

***Acan-P2A-CreERT2* Cas9-KI Strategy**

Designer:

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Reviewer

Daohua Xu

Design Date:

2021-9-10

Project Overview

Project Name

Acan-P2A-CreERT2

Project type

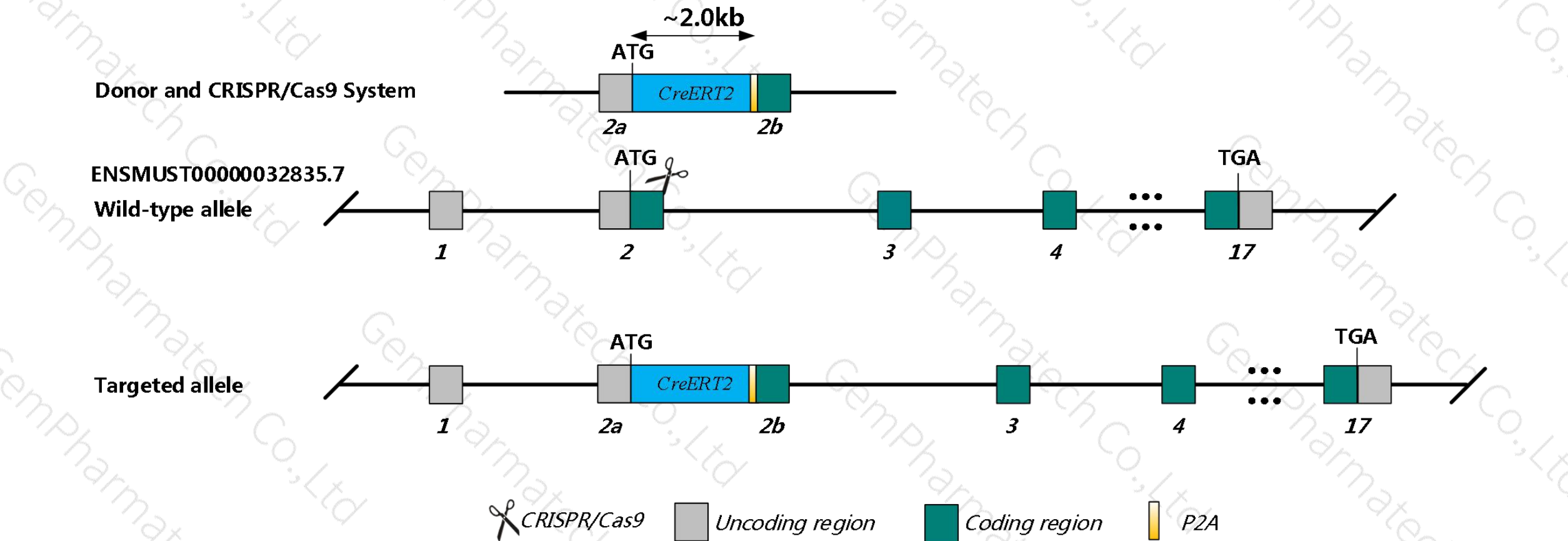
Cas9-KI

Strain background

C57BL/6JGpt

Knockin strategy

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



- The *Acan* gene has 2 transcripts. According to the structure of *Acan* gene, *Acan-201* (ENSMUST00000032835.7) transcript is selected for this strategy. *Acan-201* gene has 17 exons, with the ATG start codon in exon2 and TGA stop codon in exon17.
- We constructed CRISPR/Cas9 system targeting mouse *Acan* gene and donor vector, CreERT2-P2A will be introduced to near the ATG of mouse *Acan* gene. The CreERT2 will be expressed under the direction of endogenous regulatory mechanism.
- The project will use CRISPR/Cas9 technology to modify *Acan* 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.

- According to the existing MGI data, spontaneous mutations in this gene lead to dwarfism, cartilage, skeletal and limb anomalies, craniofacial defects, hearing loss and neonatal death due to respiratory failure. Homozygotes for an ENU-induced allele show cardiomyopathy as well as cleft palate, disproportionate dwarfism and brachypodia.
- According to the existing JAX data, When these mice are bred with mice containing loxP-flanked sequence, tamoxifen-inducible, Cre-mediated recombination will result in deletion of the floxed sequences in aggrecan-expressing cells (the growth plate and articular cartilage, fibrocartilage of the meniscus, trachea, and intervertebral disks) of the offspring.
- Insertion of CreERT2 may affect the regulation of the 5' end of the *Acan* gene.
- The 5' end of *Acan*-202 transcript is incomplete, and the influence on it is unknown.
- There will be 1 to 2 amino acid synonymous mutation in exon2 of *Acan* gene in this strategy.
- The *Acan* gene is located on the Chr7. 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.

The Sequence of *CreERT2* (1983bp)

ATGTCCAATTTACTGACCGTACACCAAATTTGCCTGCATTACCGGTTCGATGCAACGAGTGATGAGGTTTCGCAAGAACCTGATGGACATGTTTCAG
GGATCGCCAGGCGTTTTTCTGAGCATACCTGGAAAATGCTTCTGTCCGTTTGCCGGTTCGTGGGCGGCATGGTGCAAGTTGAATAACCGGAAATGGT
TTCCCGCAGAACCTGAAGATGTTTCGCGATTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAACTATCCAGCAACATTTGGGCCAGCTAA
ACATGCTTCATCGTCGGTCCGGGCTGCCACGACCAAGTGACAGCAATGCTGTTTCACTGGTTATGCGGGCGGATCCGAAAAGAAAACGTTGATGCC
GGTGAACGTGCAAAACAGGCTCTAGCGTTTGAACGCACTGATTTTCGACCAGGTTCGTTCACTCATGGAAAATAGCGATCGCTGCCAGGATATACG
TAATCTGGCATTCTGGGGATTGCTTATAACACCCTGTTACGTATAGCCGAAATTGCCAGGATCAGGGTTAAAGATATCTCACGTACTGACGGTGGG
AGAATGTTAATCCATATTGGCAGAACGAAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGCACCTAGCCTGGGGGTAATACTAACTGGTCGAGC
GATGGATTTCCGTCTCTGGTGTAGCTGATGATCCGAATAACTACCTGTTTTTGCCGGGTCAGAAAAAATGGTGTTGCCGCGCCATCTGCCACCAGCC
AGCTATCAACTCGCGCCCTGGAAGGGATTTTTGAAGCAACTCATCGATTGATTACGGCGCTAAGGATGACTCTGGTCAGAGATACCTGGCCTGGT
CTGGACACAGTGCCCGTGTTCGGAGCCGCGCGAGATATGGCCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGGCTGGACCAATGT
AAATATTGTCATGAACTATATCCGTAACCTGGATAGTGAAACAGGGGCAATGGTGCGCCTGCTGGAAGATGGCGATCTCGAGCCATCTGCTGGAG
ACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAAGAAGAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGT
CAGTGCCTTGTTGGATGCTGAGCCCCCATACTCTATTCCGAGTATGATCCTACCAGACCCTTCAGTGAAGCTTCGATGATGGGCTTACTGACCAA
CCTGGCAGACAGGGAGCTGGTTCACATGATCAACTGGGCGAAGAGGGTGCCAGGCTTTGTGGATTTGACCCTCCATGATCAGGTCCACCTTCTAG
AATGTGCCTGGCTAGAGATCCTGATGATTGGTCTCGTCTGGCGCTCCATGGAGCACCCAGTGAAGCTACTGTTTGCTCCTAACTTGCTCTTGGACA
GGAACCAGGGAAAATGTGTAGAGGGCATGGTGGAGATCTTCGACATGCTGCTGGCTACATCATCTCGGTTCCGCATGATGAATCTGCAGGGAGAG
GAGTTTGTGTGCCTCAAATCTATTATTTTGCTTAATTCTGGAGTGTACACATTTCTGTCCAGCACCTGAAGTCTCTGGAAGAGAAGGACCATATCC
ACCGAGTCCTGGACAAGATCACAGACACTTTGATCCACCTGATGGCCAAGGCAGGCCTGACCCTGCAGCAGCAGCACCAGCGGCTGGCCCAGC
TCCTCCTCATCCTCTCCCACATCAGGCACATGAGTAACAAAGGCATGGAGCATCTGTACAGCATGAAGTGCAAGAACGTGGTGCCCTCTATGAC
CTGCTGCTGGAGGGCGGCGGACGCCACCGCCTACATGCGCCCACTAGCCGTGGAGGGGCATCCGTGGAGGAGACGGACCAAAGCCACTTGGCC
ACTGCGGGCTCTACTTCATCGCATTCTTGCAAAAGTATTACATCACGGGGGAGGCAGAGGGTTTCCCTGCCACAGCTTAA

Target gene

Gene name	mouse <i>Acan</i>
Gene ID(NCBI)	11595
Gene link(NCBI)	https://www.ncbi.nlm.nih.gov/gene/11595
Gene link(Ensembl)	http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000030607;r=7:78703231-78764847
chromosome location	Chr7

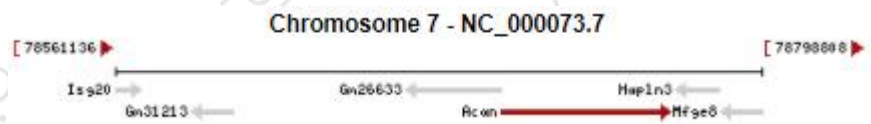
Gene information (NCBI)

Acan aggrecan [*Mus musculus* (house mouse)]

Gene ID: 11595, updated on 5-Sep-2021

Summary

Official Symbol	Acan provided by MGI
Official Full Name	aggrecan provided by MGI
Primary source	MGI:MGI:99602
See related	Ensembl:ENSMUSG00000030607
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Agc; Csp; cmd; Agc1; CSPCP; Cspg1; b2b183C; b2b183Clo
Expression	Biased expression in limb E14.5 (RPKM 40.6) and CNS E14 (RPKM 4.1) See more
Orthologs	human all

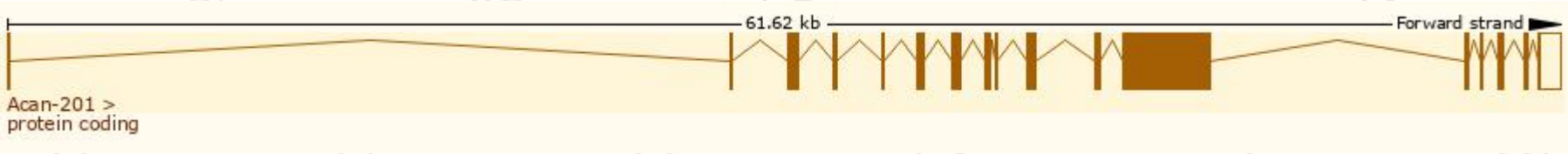


Transcript information (Ensembl)

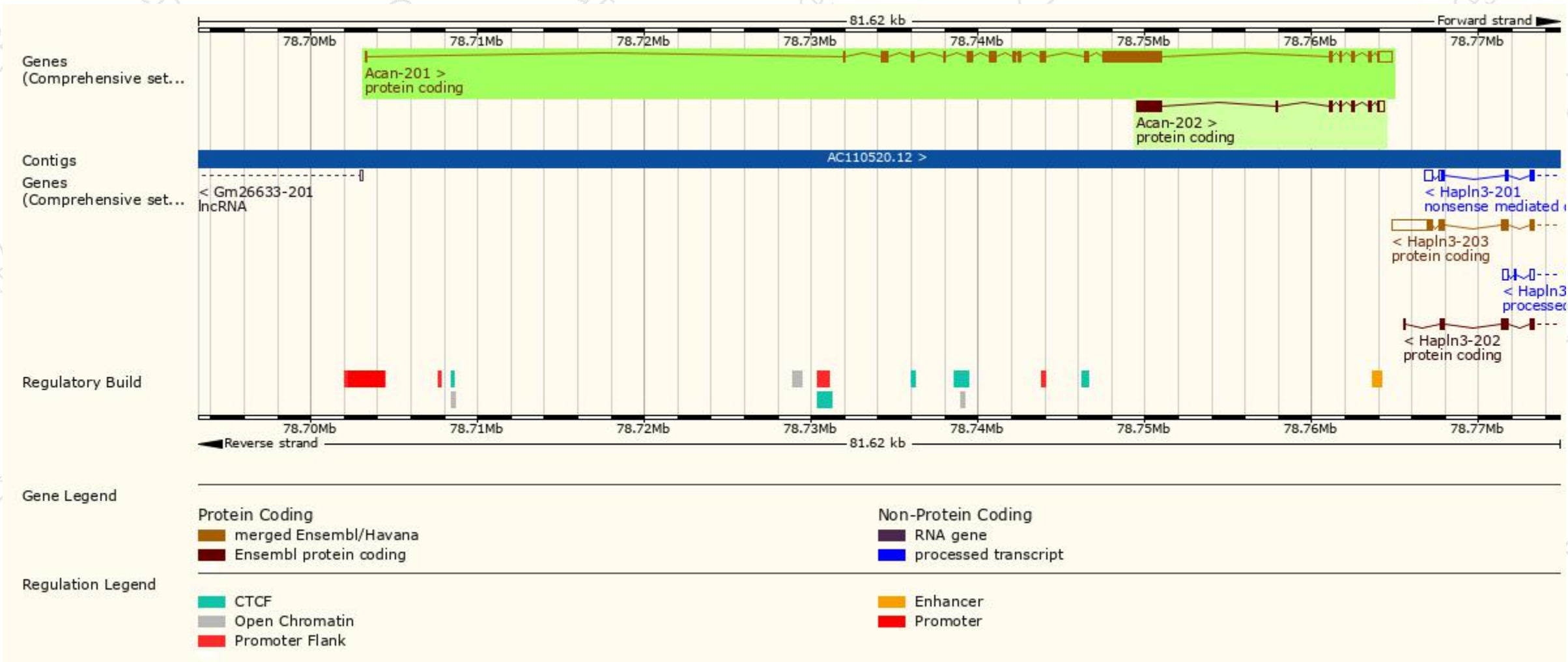
The gene has 2 transcripts, and all transcripts are shown below:

Show/hide columns (1 hidden) Filter							
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Acan-201	ENSMUST00000032835.7	7355	2132aa	Protein coding	CCDS21377	Q61282	GENCODE basic APPRIS P1 TSL:1
Acan-202	ENSMUST000000206779.2	2596	748aa	Protein coding	-	A0A0U1RQ88	TSL:1 CDS 5' incomplete

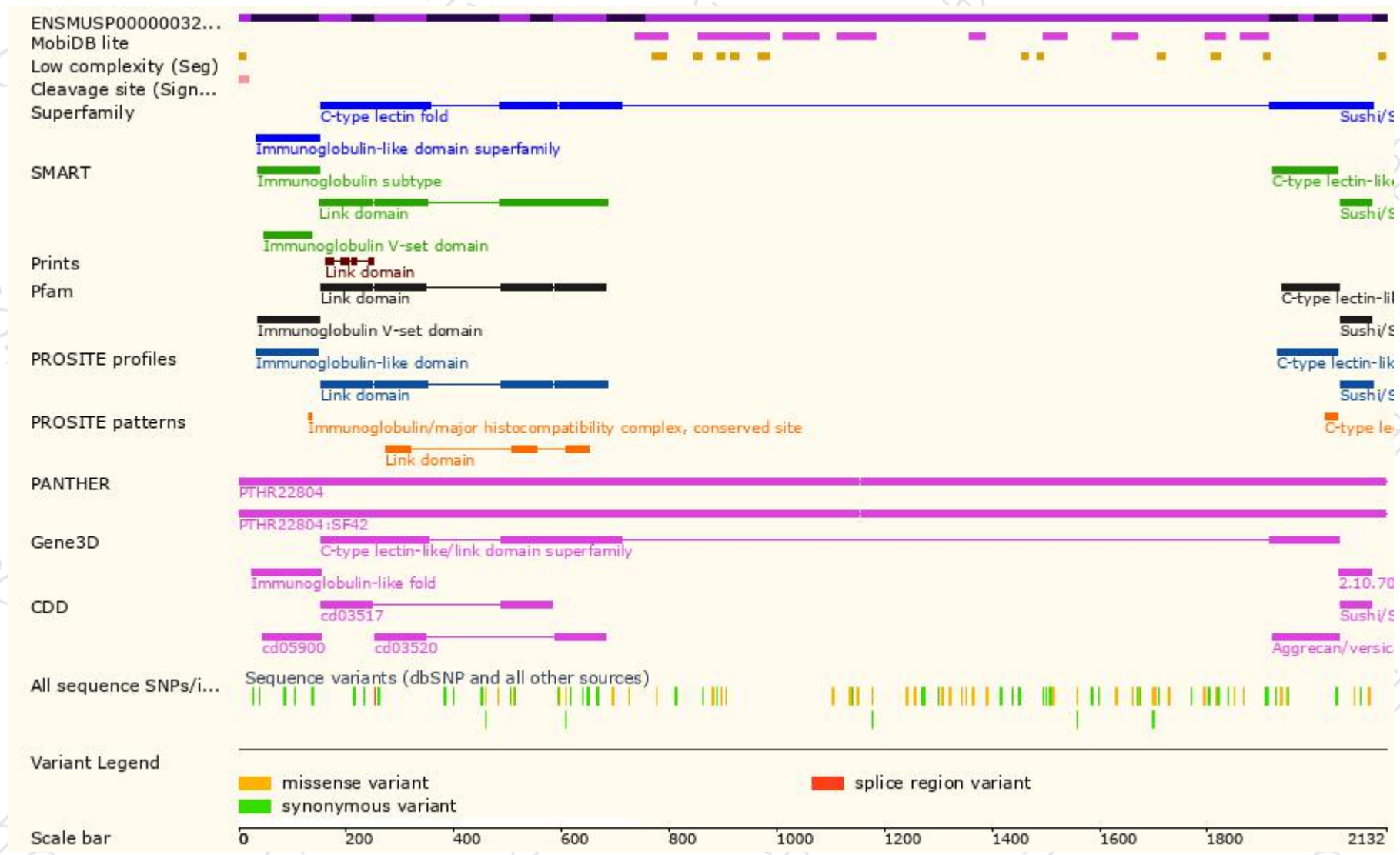
The strategy is based on the design of *Acan-201* transcript, The transcription is shown below:



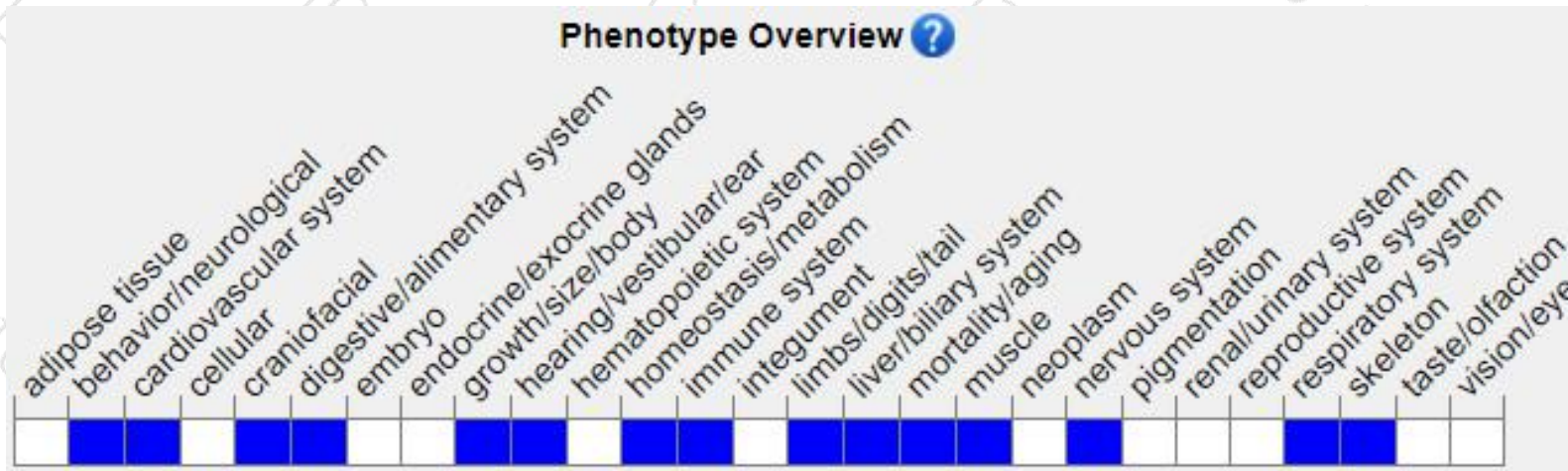
Genomic location distribution



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/marker/MGI:99602>) .

Spontaneous mutations in this gene lead to dwarfism, cartilage, skeletal and limb anomalies, craniofacial defects, hearing loss and neonatal death due to respiratory failure. Homozygotes for an ENU-induced allele show cardiomyopathy as well as cleft palate, disproportionate dwarfism and brachypodia.

Targeted Progress (from Jax)



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B6.Cg-Acan^{tm1(cre/ERT2)Crm/J}

MOUSE STRAIN DATASHEET - 019148

PLACE ORDER

3-6 week average lead time depending on quantity and age requests are not accepted

OVERVIEW DETAILS ▾ GENETICS DISEASE/PHENOTYPE ▾ TECHNICAL SUPPORT ▾ PRICING & AVAILABILITY ▾ TERMS OF USE ▾ RELATED STRAINS

Allele Type

Targeted (Inducible, Recombinase-expressing)

Allele Synonym(s)

Agc1^{CreERT2}; Agc1^{tm(IRES-creERT2)}; Agc1^{Cre}; Agc1^{tm(IRES-CreERT-Neo-pgk)}

Gene Symbol and Name

Acan , aggrecan

Gene Synonym(s)

AGC1; AGCAN; Agc; Agc1; Agc1; CSPG1; CSPGCP; Cspg1; MSK16; Mutant line 183; SEDK; SS0A0D; aggrecan 1; b2b183Clo; b2b183Clo; cartilage matrix deficiency; cmd

Expressed Gene

cre/ERT2, Cre recombinase and estrogen receptor 1 (human) fusion gene,

Site of Expression

When these mice are bred with mice containing loxP-flanked sequence, tamoxifen-inducible, Cre-mediated recombination will result in deletion of the floxed sequences in aggrecan-expressing cells (the growth plate and articular cartilage, fibrocartilage of the meniscus, trachea, and intervertebral disks) of the offspring.

Strain of Origin

(129S6/SvEvTac x C57BL/6NCrl)F1

Chromosome

7

Molecular Note

IRES-cre/ERT2 and an FRT-flanked PGK-neo selection cassette (in reverse orientation to the target gene) was inserted into the 3' UTR of the Aggrecan gene by homologous recombination in G4 ES cells. The line was established by breeding to C57BL/6 mice. The neo cassette was not removed.

<https://www.jax.org/strain/019148>

Targeted Progress (from Jax)

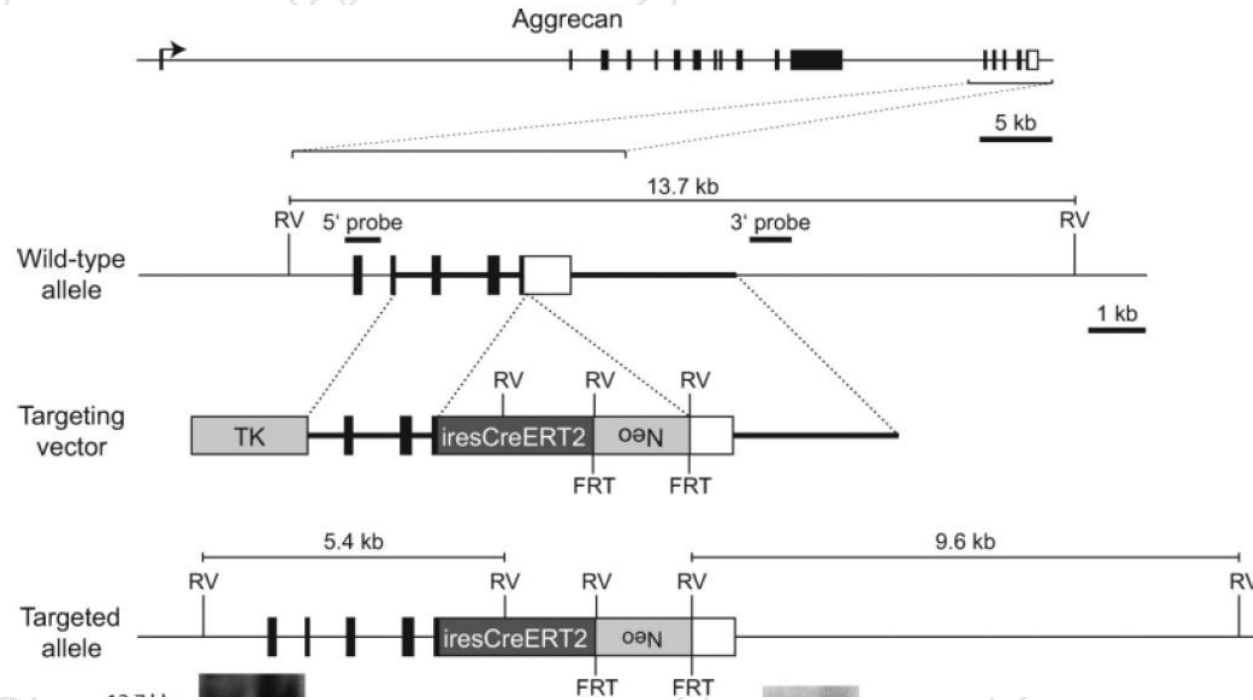


FIG. 1. Gene-targeting strategy for the AggreCan-CreERT² allele. Top panel displays the genomic structure of the mouse *aggreCan* gene. Middle panel shows the targeting vector. Bottom panel depicts the targeted allele whereby IRES-CreERT2 and an FRT-flanked Pgk-Neo cassette (in reverse transcriptional orientation to *aggreCan*) is recombined into the 3' untranslated region (3' UTR) of the *aggreCan* gene. EcoRV restriction enzyme digestion is used in Southern blotting analysis to identify correctly targeted ES cell clones. Both the 5' and 3' probes (position shown in the middle panel) detect a 13.5-kb fragment with the wild-type allele. The targeted allele generates a 5.4- or 9.6-kb fragment detected by the 5' or 3' probe, respectively. At the bottom of the Panel (a) targeted ES clone (3H11) is shown to the left in a Southern blot hybridized with the radiolabeled 5' probe. The Southern blot of the 3' probe with the same clone is shown to the right. RV: EcoRV sites. TK: MC1-TK. Neo: Pgk-Neo. FRT: FRT sites.

1. Henry SP; Jang CW; Deng JM; Zhang Z; Behringer RR; de Crombrughe B. Generation of *aggreCan*-CreERT2 knockin mice for inducible Cre activity in adult cartilage. *Genesis*.2009 47 (12) 805-14.

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