

# Zbed3 Cas9-CKO Strategy

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Reviewer: Daohua Xu

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# **Project Overview**



Project Name Zbed3

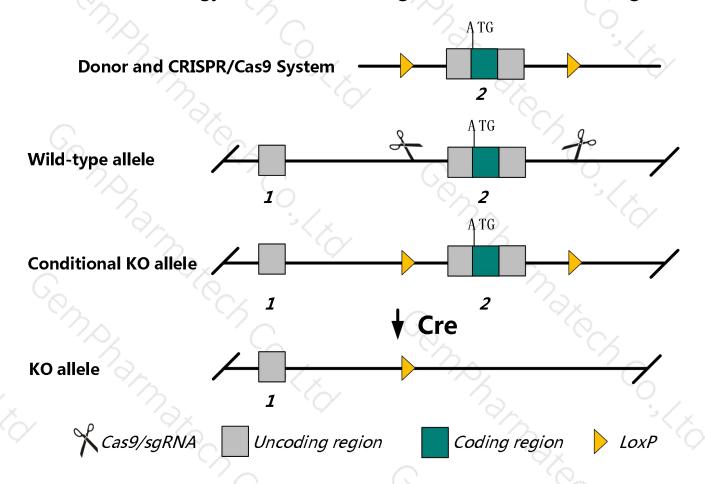
Project type Cas9-CKO

Strain background C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Zbed3* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Zbed3* gene has 3 transcripts. According to the structure of *Zbed3* gene, exon2 of *Zbed3*202(ENSMUST00000221807.1) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Zbed3* 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.
- > The flox mice was 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**



- > The *Zbed3* gene is located on the Chr13. 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)



#### Zbed3 zinc finger, BED type containing 3 [Mus musculus (house mouse)]

Gene ID: 72114, updated on 13-Mar-2020

#### Summary

↑ ?

Official Symbol Zbed3 provided by MGI

Official Full Name zinc finger, BED type containing 3 provided by MGI

Primary source MGI:MGI:1919364

See related Ensembl:ENSMUSG00000041995

Gene type protein coding
RefSeq status REVIEWED
Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as 0610037K01Rik, 2610005H11Rik, AU018975, AU024588

Summary This gene encodes a member of the zinc finger protein superfamily. This protein may regulate the Wnt/beta-catenin signaling pathway.

This protein may be involved in insulin resistance and type 2 diabetes in humans. Alternative splicing results in multiple transcript

variants. [provided by RefSeq, Oct 2014]

Expression Broad expression in ovary adult (RPKM 63.5), limb E14.5 (RPKM 22.0) and 22 other tissues See more

Orthologs <u>human</u> all

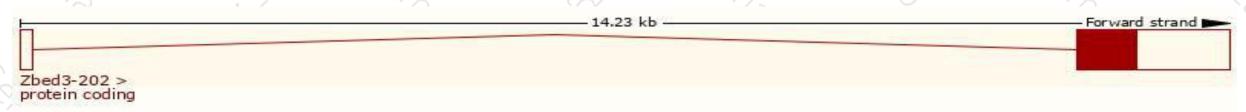
# Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

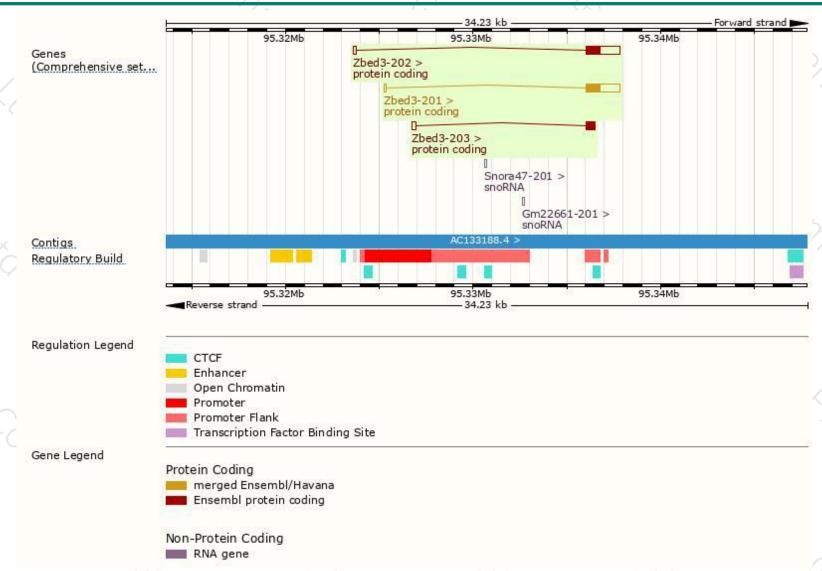
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zbed3-202	ENSMUST00000221807.1	1945	228aa	Protein coding	CCDS26696	Q9D0L1	TSL:1 GENCODE basic APPRIS P1
Zbed3-201	ENSMUST00000045909.7	1894	228aa	Protein coding	CCDS26696	Q9D0L1	TSL:1 GENCODE basic APPRIS P1
Zbed3-203	ENSMUST00000222456.1	650	<u>152aa</u>	Protein coding	2	A0A1Y7VIM9	CDS 3' incomplete TSL:2

The strategy is based on the design of *Zbed3-202* transcript, the transcription is shown below:



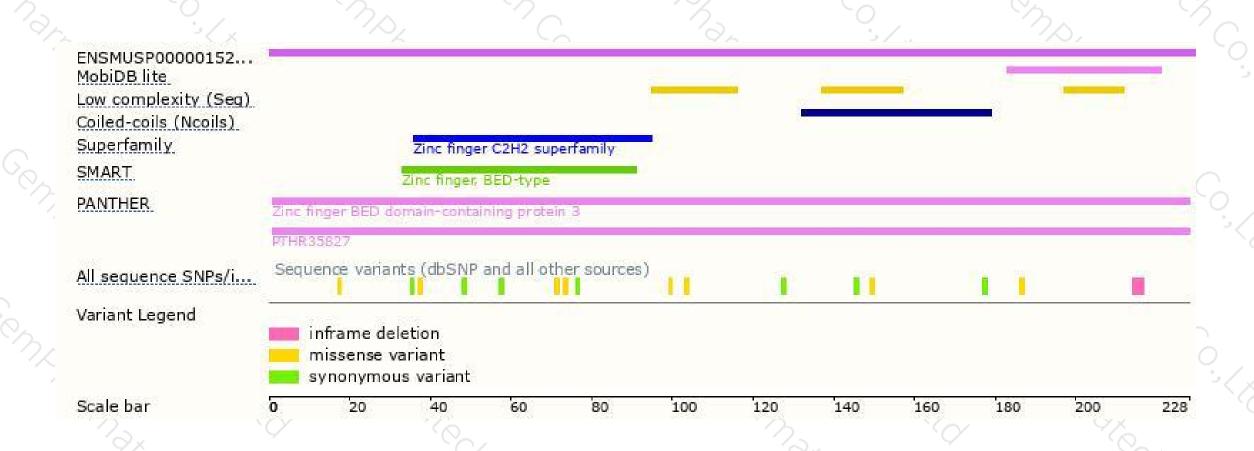
### Genomic location distribution





### Protein domain







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

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