Postn-MerCreMer cas9-ki Mouse Model Strategy

-CRISPR/Cas9 technology

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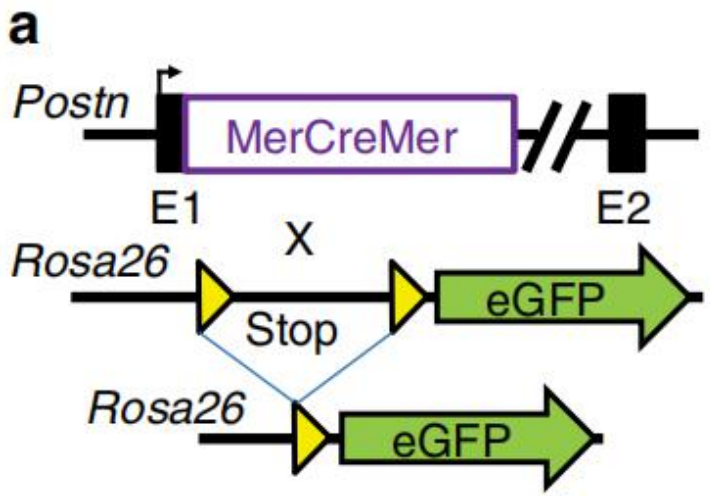
Project Overview

Project Name	Postn-MerCreMer
Project Type	cas9-ki
Background	C57BL/6JGpt
Project Cycle	6-8 months

Technical Description

- The mouse *Postn* gene has 9 transcripts.
- According to the structure of *Postn* gene and clients' requirement ,The element MerCreMer-P2A will be inserted at the translation start codon(ATG) of *Postn*-201(ENSMUST00000073012.12), the length of inserted fragment is about 3.1kb.
- In this project, *Postn* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR, sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

Reference



Methods

Mice. All experiments involving mice were approved by the Institutional Animal Care and Use Committee (IACUC) at Cincinnati Children's Hospital Medical Center. Targeted *Postn*^{MCM} mice were generated by standard gene-targeting techniques. DNA homology arms upstream and downstream of the ATG start codon of the *Postn* gene were subcloned into a plasmid backbone to create a gene-targeting construct. The plasmid also contained a diphtheria toxin A (DTA) cDNA cassette for negative selection and a frt site-flanked neomycin cDNA cassette for positive selection. A cDNA encoding the MerCreMer cDNA[45] was cloned in-frame with the *Postn* ATG start site of exon 1. Embryonic stem (ES) cells were electroporated with this linearized DNA-targeting vector and G418-resistant colonies were picked and subject to Southern blot and PCR to identify properly targeted clones. ES cell aggregation with eight-cell embryos was used to generate chimeric mice. Germline transmitting male chimeras were crossed with *Rosa26*-Flpe females (B6.129S4-*Gt(ROSA)26Sor*$^{tm1(FLP1)Dym}$/RainJ) to delete the neomycin cassette at the frt sites, and verified offspring were further backcrossed to C57Bl/6J for five generations. Reporter mice FVB.Cg-*Gt(ROSA)26Sor*$^{tm1(CAG-lacZ,EGFP)Glh}$/J (previously modified by cross-breeding to B6(C3)-Tg (Pgk1-FLPo)10Sykr/J) and B6.129(Cg)-*Gt(ROSA)26Sor*$^{tm4(ACTB-tdTomato,-EGFP)Luo}$/J were purchased from the Jackson Laboratories[30]. PCR genotyping of *Postn*^{MCM} mice used the following primers, (wt-Postn-forward: 5′-TCT GTA AGG CCA TCG CAA GCT-3′; mutant-forward: 5′-GGT GGG ACA TTT GAG TTG CT-3′ and WT intron-reverse: 5′- AAT AAG TAA AAC AGC TCC CCT-3′). Other mouse lines are as follows: *LysM*^{Cre} B6N.129P2(B6)-Lyz2tm1(cre)Ifo/J] Jax stock no: ID018956; *Cdh5*^{Cre} [B6.FVB-Tg(Cdh5-cre)7Mlia/J] Jax Stock No: 006137. *Myh11*^{CreERT2} [B6.FVB-Tg(Myh11-cre/ERT2)1Soff/J] Jax Stock No:019079. Rosa26-DTA [Gt(ROSA)26Sortm1 (DTA)Jpmb/J] Jax Stock No:006331. Rosa26-nLacZ [FVB.Cg-Gt(ROSA)26Sortm1 (CAG-lacZ,-EGFP)Glh/J] Jax Stock No:012429. *Tcf21*^{LacZ} (ref. 46); *Tcf21*^{MCM} (ref. 47); collagen1a1-GFP[24], *Postn*$^{-/-}$ (ref. 28) and Postn-ZsGreen[38] mice were previously described.

