

Atf4 Cas9-CKO Strategy

Designer:

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Design Date:

2019-8-6

Project Overview

Project Name

Atf4

Project type

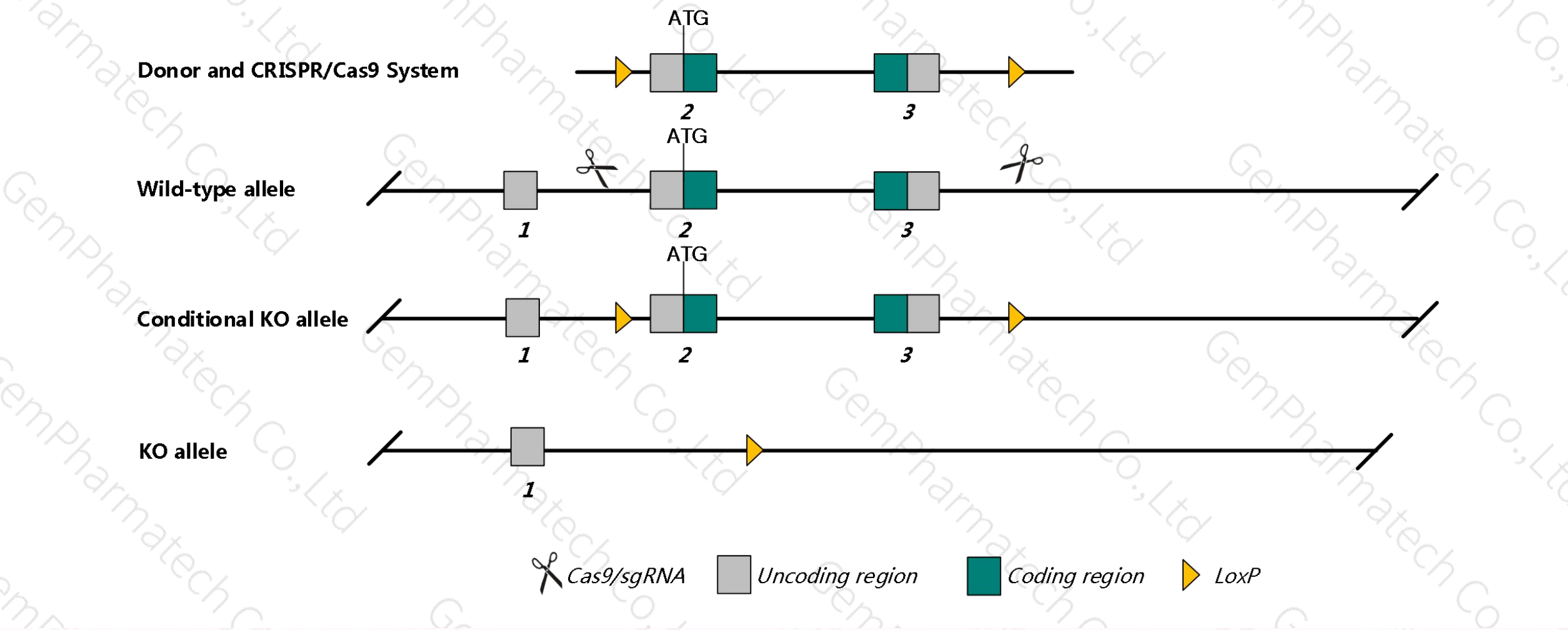
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Atf4* gene. The schematic diagram is as follows:



- The *Atf4* gene has 3 transcripts. According to the structure of *Atf4* gene, exon2-exon3 of *Atf4*-201 (ENSMUST00000109605.4) transcript is recommended as the knockout region. The region contains all coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Atf4* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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 or cell types.

- According to the existing MGI data , Mice homozygous for one knock-out allele exhibit postnatal lethality, abnormal lens development, and reduced male fertility. Mice homozygous for a different knock-out allele exhibit abnormal pancreatic and skeletal development, glucose homeostasis, and insulin homeostasis.
- The *Atf4* gene is located on the Chr15. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Atf4 activating transcription factor 4 [*Mus musculus* (house mouse)]



Gene ID: 11911, updated on 12-Mar-2019

Summary

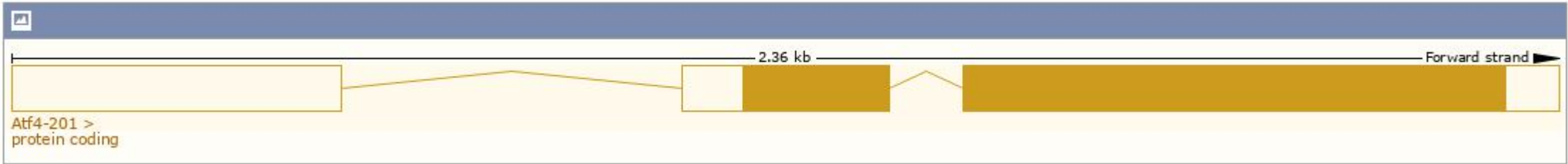
Official Symbol	Atf4 provided by MGI
Official Full Name	activating transcription factor 4 provided by MGI
Primary source	MGI:MGI:88096
See related	Ensembl:ENSMUSG000000042406
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Atf-4; C/ATF; CREB2; TAXREB67
Expression	Ubiquitous expression in liver E14 (RPKM 153.4), liver E14.5 (RPKM 138.1) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

