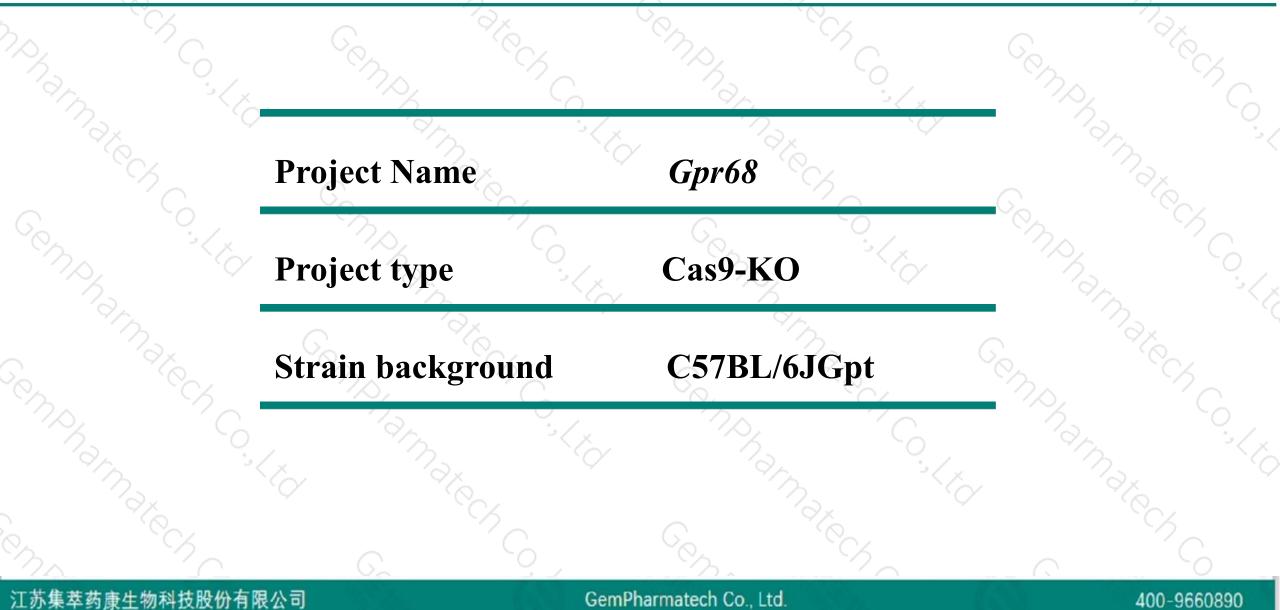


Gpr68 Cas9-KO Strategy

Designer: Reviewer: Design Date: Huan Wang Huan Fan 2019-10-10

Project Overview

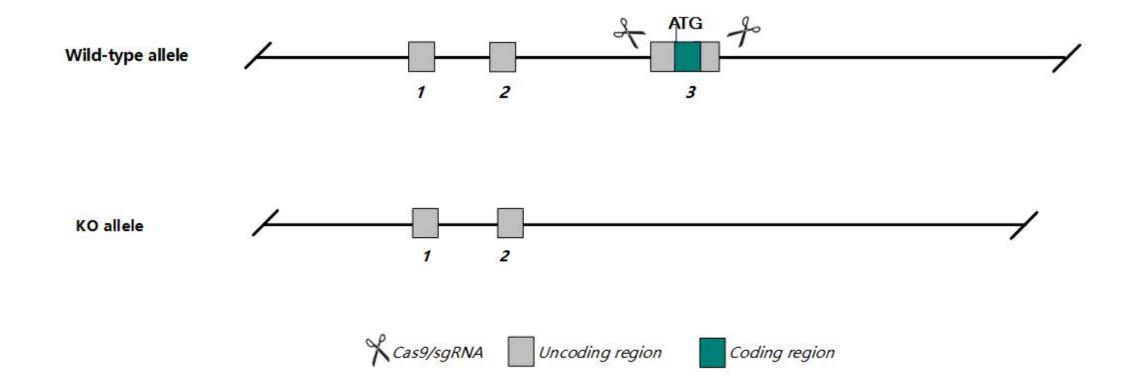




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Gpr68* gene. The schematic diagram is as follows:





The Gpr68 gene has 7 transcripts. According to the structure of Gpr68 gene, exon43 of Gpr68-203 (ENSMUST00000110066.7)transcript is recommended as the knockout region. The region contains all coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Gpr68* gene. The brief process is as follows:gRNA was transcribed in vitro.Cas9, gRNA 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.

