

# Fam20a Cas9-CKO Strategy

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**Reviewer:** Huimin Su

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# **Project Overview**



**Project Name** 

Fam20a

**Project type** 

Cas9-CKO

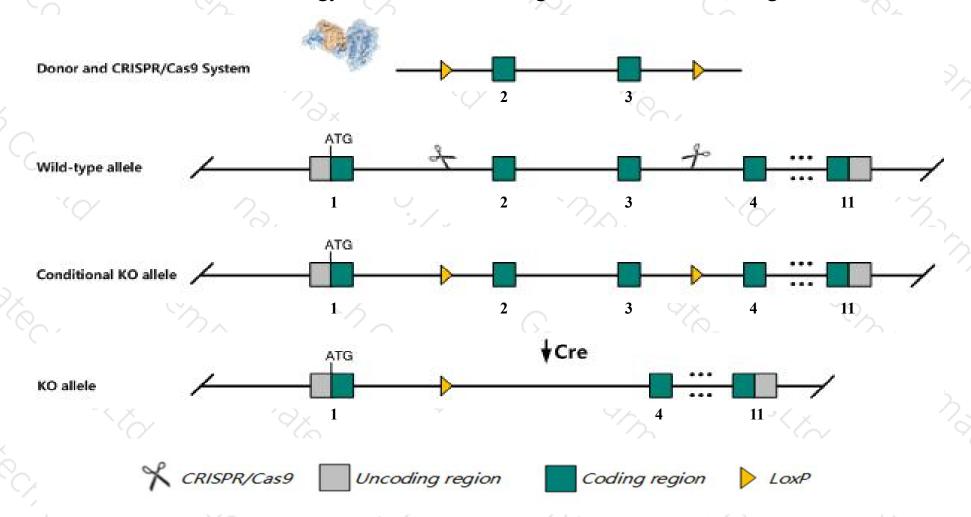
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Fam20a gene. The schematic diagram is as follows:



### Technical routes



- The Fam20a gene has 4 transcripts. According to the structure of Fam20a gene, exon2-exon3 of Fam20a-201 (ENSMUST00000020938.7) transcript is recommended as the knockout region. The region contains 236bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Fam20a* 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 abnormal ameloblast morphology, disrupted dental enamel formation in both incisor and molar teeth, abnormal kidney morphology, disseminated calcifications of muscular arteries, and intrapulmonary calcifications.
- The Fam20a gene is located on the Chr11. 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)



#### Fam20a family with sequence similarity 20, member A [ Mus musculus (house mouse) ]

Gene ID: 208659, updated on 13-Mar-2020

Summary

△ ?

Official Symbol Fam20a provided by MGI

Official Full Name family with sequence similarity 20, member A provided by MGI

Primary source MGI:MGI:2388266

See related Ensembl: ENSMUSG00000020614

Gene type protein coding
RefSeq status VALIDATED
Organism <u>Mus musculus</u>

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

Muroidea; Muridae; Murinae; Mus; Mus

Also known as Al606893

Expression Ubiquitous expression in testis adult (RPKM 14.5), duodenum adult (RPKM 11.5) and 26 other tissues See more

Orthologs human all

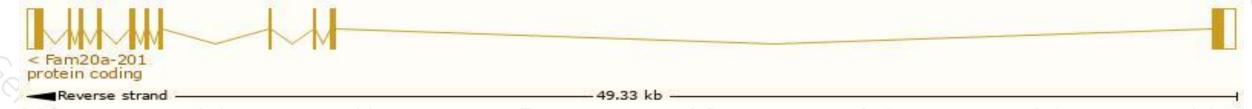
# Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

