Usf1 Cas9-CKO Strategy

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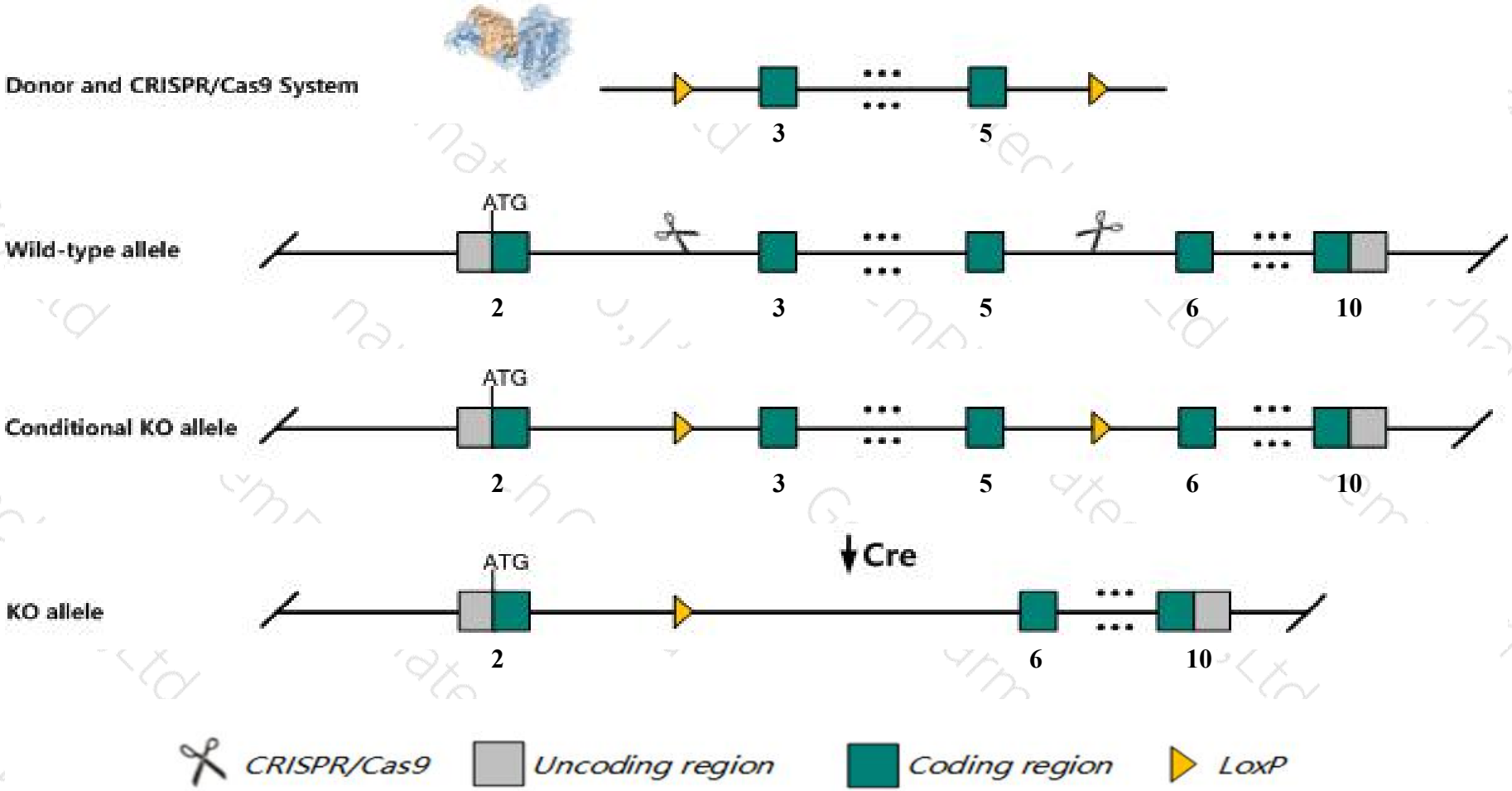
Project Overview

Project Name	***Usf1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Usf1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Usf1* gene has 10 transcripts. According to the structure of *Usf1* gene, exon3-exon5 of *Usf1-208*(ENSMUST00000161241.7) transcript is recommended as the knockout region.The region contains 268bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Usf1* 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 mutants exhibit slight behavioral abnormalities. Females exhibit barbering and some have seizures. This knockout mutation (heterozygous or homozygous) acts as an enhancer of a null mutation of Usf2, resulting in embryonic lethality.

