* gene has 7 transcripts. According to the structure of *Pparg* gene, partial sequence of exon4 of *Pparg-202* (ENSMUST00000171644.7) transcript is recommended as the knockout region. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Pparg* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice



- ➤ According to the existing MGI data, Homozygotes for targeted null mutations exhibit lethality due to placental defects. Heterozygotes show greater B cell proliferation, enhanced leptin secretion, and resistance to diet-induced adipocyte hypertrophy and insulin resistance.
- > The *Pparg* gene is located on the Chr6. 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)



Pparg peroxisome proliferator activated receptor gamma [Mus musculus (house mouse)]

Gene ID: 19016, updated on 9-Apr-2019

Summary

☆ ?

Official Symbol Pparg provided by MGI

Official Full Name peroxisome proliferator activated receptor gamma provided by MGI

Primary source MGI:MGI:97747

See related Ensembl:ENSMUSG00000000440

Gene type protein coding
RefSeq status REVIEWED

Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as Nr1c3, PPAR-gamma, PPAR-gamma2, PPARgamma, PPARgamma2

Summary This gene encodes a nuclear receptor protein belonging to the peroxisome proliferator-activated receptor (Ppar) family. The encoded protein

is a ligand-activated transcription factor that is involved in the regulation of adipocyte differentiation and glucose homeostasis. The encoded

protein forms a heterodimer with retinoid X receptors and binds to DNA motifs termed "peroxisome proliferator response elements" to either

activate or inhibit gene expression. Mice lacking the encoded protein die at an embryonic stage due to severe defects in placental vascularization. When the embryos lacking this gene are supplemented with healthy placentas, the mutants survive to term, but succumb to

lipodystrophy and multiple hemorrhages. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by

RefSeq, Apr 2015]

Expression Biased expression in subcutaneous fat pad adult (RPKM 32.5), mammary gland adult (RPKM 26.1) and 13 other tissues See more

Orthologs <u>human all</u>

Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

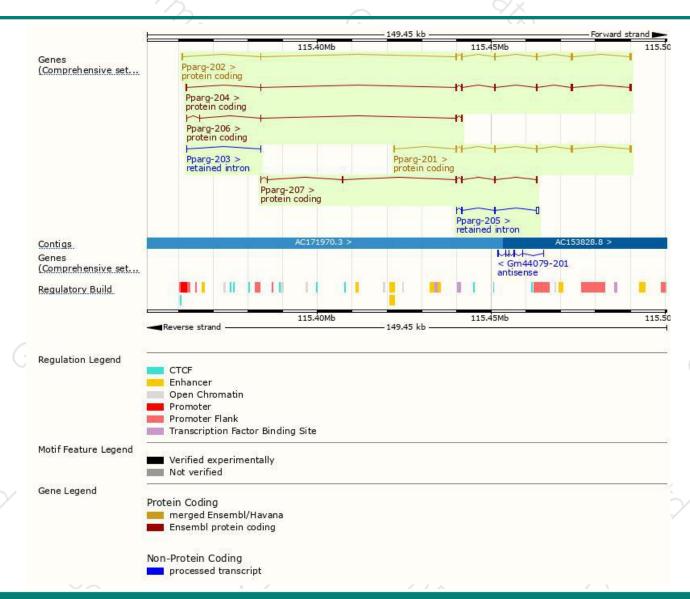
<u> </u>	- Million	<u> </u>					"/) ₁₁	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Pparg-202	ENSMUST00000171644.7	2125	475aa	Protein coding	CCDS51876	M1VPI1	TSL:1 GENCODE basic APPRIS ALT1	
Pparg-204	ENSMUST00000203732.2	1826	<u>475aa</u>	Protein coding	CCDS51876	M1VPI1	TSL:1 GENCODE basic APPRIS ALT1	
Pparg-201	ENSMUST00000000450.4	1767	<u>505aa</u>	Protein coding	CCDS20439	Q6GU14	TSL:1 GENCODE basic APPRIS P3	
Pparg-207	ENSMUST00000205213.2	835	222aa	Protein coding	-	A0A0N4SV67	CDS 3' incomplete TSL:3	
Pparg-206	ENSMUST00000204305.2	497	96aa	Protein coding	-5	A0A0N4SVF8	CDS 3' incomplete TSL:3	
Pparg-205	ENSMUST00000203896.1	915	No protein	Retained intron	ě	8 5	TSL:2	
Pparg-203	ENSMUST00000203308.1	213	No protein	Retained intron		34	TSL:5	

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



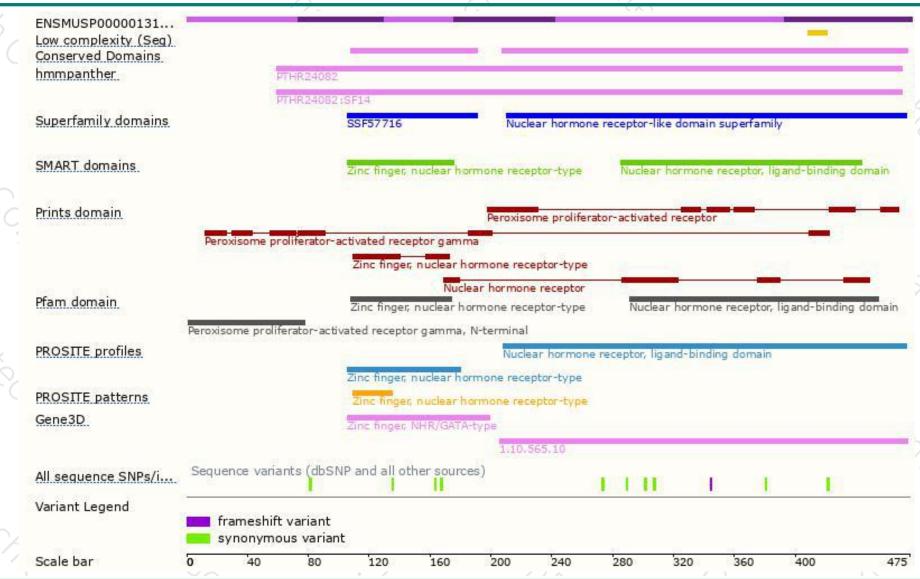
Genomic location distribution





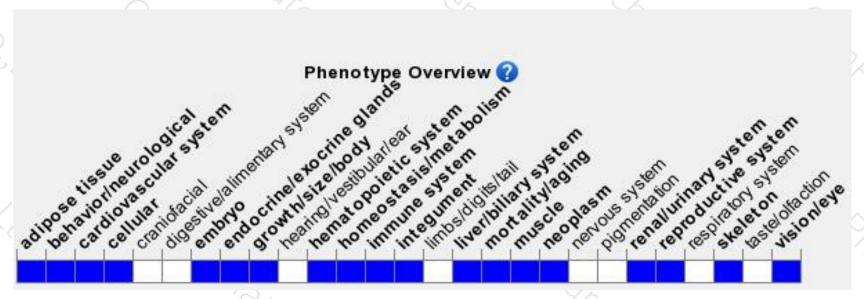
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Homozygotes for targeted null mutations exhibit lethality due to placental defects. Heterozygotes show greater B cell proliferation, enhanced leptin secretion, and resistance to diet-induced adipocyte hypertrophy and insulin resistance.



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

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