Notice



- > According to the existing MGI data, mice homozygous for a null allele exhibit decreased osteoclastogenesis, abnormal pH-sensitive osteoclast survival, and background sensitive alterations in brown adipose tissue, monocytes, and macrophages. Mice homozygous for a different allele exhibit attenuated glucose-stimulated insulin secretion.
- The Gpr68 gene is located on the Chr12. 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.
- > The KO region contains functional region of the Dglucy gene. Knockout the region may affect the function of Dglucy gene.
- > 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)



Gpr68 G protein-coupled receptor 68 [Mus musculus (house mouse)]

Gene ID: 238377, updated on 12-Aug-2019	
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Orthologs human all

Summary

 Official Symbol
 Gpr68 provided by MGI

 Official Full Name
 G protein-coupled receptor 68 provided by MGI

 Primary source
 MGI:MGI:2441763

 See related
 Ensembl:ENSMUSG0000047415

 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; Muridae; Muridae; Musidus

 Also known as
 Ogr1; BB131428

 Expression
 Broad expression in spleen adult (RPKM 5.7), thymus adult (RPKM 5.5) and 20 other tissues See more

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Transcript information (Ensembl)



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

1		-			a 11 (i la ha		
Name 🍦	Transcript ID v	bp 🖕	Protein 🖕	Biotype 💧	CCDS	UniProt 🖕	Flags		
Gpr68-207	ENSMUST00000222453.1	3817	No protein	Retained intron	-	2	TSL:NA		
Gpr68-206	ENSMUST00000135684.1	444	No protein	IncRNA		2	TSL:2		
Gpr68-205	ENSMUST00000128306.1	483	No protein	IncRNA		2	TSL:2		
Gpr68-204	ENSMUST00000124459.1	567	No protein	IncRNA		4	TSL:3		
Gpr68-203	ENSMUST00000110066.7	3223	<u>365aa</u>	Protein coding	<u>CCDS26109</u> @	<u> </u>	TSL:3 GENCODE basic APPRIS P		
Gpr68-202	ENSMUST00000110065.7	2900	<u>365aa</u>	Protein coding	<u>CCDS26109</u> @	<u> </u>	TSL:1 GENCODE basic APPRIS P		
Gpr68-201	ENSMUST0000053668.9	3099	<u>365aa</u>	Protein coding	<u>CCDS26109</u> &	<u> </u>	TSL:2 GENCODE basic APPRIS P		

