Otof-L1010P Mouse Model Strategy

-CRISPR/Cas9 technology

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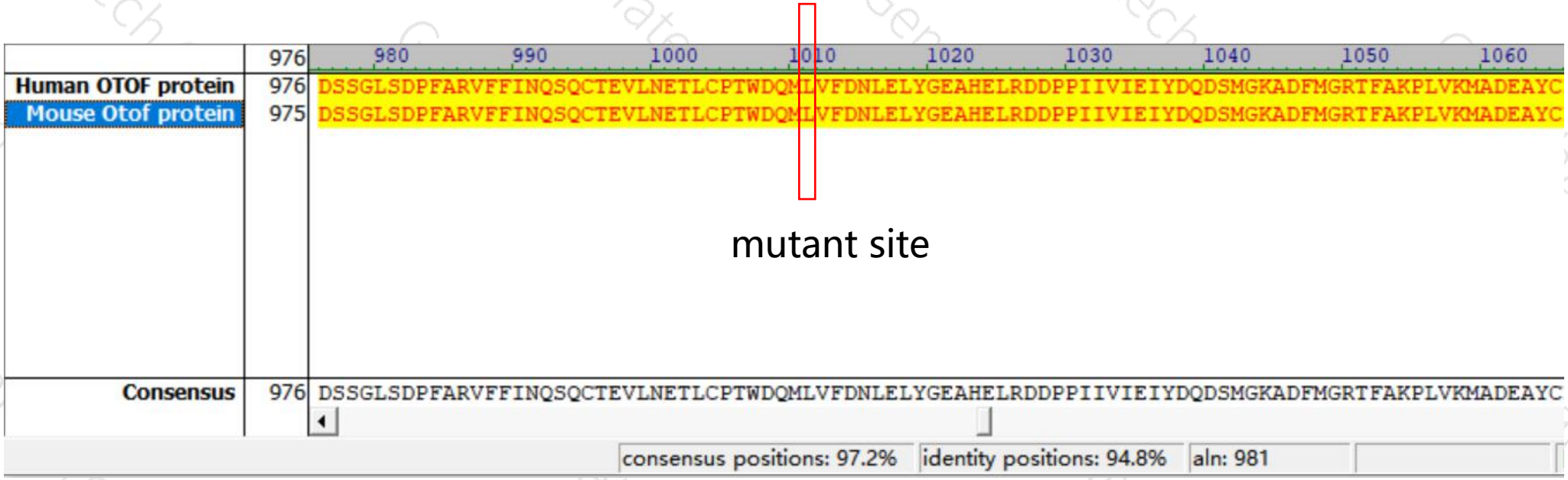
Project Overview

Project Name	**Otof-L1010P**
Project type	**Cas9-ki(PM)**
Strain background	**C57BL/6JGpt**

Technical Description

- The mouse *Otof* gene has 5 transcripts.The human *OTOF* gene has 7 transcripts.
- According to the structure of *Otof* gene and requirements of customer, the 1011th amino acid(Leu) of human *OTOF* gene corresponds to the 1010th amino acid(Leu) of mouse *Otof* gene after comparing homology of mouse *Otof* gene and human *Otof* gene.This project produced *Otof*-L1010P point mutation on exon25 of the transcript of *Otof*-201(ENSMUST00000074171.9, NM_031875.2). The 1010th amino acids will be mutated from L(Leu) to P(Pro), and the corresponding codon will be mutated to CCG by the CTG.
- The mouse *Otof*-201 transcript contains 47 exons. The translation initiation site ATG is located at exon1, and the translation termination site TGA is located at exon46, encoding 1997aa.
- In this project, *Otof* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

A comparison of the aa homology of human and mouse *Otof* gene



The 1011th amino acid(Leu) of human *Otof* gene corresponds to the 1010th amino acid(Leu) of mouse *Otof* gene after comparing homology of mouse *Otof* gene and human *OTOF* gene.

Mutation Site

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Before mutation

```
     +1                                                                                                V  L  N  E  T  L  C  P  T  W  D  Q  M  L  V  F?
81401  CAGCATGGGA ACAGTGGCTG CCACTTCTTC CCCATCCCTC ACCCCATGAT CAGGTTCTAA ACGAGACACT GTGTCCCACC TGGGACCAGA TGCTGGTATT
       GTCGTACCCT TGTCACCGAC GGTGAAGAAG GGGTAGGGAG TGGGGTACTA GTCCAAGATT TGCTCTGTGA CACAGGGTGG ACCCTGGTCT ACGACCATAA
     +1 ?F  D  N  L  E  L  Y  G  E  A  H  E  L  R  D  D  P  P  I  I  V  I  E  I  Y  D  Q  D  S  M
81501  TGACAACCTG GAGCTGTACG GTGAAGCTCA CGAGTTACGA GATGATCCCC CCATCATTGT CATTGAAATC TACGACCAGG ACAGCATGGT ATGGTGGGCA
       ACTGTTGGAC CTCGACATGC CACTTCGAGT GCTCAATGCT CTACTAGGGG GGTAGTAACA GTAACTTTAG ATGCTGGTCC TGTCGTACCA TACCACCCGT
```

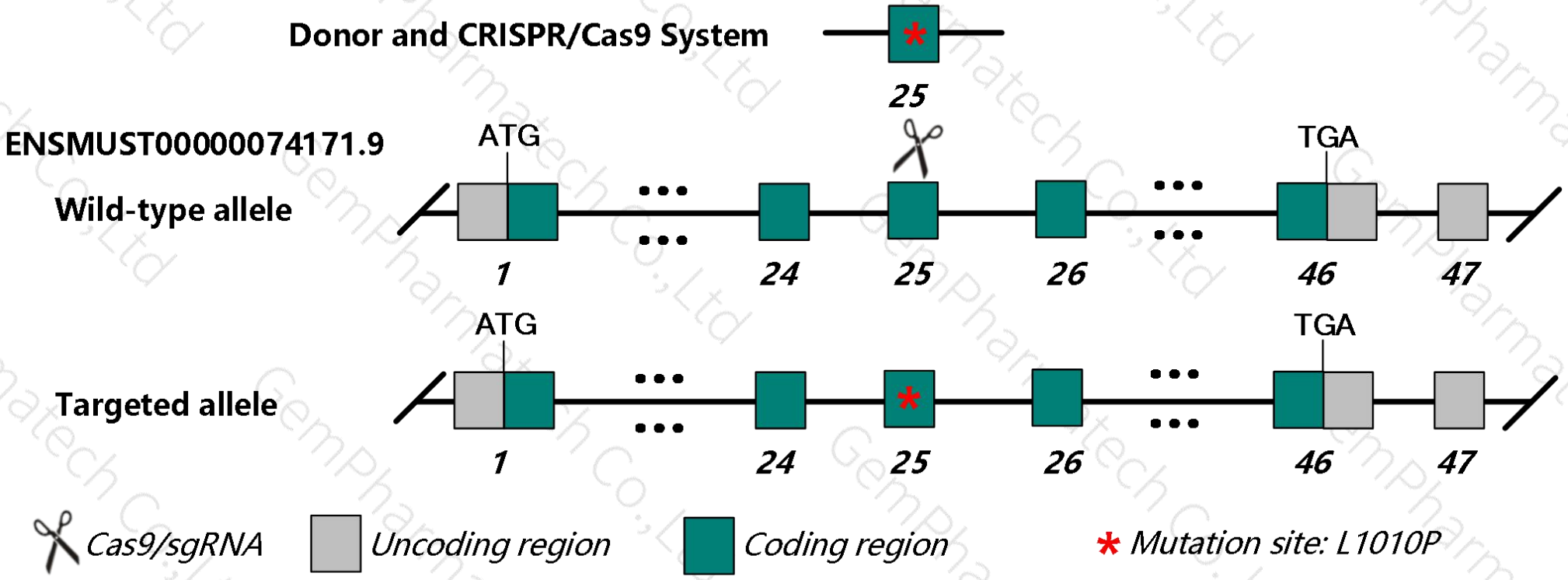
After mutation

```
     +1                                                                                                V  L  N  E  T  L  C  P  T  W  D  Q  M  P  V  F?
81401  CAGCATGGGA ACAGTGGCTG CCACTTCTTC CCCATCCCTC ACCCCATGAT CAGGTTCTAA ACGAGACACT GTGTCCCACC TGGGACCAGA TGCCGGTATT
       GTCGTACCCT TGTCACCGAC GGTGAAGAAG GGGTAGGGAG TGGGGTACTA GTCCAAGATT TGCTCTGTGA CACAGGGTGG ACCCTGGTCT ACGGCCATAA
     +1 ?F  D  N  L  E  L  Y  G  E  A  H  E  L  R  D  D  P  P  I  I  V  I  E  I  Y  D  Q  D  S  M
81501  TGACAACCTG GAGCTGTACG GTGAAGCTCA CGAGTTACGA GATGATCCCC CCATCATTGT CATTGAAATC TACGACCAGG ACAGCATGGT ATGGTGGGCA
       ACTGTTGGAC CTCGACATGC CACTTCGAGT GCTCAATGCT CTACTAGGGG GGTAGTAACA GTAACTTTAG ATGCTGGTCC TGTCGTACCA TACCACCCGT
```

The yellow region is exon25 of *Otof-201,* the red region represents the mutation site.

