

Lgr5 Cas9-CKO Strategy

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Design Date: 2019-7-17

Project Overview



Project Name

Lgr5

Project type

Cas9-CKO

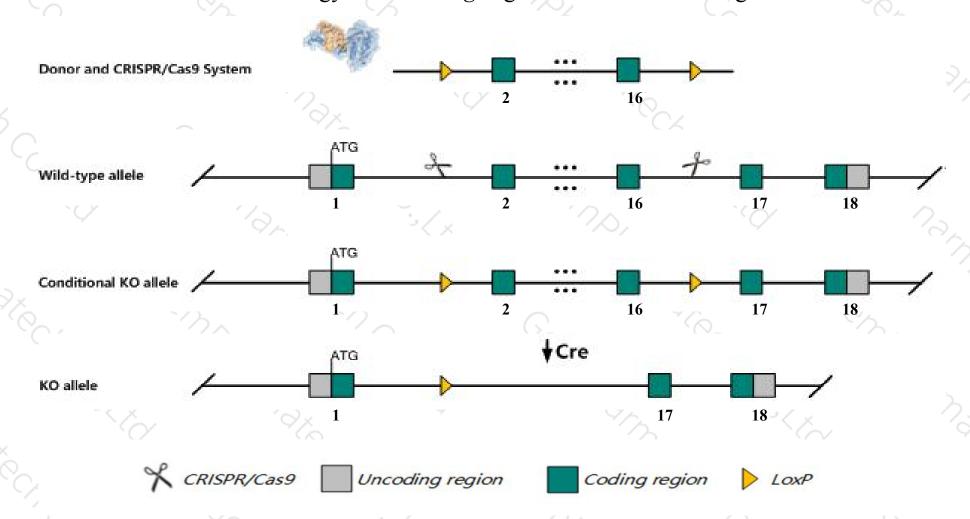
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Lgr5* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Lgr5* gene has 8 transcripts. According to the structure of *Lgr5* gene, exon2-exon16 of *Lgr5-201*(ENSMUST0000020350.14) transcript is recommended as the knockout region. The region contains 1340bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Lgr5* 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, Mice homozygous for a knock-out allele display 100% neonatal lethality associated with ankyloglossia, gastrointestinal distension, cyanosis and respiratory failure.
- > The *Lgr5* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)



Lgr5 leucine rich repeat containing G protein coupled receptor 5 [Mus musculus (house mouse)]

Gene ID: 14160, updated on 9-Apr-2019

Summary

↑ ?

Official Symbol Lgr5 provided by MGI

Official Full Name leucine rich repeat containing G protein coupled receptor 5 provided by MGI

Primary source MGI:MGI:1341817

See related Ensembl:ENSMUSG00000020140

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 FEX, Gpr49

Summary The protein encoded by this gene is a leucine-rich repeat-containing receptor (LGR) and member of the G protein-coupled, 7-

transmembrane receptor (GPCR) superfamily. The encoded protein is a receptor for R-spondins and is involved in the canonical Wnt signaling pathway. This protein plays a role in the formation and maintenance of adult intestinal stem cells during postembryonic

development. [provided by RefSeq, Sep 2015]

Expression Broad expression in frontal lobe adult (RPKM 8.3), limb E14.5 (RPKM 3.7) and 18 other tissues See more

Orthologs <u>human</u> all

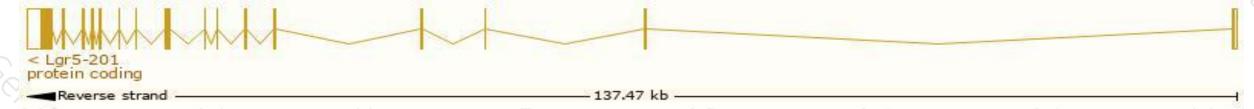
Transcript information (Ensembl)



The gene has 8 transcripts, all transcripts are shown below:

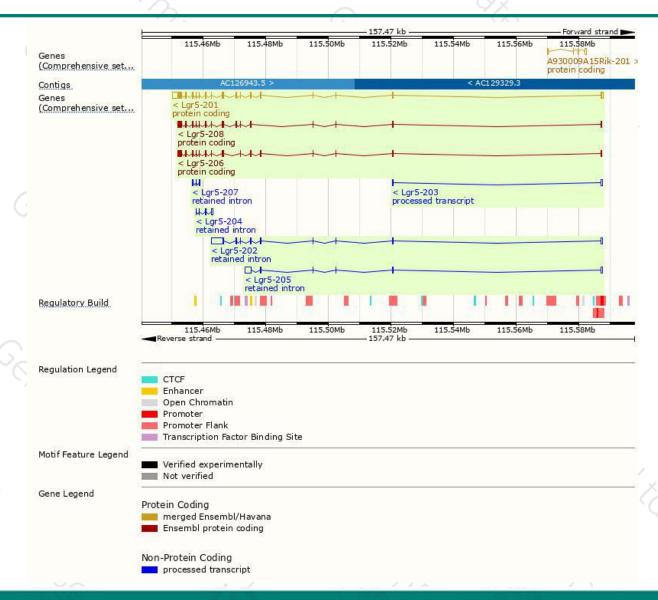
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lgr5-201	ENSMUST00000020350.14	4714	<u>907aa</u>	Protein coding	CCDS24180	Q9Z1P4	TSL:1 GENCODE basic APPRIS P1
Lgr5-206	ENSMUST00000172806.7	2652	883aa	Protein coding		G3UXX4	TSL:5 GENCODE basic
Lgr5-208	ENSMUST00000173740.1	2508	835aa	Protein coding	828	G3UXI8	TSL:5 GENCODE basic
Lgr5-203	ENSMUST00000129309.1	607	No protein	Processed transcript	1528	-	TSL:2
Lgr5-202	ENSMUST00000105272.7	4793	No protein	Retained intron	1871	-	TSL:2
Lgr5-205	ENSMUST00000149008.1	2781	No protein	Retained intron	-		TSL:2
Lgr5-207	ENSMUST00000173619.1	631	No protein	Retained intron	828	2	TSL:5
Lgr5-204	ENSMUST00000144732.1	560	No protein	Retained intron	7527	-	TSL:3

The strategy is based on the design of Lgr5-201 transcript, The transcription is shown below



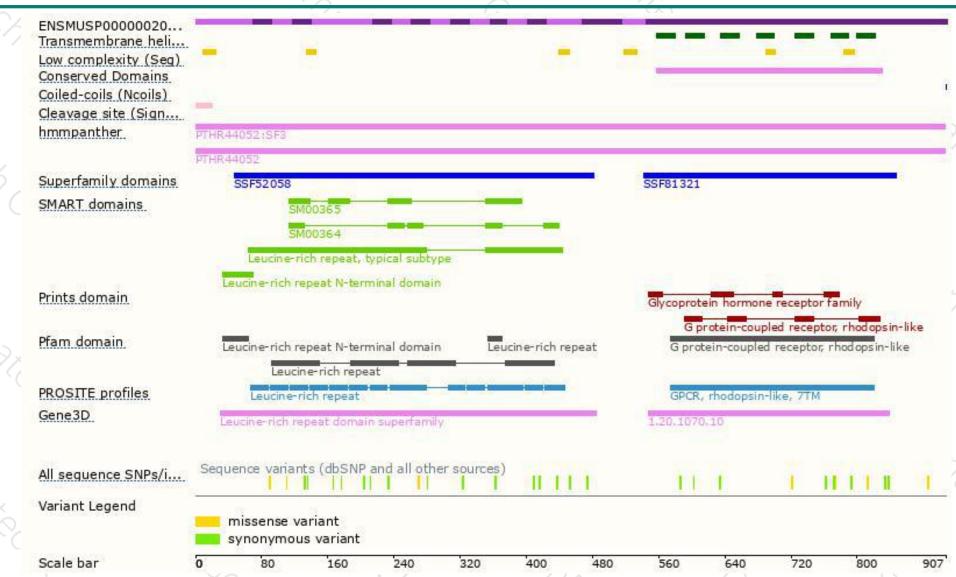
Genomic location distribution





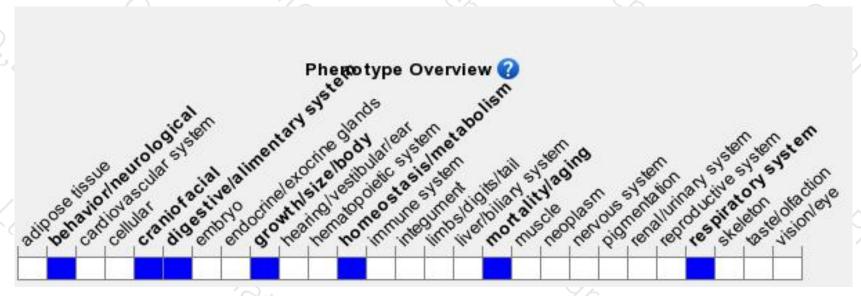
Protein domain





Mouse phenotype description(MGI)





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

According to the existing MGI data, Mice homozygous for a knock-out allele display 100% neonatal lethality associated with ankyloglossia, gastrointestinal distension, cyanosis and respiratory failure.



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





