Hes3 Cas9-CKO Strategy

Designer:Xueting Zhang
Reviewer:Yanhua Shen
Date:2020-02-05

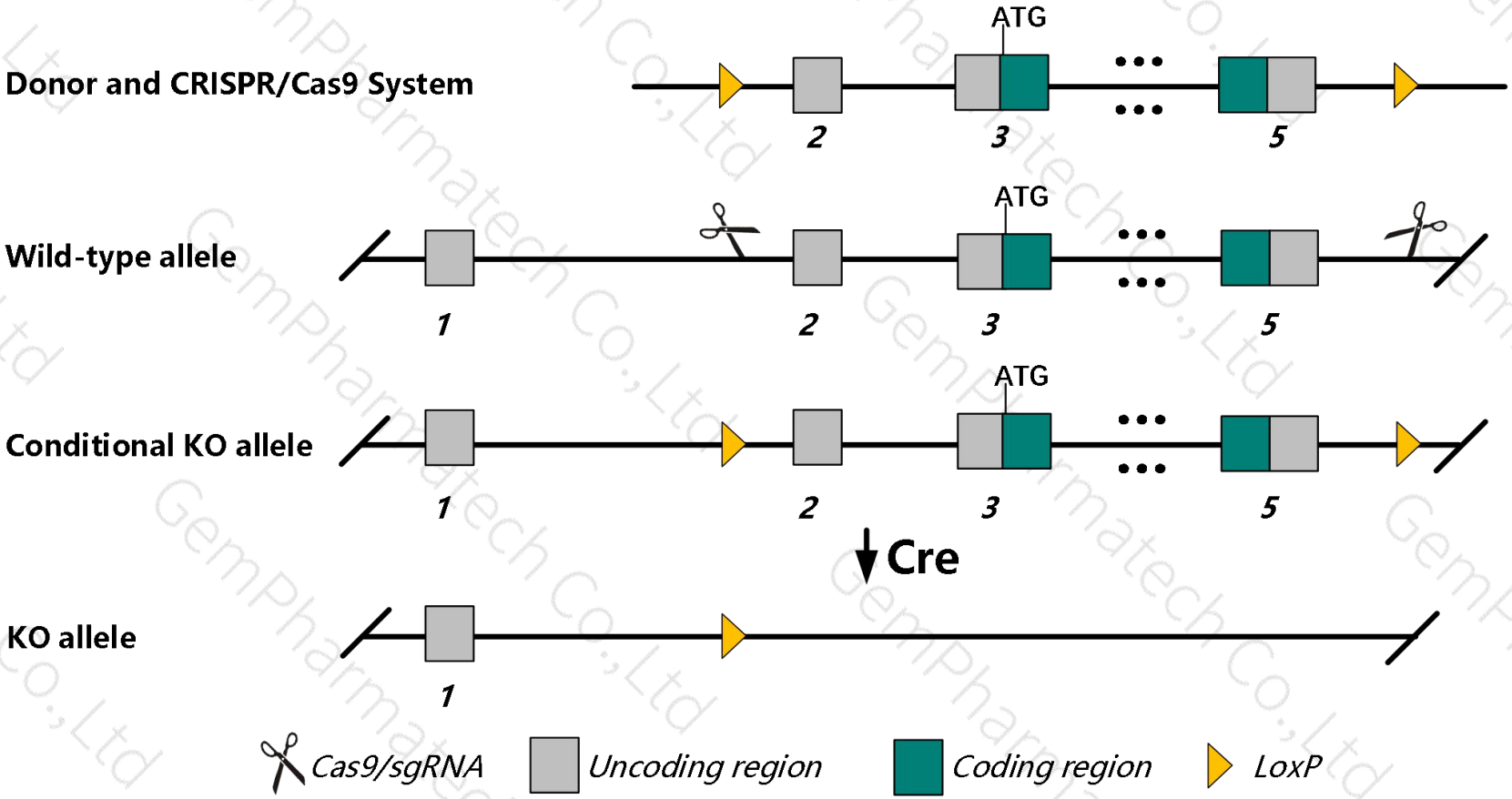
Project Overview

Project Name	***Hes3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

集萃药康 GemPharmatech

This model will use CRISPR/Cas9 technology to edit the *Hes3* gene. The schematic diagram is as follows:



Technical routes

- The *Hes3* gene has 2 transcripts. According to the structure of *Hes3* gene, exon2-exon5 of *Hes3-201* (ENSMUST00000094438.1) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Hes3* 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,Homozygous null mice are fertile and display no obvious abnormalities.

➢The floxed region is near to the N-terminal of *Icmt* gene and the C-terminal of *Gpr153* gene,this strategy may influence the regulatory function of the N-terminal of *Icmt* gene and the C-terminal of *Gpr153* gene.

