

Hoxa5 Cas9-CKO Strategy

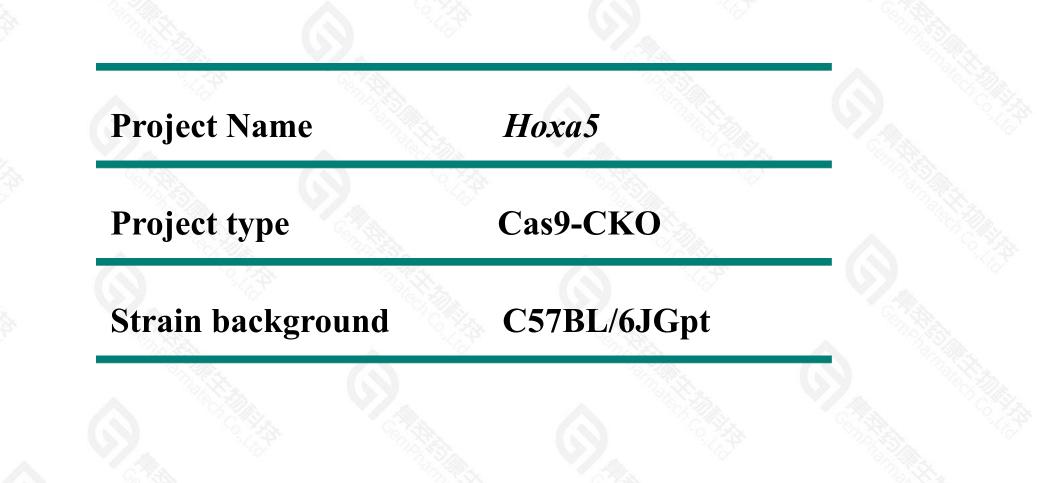
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Reviewer: Xueting Zhang

Design Date: 2021-4-15

Project Overview



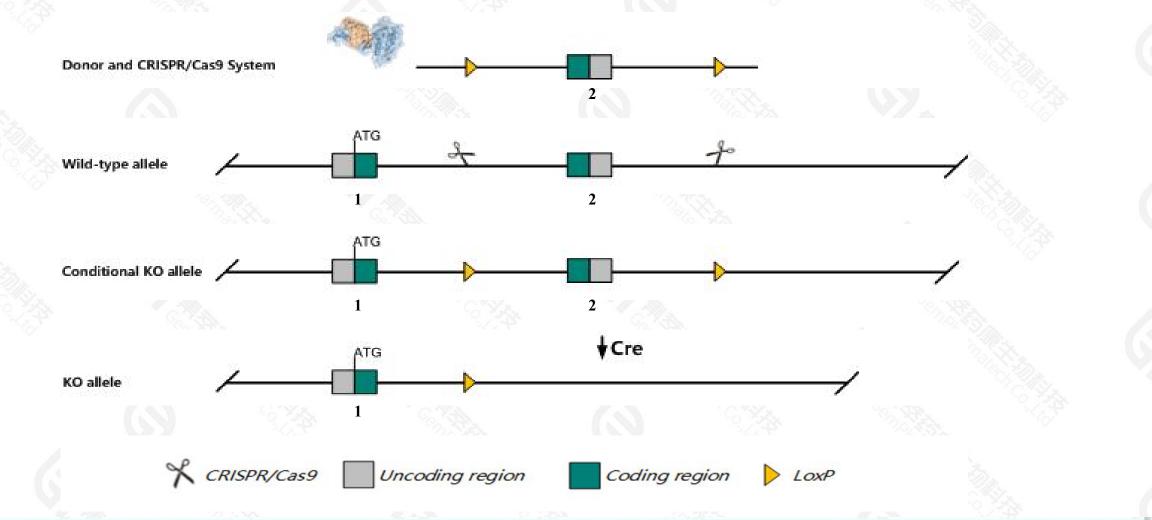


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Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the Hoxa5 gene. The schematic diagram is as follows:



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Technical routes



The Hoxa5 gene has 1 transcript. According to the structure of Hoxa5 gene, exon2 of Hoxa5-201(ENSMUST00000048794.7) transcript is recommended as the knockout region. The region contains 251bp coding sequence. Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify *Hoxa5* 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,nullizygous mice show skeletal defects, tracheal dysmorphology, reduced surfactant production, emphysema, and partial neonatal lethality. Survivors show stunted growth, delayed ear elevation and eyelid opening, and altered thyroid development, digestive secretion, and ovarian biology.
- > The KO region contains functional region of the *Hoxa3,Hoxaas3,Gm28308* gene.Knockout the region may affect the function of *Hoxa3,Hoxaas3,Gm28308* gene.
- > The *Hoxa5* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)



☆ ?

