H2ax Cas9-CKO Strategy

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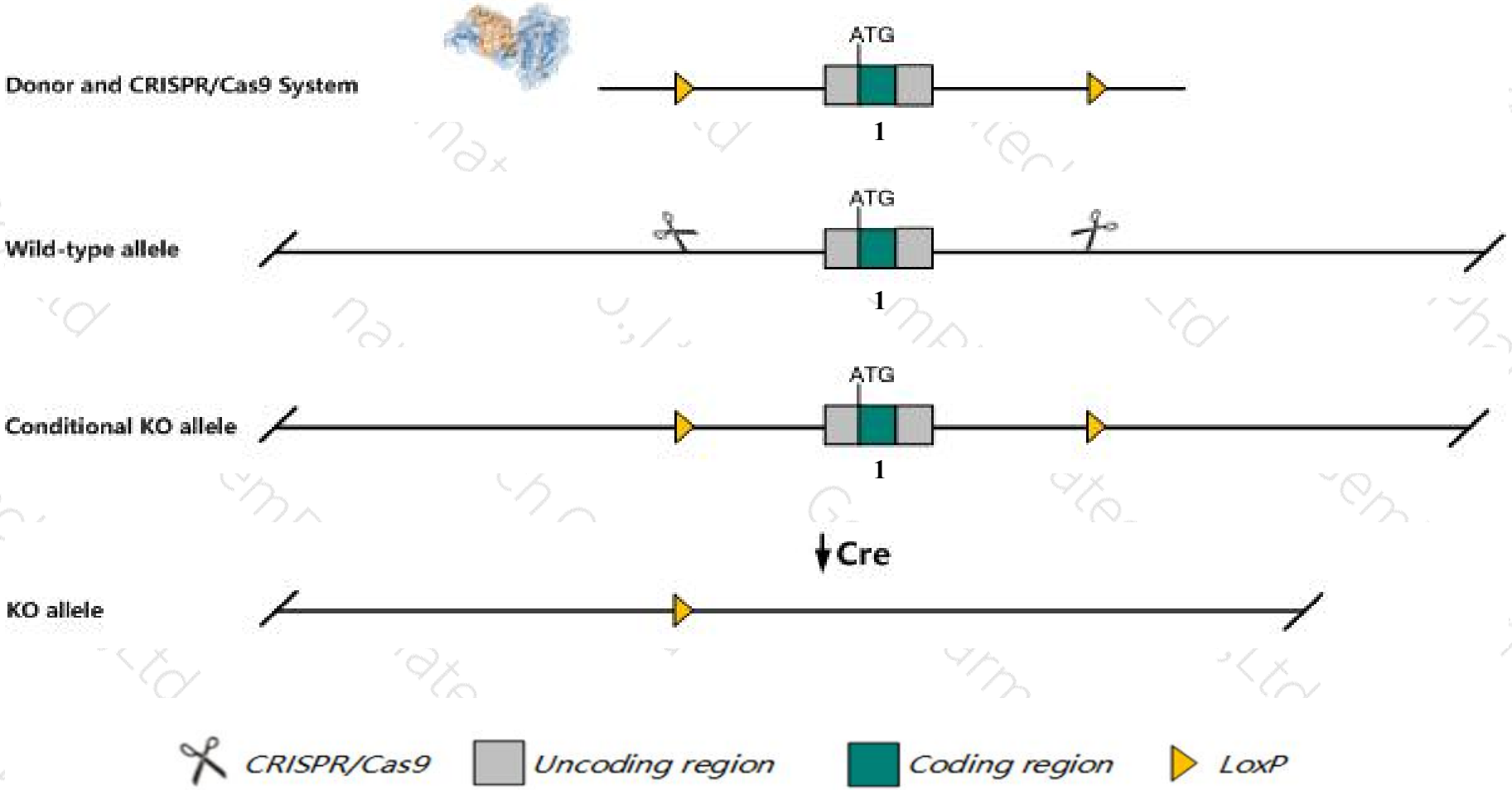
Project Overview

Project Name	***H2ax***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *H2ax* gene. The schematic diagram is as follows:



Technical routes

- The *H2ax* gene has 1 transcript. According to the structure of *H2ax* gene, exon1 of *H2ax-201* (ENSMUST00000052686.3) 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 *H2ax* 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 smaller and display increased susceptibility to ionizing radiation, male infertility, t and b cell abnormalities, and increased genomic instability.
- The flox region is about 0.2kb away from the 3th end of the *Hmbs* gene, and its effect is unknown.
- The flox region is about 0.7kb away from the 3th end of the *Dpagt1* gene, and its effect is unknown.
- The flox region is about 0.3kb away from the 5th end of the *Gm48853-201* gene, and its effect is unknown.
- *Gm44335*-201 gene may be destroyed.
- The *H2ax* gene is located on the Chr9. 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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H2ax H2A.X variant histone [Mus musculus (house mouse)]

Gene ID: 15270, updated on 13-Mar-2020

Summary

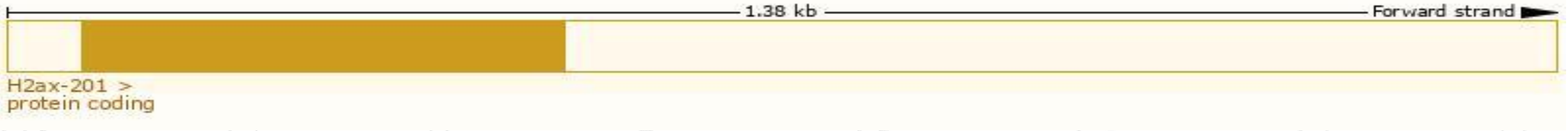
Official Symbol	H2ax provided by MGI
Official Full Name	H2A.X variant histone provided by MGI
Primary source	MGI:MGI:102688
See related	Ensembl:ENSMUSG00000049932
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW228881, H2A.X, H2afx, Hist5-2ax, gammaH2ax
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 encodes a replication-independent histone that is a member of the histone H2A family. [provided by RefSeq, Nov 2015]
Orthologs	human all

Transcript information（Ensembl）

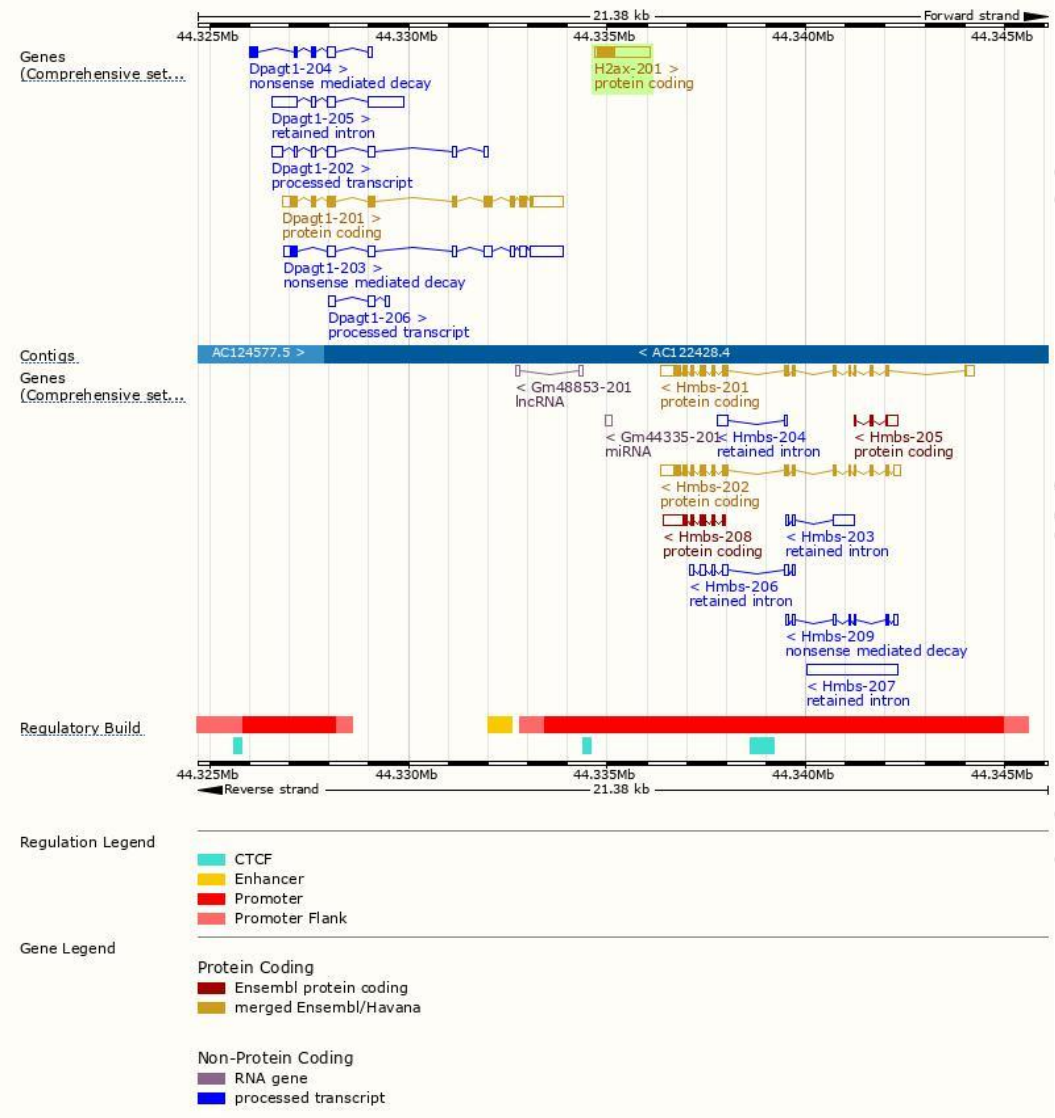
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
H2ax-201	ENSMUST00000052686.3	1384	143aa	Protein coding	CCDS23105	P27661	TSL:NA GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1

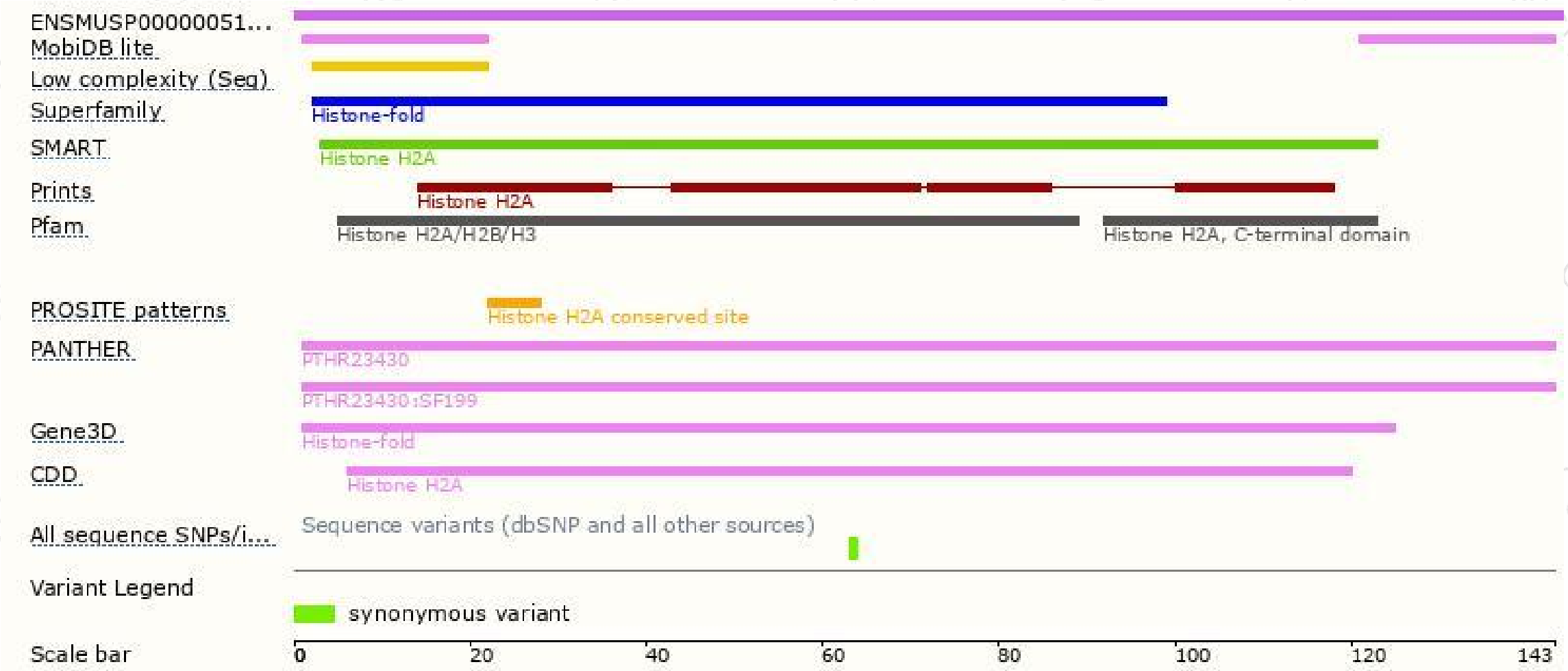
The strategy is based on the design of *H2ax-201* transcript,The transcription is shown below



