

Otof Cas9-CKO Strategy

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Project Overview

Project Name

Otof

Project type

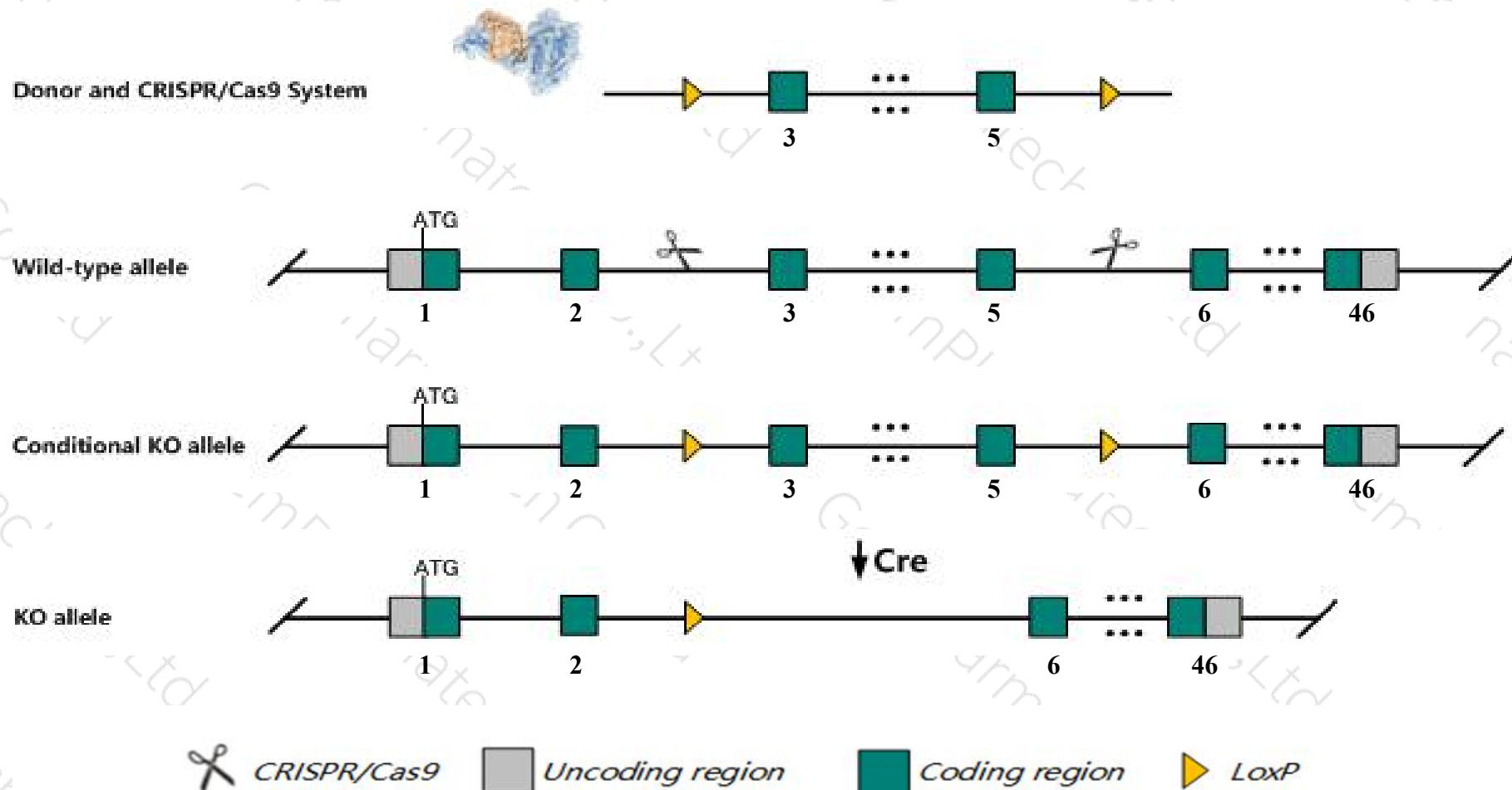
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Otof* gene has 5 transcripts. According to the structure of *Otof* gene, exon3-exon5 of *Otof-201* (ENSMUST00000074171.9) transcript is recommended as the knockout region. The region contains 368bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Otof* 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, 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.
- Transcript *Otof*-203 may not be affected.
- The *Otof* gene is located on the Chr5. 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)

