H11-CAG-LSL-mHoxa5-HA-PolyA cas9-ki(H11) Strategy

Designer: Daohua Xu

Reviewer: Jia Yu

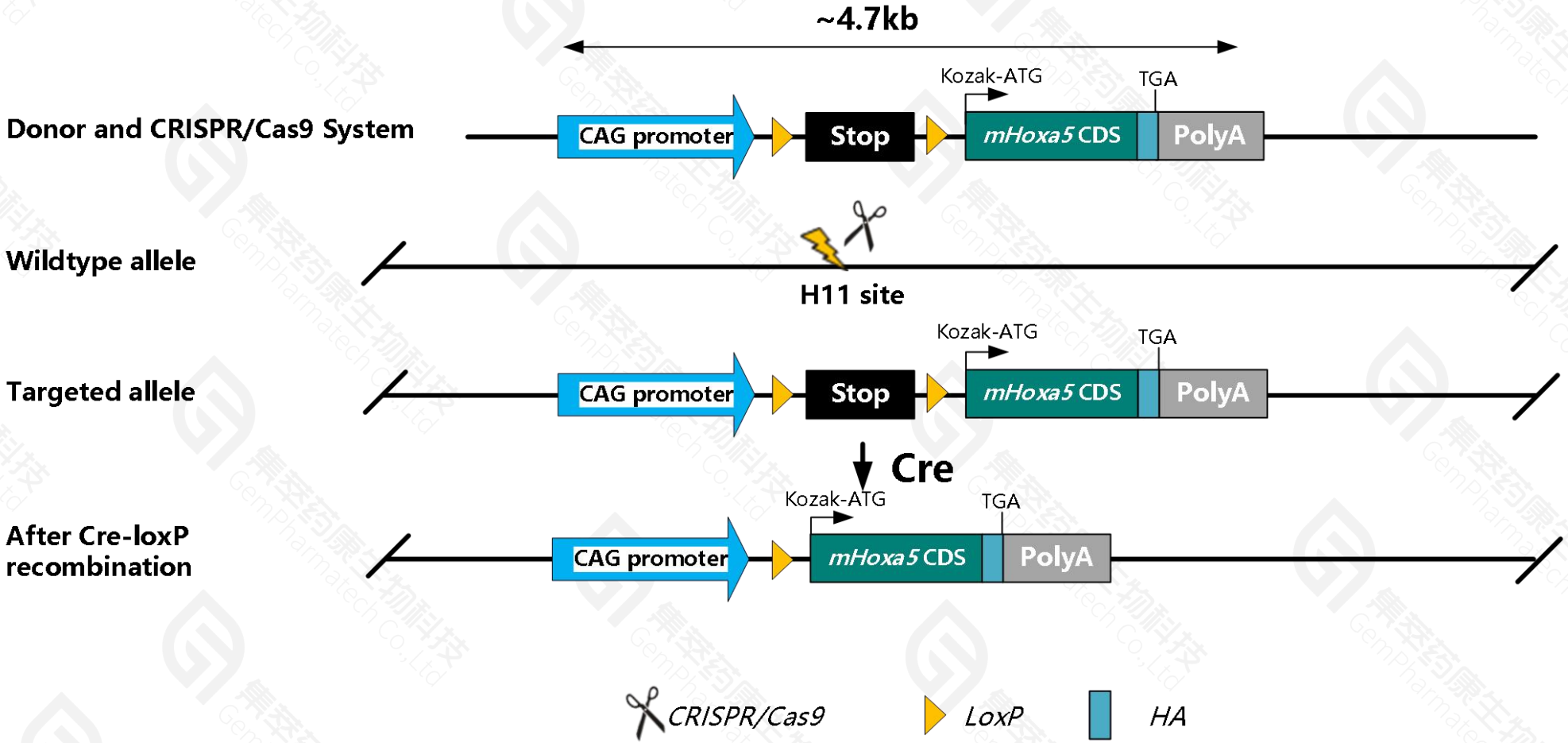
Design Date: 2021-9-9

Project Overview

Project Name	**CAG-LSL-mHoxa5-HA-PolyA**
Project type	**cas9-ki(H11)**
Strain background	**C57BL/6JGpt**

Knockin strategy

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



Technical routes

➢ Mouse *Hoxa5* gene has 1 transcript, *Hoxa5*-201(ENSMUST00000048794.7), which encodes all amino acids, was selected for this strategy.

➢ *Hoxa5*-201 CDS is expressed by the promoter *CAG*[1], Kozak is used to enhance the translation of *Hoxa5*.

➢ Before breeding with Cre mice in this model, the expression of *Hoxa5* was turned off. After breeding with Cre mice, the STOP element would be deleted to turn on the expression.

➢ H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.

➢ In this project we use CRISPR/Cas9 technology to modify H11 localization. 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.

Notice

- The expression of *Hoxa5* gene in mice was turned off before breeding with Cre mice. After breeding with Cre mice, *Hoxa5* gene could be expressed in specific tissues or cells.
- According to existing validation results, the Stop transcription termination element used in this strategy cannot achieve 100% gene transcription termination in the constructed mouse model.
- Please confirm the CDS sequence of *Hoxa5* gene, and the CDS needs to be synthesized.
- The H11 localization 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.

Mouse *Hoxa5* CDS (813bp)

https://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi?REQUEST=CCDS&DATA=CCDS20143

ATGAGCTCTTATTTTGTAAACTCATTTTGCGGTCGCTATCCAAATGGCCCGGACTACCAGTTGCATAATTATGGAGA
TCATAGTTCCGTGAGCGAACAATTCAGGGACTCGGCGAGCATGCACTCCGGCAGGTACGGCTACGGCTACAATGG
CATGGATCTCAGCGTCGGCCGTTCGGGTTCCGGCCACTTTGGCTCCGGCGAGCGCGCCCGCAGCTACGCGGCTGG
GGCCAGTGCGGCGCCCGCCGAGCCCAGGTACAGCCAGCCGGCCACGTCCACGCACTCGCCACCGCCCGACCCGC
TGCCCTGCTCAGCGGTGGCCCCCTCGCCCGGCAGCGACAGCCACCACGGCGGGAAAAACTCCCTGGGCAACTCC
AGCGGCGCCTCGGCCAACGCCGGCAGCACCCACATCAGCAGCAGAGAGGGGGTTGGCACGGCGTCCGCAGCCG
AGGAGGACGCCCCTGCCAGCAGCGAGCAGGCGGGCGCCCAGAGCGAGCCGAGCCCGGCGCCGCCCGCTCAGCC
CCAGATCTACCCCTGGATGCGCAAGCTGCACATTAGTCACGACAATATAGGTGGCCCAGAAGGCAAAAGGGCCCG
GACGGCCTACACTCGCTACCAGACCCTGGAGCTGGAGAAAGAATTCCACTTCAACCGCTACCTGACCCGCCGAA
GAAGGATCGAAATAGCTCATGCCCTTTGCCTCTCCGAGAGACAAATTAAAATCTGGTTCCAAAACAGGAGGATGA
AGTGGAAAAAAGATAATAAGCTGAAAAGCATGAGTATGGCCGCGGCAGGGGGGGCTTTCCGCCCCTGA

Gene information(NCBI)

集萃药康 GemPharmatech

Hoxa5 homeobox A5 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 15402, updated on 6-May-2021

Summary

Official Symbol	Hoxa5 provided by MGI
Official Full Name	homeobox A5 provided by MGI
Primary source	MGI:MGI:96177
See related	Ensembl:ENSMUSG00000038253
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 tissues See more
Orthologs	human all

NEW **Try the new Gene table**

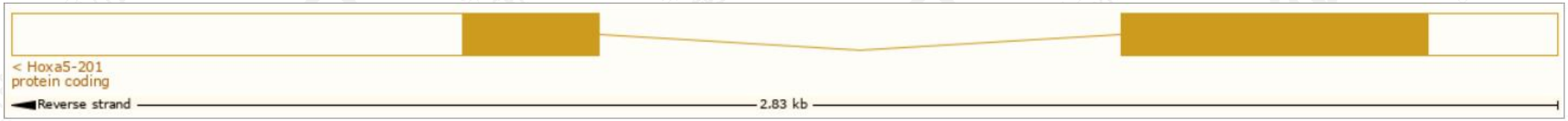
Try the new Transcript table

Transcript information(Ensembl)

