

# ***Fto Cas9-CKO Strategy***

**Designer:**

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**Design Date:**

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# Project Overview

**Project Name**

*Fto*

**Project type**

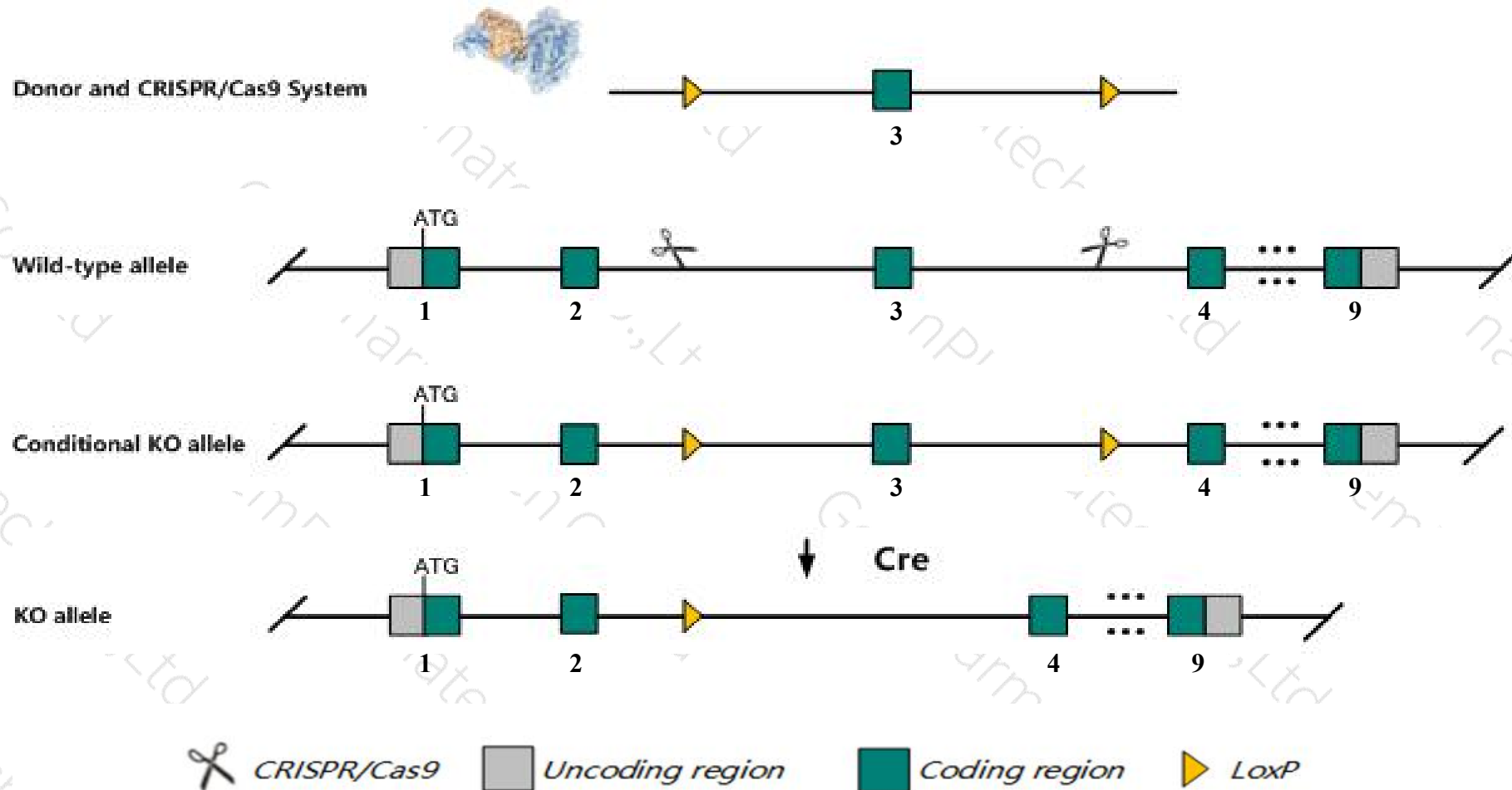
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

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



# Technical routes

- The *Fto* gene has 7 transcripts. According to the structure of *Fto* gene, exon3 of *Fto-201* (ENSMUST00000069718.14) transcript is recommended as the knockout region. The region contains 619bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Fto* 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.

- According to the existing MGI data, Mice homozygous for an ENU-induced or targeted knock-out allele exhibit decreased body weight, adipose tissue, and body fat and increased metabolism, serum lipids, and serum glucagon that may be gender and diet dependent.
- The *Fto* gene is located on the Chr8. 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)

## Fto fat mass and obesity associated [Mus musculus (house mouse)]

Gene ID: 26383, updated on 2-Apr-2019

### Summary



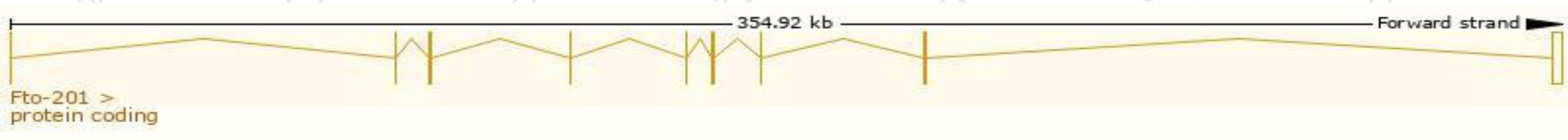
<b>Official Symbol</b>	Fto provided by <a href="#">MGI</a>
<b>Official Full Name</b>	fat mass and obesity associated provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1347093</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000055932</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	AW743446, mKIAA1752
<b>Expression</b>	Ubiquitous expression in cerebellum adult (RPKM 31.5), whole brain E14.5 (RPKM 30.4) and 28 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

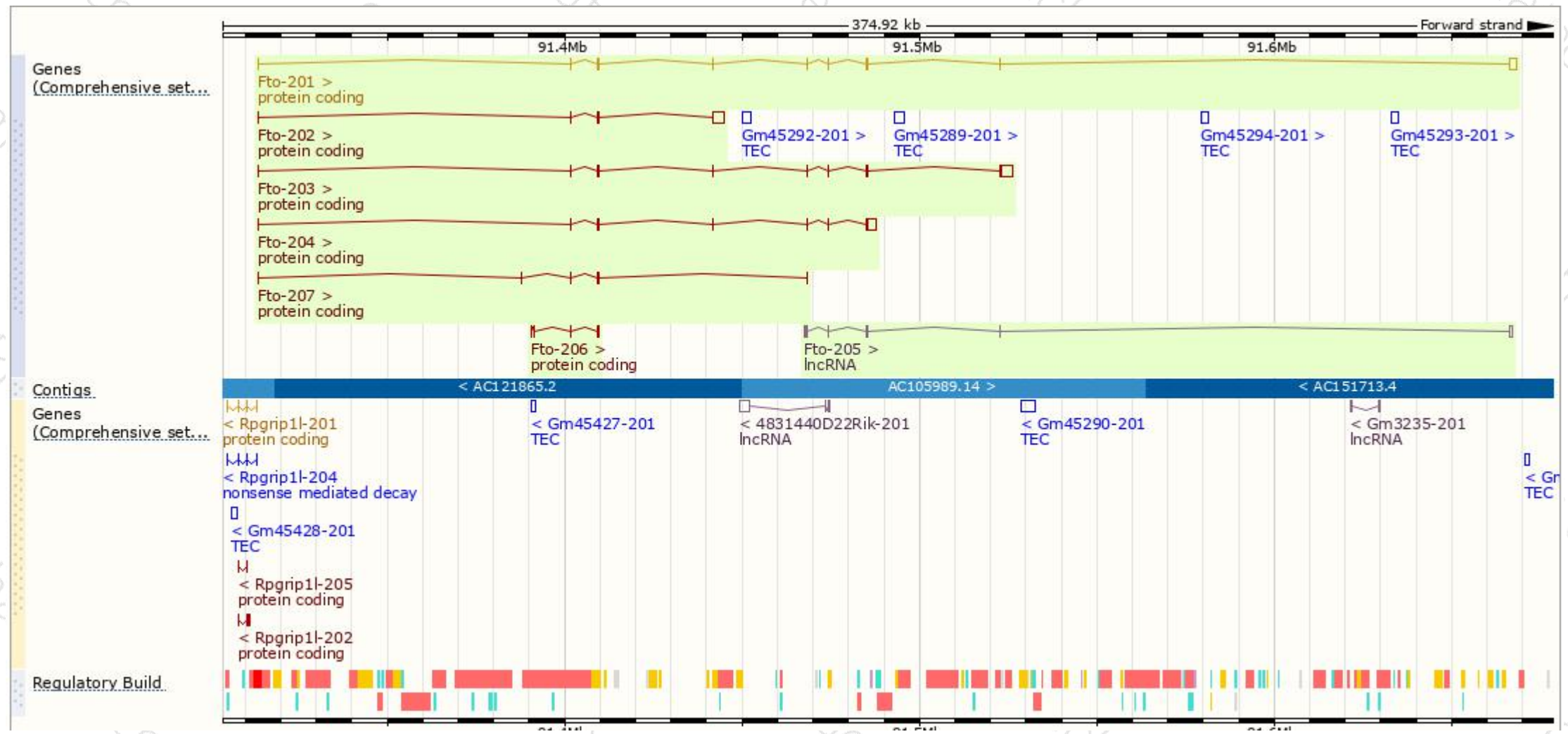
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fto-201	<a href="#">ENSMUST00000069718.14</a>	3564	<a href="#">502aa</a>	Protein coding	<a href="#">CCDS22521</a>	<a href="#">Q8BGW1</a>	TSL:1 Gencode basic APPRIS P1
Fto-203	<a href="#">ENSMUST00000128081.7</a>	4707	<a href="#">481aa</a>	Protein coding	-	<a href="#">Q8BGW1</a>	TSL:1 Gencode basic
Fto-202	<a href="#">ENSMUST00000125471.7</a>	3887	<a href="#">316aa</a>	Protein coding	-	<a href="#">Q8BGW1</a>	TSL:1 Gencode basic
Fto-204	<a href="#">ENSMUST00000136802.7</a>	3751	<a href="#">413aa</a>	Protein coding	-	<a href="#">Q8BGW1</a>	TSL:1 Gencode basic
Fto-207	<a href="#">ENSMUST00000166548.8</a>	819	<a href="#">270aa</a>	Protein coding	-	<a href="#">E9Q1E4</a>	CDS 3' incomplete TSL:5
Fto-206	<a href="#">ENSMUST00000149913.1</a>	762	<a href="#">149aa</a>	Protein coding	-	<a href="#">D3Z127</a>	CDS 3' incomplete TSL:3
Fto-205	<a href="#">ENSMUST00000140276.1</a>	1312	No protein	lncRNA	-	-	TSL:1

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

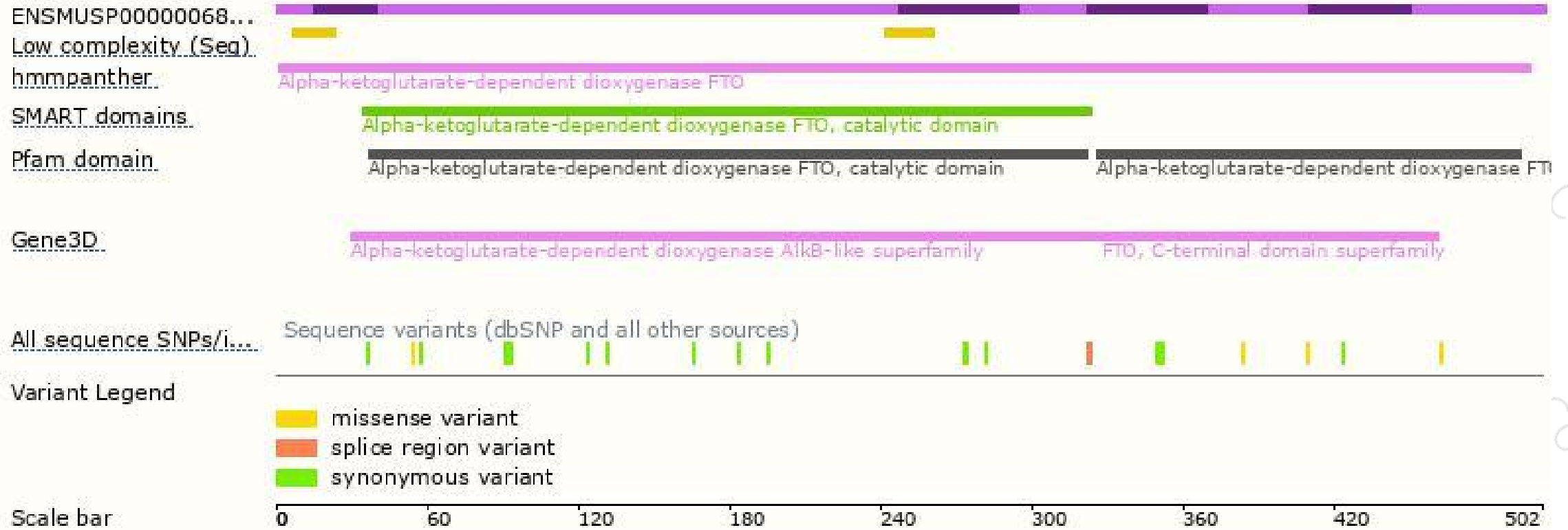


# Genomic location distribution

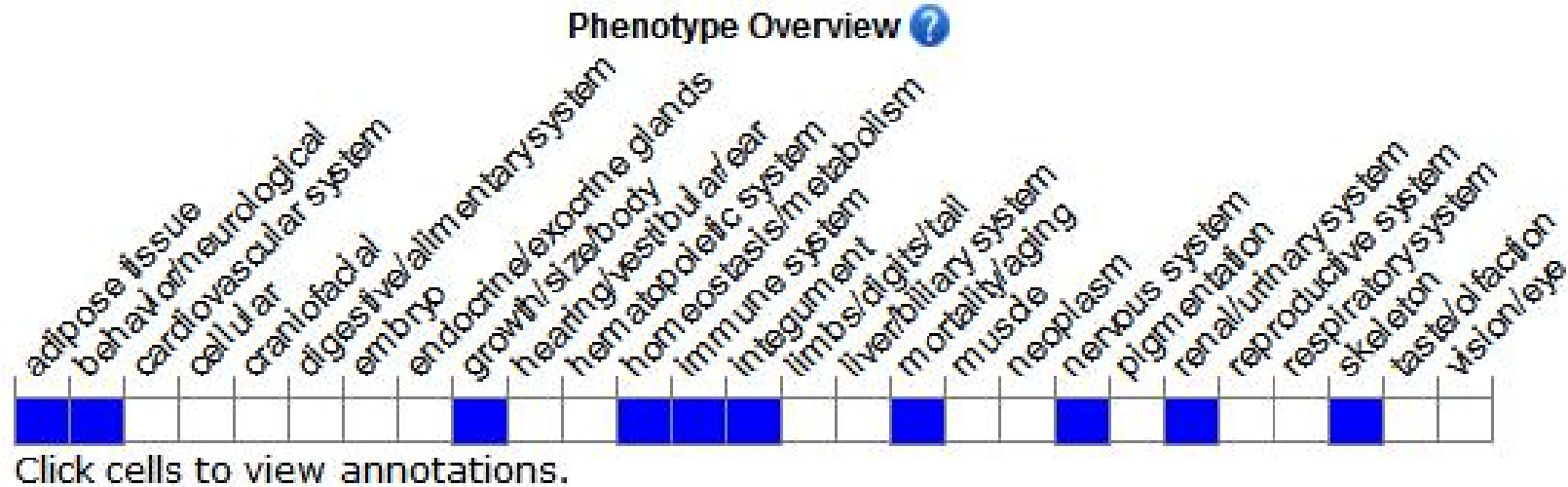




# Protein domain



# 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 an ENU-induced or targeted knock-out allele exhibit decreased body weight, adipose tissue, and body fat and increased metabolism, serum lipids, and serum glucagon that may be gender and diet dependent.

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

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