Ncam1-P2A-CreERT2 Cas9-ki Strategy

Designer: Lu Chen

Reviewer: Xueting Zhang

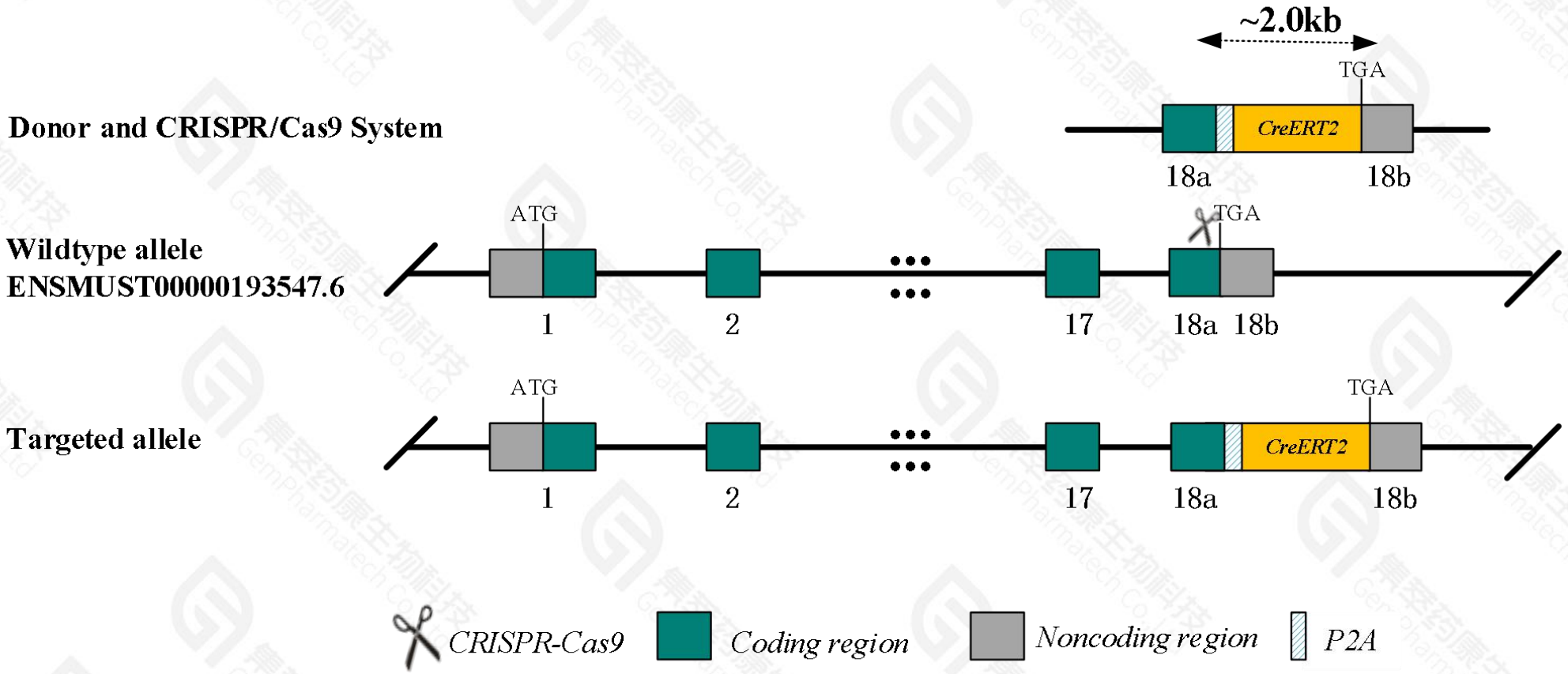
Design Date: 2022-1-10

Project Overview

Project Name	***Ncam1-P2A-CreERT2***
Project type	**Cas9-KI**
Strain background	**C57BL/6JGpt**

Knockin strategy

This model will use CRISPR-Cas9 technology to edit the *Ncam1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ncam1* gene has 7 transcripts. According to the structure of *Ncam1* gene, *Ncam1*-204 ((ENSMUST00000193547.6) transcript is recommended for this strategy.

➢ *Ncam1*-204 transcript has 18 exons, with the ATG start codon in exon 1 and TGA stop codon in exon 18, encode 848 aa.

