# Aspn Cas9-CKO Strategy

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# **Project Overview**



**Project Name** 

Aspn

**Project type** 

Cas9-CKO

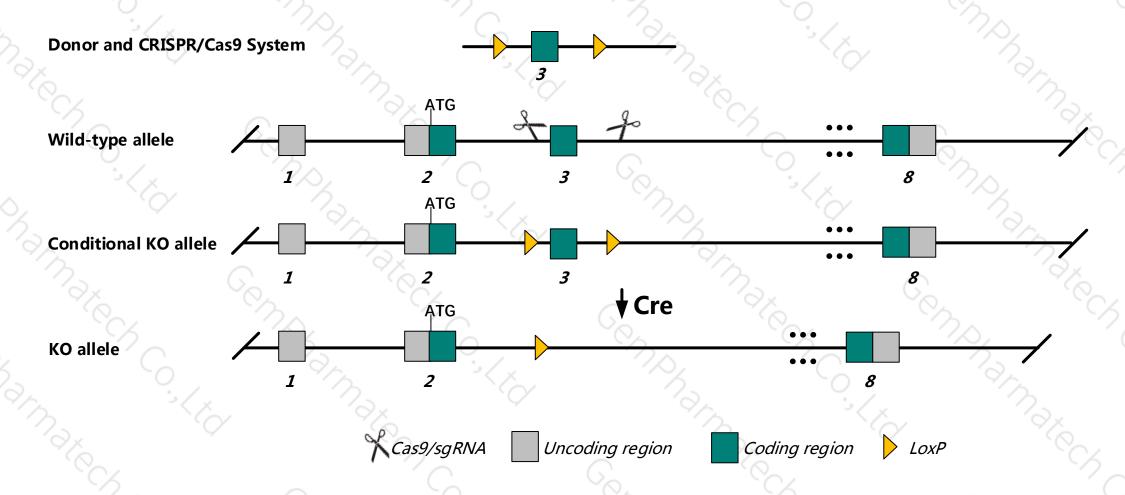
Strain background

C57BL/6JGpt

## **Conditional Knockout strategy**



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



### **Technical routes**



- The Aspn gene has 2 transcripts. According to the structure of Aspn gene, exon 3 of Aspn-201 (
- ➤ ENSMUST00000021820.13) transcript is recommended as the knockout region. The region contains 113bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Aspn* 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 or cell types.

### **Notice**



- > The KO region contains partial intron of the *Cenpp* gene.Knockout the region may affect the function of *Cenpp* gene.
- The *Aspn* 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Gene information (NCBI)



#### Aspn asporin [ Mus musculus (house mouse) ]

Gene ID: 66695, updated on 14-Jan-2020

#### Summary

☆ ?

Official Symbol Aspn provided by MGI
Official Full Name asporin provided by MGI
Primary source MGI:MGI:1913945

See related Ensembl:ENSMUSG00000021388

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 Plap1; Slrr1c; AA986886; 4631401G09Rik

Summary This gene encodes a member of the small leucine-rich proteoglycan family. The encoded protein is an extracellular matrix protein that

modulates the transforming growth factor-beta signaling pathway, regulating cartilage matrix gene expression and cartilage formation. The

protein plays a role in the pathology of osteoarthritis. Alternative splicing results in multiple transcript variants.[provided by RefSeq, Feb 2010]

Expression Biased expression in limb E14.5 (RPKM 23.1), CNS E14 (RPKM 4.5) and 6 other tissues See more

Orthologs <u>human</u> <u>all</u>

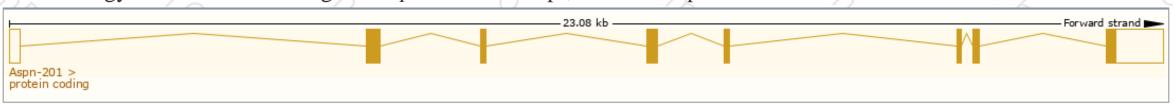
# Transcript information (Ensembl)



The gene has 2 transcripts, and all transcripts are shown below:

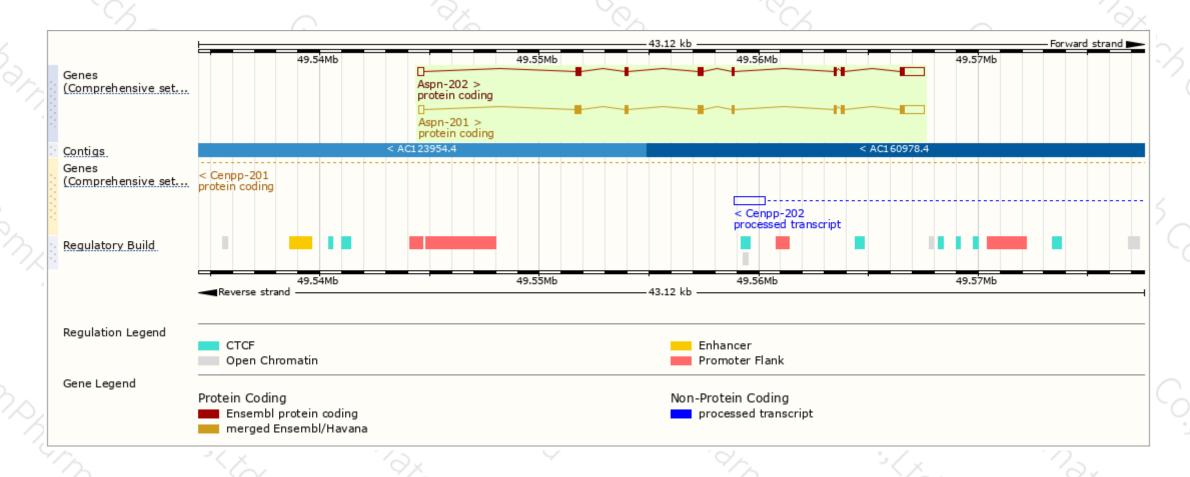
	Name 🍦	Transcript ID	bp 🌲	Protein	Biotype 🍦	CCDS 🍦	UniProt	Flags 🛊
I	Aspn-202	ENSMUST00000177948.1	2329	<u>373aa</u>	Protein coding	CCDS26503 ₽	<u>A6H6K1</u> & <u>Q99MQ4</u> &	TSL:1 GENCODE basic APPRIS P1
I	Aspn-201	ENSMUST00000021820.13	2310	<u>373aa</u>	Protein coding	CCDS26503 ₽	<u>A6H6K1</u> & <u>Q99MQ4</u> &	TSL:1 GENCODE basic APPRIS P1

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



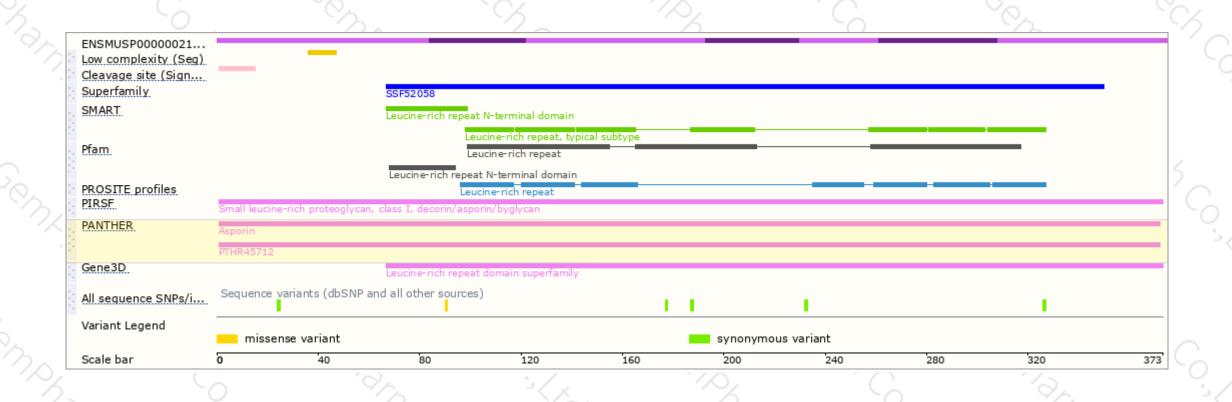
### Genomic location distribution





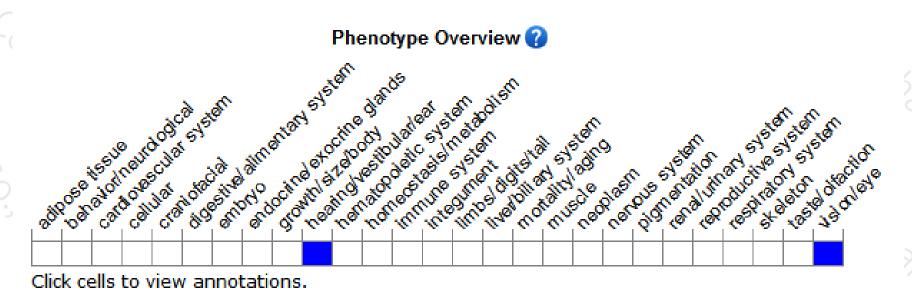
### Protein domain





## Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534





