

# **Bsnd** Cas9-CKO Strategy

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Reviewer: Xiaojing Li

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# **Project Overview**



**Project Name** 

**Bsnd** 

**Project type** 

Cas9-CKO

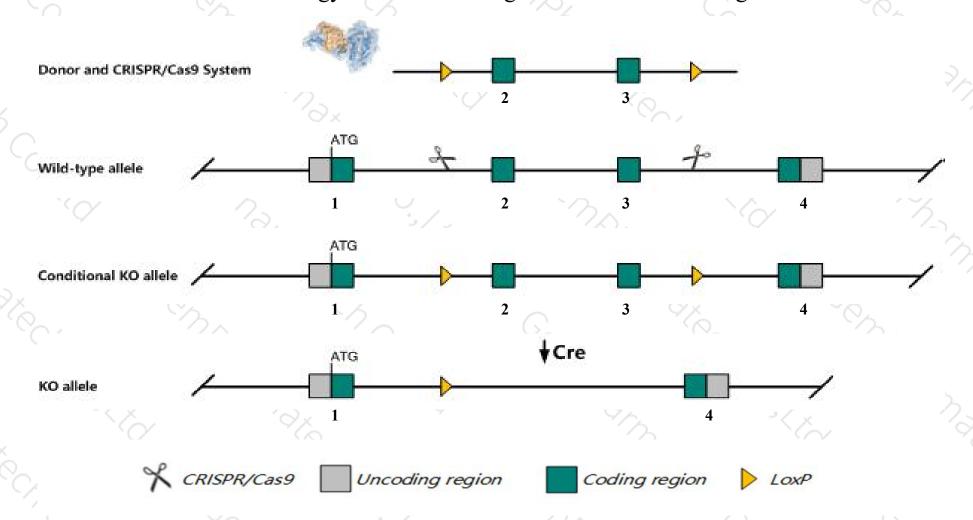
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



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



### Technical routes



- ➤ The *Bsnd* gene has 1 transcript. According to the structure of *Bsnd* gene, exon2-exon3 of *Bsnd*201(ENSMUST0000054472.3) transcript is recommended as the knockout region. The region contains 347bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Bsnd* 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.

### **Notice**



- > According to the existing MGI data, mice homozygous for a knock-out allele exhibit severe dehydration and postnatal lethality. Mice homozygous for a cre-activated conditional allele exhibit hearing loss with outer hair cell and stria vascularis degeneration.
- The flox region overlap with part of the AL954352.1 gene, which may affect the regulation of this gene.
- > The *Bsnd* gene is located on the Chr4. 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)



#### Bsnd barttin CLCNK type accessory beta subunit [Mus musculus (house mouse)]

Gene ID: 140475, updated on 13-Mar-2020

#### Summary

↑ ?

Official Symbol Bsnd provided by MGI

Official Full Name barttin CLCNK type accessory beta subunit provided by MGI

Primary source MGI:MGI:2153465

See related Ensembl: ENSMUSG00000025418

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 Restricted expression toward kidney adult (RPKM 30.7)See more

Orthologs <u>human</u> all

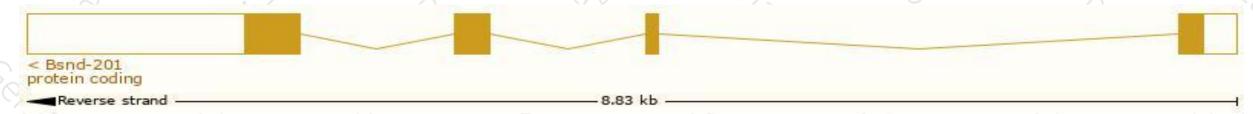
# Transcript information (Ensembl)



The gene has 1 transcript, and the transcript is shown below:

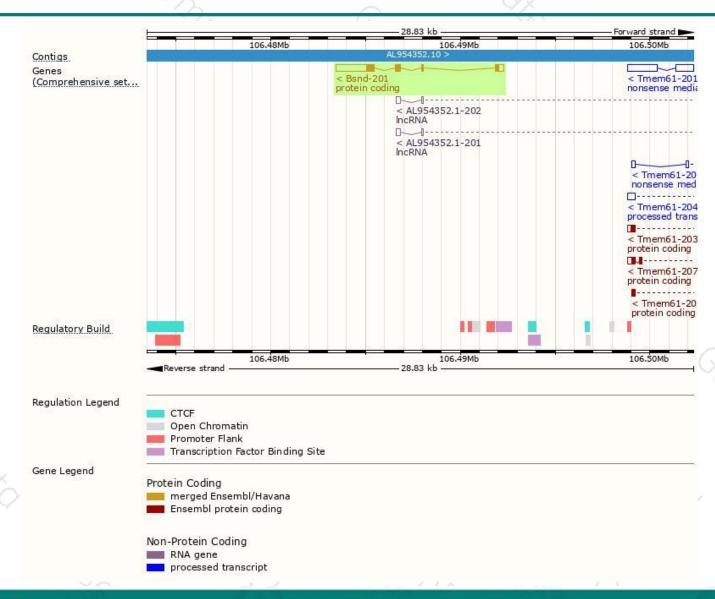
| Name     | Transcript ID        | bp   | Protein | Biotype        | CCDS      | UniProt | Flags                         |   |
|----------|----------------------|------|---------|----------------|-----------|---------|-------------------------------|---|
| Bsnd-201 | ENSMUST00000054472.3 | 2756 | 307aa   | Protein coding | CCDS18419 | Q8VIM4  | TSL:1 GENCODE basic APPRIS P1 | L |

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



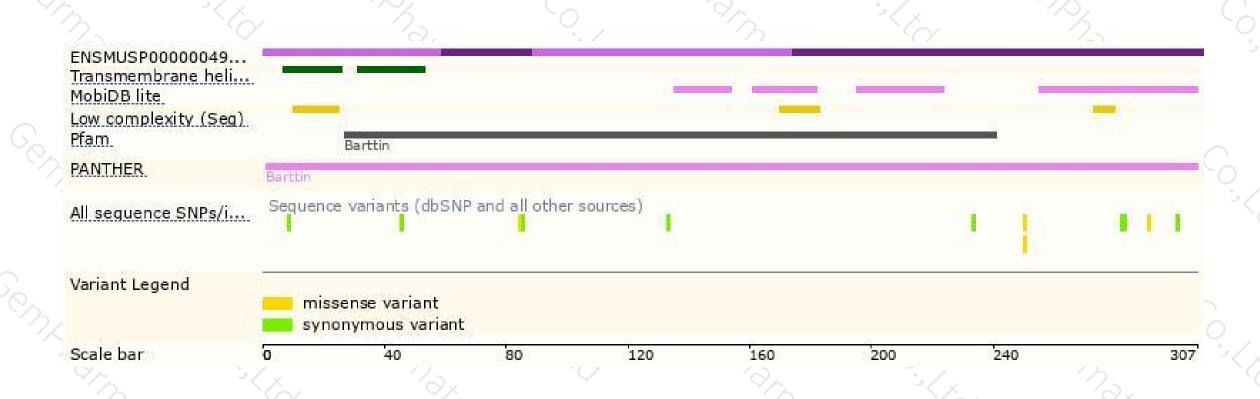
### Genomic location distribution





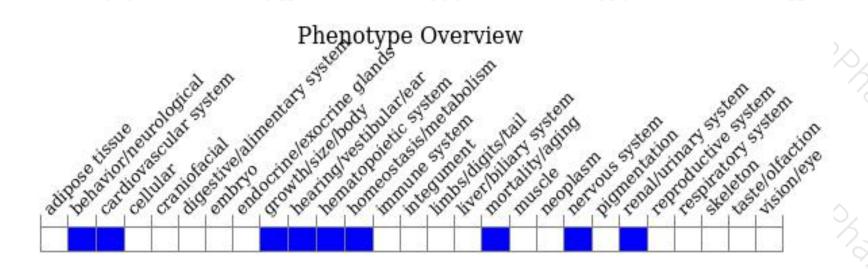
### Protein domain





# 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 exhibit severe dehydration and postnatal lethality. Mice homozygous for a cre-activated conditional allele exhibit hearing loss with outer hair cell and stria vascularis degeneration.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