➢ In this project we make *Ncam1-P2A-CreERT2* knockin mice via CRISPR/Cas9 system.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.

Notice

- According to the existing MGI data, *Ncam1* homozygous mutants show impairment in Morris water maze test, reduced brain and olfactory bulb size, hypoplasic corticospinal tract, abnormally distributed anterior pituitary cell types, and neuromuscular junctions defects. Homozygotes for a null allele exhibit age-dependent retinal abnormalities.
- There may be 1 to 2 synonymous mutations in exon 18 of *Ncam1* gene in this strategy.
- The effect of transcript *Ncam1*-201, *Ncam1*-205, *Ncam1*-203, *Ncam1*-206 and *Ncam1*-207 is unknown.
- There may be base mutations in the modeling process because of the repetitive sequences(TA) upstream of the insertion site.
- The *CreERT2* and *Ncam1* linked by P2A will expressed under the regulation of endogenous *Ncam1*, and protein products will be divided into two protein molecules. The anterior protein will carry the peptide translated from the P2A sequence.
- The *Ncam1* gene is located on the Chr9. 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.

Targeted progress（MGI）

http://www.informatics.jax.org/allele/summary?markerId=MGI:97281

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Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems	Human Disease Models
Ncam1$^{tm1.1Mag}$ neural cell adhesion molecule 1; targeted mutation 1.1, Terry Magnuson	9	Ncam$^{tm1.1Ciw}$, NCAM^{tm3Ciw}	Targeted (Null/knockout)	no abnormal phenotype observed	
Ncam1^{tm1Cgn} neural cell adhesion molecule 1; targeted mutation 1, University of Cologne	9	Ncam-, N-CAM-, NCAM^{-}, N-CAM-1-	Targeted (Null/knockout)	behavior, cellular, nervous system, vision/eye	
Ncam1^{tm1Klc} neural cell adhesion molecule 1; targeted mutation 1, Kathryn L Crossin	9		Targeted (Null/knockout, Reporter)	nervous system	
Ncam1^{tm1Mag} neural cell adhesion molecule 1; targeted mutation 1, Terry Magnuson	9	Ncam-180-, Ncam^{tm1Cwr}	Targeted (Null/knockout)	behavior, nervous system, vision/eye	
Ncam1^{tm1Msch} neural cell adhesion molecule 1; targeted mutation 1, Melitta Schachner	9	NCAMff	Targeted (Conditional ready, No functional change)	nervous system	
Ncam1^{tm2Mag} neural cell adhesion molecule 1; targeted mutation 2, Terry Magnuson	9		Targeted (Null/knockout)	mortality/aging	
Tg(Rip1NCAM120)1Gcr transgene insertion 1, Gerhard Christofori; transgene insertion 1, Gerhard Christofori	UN	Rip1/NCAM 120	Transgenic (Inserted expressed sequence)		
Ncam1$^{Gt(D182G11)Wrst}$ neural cell adhesion molecule 1; gene trap D182G11, German Gene Trap Consortium	9		Gene trapped **(Cell Line)**		

Targeted gene

基因名称	*Ncam1*
基因ID（NCBI）	17967
基因NCBI链接（NCBI）	https://www.ncbi.nlm.nih.gov/gene/17967
基因Ensembl链接	http://uswest.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000039542;r=9:49413436-49710225
基因组位置	Chr9

Gene information（NCBI）

Ncam1 neural cell adhesion molecule 1 [*Mus musculus* (house mouse)]

Gene ID: 17967, updated on 5-Jan-2022

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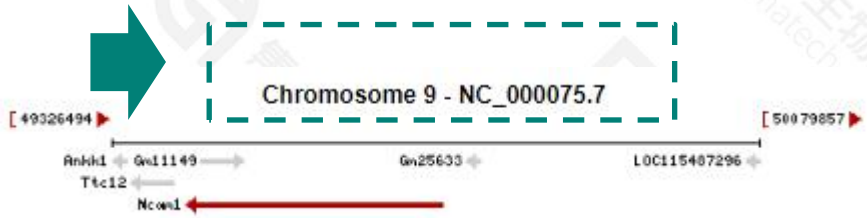
Summary