Figure 1 | *Postn*^{MCM} allele activity *in vivo*. (**a**) Schematic representation of the *Postn* genetic locus with a tamoxifen-regulated MerCreMer cDNA cassette inserted into exon 1 (E1), which was crossed with *Rosa26* reporter mice (R26-eGFP) containing loxP sites flanking a stop cassette upstream of eGFP to

Sequence of MerCreMer(3012bp)

集萃药康 GemPharmatech

ATGGGAGATCCACGAAATGAAATGGGTGCTTCAGGAGACATGAGGGCTGCCAACCTTTGGCCAAGCCCTCTTGTGATTAAGCACACTAAGAAGAATAGCCCTGCCTTGTCCTTGACAGCTGACCAGATG
GTCAGTGCCTTGTTGGATGCTGAACCGCCCATGATCTATTCTGAATATGATCCTTCTAGACCCTTCAGTGAAGCCTCAATGATGGGCTTATTGACCAACCTAGCAGATAGGGAGCTGGTTCATATGATCAA
CTGGGCAAAGAGAGTGCCAGGCTTTGGGGACTTGAATCTCCATGATCAGGTCCACCTTCTCGAGTGTGCCTGGCTGGAGATTCTGATGATTGGTCTCGTCTGGCGCTCCATGGAACACCCGGGGAAGCT
CCTGTTTGCTCCTAACTTGCTCCTGGACAGGAATCAAGGTAAATGTGTGGAAGGCATGGTGGAGATCTTTGACATGTTGCTTGCTACGTCAAGTCGGTTCCGCATGATGAACCTGCAGGGTGAAGAGTTT
GTGTGCCTCAAATCCATCATTTTGCTTAATTCCGGAGTGTACACGTTTCTGTCCAGCACCTTGAAGTCTCTGGAAGAGAAGGACCACATCCACCGTGTCCTGGACAAGATCACAGACACTTTGATCCACCT
GATGGCCAAAGCTGGCCTGACTCTGCAGCAGCAGCATCGCCGCCTAGCTCAGCTCCTTCTCATTCTTTCCCATATCCGGCATATGAGTAACAAACGCATGGAGCATCTCTACAACATGAAATGCAAGAAC
GTGGTACCCCTCTATGACCTGCTCCTGGAGATGTTGGATGCCCACCGCCTTCATGCCCCAGCCAGTCGCATGGGAGTGCCCCCAGAGGAGCCCAGCCAGACCCAGCTGGCCACCACCAGCTCCACTTCA
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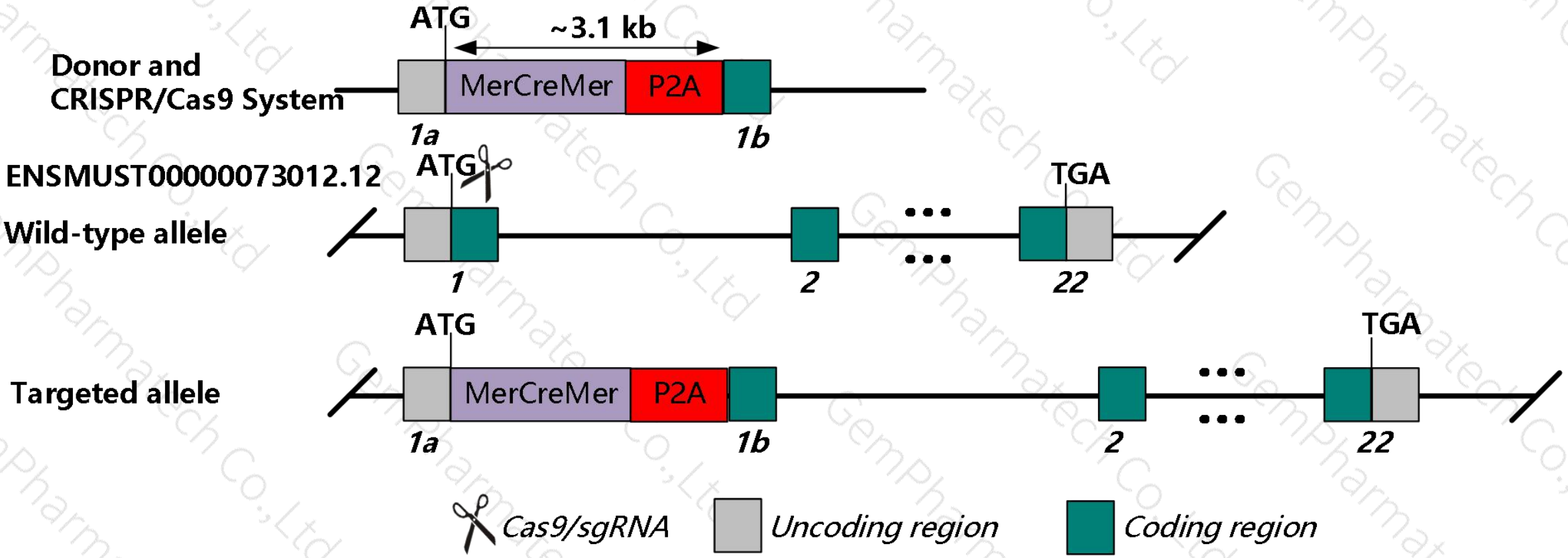
GTCTCTGGTGTAGCTGATGATCCGAATAACTACCTGTTTTGCCGGGTCAGAAAAAATGGTGTTGCCGCGCCATCTGCCACCAGCCAGCTATCAACTCGCGCCCTGGAAGGGATTTTTGAAGCAACTCATC
GATTGATTTACGGCGCTAAGGATGACTCTGGTCAGAGATACCTGGCCTGGTCTGGACACAGTGCCCGTGTCGGAGCCGCGCGAGATATGGCCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCT
GGTGGCTGGACCAATGTAAATATTGTCATGAACTATATCCGTAACCTGGATAGTGAAACAGGGGCAATGGTGCGCCTGCTGGAAGATGGCGATGGAGGTTCTGGAGATCCACGAAATGAAATGGGTGC
TTCAGGAGACATGAGGGCTGCCAACCTTTGGCCAAGCCCTCTTGTGATTAAGCACACTAAGAAGAATAGCCCTGCCTTGTCCTTGACAGCTGACCAGATGGTCAGTGCCTTGTTGGATGCTGAACCGCCC
ATGATCTATTCTGAATATGATCCTTCTAGACCCTTCAGTGAAGCCTCAATGATGGGCTTATTGACCAACCTAGCAGATAGGGAGCTGGTTCATATGATCAACTGGGCAAAGAGAGTGCCAGGCTTTGGGG
ACTTGAATCTCCATGATCAGGTCCACCTTCTCGAGTGTGCCTGGCTGGAGATTCTGATGATTGGTCTCGTCTGGCGCTCCATGGAACACCCGGGGAAGCTCCTGTTTGCTCCTAACTTGCTCCTGGACAGG
AATCAAGGTAAATGTGTGGAAGGCATGGTGGAGATCTTTGACATGTTGCTTGCTACGTCAAGTCGGTTCCGCATGATGAACCTGCAGGGTGAAGAGTTTGTGTGCCTCAAATCCATCATTTTGCTTAATTC
CGGAGTGTACACGTTTCTGTCCAGCACCTTGAAGTCTCTGGAAGAGAAGGACCACATCCACCGTGTCCTGGACAAGATCACAGACACTTTGATCCACCTGATGGCCAAAGCTGGCCTGACTCTGCAGCA
GCAGCATCGCCGCCTAGCTCAGCTCCTTCTCATTCTTTCCCATATCCGGCATATGAGTAACAAACGCATGGAGCATCTCTACAACATGAAATGCAAAAACGTGGTACCCCTCTATGACCTGCTCCTGGAG
ATGTTGGATGCCCACCGCCTTCATGCCCCAGCCAGTCGCATGGGAGTGCCCCCAGAGGAGCCCAGCCAGACCCAGCTGGCCACCACCAGCTCCACTTCAGCACATTCCTTACAAACCTACTACATACCC
CCGGAAGCAGAGGGCTTCCCCAACACGATCTGA

http://www.addgene.org/browse/sequence/239589/
http://www.addgene.org/124184/sequences/

Strategy

This model uses CRISPR/Cas9 technology to edit the *Postn* gene and the schematic diagram is as follow:



Notice

- According to the existing MGI data, homozygous null mice display abnormalities of the enamel, periodontal ligament, ameloblasts, and incisors. For one allele changing the hardness of the food alters the severity of the abnormalities.
- There may be 1 to 2 amino acid synonymous mutation in exon1 of *Postn* gene in this strategy.
- The effect on transcript 206 is unknown.
- If the two genes are linked with P2A,and these two genes will be transcripted together and then be translated two protein separately. Otherwise, P2A may produce lower amounts of the downstream protein in relation to the upstream protein.
- Mouse *Postn* gene is located on Chr3. Please take the loci in consideration when breeding this knockin mice with other gene modified (e.g., Tg, iCre) strains, if the other gene is also on Chr3, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 5'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene name and location (NCBI)

Postn periostin, osteoblast specific factor [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 50706, updated on 19-Jan-2021

Summary

Official Symbol	Postn provided by MGI
Official Full Name	periostin, osteoblast specific factor provided by MGI
Primary source	MGI:MGI:1926321
See related	Ensembl:ENSMUSG00000027750
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	p; Os; PN; Pe; OSF; PLF; Osf2; OSF-2; AI747096; A630052E07Rik
Summary	This gene encodes a secreted extracellular matrix protein that functions in tissue development and regeneration, including wound healing and ventricular remodeling following myocardial infarction. The encoded protein binds to integrins to support adhesion and migration of epithelial cells. This protein plays a role in cancer stem cell maintenance and metastasis. Mice lacking this gene exhibit cardiac valve disease, and skeletal and dental defects. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Sep 2015]
Expression	Biased expression in limb E14.5 (RPKM 151.8), placenta adult (RPKM 112.7) and 12 other tissues See more
Orthologs	human all

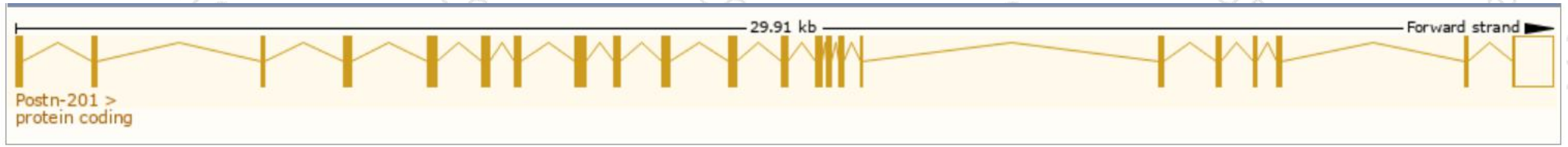
NEW **Try the new Data Table view**

Transcript information （Ensembl）

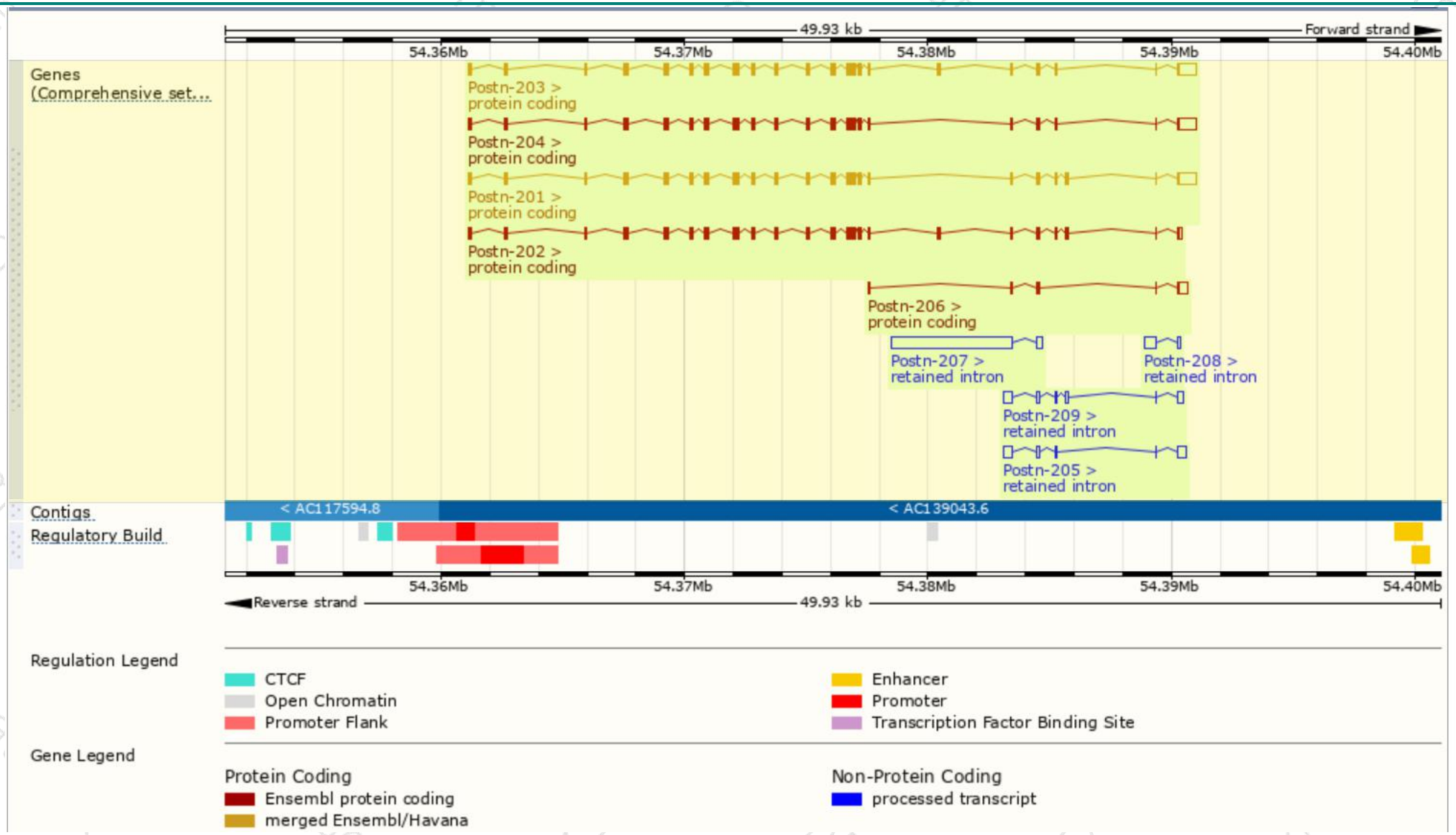
The gene has 9 transcripts, and the transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match ▲	Flags
Postn-206	ENSMUST00000143258.1	655	102aa	Protein coding	-	F7C9H0	CDS 5' incomplete TSL:2
Postn-203	ENSMUST00000107985.9	3205	810aa	Protein coding	CCDS57211	Q62009	TSL:1 GENCODE basic APPRIS ALT2
Postn-201	ENSMUST00000073012.12	3189	811aa	Protein coding	CCDS17351	Q62009	TSL:1 GENCODE basic APPRIS P3
Postn-204	ENSMUST00000117373.7	3121	783aa	Protein coding	CCDS57212	Q62009	TSL:1 GENCODE basic APPRIS ALT2
Postn-202	ENSMUST00000081564.12	2670	838aa	Protein coding	-	Q62009	TSL:5 GENCODE basic APPRIS ALT2
Postn-207	ENSMUST00000145036.2	5223	No protein	Retained intron	-	-	TSL:1
Postn-205	ENSMUST00000127452.1	900	No protein	Retained intron	-	-	TSL:2
Postn-209	ENSMUST00000154157.7	891	No protein	Retained intron	-	-	TSL:3
Postn-208	ENSMUST00000150868.1	624	No protein	Retained intron	-	-	TSL:2

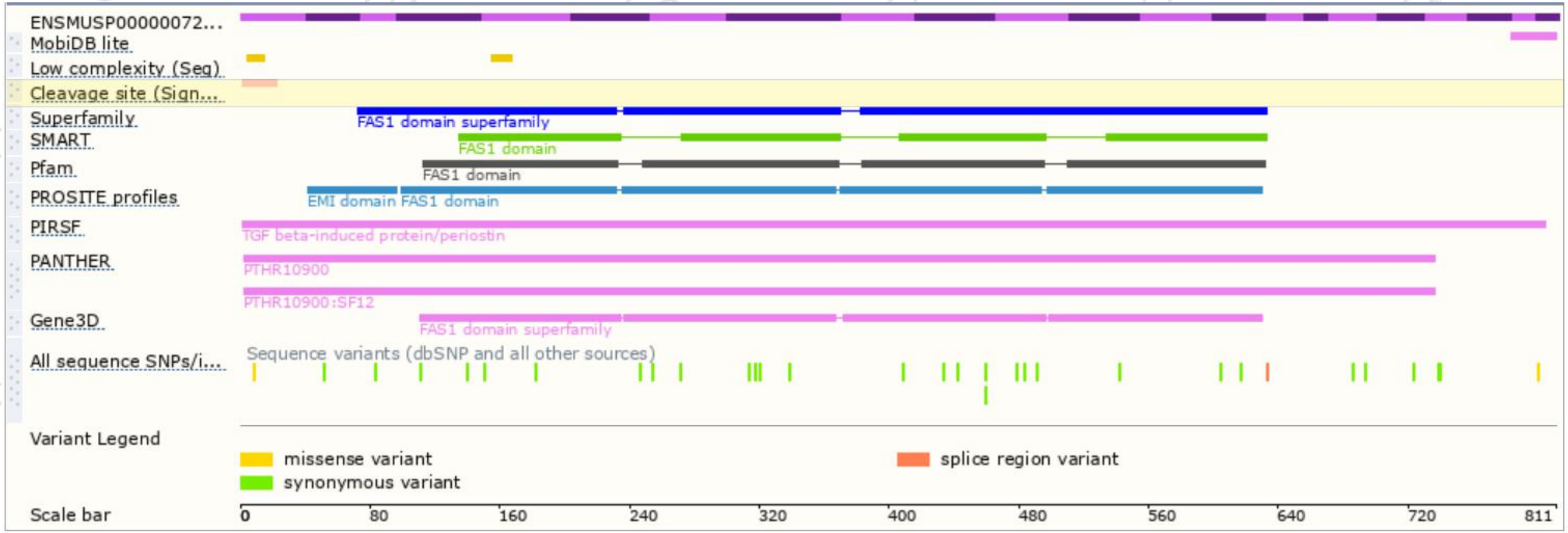
The strategy is based on the design of *Postn-201* transcript,the transcription is shown below:



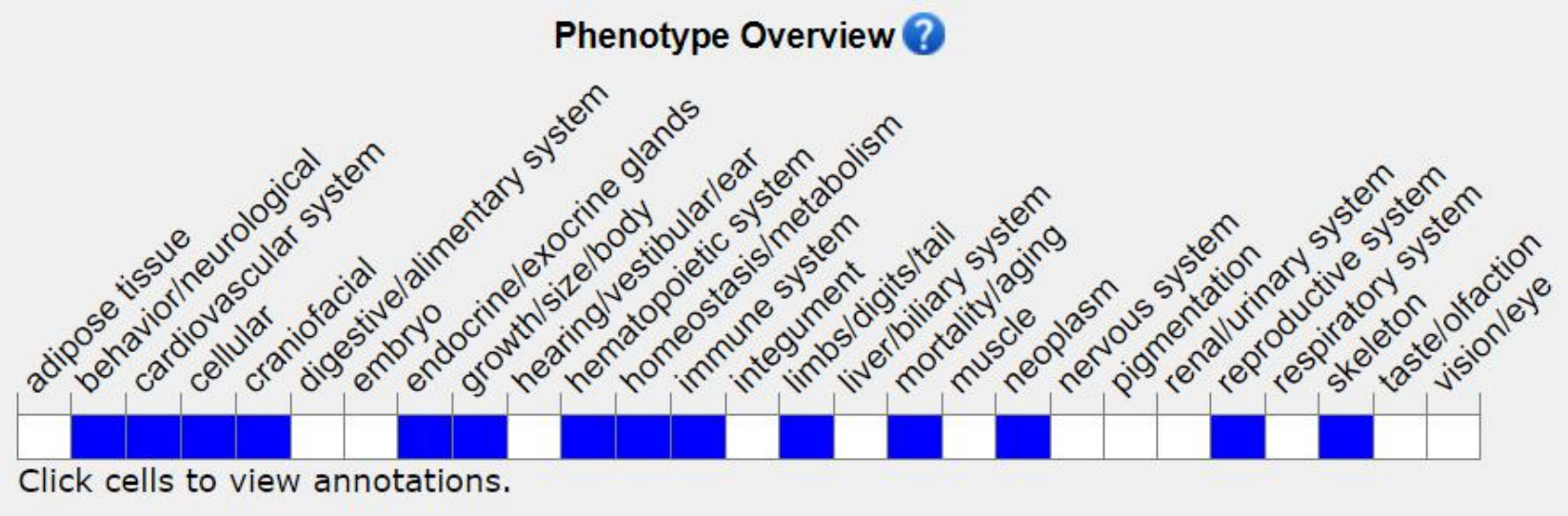
Genomic location distribution



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:1926321) .

Homozygous null mice display abnormalities of the enamel, periodontal ligament, ameloblasts, and incisors.

For one allele changing the hardness of the food alters the severity of the abnormalities.

Additional charge

Content	Cycle(month)	Charge(RMB)
MerCreMer Sequence	1	4518

The sequence synthesis cycle is not included in the project cycle. If the customer can provide the amplification template, please inform us in time.

If you have any questions, please feel free to contact us.
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