Ush2a-N2551S Mouse Model Strategy

-CRISPR/Cas9 technology

Designer: Yun Li

Reviewer: Longyun Hu

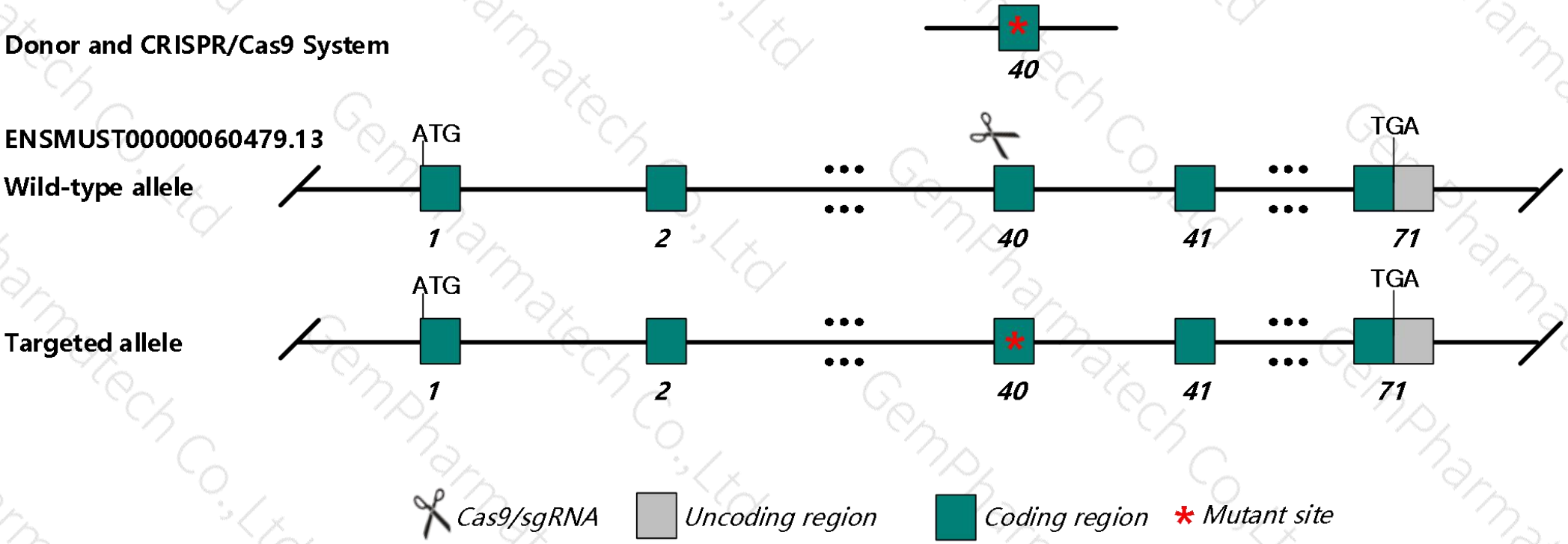
Design Date: 2021-1-25

Project Overview

Project Name	**Ush2a-N2551S**
Project type	**Cas9-ki(PM)**
Strain background	**C57BL/6JGpt**

Strategy

This model will use CRISPR/Cas9 technology to edit the *Ush2a* gene. The schematic diagram is as follows:



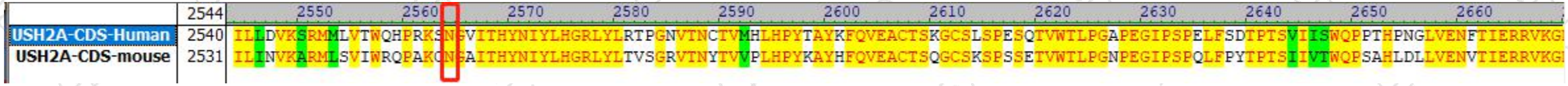
Technical routes

➢ The *Ush2a* gene has 5 transcripts.

➢ This project produced *Ush2a*-c.7652A＞G point mutation on exon 40 of the transcript of *Ush2a*-201(ENSMUST00000060479.13). The7652th nucleotide of *Ush2a* CDS is mutated from A to G, The 2551th amino acid will be mutated from N(Asn) to S(Ser).This mutation may cause congenital deafness.

➢ In this project we use CRISPR/Cas9 technology to modify *Ush2a* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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.