Official Symbol	Ncam1 provided by MGI
Official Full Name	neural cell adhesion molecule 1 provided by MGI
Primary source	MGI:MGI:97281
See related	Ensembl:ENSMUSG00000039542
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	CD56; Ncam; E-NCAM; NCAM-1
Summary	Predicted to enable LRR domain binding activity and phosphatase binding activity. Involved in commissural neuron axon guidance and regulation of semaphorin-plexin signaling pathway. Acts upstream of or within several processes, including homotypic cell-cell adhesion; positive regulation of calcium-mediated signaling; and regulation of exocyst assembly. Located in several cellular components, including external side of plasma membrane; growth cone; and neuronal cell body. Is expressed in several structures, including embryo mesenchyme; nervous system; sensory organ; skin; and urinary system. Human ortholog(s) of this gene implicated in bipolar disorder; middle cerebral artery infarction; and pancreatic cancer. Orthologous to human NCAM1 (neural cell adhesion molecule 1). [provided by Alliance of Genome Resources, Nov 2021]
Expression	Biased expression in CNS E18 (RPKM 29.9), whole brain E14.5 (RPKM 28.6) and 10 other tissues See more
Orthologs	human all

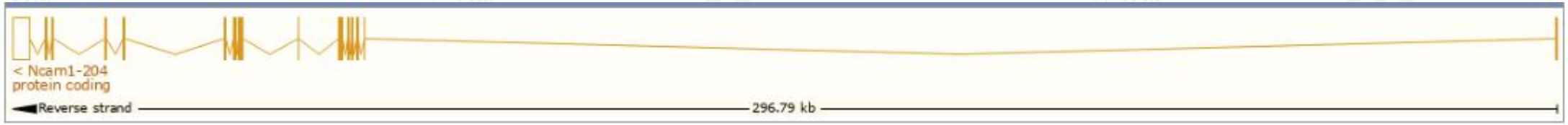


Transcript information（Ensembl）

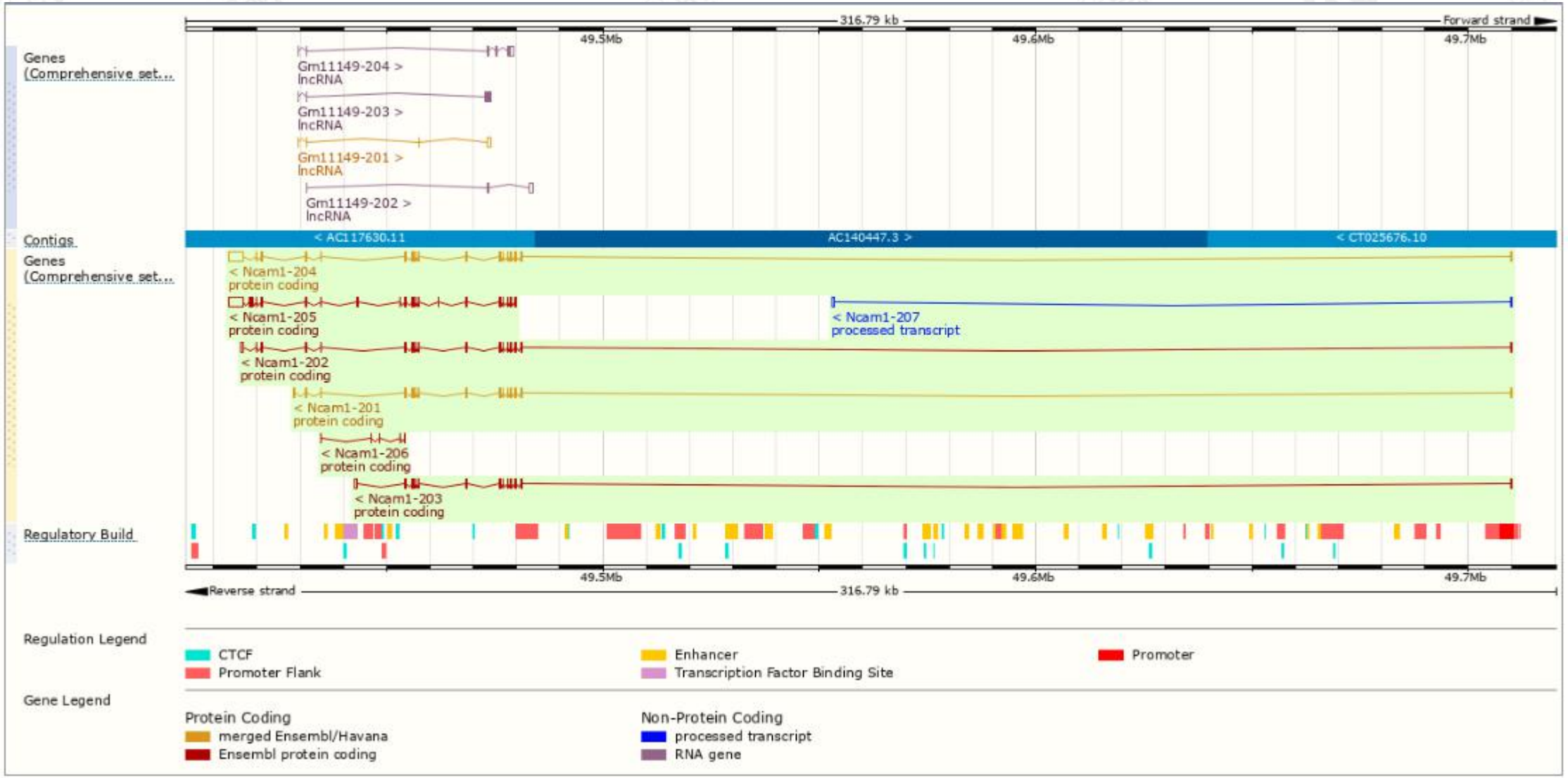
The gene has 7 transcripts, and the transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000193547.6	Ncam1-204	6050	848aa	Protein coding	CCDS40617	A0A0A6YY47	GENCODE basic APPRIS P3 TSL:1
ENSMUST00000166811.9	Ncam1-202	3088	849aa	Protein coding	CCDS80993	E9QB01	GENCODE basic APPRIS ALT1 TSL:1
ENSMUST00000114476.8	Ncam1-201	2567	725aa	Protein coding	CCDS40618	E9Q589	GENCODE basic TSL:1
ENSMUST00000194252.6	Ncam1-205	6564	1089aa	Protein coding	-	A0A0A6YY91	TSL:5 CDS 5' incomplete
ENSMUST00000192584.2	Ncam1-203	2525	605aa	Protein coding	-	A0A0A6YWU2	GENCODE basic TSL:1
ENSMUST00000194844.2	Ncam1-206	146	49aa	Protein coding	-	A0A0A6YWW5	TSL:5 CDS 5' and 3' incomplete
ENSMUST00000217121.2	Ncam1-207	914	No protein	Processed transcript	-	-	TSL:1

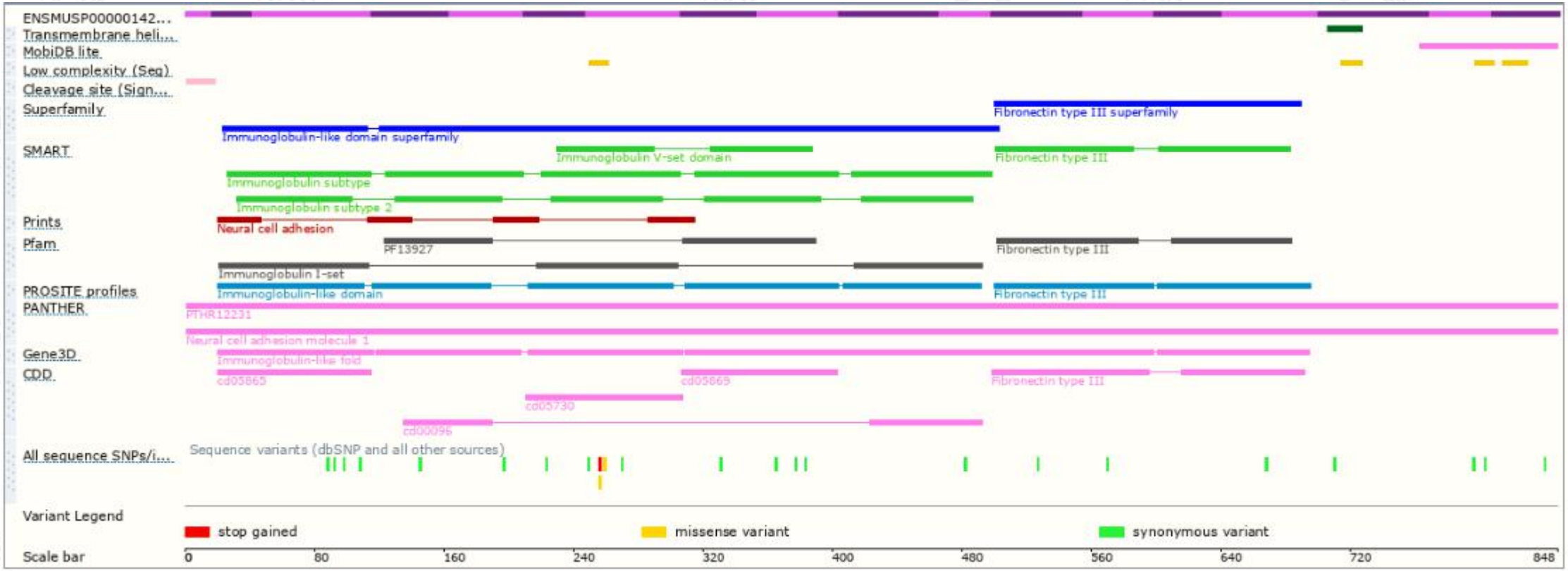
The strategy is based on the design of *Ncam1*-204 transcript,the transcription is shown below:



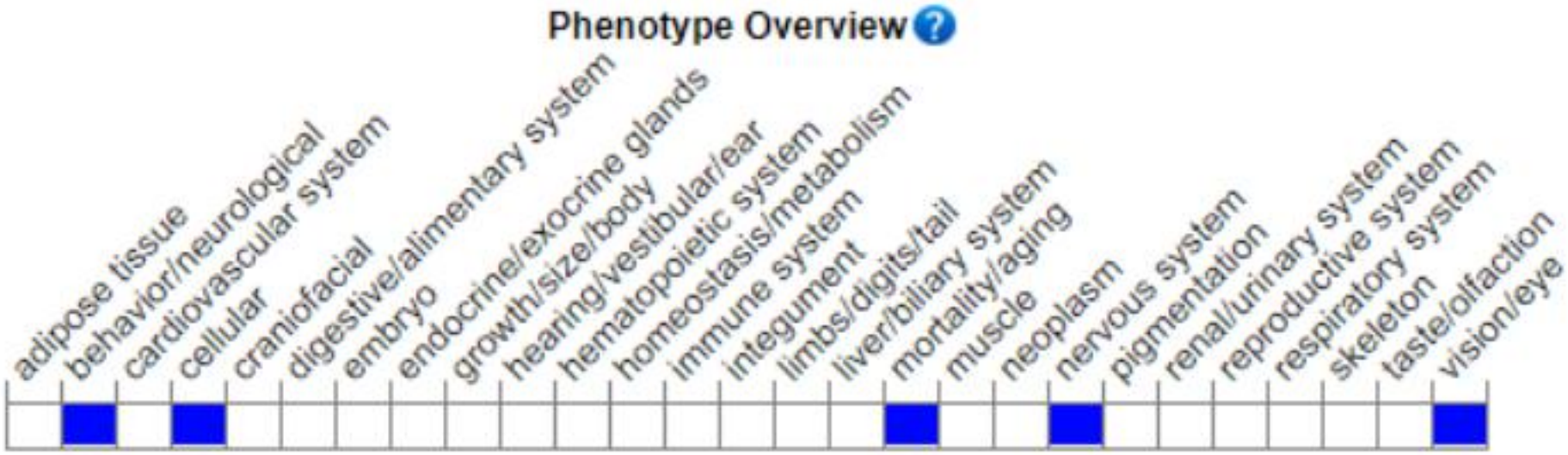
Genomic location distribution



Protein domain



Phenotype Overview(MGI)



http://www.informatics.jax.org/marker/MGI:97281

Homozygous mutants show impairment in Morris water maze test, reduced brain and olfactory bulb size, hypoplasic corticospinal tract, abnormally distributed anterior pituitary cell types, and neuromuscular junctions defects. Homozygotes for a null allele exhibit age-dependent retinal abnormalities.

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

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