Muc2-P2A-iCre Cas9-KI Strategy

Designer: Xueting Zhang

Design Date: 2019-8-6

Project Overview



Project Name

Muc2-P2A-iCre

Project type

Cas9-KI

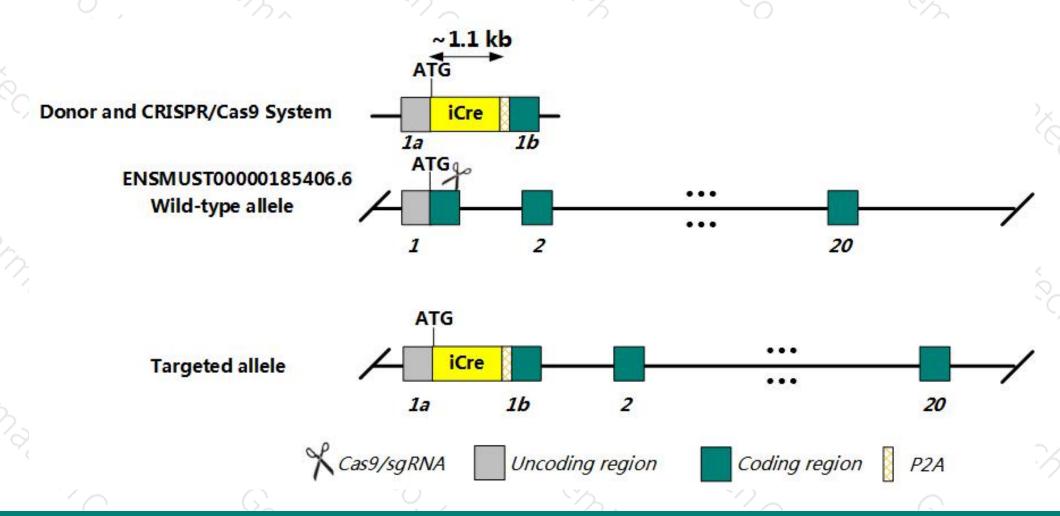
Strain background

C57BL/6JGpt

Knockin strategy



This model will use CRISPR/Cas9 technology to edit the *Muc2* gene. The schematic diagram is as follows:



Technical routes



- The *Muc2* gene has 8 transcripts. According to the structure of *Muc2* gene, *Muc2-204*(ENSMUST00000185406.6) is selected for presentation of the recommended strategy.
- > Muc2-204 gene has 20 exons, with the ATG start codon in exon1.
- ➤ We make *Muc2-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start coding(ATG) of Muc2 gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre after start coding(ATG) of Muc2 gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice



- According to the existing MGI data, Homozygotes for a point mutation have soft feces at weaning and develop diarrhea associated with malapsorption syndrome. Homozygous null mutants pass blood in their feces at 6 months, and 65% of null mutants have intestinal tumors at 1 year.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of iCre may affect the regulation of the 5' end of the *Muc2* gene.
- ➤ There will be 1 to 2 amino acid synonymous mutation in exon1 of *Muc2* gene in this strategy.
- The *Muc2* gene is located on the Chr15. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)



Muc2 mucin 2 [Mus musculus (house mouse)]

Gene ID: 17831, updated on 9-Apr-2019

Summary

☆ ?

Official Symbol Muc2 provided by MGI
Official Full Name mucin 2 provided by MGI
Primary source MGI:MGI:1339364
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 MCM; wnn; 2010015E03Rik

Annotation information Annotation category: suggests misassembly

Annotation category: partial on reference assembly

Expression Biased expression in colon adult (RPKM 1294.7), large intestine adult (RPKM 306.2) and 2 other tissues See more

Orthologs human all

Genomic context

↑ ?

Location: 7 F5; 7 87.1 cM

See Muc2 in Genome Data Viewer

Exon count: 46

Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	7	NC_000073.6 (141690340141754694)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	7	NC_000073.5 (148876261148890250), (148930517148940598)

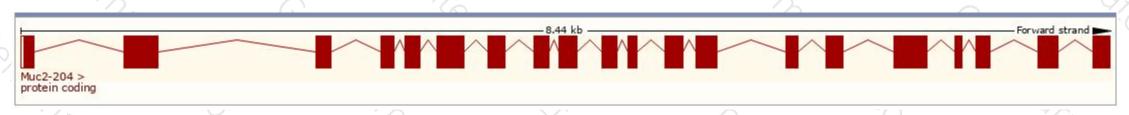
Transcript information (Ensembl)



The gene has 8 transcripts, and all transcripts are shown below:

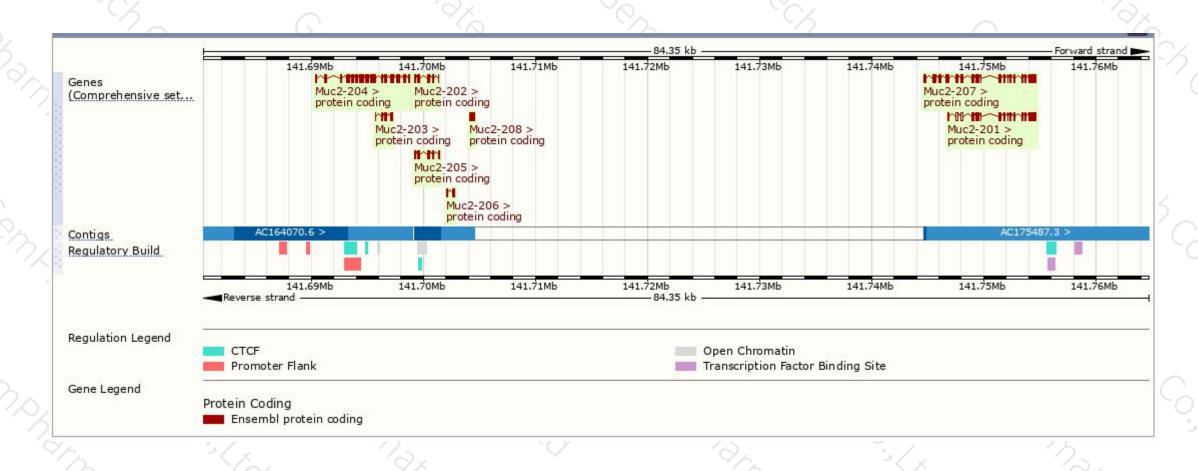
Name 🍦	Transcript ID A	bp 🍦	Protein 4	Biotype	CCDS	UniProt 🍦	Flags
Muc2-201	ENSMUST00000026590.8	2032	483aa	Protein coding	Ε.	A0A140T8I8₺	TSL:1 GENCODE basic APPRIS P1
Muc2-202	ENSMUST00000167366.2	711	237aa	Protein coding	12	F6QGV1₽	TSL:5 GENCODE basic
Muc2-203	ENSMUST00000179227.1	399	<u>132aa</u>	Protein coding	Ε.	Q9R1L0@	TSL:1 GENCODE basic
Muc2-204	ENSMUST00000185406.6	2690	887aa	Protein coding	Ε.	A0A087WSG8@	CDS 3' incomplete TSL:5
Muc2-205	ENSMUST00000185823.6	823	274aa	Protein coding	Έ.	A0A087WS13₽	CDS 5' and 3' incomplete TSL:3
Muc2-206	ENSMUST00000187789.1	241	80aa	Protein coding	12	A0A087WQU6@	CDS 5' and 3' incomplete TSL:5
Muc2-207	ENSMUST00000187945.6	2926	923aa	Protein coding	=	A0A087WSP1₽	CDS 5' incomplete TSL:1
Muc2-208	ENSMUST00000191587.1	447	149aa	Protein coding	=	A0A087WP72₽	CDS 5' and 3' incomplete TSL:NA

The strategy is based on the design of Muc2-204 transcript, The transcription is shown below



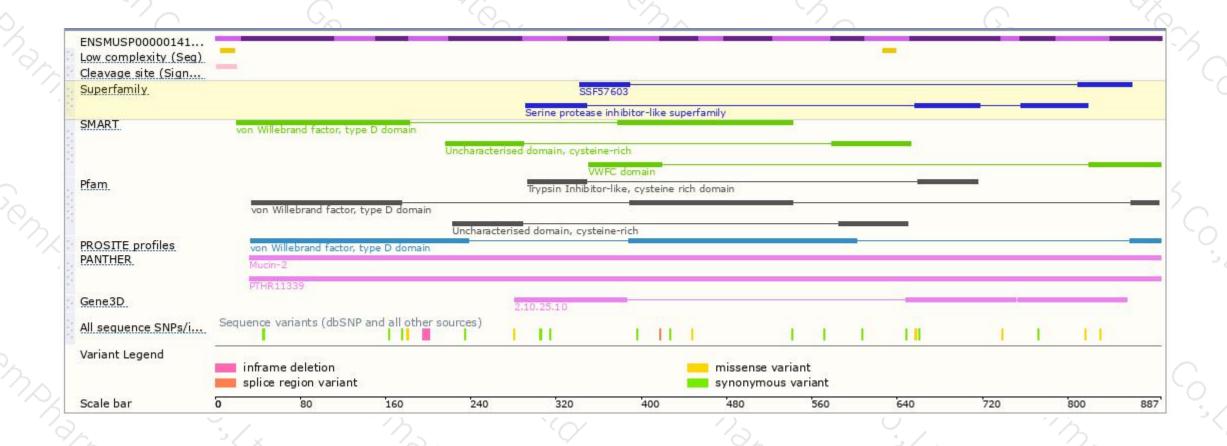
Genomic location distribution





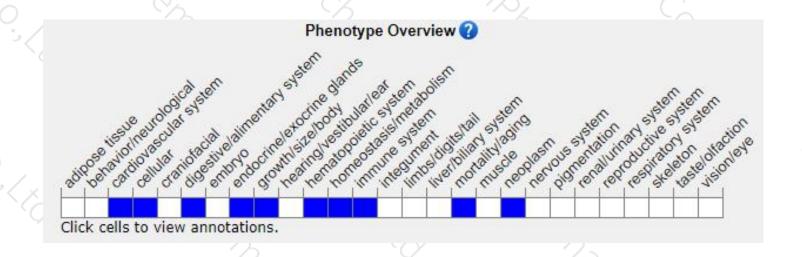
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/marker/MGI:109517).

Homozygotes for a point mutation have soft feces at weaning and develop diarrhea associated with malapsorption syndrome. Homozygous null mutants pass blood in their feces at 6 months, and 65% of null mutants have intestinal tumors at 1 year.

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