Analysis of Homology



Human *USH2A* p.N2560S-Mouse *Ush2a* p.N2551S

consensus positions: 79.0% identity positions: 71.2%

Mutation Site

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Before mutation

```
485001  TTCATTTGAG TTTTATTGTG GAGAGTCTGT GGGAAGTTTG TAAGTCCATG ACTTCCTGGC TGTGTTCTTC TTTCTAGAGC CTGGACCTAT AGATGCTCCA
        AAGTAAACTC AAAATAACAC CTCTCAGACA CCCTTCAAAC ATTCAGGTAC TGAAGGACCG ACACAAGAAG AAAGATCTCG GACCTGGATA TCTACGAGGT

485101  ATTCTTATCA ATGTGAAAGC TAGGATGTTG TCGGTCATCT GGAGGCAGCC TGCAAAGTGC AATGGGGCCA TTACCCATTA TAACATCTAC CTGCATGGCC
        TAAGAATAGT TACACTTTCG ATCCTACAAC AGCCAGTAGA CCTCCGTCGG ACGTTTCACG TTACCCCGGT AATGGGTAAT ATTGTAGATG GACGTACCGG
```

After mutation

```
485001  TTCATTTGAG TTTTATTGTG GAGAGTCTGT GGGAAGTTTG TAAGTCCATG ACTTCCTGGC TGTGTTCTTC TTTCTAGAGC CTGGACCTAT AGATGCTCCA
        AAGTAAACTC AAAATAACAC CTCTCAGACA CCCTTCAAAC ATTCAGGTAC TGAAGGACCG ACACAAGAAG AAAGATCTCG GACCTGGATA TCTACGAGGT

485101  ATTCTTATCA ATGTGAAAGC TAGGATGTTG TCGGTCATCT GGAGGCAGCC TGCAAAGTGC AGTGGGGCCA TTACCCATTA TAACATCTAC CTGCATGGCC
        TAAGAATAGT TACACTTTCG ATCCTACAAC AGCCAGTAGA CCTCCGTCGG ACGTTTCACG TCACCCCGGT AATGGGTAAT ATTGTAGATG GACGTACCGG
```

The red region is exon40 of *Ush2a*-201, and the blue region represents the c.7652A＞G mutation site.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-out allele display progressive retinal photoreceptor degeneration along with significantly reduced a- and b-wave amplitudes, and a moderate but nonprogressive high-frequency hearing loss associated with widespread loss of outer hair cells in the basal turn of the cochlea.

➢ One or two synonymous mutations of amino acids will be intronduced on exon40 of *Ush2a*.

➢ The *Ush2a* gene is located on the Chr1. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr1, 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 information （NCBI）

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Ush2a usherin [Mus musculus (house mouse)]

Gene ID: 22283, updated on 13-Mar-2020

Summary

Official Symbol	Ush2a provided by MGI
Official Full Name	usherin provided by MGI
Primary source	MGI:MGI:1341292
See related	Ensembl:ENSMUSG00000026609
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	A930011D15Rik, A930037M10Rik, Gm676, Gm983, Mush2a, Ushrn
Expression	Biased expression in testis adult (RPKM 1.3) and ovary adult (RPKM 0.0) See more
Orthologs	human all