➢ The *Hes3* gene is located on the Chr4. 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）

集萃药康 GemPharmatech

Hes3 hes family bHLH transcription factor 3 [*Mus musculus* (house mouse)]

Gene ID: 15207, updated on 3-Sep-2019

Summary

Official Symbol	Hes3 provided by MGI
Official Full Name	hes family bHLH transcription factor 3 provided by MGI
Primary source	MGI:MGI:104877
See related	Ensembl:ENSMUSG00000028946
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	Hes-3; bHLHb43
Expression	Biased expression in cerebellum adult (RPKM 9.7) and CNS E11.5 (RPKM 0.6) See more
Orthologs	human all

Genomic context

Location: 4 E2; 4 83.01 cM

See Hes3 in Genome Data Viewer

Exon count: 8

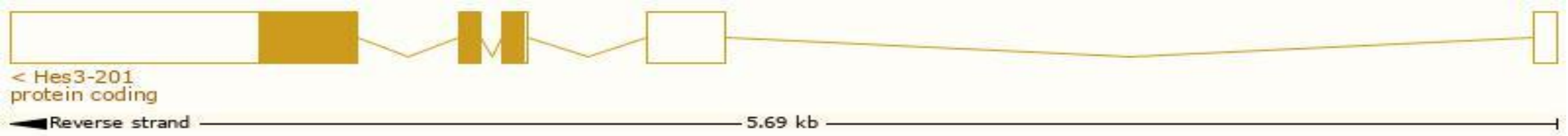
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	4	NC_000070.6 (152285250..152291911, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	4	NC_000070.5 (151660081..151665771, complement)

Transcript information（Ensembl）

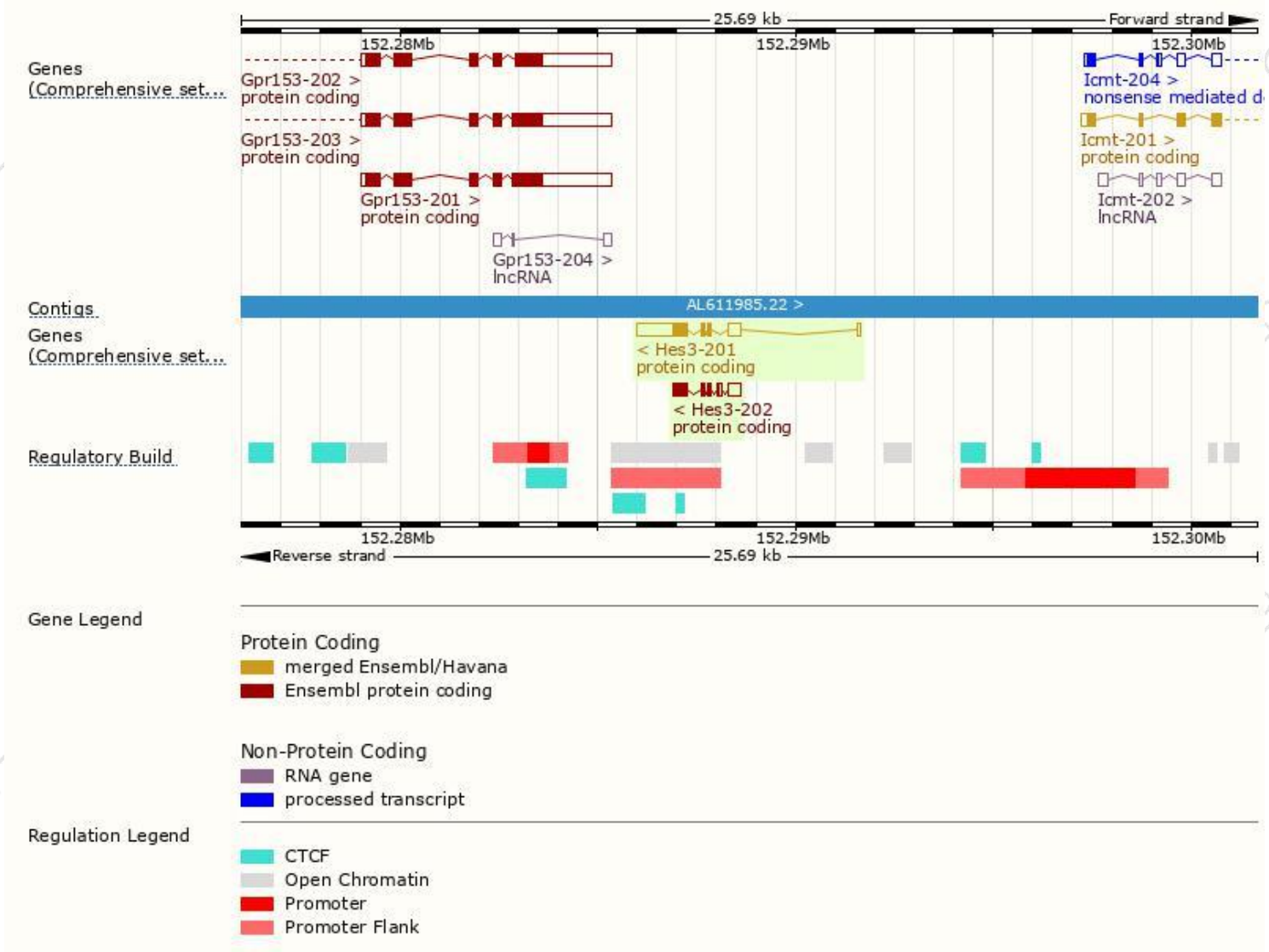
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hes3-201	ENSMUST00000094438.1	1829	175aa	Protein coding	CCDS18997	Q61657	TSL:1 GENCODE basic APPRIS P2
Hes3-202	ENSMUST00000218045.1	962	200aa	Protein coding	-	A0A1W2P7Y8	TSL:5 GENCODE basic APPRIS ALT2