➢ The Intron5 is only 573bp,loxp insertion may affect mRNA splicing.

➢ The floxed region is near to the N-terminal of *Tstd1* gene ,this strategy may influence the regulatory function of the N-terminal of *Tstd1* gene.

➢ Transcript *Usf1*-203&205 may not be affected.

➢ The *Usf1* 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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Usf1 upstream transcription factor 1 [Mus musculus (house mouse)]

Gene ID: 22278, updated on 13-Mar-2020

Summary

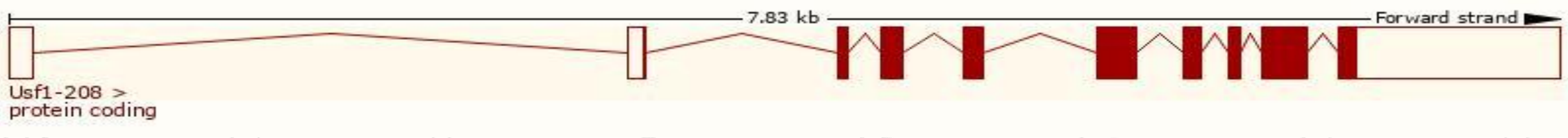
Official Symbol	Usf1 provided by MGI
Official Full Name	upstream transcription factor 1 provided byMGI
Primary source	MGI:MGI:99542
See related	Ensembl:ENSMUSG00000026641
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	bHLHb11
Summary	This protein encoded by this gene is a member of the basic-Helix-Hoop-Helix-Leucine zipper (bHLH-LZ) family and encodes a protein that can act as a transcription factor. Studies indicate that the basic region interacts with DNA at E-Box motifs, while the helix-loop-helix and leucine zipper domains are involved in dimerization with different partners. This protein is involved in a wide array of biological pathways, including cell cycle regulation, immune response, and responses to ultraviolet radiation. Mice lacking most of the coding exons of this gene often lacked both whiskers and nasal fur, and were prone to epileptic seizures, while mice lacking both this gene and another family member, Usf2, displayed embryonic lethality (PMID:9520440). Mutations in the human ortholog of this gene have been associated with Familial Combined Hyperlipidemia (FCHL) in humans. Pseudogenes of this gene are found on chromosome 11 and the X chromosome. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Mar 2015]
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 35.2), ovary adult (RPKM 33.6) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