Hoxa5 homeobox A5 [Mus musculus (house mouse)]

Gene ID: 15402, updated on 23-Feb-2021

Summary

Official Symbol	Hoxa5 provided by MGI
Official Full Name	homeobox A5 provided byMGI
Primary source	MGI:MGI:96177
See related	Ensembl:ENSMUSG0000038253
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; Murinae; Mus; Mus
Also known as	Hox-1., Hox-1.3
Expression	Broad expression in lung adult (RPKM 17.3), CNS E14 (RPKM 14.7) and 16 other tissuesSee more
Orthologs	human all

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Transcript information (Ensembl)

The gene has 1 transcript, and the transcript is shown below:

NameTranscript IDbpProteinBiotypeCCDSUniProtFlagsHoxa5-201ENSMUST0000048794.71877270aaProtein codingCCDS20143TSL:1, GENCODE basic, APPRIS P1,

The strategy is based on the design of *Hoxa5-201* transcript, the transcription is shown below:

Hoxa5-201 rotein coding				
Reverse strand				
	NOL INFA	Sec. 7. 19	MO. NAN	50 BEA

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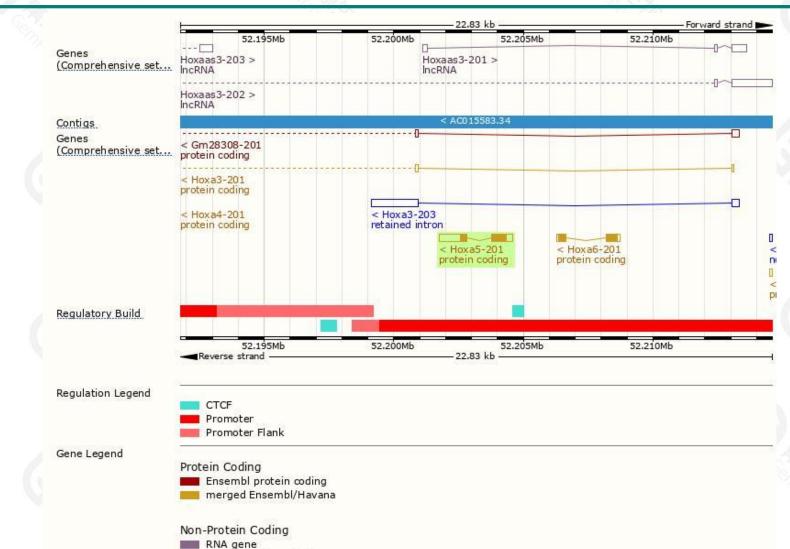
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Genomic location distribution

processed transcript



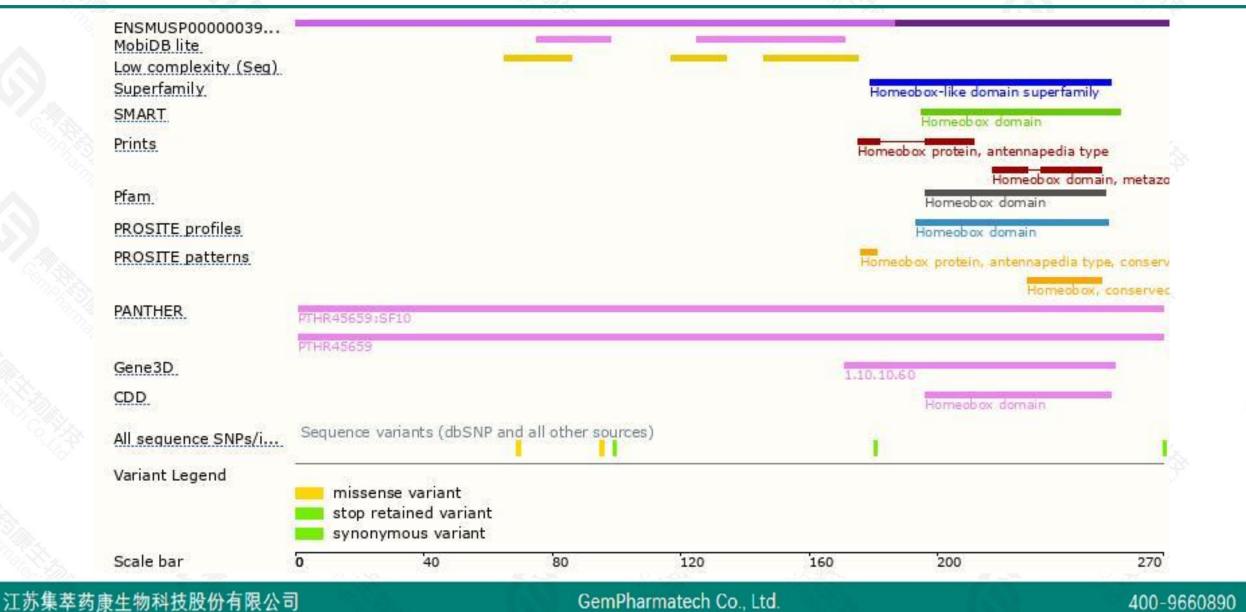


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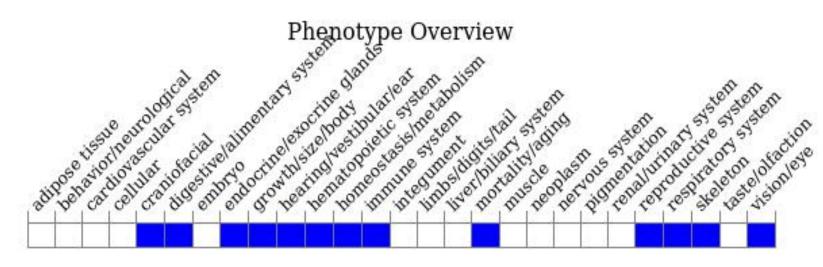
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Protein domain





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,nullizygous mice show skeletal defects, tracheal dysmorphology, reduced surfactant production, emphysema, and partial neonatal lethality. Survivors show stunted growth, delayed ear elevation and eyelid opening, and altered thyroid development, digestive secretion, and ovarian biology.

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