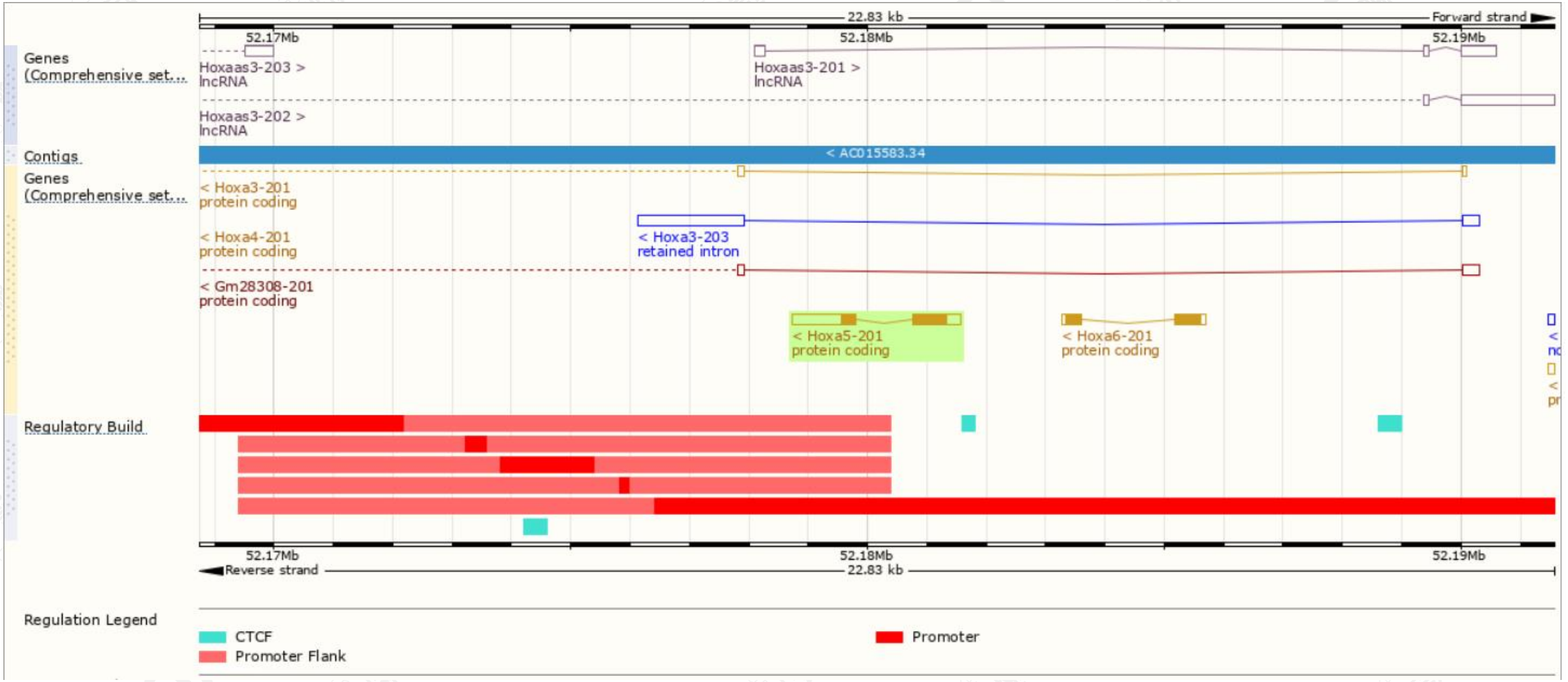
The gene has 1 transcript,the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Hoxa5-201	ENSMUST00000048794.7	1877	270aa	Protein coding	CCDS20143	P09021	GENCODE basic APPRIS P1 TSL:1

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



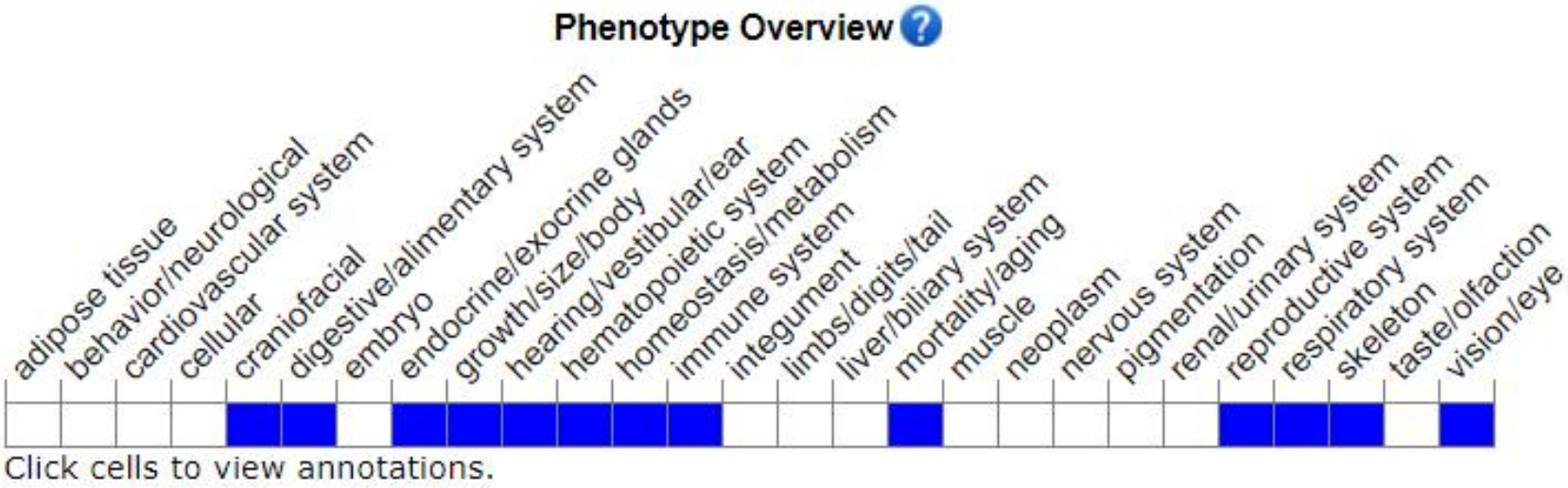
Genomic location distribution



Protein domain

Mouse phenotype description(MGI)

URL link is as follows:
http://www.informatics.jax.org/marker/MGI:96177



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.

References

[1] Alexopoulou, A. N., J. R. Couchman, et al. (2008). "The CMV early en3*flagncer/chicken beta actin (CAG) promoter can be used to drive transgene expression during the differentiation of murine embryonic stem cells into vascular progenitors." BMC Cell Biol 9: 2.

Additional cycles and costs

Additional itmes	cycle（month）	cost（¥）
Mouse *Hoxa5* CDS	0.5	1220

The CDS synthesis cycle is not included in this project cycle.

If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd