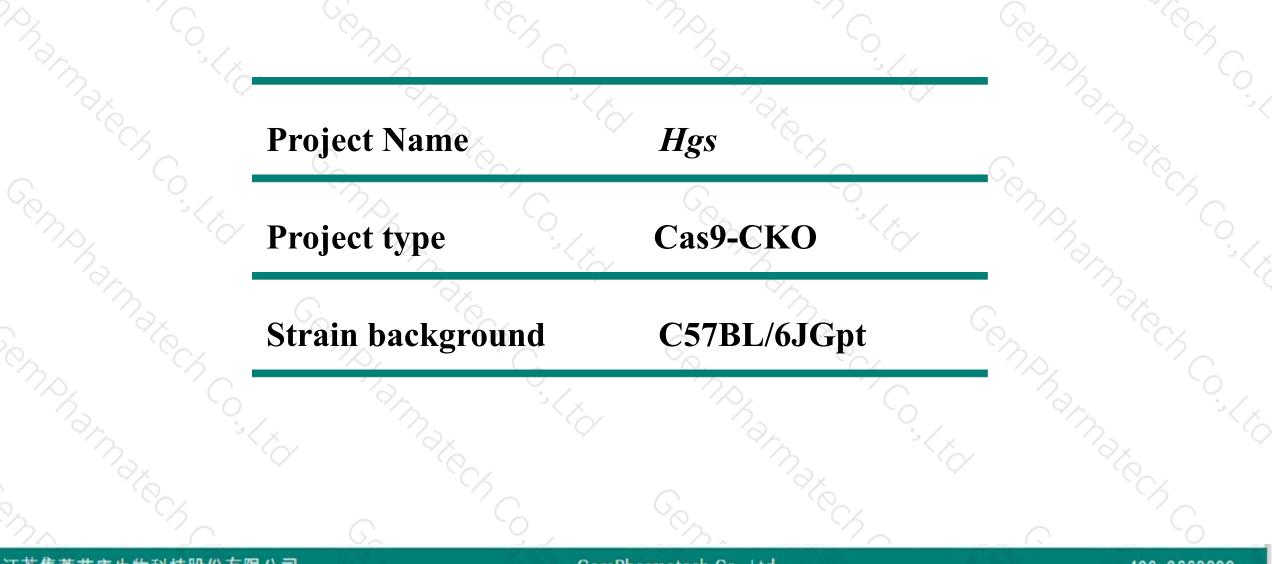


Hgs Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2019-10-19

Project Overview





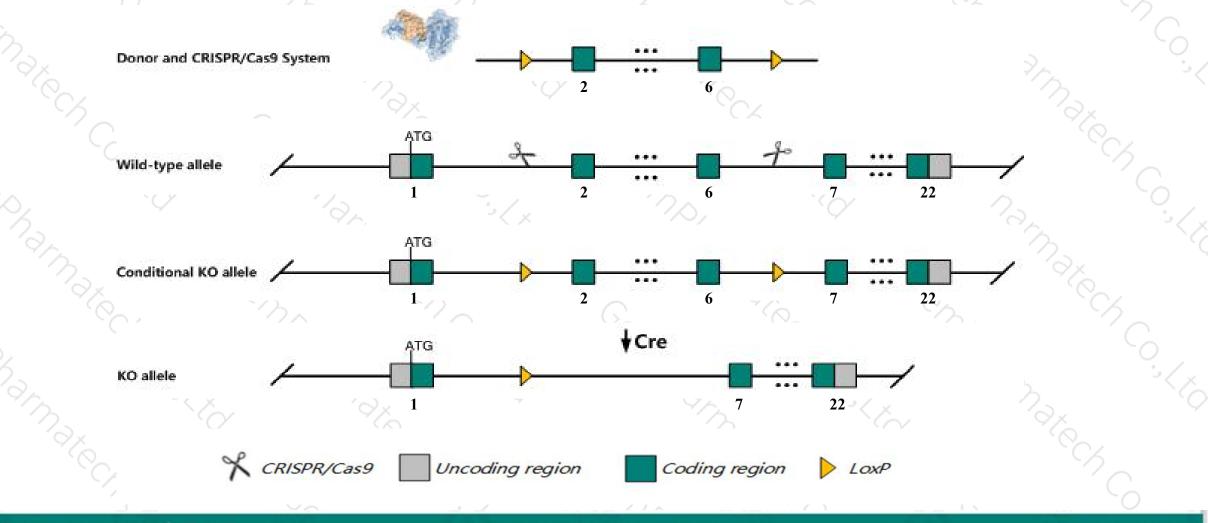
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Conditional Knockout strategy



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



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The Hgs gene has 5 transcripts. According to the structure of Hgs gene, exon2-exon6 of Hgs-201 (ENSMUST00000106203.8) transcript is recommended as the knockout region. The region contains 431bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Hgs* 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.