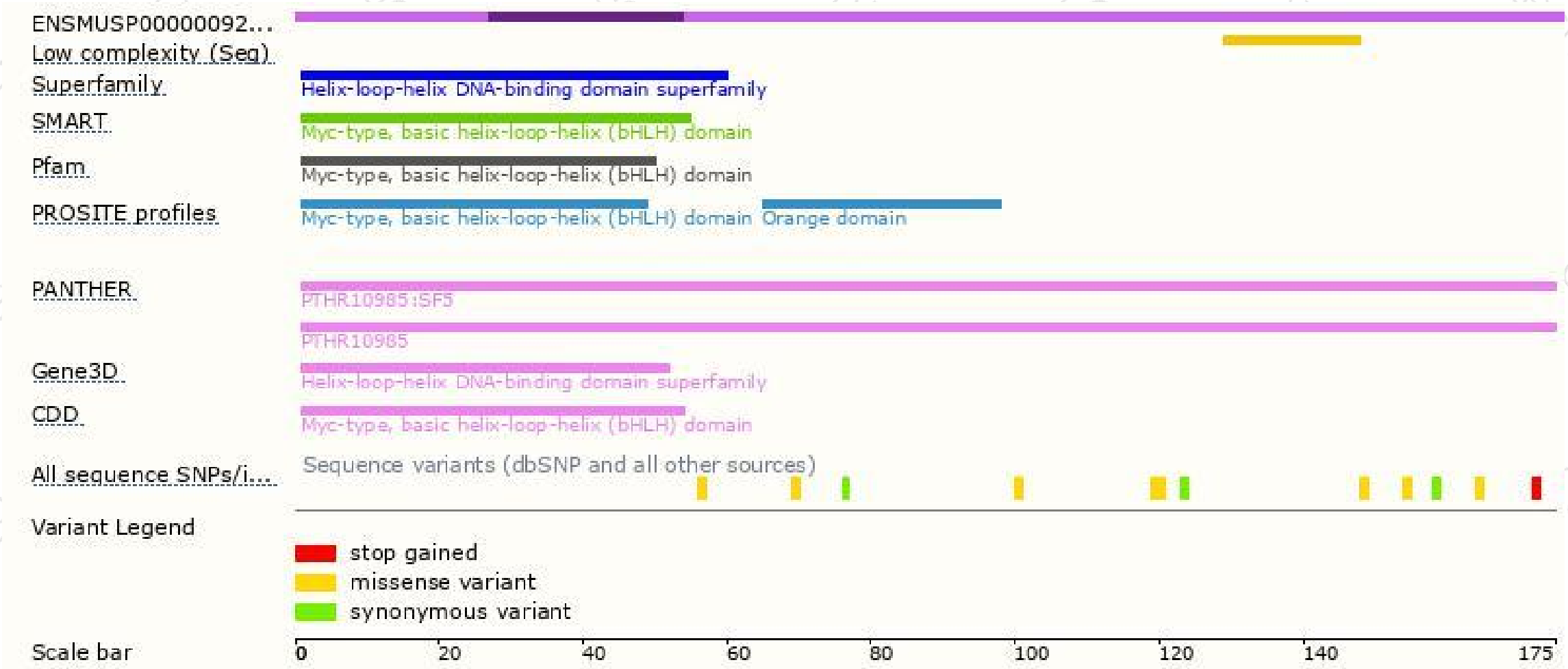
The strategy is based on the design of *Hes3-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd