

# Ghrh Cas9-KO Strategy

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# **Project Overview**



Project Name Ghrh

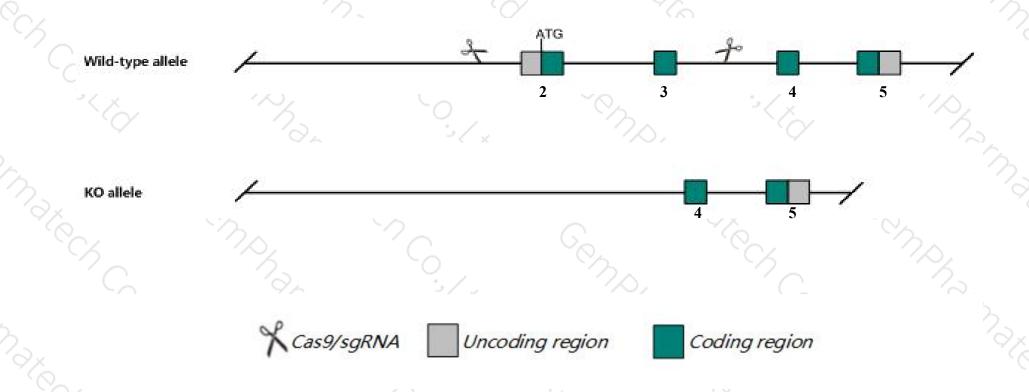
Project type Cas9-KO

Strain background C57BL/6J

# **Knockout strategy**



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



### **Technical routes**



- ➤ The *Ghrh* gene has 3 transcripts. According to the structure of *Ghrh* gene, exon2-exon3 of *Ghrh-201* (ENSMUST00000029172.1) 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 *Ghrh* 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, Mice homozygoous for a knock-out allele exhibit postnatal growth retardation, decreased body weight and body length, reduced IGF-I production and serum IGF-I levels, pituitary hypoplasia, decreased growth hormone level, and decreased litter size.
- > The *Ghrh* gene is located on the Chr2. 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)



#### Ghrh growth hormone releasing hormone [Mus musculus (house mouse)]

Gene ID: 14601, updated on 31-Jan-2019

#### Summary

☆ ?

Official Symbol Ghrh provided by MGI

Official Full Name growth hormone releasing hormone provided by MGI

Primary source MGI:MGI:95709

See related Ensembl: ENSMUSG00000027643

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 GRF, Ghrf

Summary This gene encodes a hormone that has stimulatory effects on pituitary growth hormone synthesis and release, and somatotrope expansion.

The encoded preproprotein undergoes proteolytic processing to generate the mature peptide that is secreted by hypothalamus. Mice lacking the encoded protein are deficient in the growth hormone, live longer and exhibit growth retardation, enhanced insulin sensitivity and increased

xenobiotic metabolism. [provided by RefSeq, Jul 2016]

Expression Restricted expression toward placenta adult (RPKM 10.5)See more

Orthologs human all

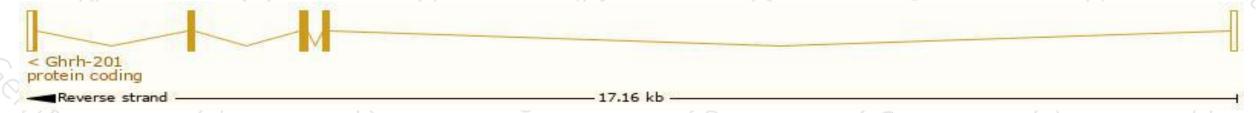
# Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