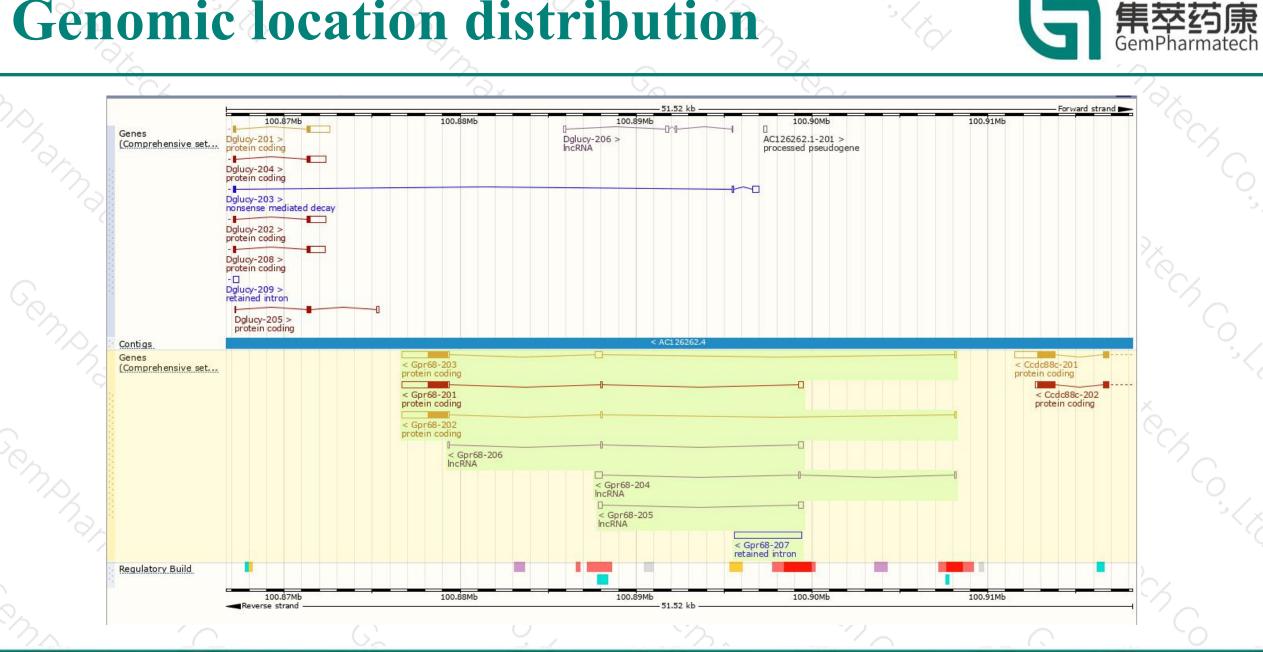
The strategy is based on the design of Gpr68-203 transcript, The transcription is shown below

< Gpr68-203 protein coding			- 31.52 kb			
- 	30	G.	6	`Ч_	C	

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Genomic location distribution



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Protein domain



400-9660890

Protein domains for ENSMUSP00000105693.1

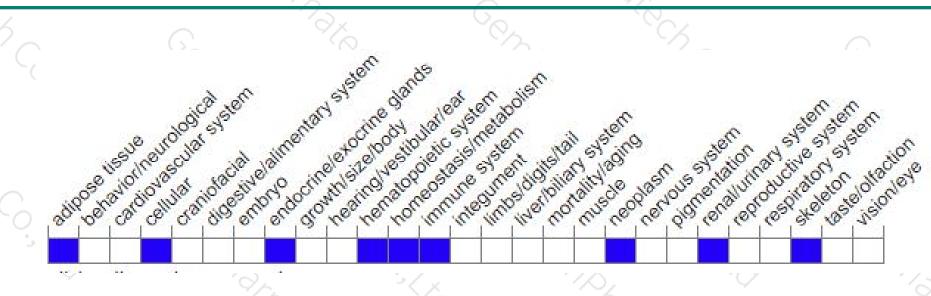
All sequence SNPs/i Variant Legend Scale bar	missense variant	80	synonym 120	ous variant 160	200	240	280	320	365
			synonym	ious variant					
	Sequence variants (dbSNP and all ot	ther sources)	100		н	049 10		D.	
CDD.	cd15367								
Gene3D	PTHR24234 1.20.1070.10								
PANTHER	PTHR24234:SF5		- Frankrin exemption (erehe	and a second					-
PROSITE profiles PROSITE patterns	GPCR, rhodopsin	n-like, 7TM	G protein-coupled recept	or rhodopsin-like					(
Pfam.	G protein-couple	ed receptor, rhodopsin-lik	2						
rinta	G protein-coupled recepto	CARLON AND ADDRESS OF COMPANY		-					-
Superfamily Prints	SSF81321							-	
Transmembrane heli Low complexity (Seg)									

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Mouse phenotype description(MGI)





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

Mice homozygous for a null allele exhibit decreased osteoclastogenesis, abnormal pH-sensitive osteoclast survival, and background sensitive alterations in brown adipose tissue, monocytes, and macrophages. Mice homozygous for a different allele exhibit attenuated glucose-stimulated insulin secretion.

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