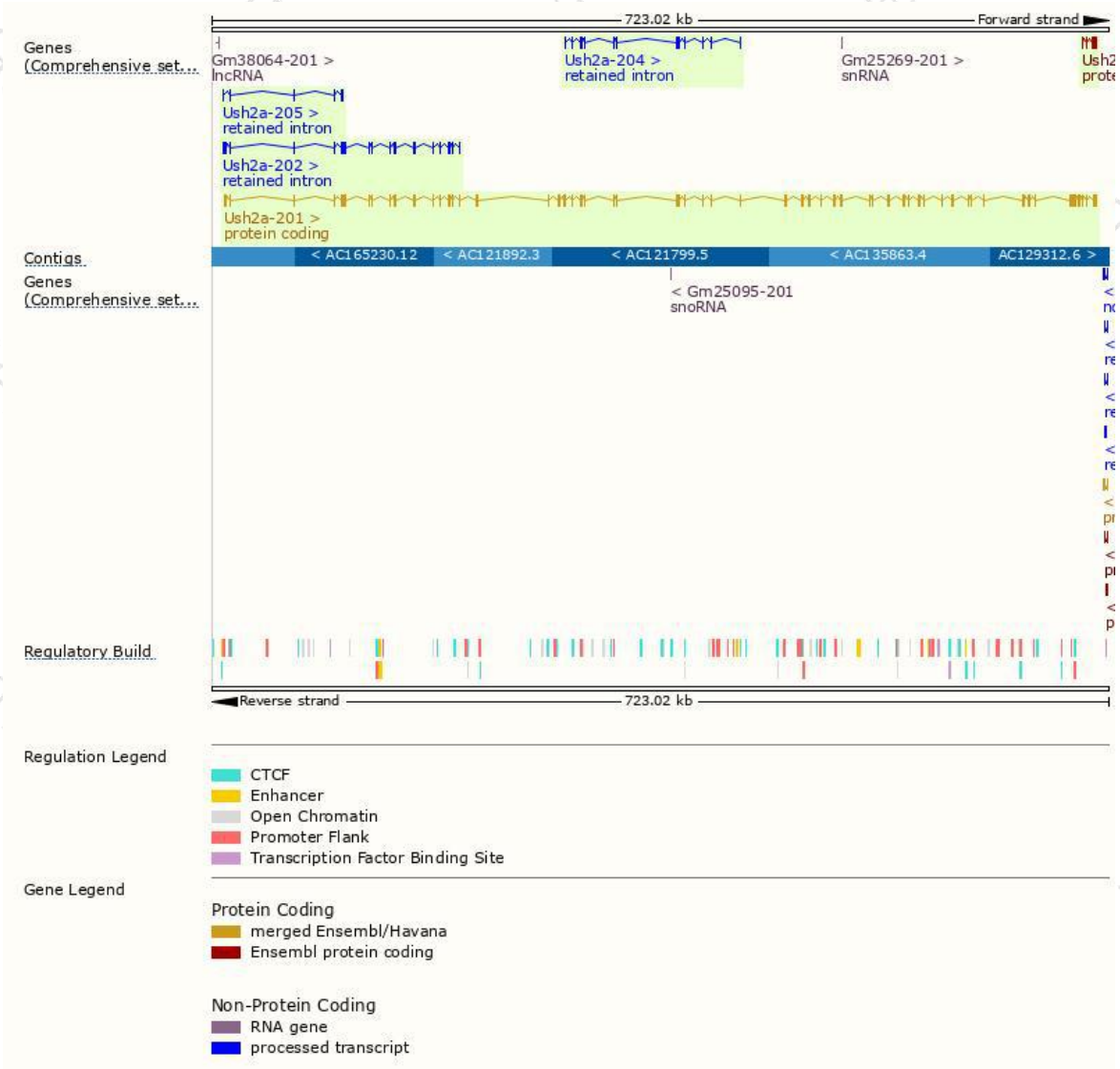
Transcript information（Ensembl）

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

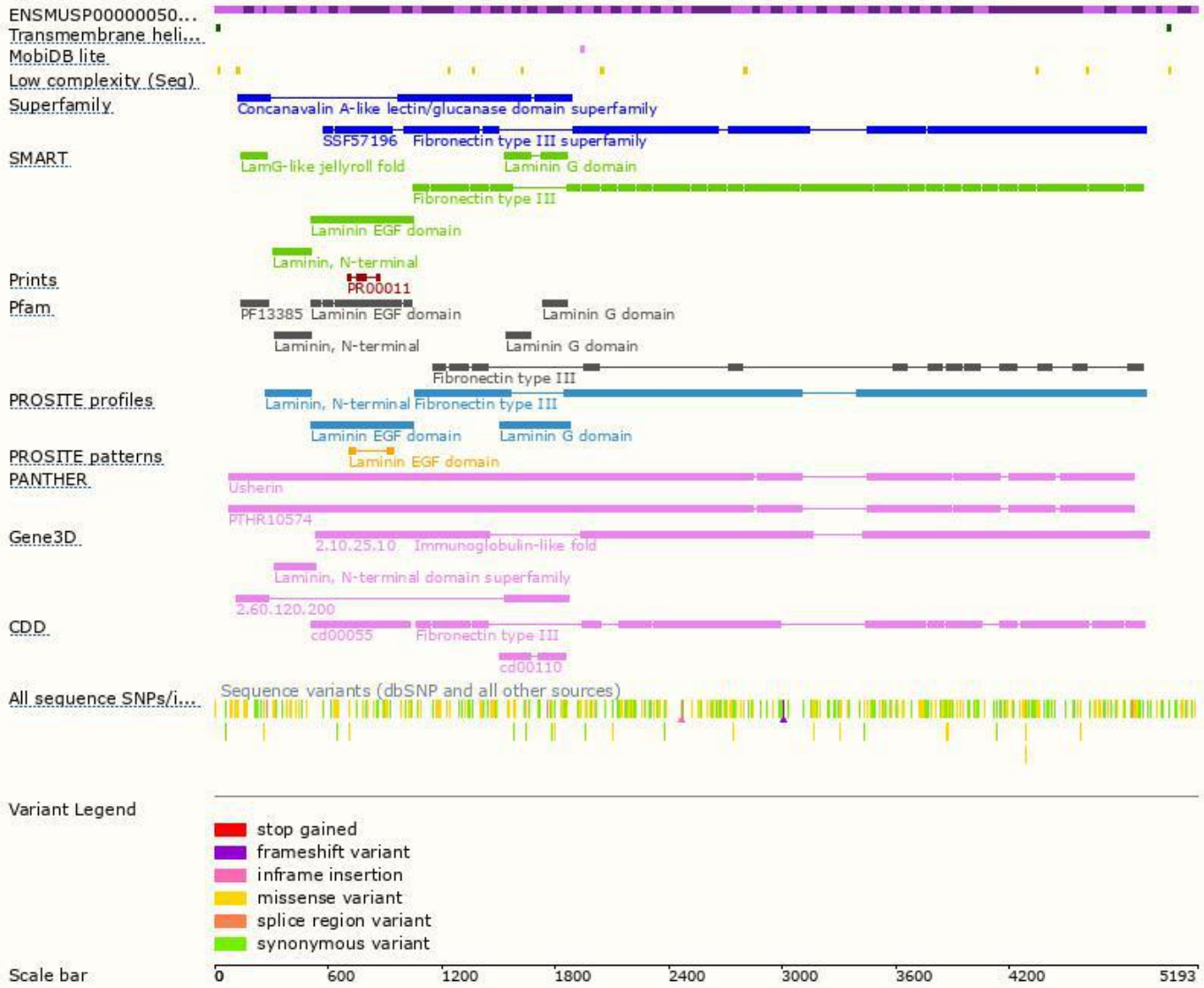
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ush2a-201	ENSMUST00000060479.13	15695	5193aa	Protein coding	CCDS15607	Q2QI47	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Ush2a-203	ENSMUST00000127077.1	750	237aa	Protein coding	-	F6TQ19	CDS 5' incomplete TSL:5
Ush2a-202	ENSMUST00000124358.1	5126	No protein	Retained intron	-	-	TSL:1
Ush2a-204	ENSMUST00000142159.1	2282	No protein	Retained intron	-	-	TSL:1
Ush2a-205	ENSMUST00000142189.7	1236	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Ush2a-201* transcript,the transcription is shown below:

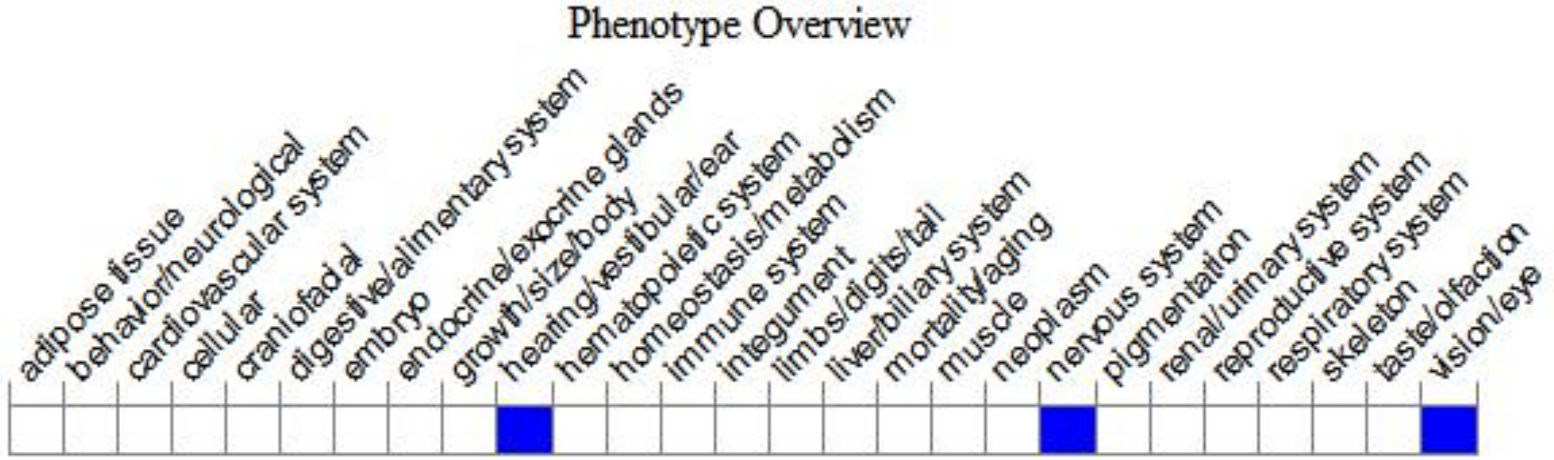
Genomic location distribution



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 a knock-out allele display progressive retinal photoreceptor degeneration along with significantly reduced a- and b-wave amplitudes, and a moderate but nonprogressive high-frequency hearing loss associated with widespread loss of outer hair cells in the basal turn of the cochlea.

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

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