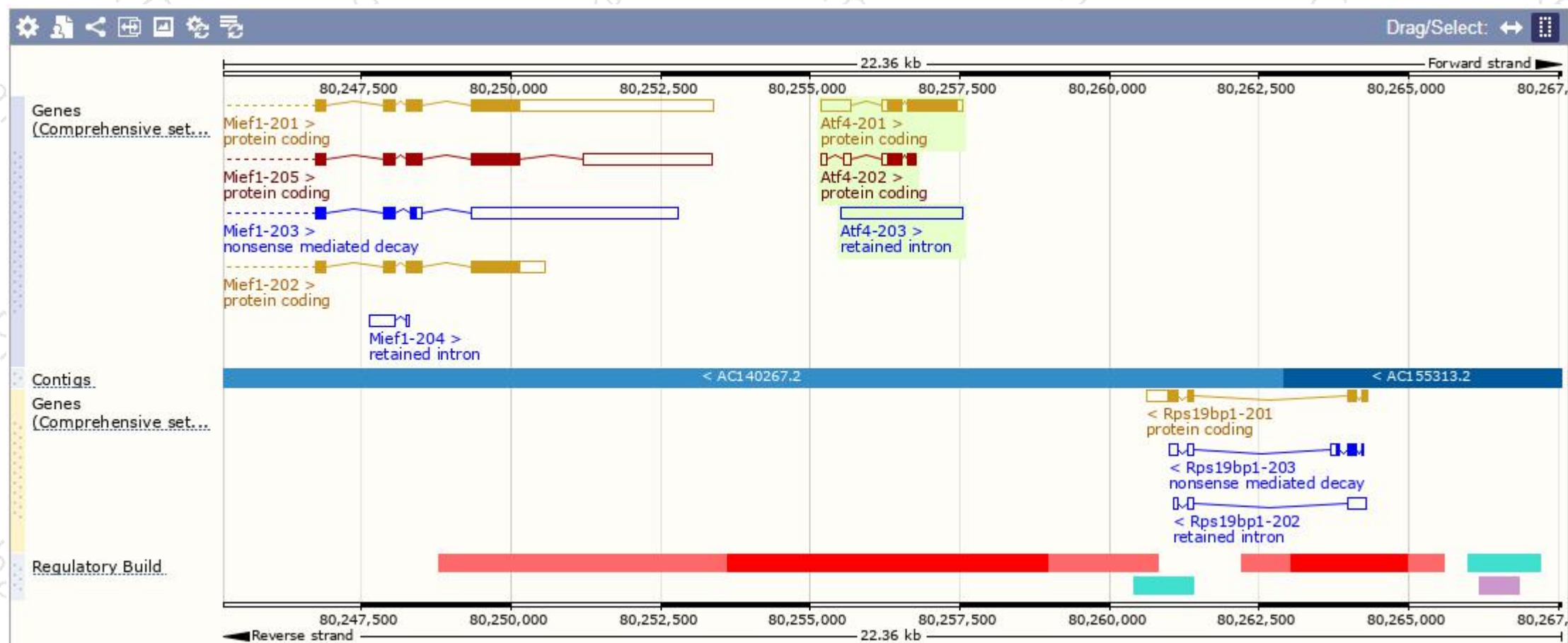
The gene has 3 transcripts, and all transcripts are shown below:

Show/hide columns (1 hidden)								Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags	
Atf4-201	ENSMUST00000109605.4	1725	349aa	 Protein coding	CCDS37145	Q06507	NM_001287180 NM_009716 NP_001274109 NP_033846	TSL:1	GENCODE basic APPRIS P1
Atf4-202	ENSMUST00000229828.1	659	116aa	 Protein coding	-	A0A2R8VI82	-	CDS 3' incomplete	
Atf4-203	ENSMUST00000230434.1	2019	No protein	 Retained intron	-	-	-		

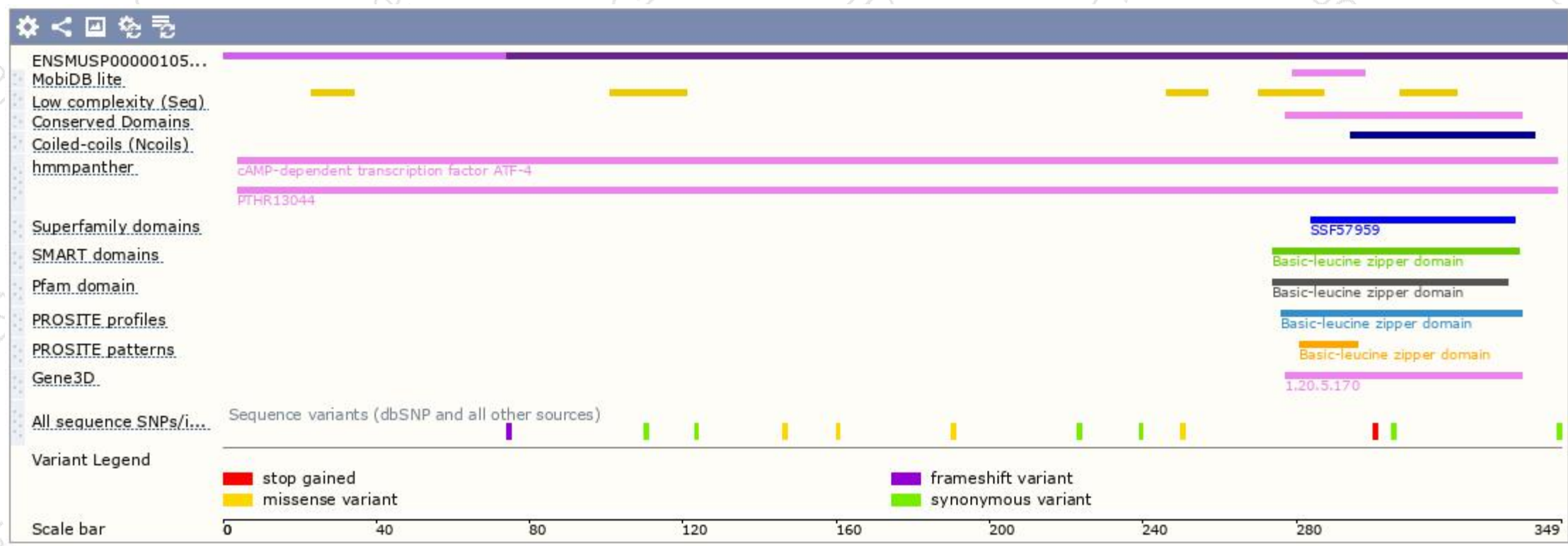
The strategy is based on the design of *Atf4*-201 transcript, The transcription is shown below



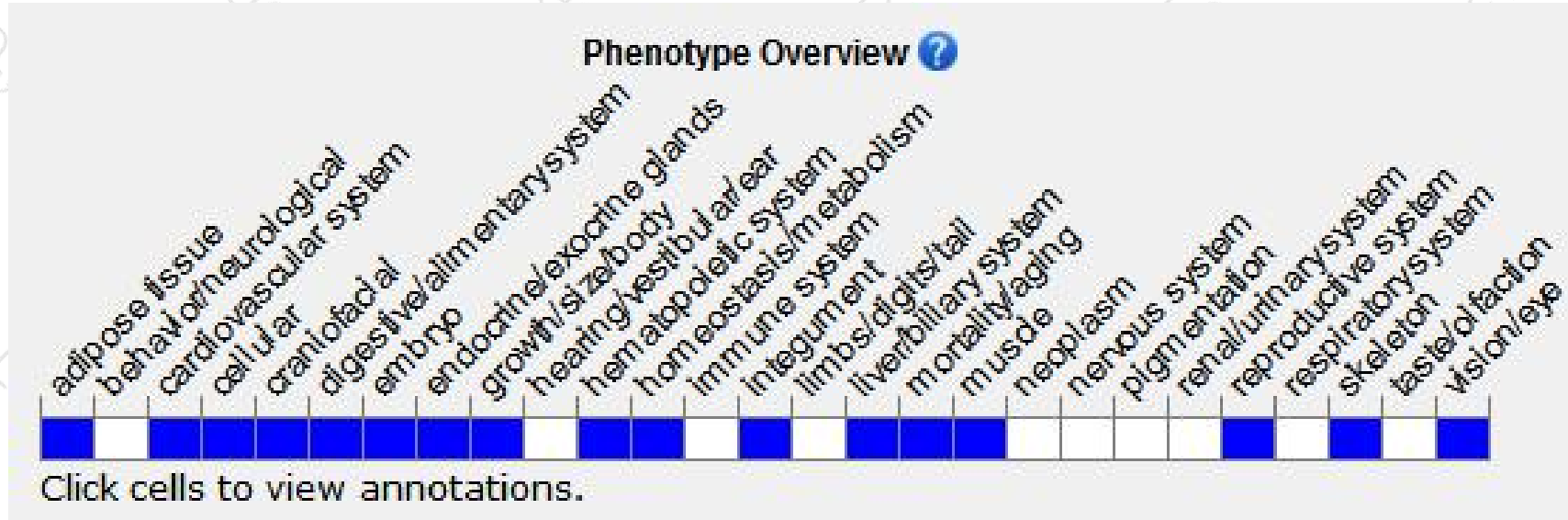
Genomic location distribution



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/>) .

According to the existing MGI data, Mice homozygous for one knock-out allele exhibit postnatal lethality, abnormal lens development, and reduced male fertility. Mice homozygous for a different knock-out allele exhibit abnormal pancreatic and skeletal development, glucose homeostasis, and insulin homeostasis.

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