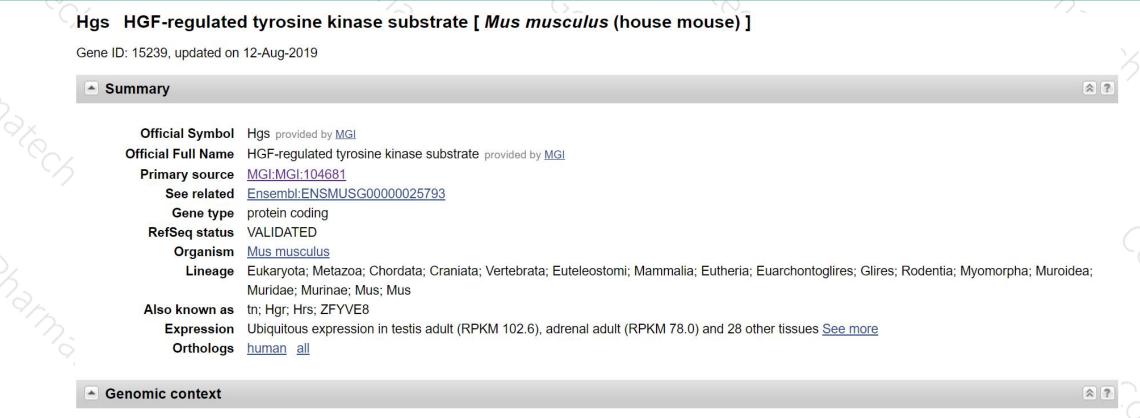
The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.



- According to the existing MGI data, Mice homozygous for disruptions in this gene display embryonic lethality during organogenesis with decreased size and no embryo turning. In addition, one allele shows cardia bifida, no foregut formation, failure of chorioallantoic fusion and neural tube, somite and allantois defects.
- > The effect on transcript *Hgs*-203 is unknown.
- ➤The floxed region is near to the N-terminal of *Arl16* gene, this strategy may influence the regulatory function of the N-terminal of *Arl16* gene.
- The Hgs gene is located on the Chr11. 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)





Location: 11 E2; 11 84.16 cM

See Hgs in Genome Data Viewer

Exon count: 23

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| Annotation release | Status | Assembly | Chr | Location |
|--------------------|-------------------|------------------------------|------------------|----------------------------------|
| 108 | current | GRCm38.p6 (GCF_000001635.26) | <mark>1</mark> 1 | NC_000077.6 (120467605120483984) |
| Build 37.2 | previous assembly | MGSCv37 (GCF_000001635.18) | <mark>1</mark> 1 | NC_000077.5 (120328949120345298) |
| (| × 2 | * / J A | - | |



Transcript information (Ensembl)

The gene has 5 transcripts, all transcripts are shown below:

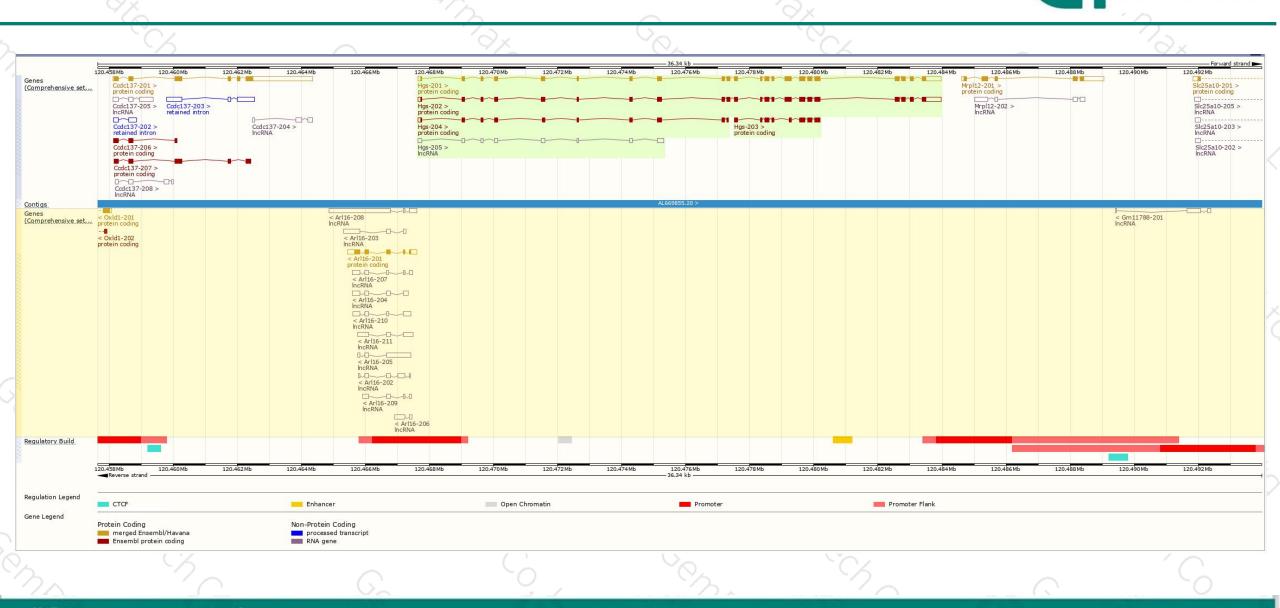
| Name 🍦 | Transcript ID | bp 🖕 | Protein 🖕 | Biotype 💧 | CCDS 🖕 | UniProt 🖕 | Flags |
|---------|----------------------|------|--------------|----------------|--------------------|-------------------------------|---------------------------------|
| Hgs-201 | ENSMUST00000106203.8 | 2903 | <u>776aa</u> | Protein coding | <u>CCDS49005</u> & | B1ATZ1₽ Q3UMA3₽ | TSL:5 GENCODE basic APPRIS P2 |
| Hgs-202 | ENSMUST00000106205.8 | 2900 | <u>775aa</u> | Protein coding | 1720 | <u>B1ATZ0</u> & <u>Q99L18</u> | TSL:5 GENCODE basic APPRIS ALT2 |
| Hgs-203 | ENSMUST00000135231.2 | 882 | <u>294aa</u> | Protein coding | 7520 | <u>F6VV02</u> | CDS 5' and 3' incomplete TSL:5 |
| Hgs-204 | ENSMUST00000140862.6 | 829 | <u>245aa</u> | Protein coding | 7520 | <u>B1ATY9</u> & | CDS 3' incomplete TSL:5 |
| Hgs-205 | ENSMUST00000141826.1 | 829 | No protein | IncRNA | 1729 | <u>6</u> | TSL:2 |

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

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Genomic location distribution



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Protein domain

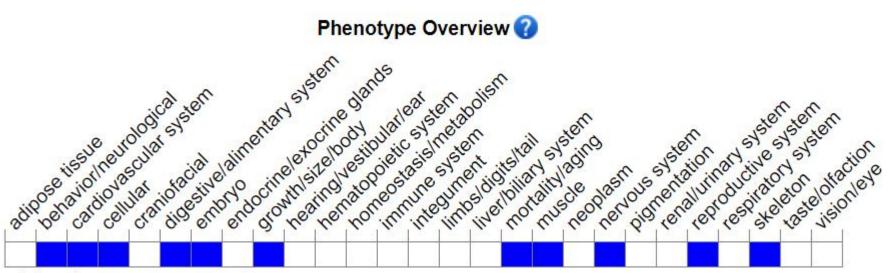


| 12harrs | | Chop | C'AC , | | | CAR | °°° Co |
|---------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------|-----------------------------|------------------------------------|----------------------------------------------|-----------|-----------|
| ENSMUSP00000101 MobiDB lite Low complexity.(Sea). Coiled-coils (Ncoils). Superfamily. | | Zinc finger, FYVE/PHD-type | | | | | |
| SMART. Pfam. PROSITE profiles. | VHS domain VHS domain VHS domain | Prve zinc finger Prve zinc finger Zinc finger, Prve-related | Ubiquitin interacting motif | Hepatocyte growth factor-regulated | ed tyrosine kinase substrate, helical domain | | |
| PIRSE PANTHER Gene3D. CDD. | Ubiquitin binding protein, Hrs/VPS27 PTHR46275 ENTH/VHS cd03569 | Zinc finges RING/FWE/PHD-type | | 2305302 | 03 | | |
| Variant Legend | | plice region variant '160 | synonymous variant | 400 | 480 560 | 640 | 776 |
| | N. | 1 pm | · / | | | | |
| MBH3 | | n pharma | × Co | Consharry | | Moharmare | |

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Mouse phenotype description(MGI)



Click cells to view annotations.

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

Mice homozygous for disruptions in this gene display embryonic lethality during organogenesis with decreased size and no embryo turning. In addition, one allele shows cardia bifida, no foregut formation, failure of chorioallantoic fusion and neural tube, somite and allantois defects.

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



