

# *Gfap-P2A-Dre* cas9-ki Strategy

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**Reviewer**

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# Project Overview

**Project Name**

***Gfap-P2A-Dre***

**Project type**

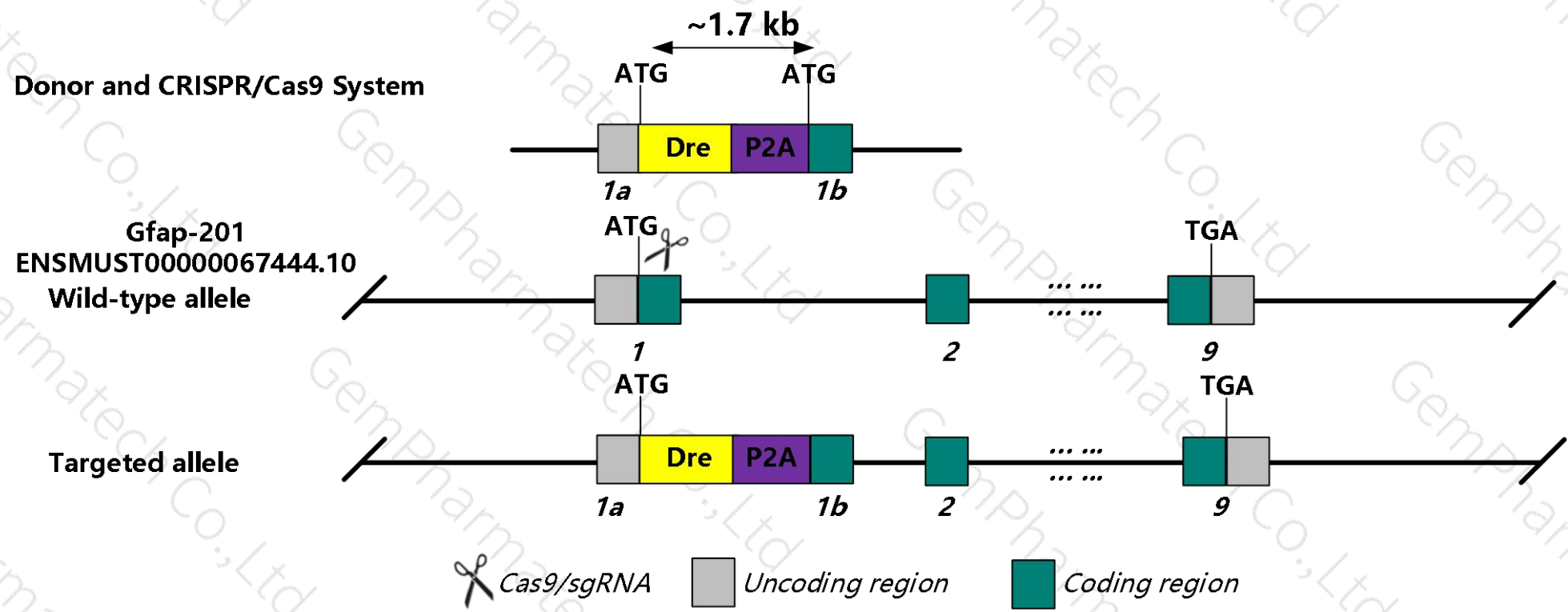
**cas9-ki**

**Strain background**

**C57BL/6JGpt**

# Knockin strategy

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



- The *Gfap* gene has 3 transcripts. According to the reference and structure of *Gfap* gene, *Gfap-201*(ENSMUST00000067444.10) is selected for presentation of the recommended strategy.
- *Gfap-201* gene has 9 exons, with the ATG start codon in exon1 and TGA stop codon in exon9.
- We make *Gfap-P2A-Dre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start codon(ATG) of *Gfap* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in Dre-P2A after start codon(ATG) of *Gfap* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

- According to the existing MGI data, homozygotes for targeted null mutations show reduced astrocyte-associated intermediate filaments, enhanced long-term potentiation and impaired eye-blink conditioning. Aged mutants may show hydrocephaly, reduced myelination and impaired blood-brain barrier.
- 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 Dre-P2A may affect the regulation of the 5' end of the *Gfap* gene.
- The influence of 203 transcript is unknown.
- There may be 1 to 2 amino acid synonymous mutation in exon1 of *Gfap* gene in this strategy.
- The *Gfap* gene is located on the Chr11. 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.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene after 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 information (NCBI)

## Gfap glial fibrillary acidic protein [ *Mus musculus* (house mouse) ]

[Download Datasets](#)

Gene ID: 14580, updated on 27-Apr-2021

### Summary

Official Symbol	Gfap provided by <a href="#">MGI</a>
Official Full Name	glial fibrillary acidic protein provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:95697</a>
See related	<a href="#">Ensembl:ENSMUSG00000020932</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<a href="#">Mus musculus</a>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI836096
Expression	Biased expression in cerebellum adult (RPKM 34.4), frontal lobe adult (RPKM 32.7) and 4 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>
<b>NEW</b>	Try the new <a href="#">Gene table</a> Try the new <a href="#">Transcript table</a>

### Genomic context

Location: 11 E1; 11 66.48 cM

See Gfap in [Genome Data Viewer](#)