Strategy

This model uses CRISPR/Cas9 technology to edit the *Otof* gene and the schematic diagram is as follow:



Notice

- According to the data of MGI, Homozygous mutants have no detectable auditory brainstem response at any frequency tested. Otoacoustic transmission distortion products are detected. Direct electrical stimulation of cochlear ganglia elicits brainstem responses. On depolarization, inner hair cells release almost no neurotransmitter.
- One or Two synonymous mutations of amino acids will be intronduced on exon25 of *Otof*.
- Mouse *Otof* gene is located on Chr5. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr5, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene name and location （NCBI）

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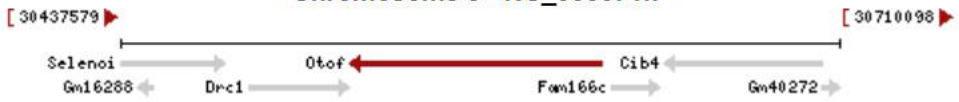
Otof otoferlin [*Mus musculus* (house mouse)]

Gene ID: 83762, updated on 17-Dec-2020

Summary

Official Symbol	Otof provided by MGI
Official Full Name	otoferlin provided by MGI
Primary source	MGI:MGI:1891247
See related	Ensembl:ENSMUSG00000062372
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
Expression	Biased expression in CNS E18 (RPKM 4.0), cortex adult (RPKM 3.4) and 9 other tissues See more
Orthologs	human all

Chromosome 5 - NC_000071.7

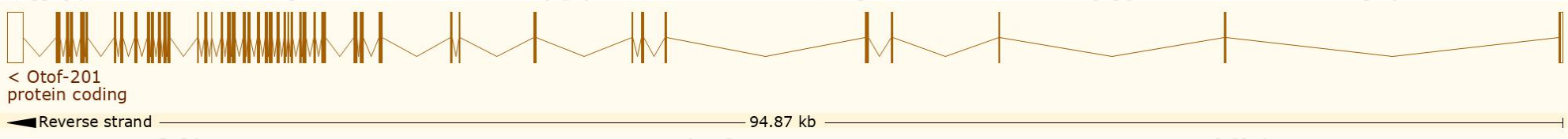


Transcript information（Ensembl）

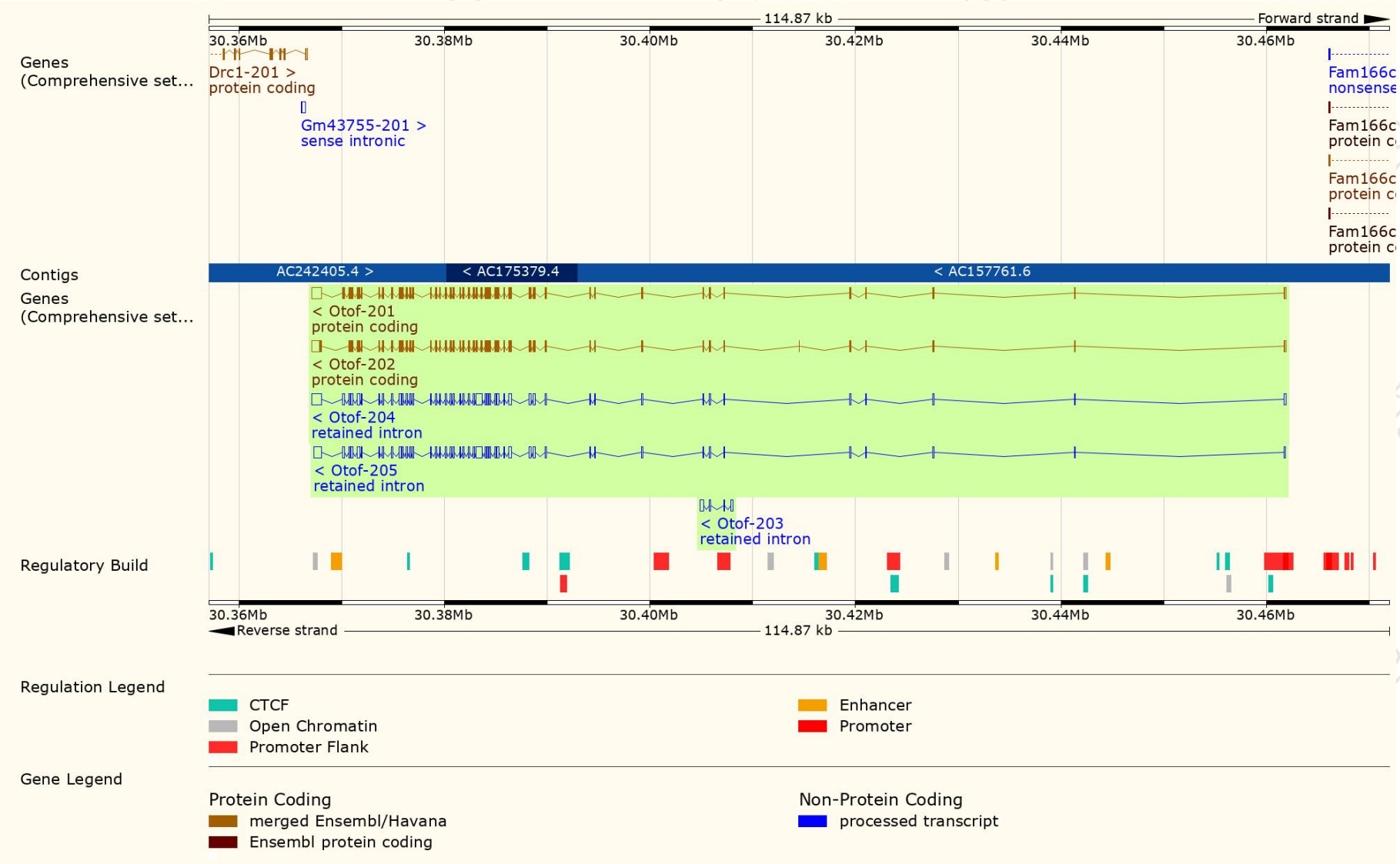
The gene has 5 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Otof-201	ENSMUST00000074171.9	7129	1997aa	Protein coding	CCDS19157	E9PYR6	TSL:1 GENCODE basic APPRIS P1
Otof-202	ENSMUST00000114747.8	6881	1992aa	Protein coding	CCDS51452	A0A0R4J1K2	TSL:1 GENCODE basic
Otof-203	ENSMUST00000128177.1	686	No protein	Retained intron	-	-	TSL:2
Otof-204	ENSMUST00000144125.7	7419	No protein	Retained intron	-	-	TSL:2
Otof-205	ENSMUST00000150734.7	7063	No protein	Retained intron	-	-	TSL:1

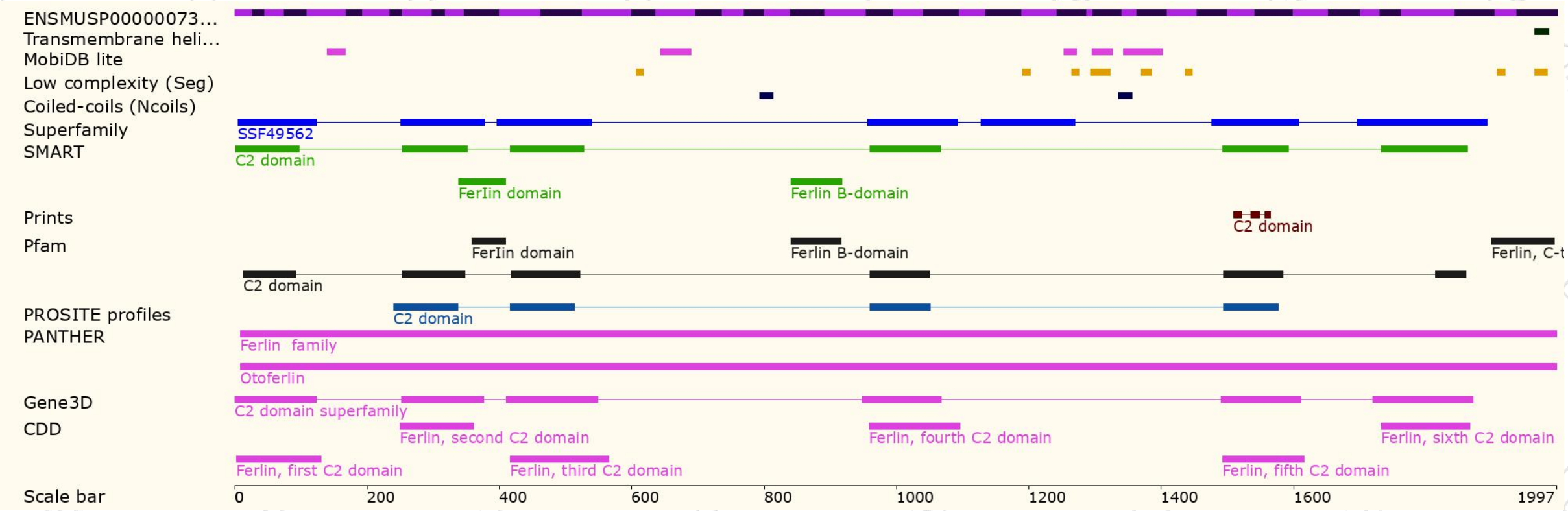
The strategy is based on the design of *Otof*-201 transcript, the transcription is shown below:



Genomic location distribution

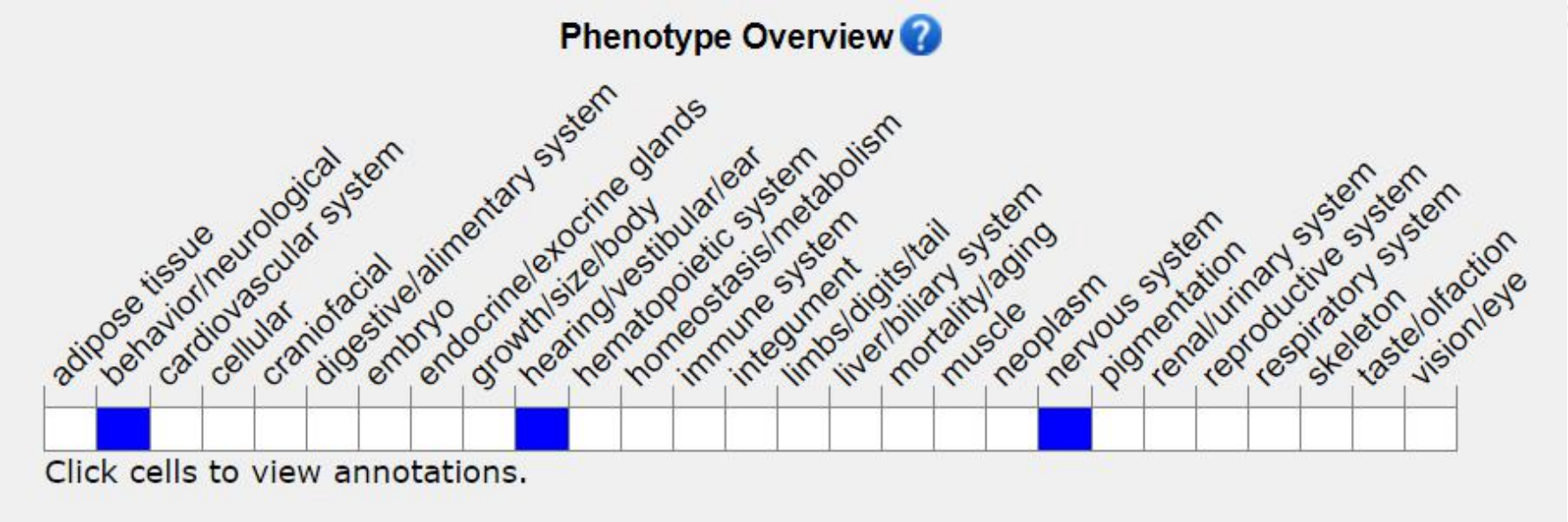


Protein domain



Mouse phenotype description(MGI)

URL link is as follows:
http://www.informatics.jax.org/marker/MGI:1891247



Homozygous mutants have no detectable auditory brainstem response at any frequency tested. Otoacoustic transmission distortion products are detected. Direct electrical stimulation of cochlear ganglia elicits brainstem responses. On depolarization, inner hair cells release almost no neurotransmitter.

If you have any questions, please feel free to contact us.
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