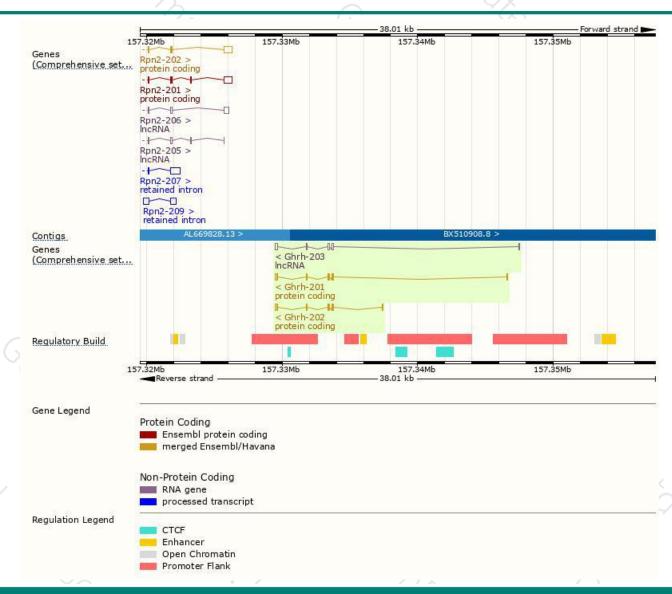
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ghrh-201	ENSMUST00000029172.1	516	<u>103aa</u>	Protein coding	CCDS16976	P16043 Q544X5	TSL:1 GENCODE basic APPRIS P1
Ghrh-202	ENSMUST00000109536.7	503	<u>103aa</u>	Protein coding	CCDS16976	P16043 Q544X5	TSL:1 GENCODE basic APPRIS P1
Ghrh-203	ENSMUST00000126630.1	512	No protein	IncRNA	#8	(4)	TSL:5

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



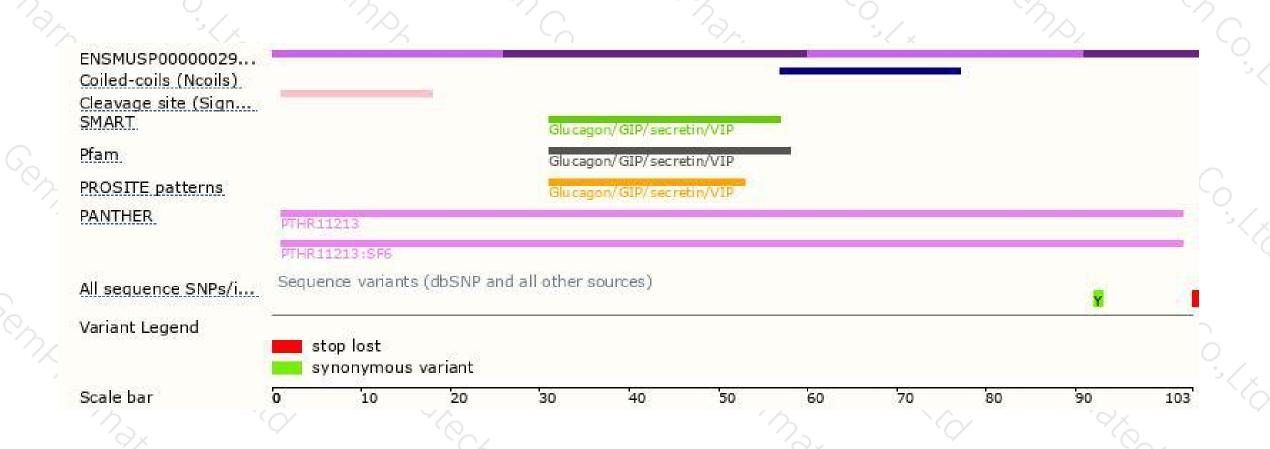
### Genomic location distribution





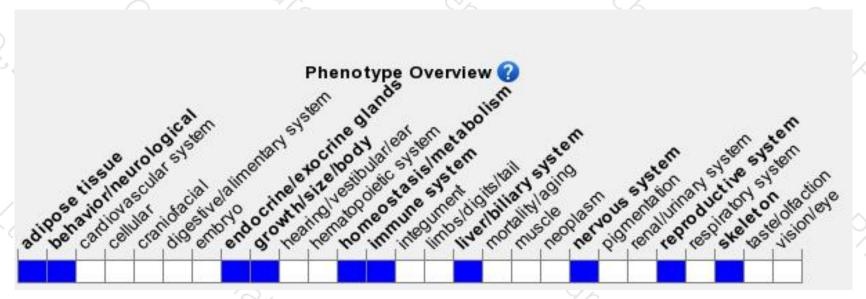
# Protein domain





# 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 homozygoous for a knock-out allele exhibit postnatal growth retardation, decreased body weight and body length, reduced IGF-I production and serum IGF-I levels, pituitary hypoplasia, decreased growth hormone level, and decreased litter size.



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

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