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

Gene ID: 83762, updated on 19-Nov-2019

Summary

Official Symbol	Otof provided by MGI
Official Full Name	otoferlin provided by MGI
Primary source	MGI:MGI:1891247
See related	Ensembl:ENSMUSG000000062372
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

Genomic context

Location: 5; 5 B1

See Otof in [Genome Data Viewer](#)

Exon count: 49

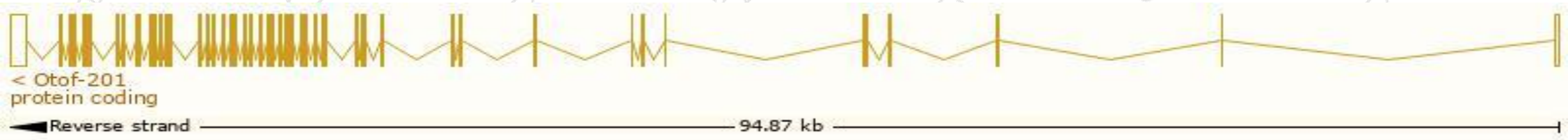
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	5	NC_000071.6 (30367066..30462730, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	5	NC_000071.5 (30669351..30764305, complement)

Transcript information (Ensembl)

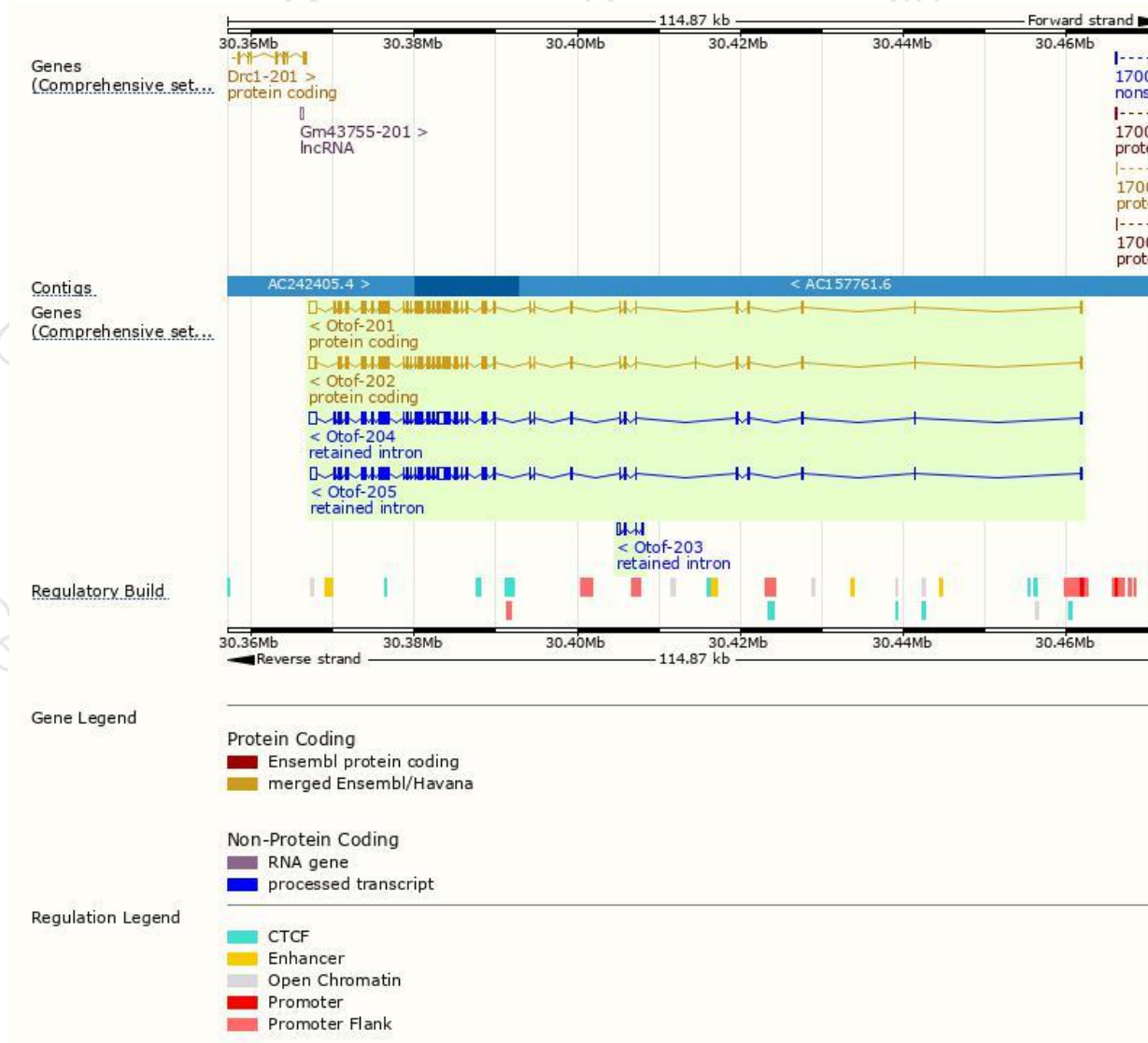
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	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-204	ENSMUST00000144125.7	7419	No protein	Retained intron	-	-	TSL:2
Otof-205	ENSMUST00000150734.7	7063	No protein	Retained intron	-	-	TSL:1
Otof-203	ENSMUST00000128177.1	686	No protein	Retained intron	-	-	TSL:2

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



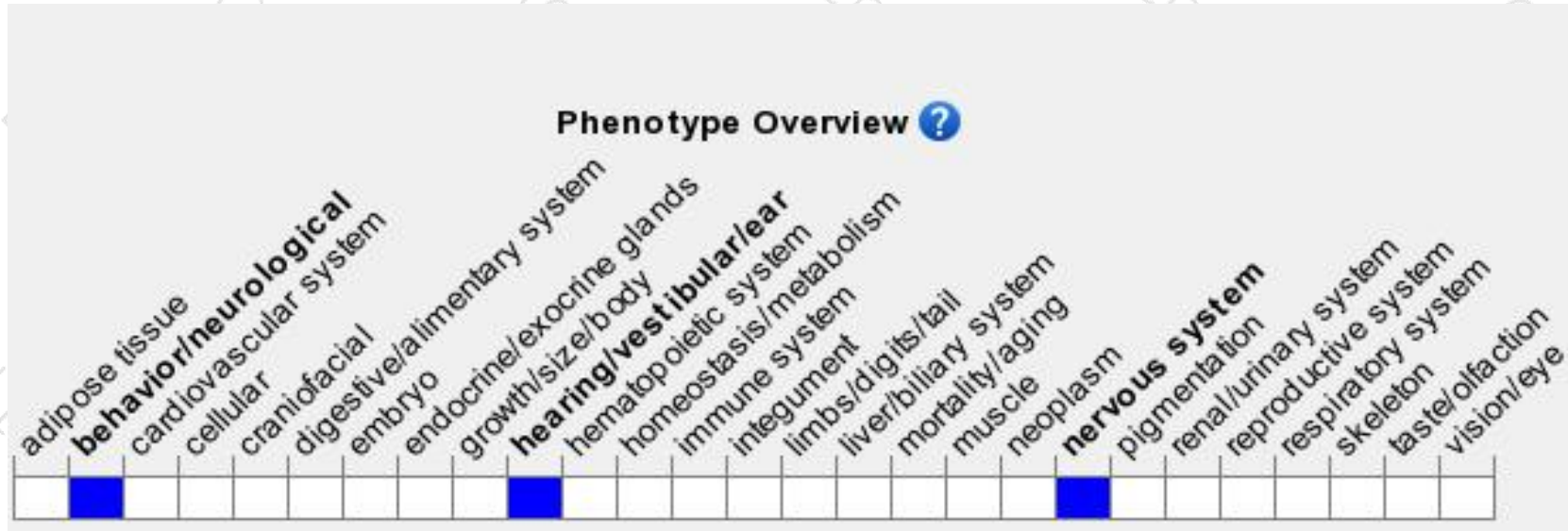
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



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, 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, you are welcome to inquire.

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