H3f3a Cas9-CKO Strategy

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Project Overview

Project Name	***H3f3a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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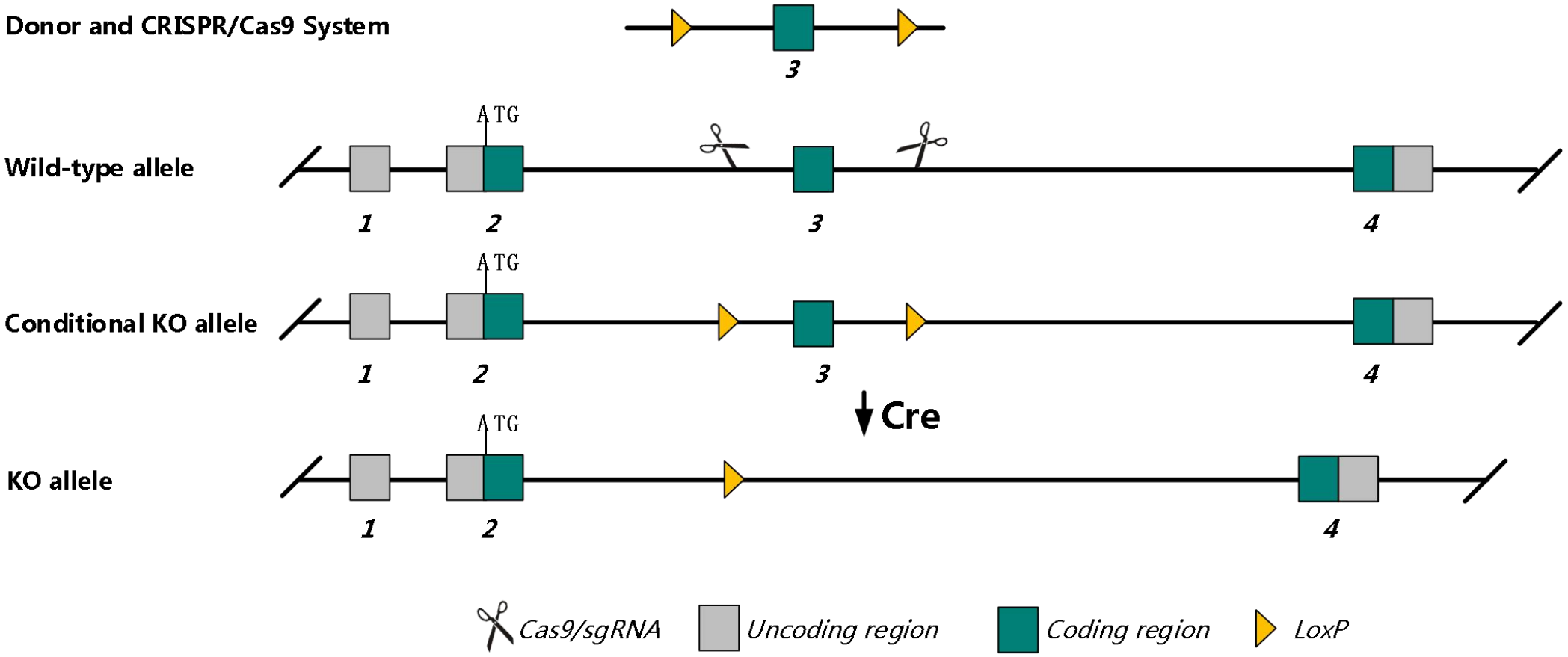
This model will use CRISPR/Cas9 technology to edit the *H3f3a* gene. The schematic diagram is as follows:



Technical routes

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- The *H3f3a* gene has 7 transcripts. According to the structure of *H3f3a* gene, exon3 of *H3f3a-205* (ENSMUST00000161308.7) transcript is recommended as the knockout region.The region contains 154bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *H3f3a* 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 mutants for a hypomorphic gene trap allele display partial neonatal lethality, reduced fertility, growth abnormalities and neuromuscular defects. Mice homozygous for a reporter allele exhibit reduced body size and male fertility.

- The *H3f3a* gene is located on the Chr1. 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）

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H3f3a H3 histone, family 3A [Mus musculus (house mouse)]

Gene ID: 15078, updated on 31-Jan-2019

Summary

Official Symbol	H3f3a provided by MGI
Official Full Name	H3 histone, family 3A provided byMGI
Primary source	MGI:MGI:1097686
See related	Ensembl:ENSMUSG00000060743
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	EyeLinc14, H3.3A
Summary	Histones are basic nuclear proteins that are responsible for the nucleosome structure of the chromosomal fiber in eukaryotes. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form an octamer, around which approximately 146 bp of DNA is wrapped in repeating units, called nucleosomes. The linker histone, H1, interacts with linker DNA between nucleosomes and functions in the compaction of chromatin into higher order structures. This gene contains introns and its mRNA is polyadenylated, unlike most histone genes. The protein encoded is a replication-independent member of the histone H3 family. [provided by RefSeq, Nov 2015]
Expression	Broad expression in liver E14 (RPKM 493.6), CNS E14 (RPKM 457.6) and 26 other tissuesSee more
Orthologs	human all

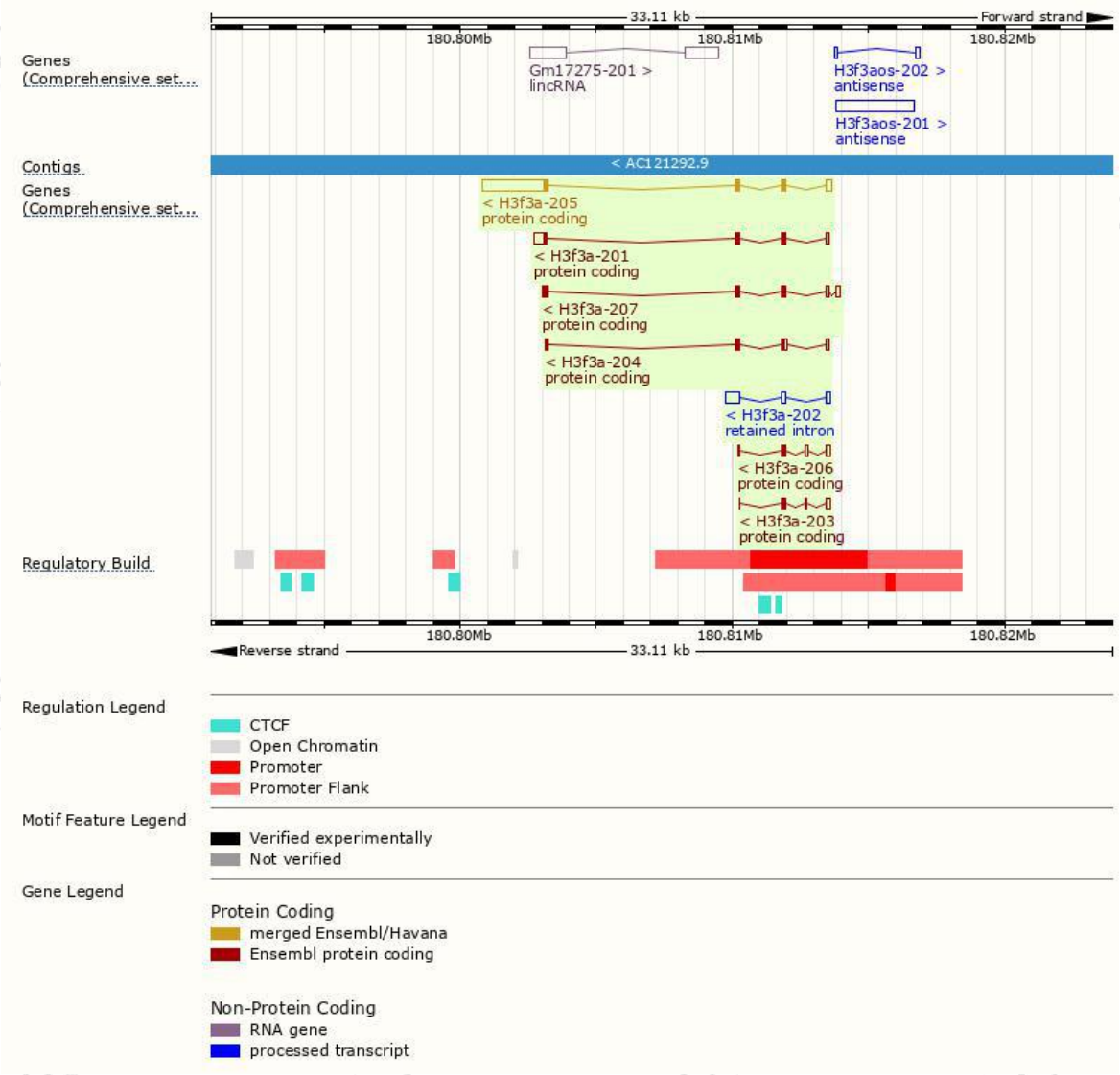
Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

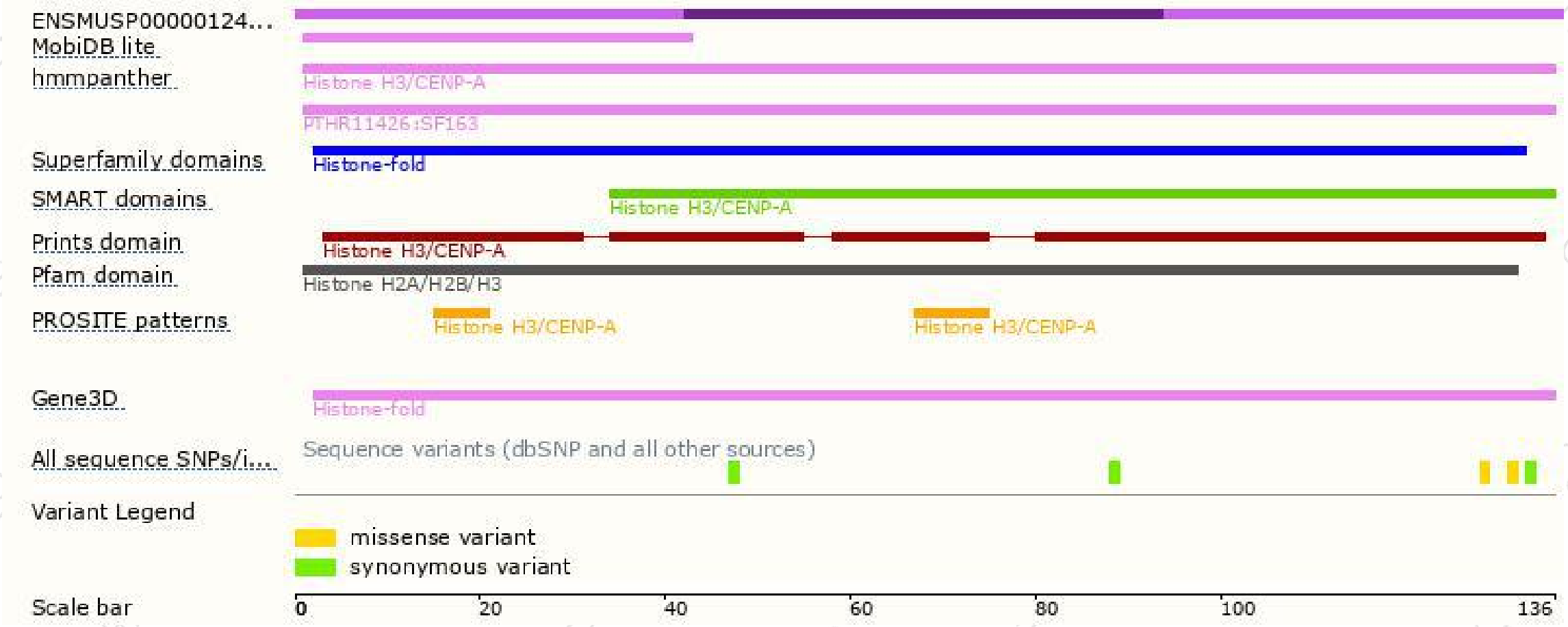
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
H3f3a-205	ENSMUST00000161308.7	2841	136aa	Protein coding	CCDS15573	P84244	TSL:1 GENCODE basic APPRIS P2
H3f3a-207	ENSMUST00000162814.7	724	136aa	Protein coding	CCDS15573	P84244	TSL:3 GENCODE basic APPRIS P2
H3f3a-201	ENSMUST00000081026.10	888	135aa	Protein coding	-	F8WI35	TSL:5 GENCODE basic APPRIS ALT1
H3f3a-204	ENSMUST00000159789.1	481	119aa	Protein coding	-	E0CZ27	CDS 3' incomplete TSL:3
H3f3a-206	ENSMUST00000162118.7	419	55aa	Protein coding	-	E0CYR7	CDS 3' incomplete TSL:3
H3f3a-203	ENSMUST00000159685.1	371	52aa	Protein coding	-	E0CYN1	CDS 3' incomplete TSL:2
H3f3a-202	ENSMUST00000159441.7	807	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *H3f3a-205* transcript,The transcription is shown below

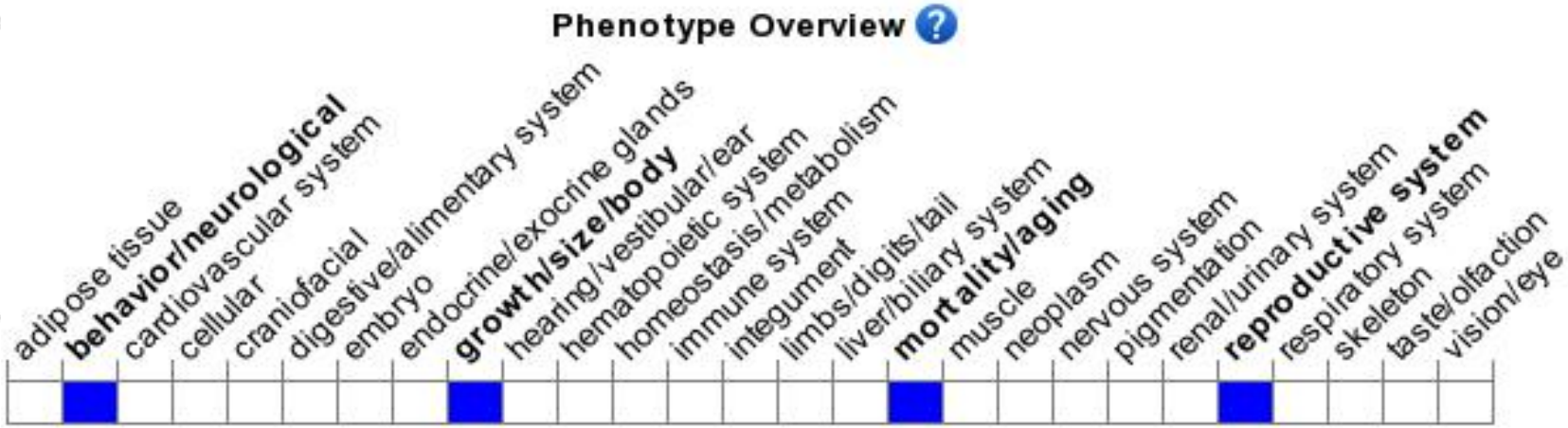
Genomic location distribution



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,Homozygous mutants for a hypomorphic gene trap allele display partial neonatal lethality, reduced fertility, growth abnormalities and neuromuscular defects. Mice homozygous for a reporter allele exhibit reduced body size and male fertility.

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

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