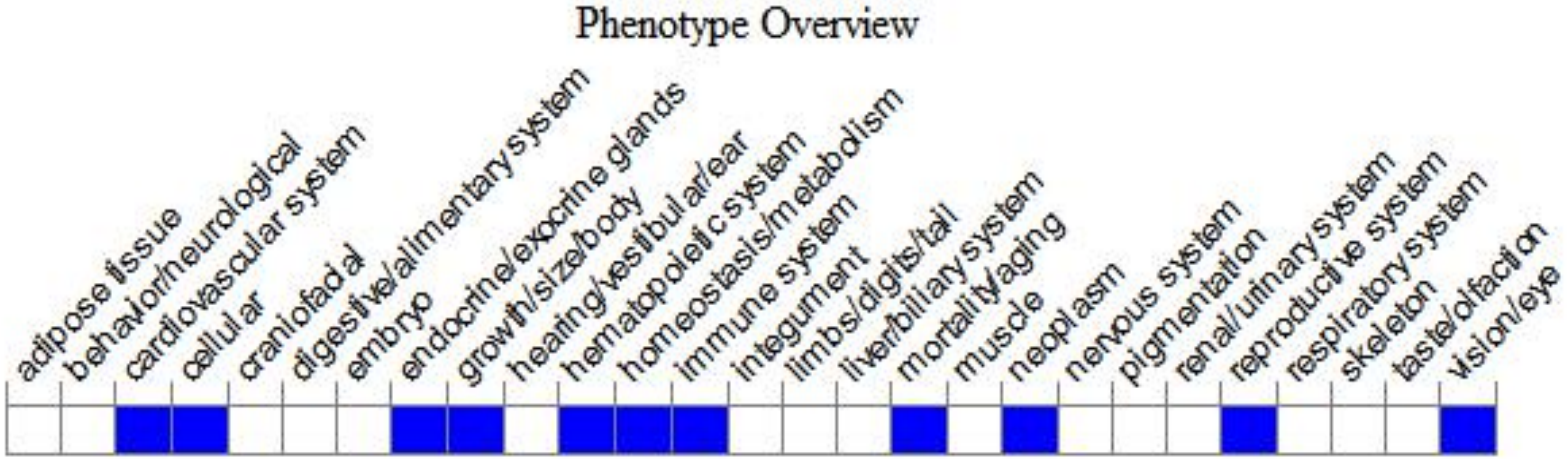
Genomic location distribution



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/).

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

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