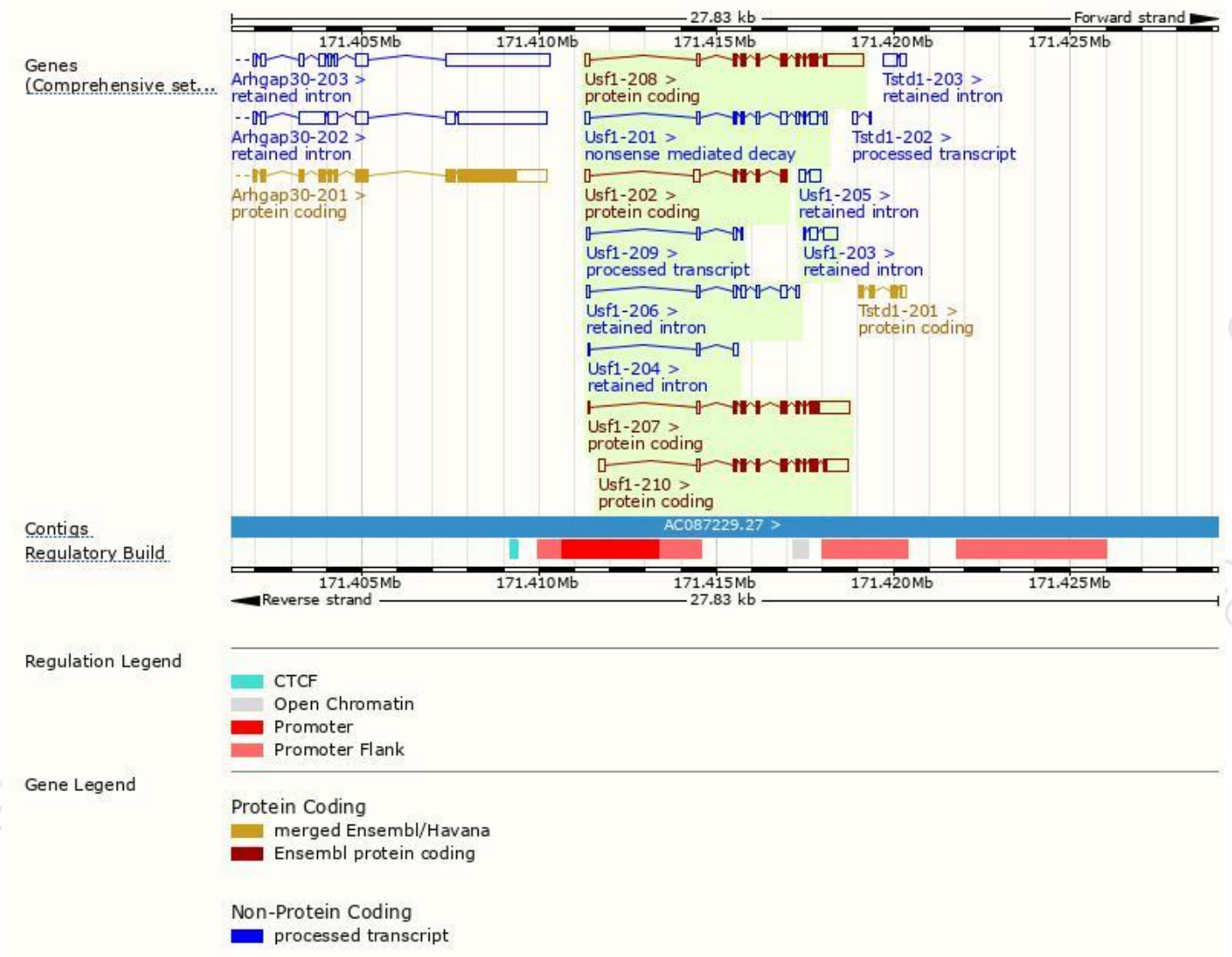
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Usf1-208	ENSMUST00000161241.7	2167	310aa	Protein coding	CCDS15495	Q3UQH7 Q61069	TSL:1 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
Usf1-210	ENSMUST00000167546.1	1822	310aa	Protein coding	CCDS15495	Q3UQH7 Q61069	TSL:5 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
Usf1-207	ENSMUST00000160486.7	1878	296aa	Protein coding	-	E9Q722	TSL:2 GENCODE basic
Usf1-202	ENSMUST00000159207.7	721	148aa	Protein coding	-	E0CYP7	CDS 3' incomplete TSL:2
Usf1-201	ENSMUST00000001284.12	1115	48aa	Nonsense mediated decay	-	F8WGH6	TSL:5
Usf1-209	ENSMUST00000161297.7	305	No protein	Processed transcript	-	-	TSL:3
Usf1-206	ENSMUST00000160335.7	779	No protein	Retained intron	-	-	TSL:3
Usf1-203	ENSMUST00000159371.1	696	No protein	Retained intron	-	-	TSL:3
Usf1-205	ENSMUST00000159929.1	492	No protein	Retained intron	-	-	TSL:3
Usf1-204	ENSMUST00000159466.1	269	No protein	Retained intron	-	-	TSL:3

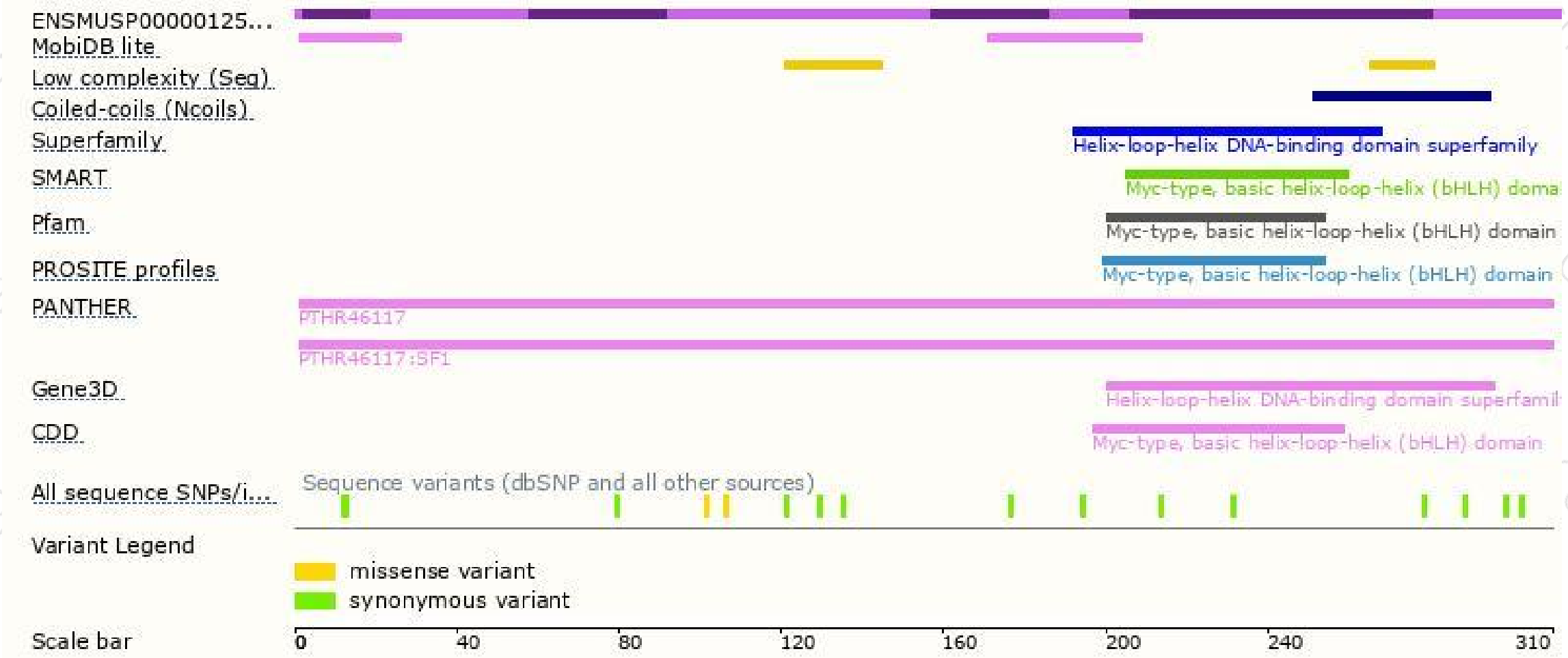
The strategy is based on the design of *Usf1-208* transcript,the transcription is shown below:



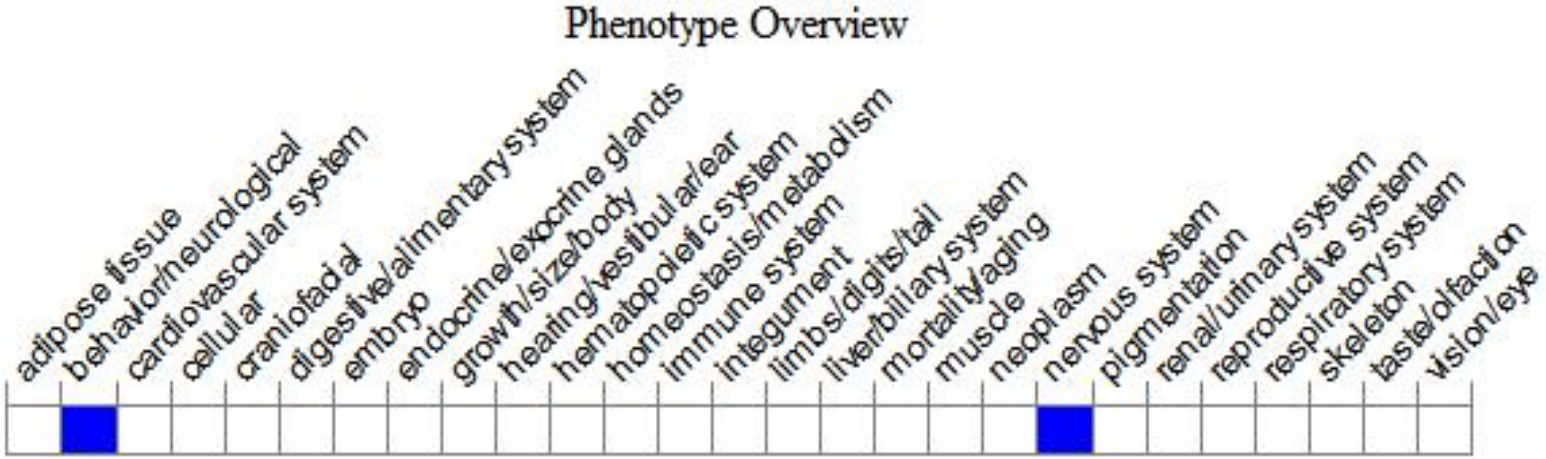
Genomic location distribution



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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