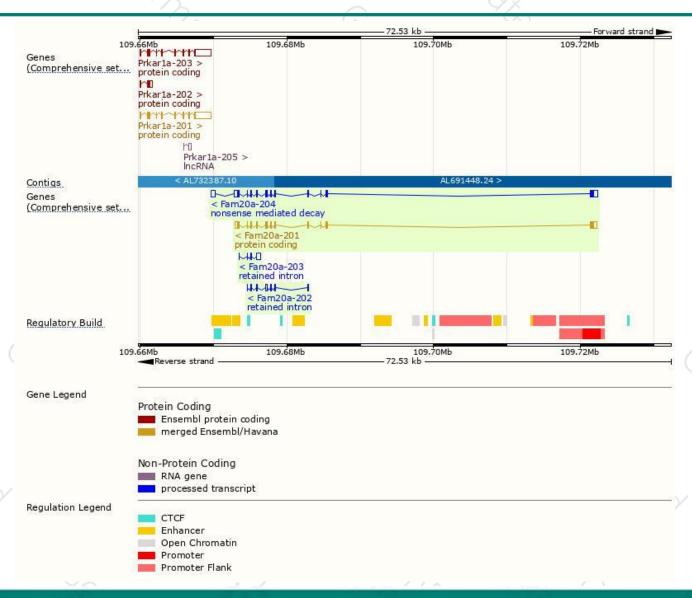
Name Fam20a-201	Transcript ID   ENSMUST00000020938.7	bp \( \phi \) 2541	Protein ♦ 541aa	Biotype  Protein coding	CCDS ♦	UniProt ⊕ Q8CID3 €	Flags		
							TSL:1	GENCODE basic	APPRIS P1
Fam20a-204	ENSMUST00000155559.7	3085	541aa	Nonsense mediated decay		Q8CID3∉	TSL:1		
Fam20a-203	ENSMUST00000146408.7	828	No protein	Retained intron	-	- 2	TSL:3		
Fam20a-202	ENSMUST00000144972.1	794	No protein	Retained intron	5-2	- 2		TSL:3	

The strategy is based on the design of Fam20a-201 transcript, The transcription is shown below



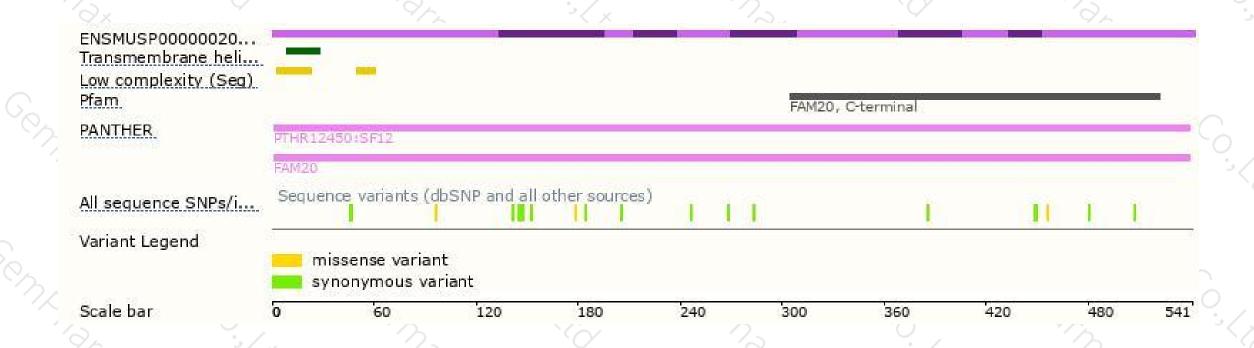
### Genomic location distribution





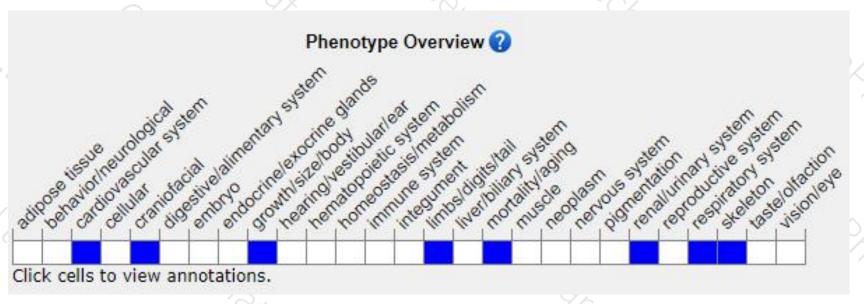
### Protein domain





# 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 abnormal ameloblast morphology, disrupted dental enamel formation in both incisor and molar teeth, abnormal kidney morphology, disseminated calcifications of muscular arteries, and intrapulmonary calcifications.



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