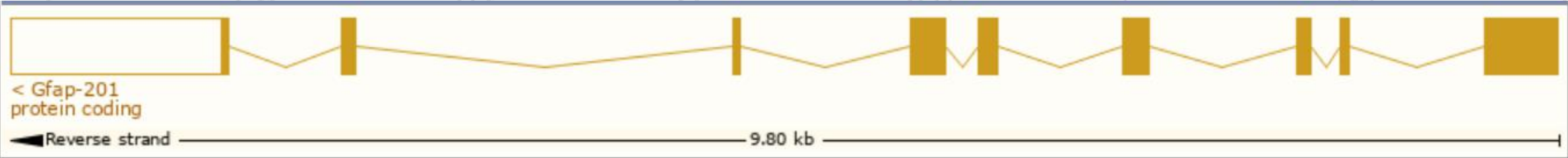
Exon count: 11

# Transcript information (Ensembl)

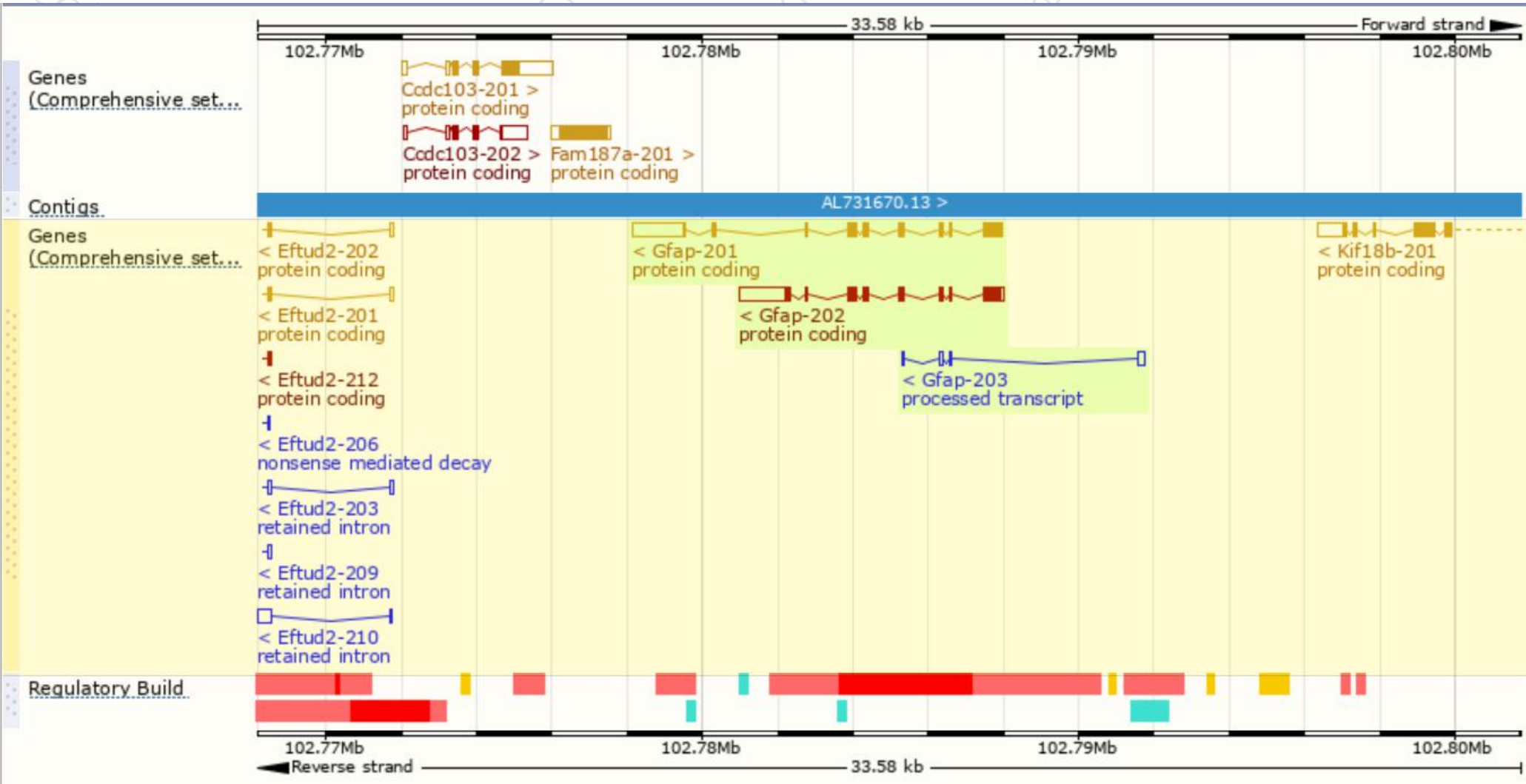
The gene has 3 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Gfap-201	<a href="#">ENSMUST00000067444.10</a>	2648	<a href="#">430aa</a>	Protein coding	<a href="#">CCDS25507</a>	<a href="#">P03995-1</a>	GENCODE basic APPRIS P3 TSL:1
Gfap-202	<a href="#">ENSMUST00000077902.5</a>	2600	<a href="#">428aa</a>	Protein coding	<a href="#">CCDS48950</a>	<a href="#">P03995-2</a>	GENCODE basic APPRIS ALT2 TSL:1
Gfap-203	<a href="#">ENSMUST00000127909.2</a>	356	No protein	Processed transcript	-	-	TSL:3

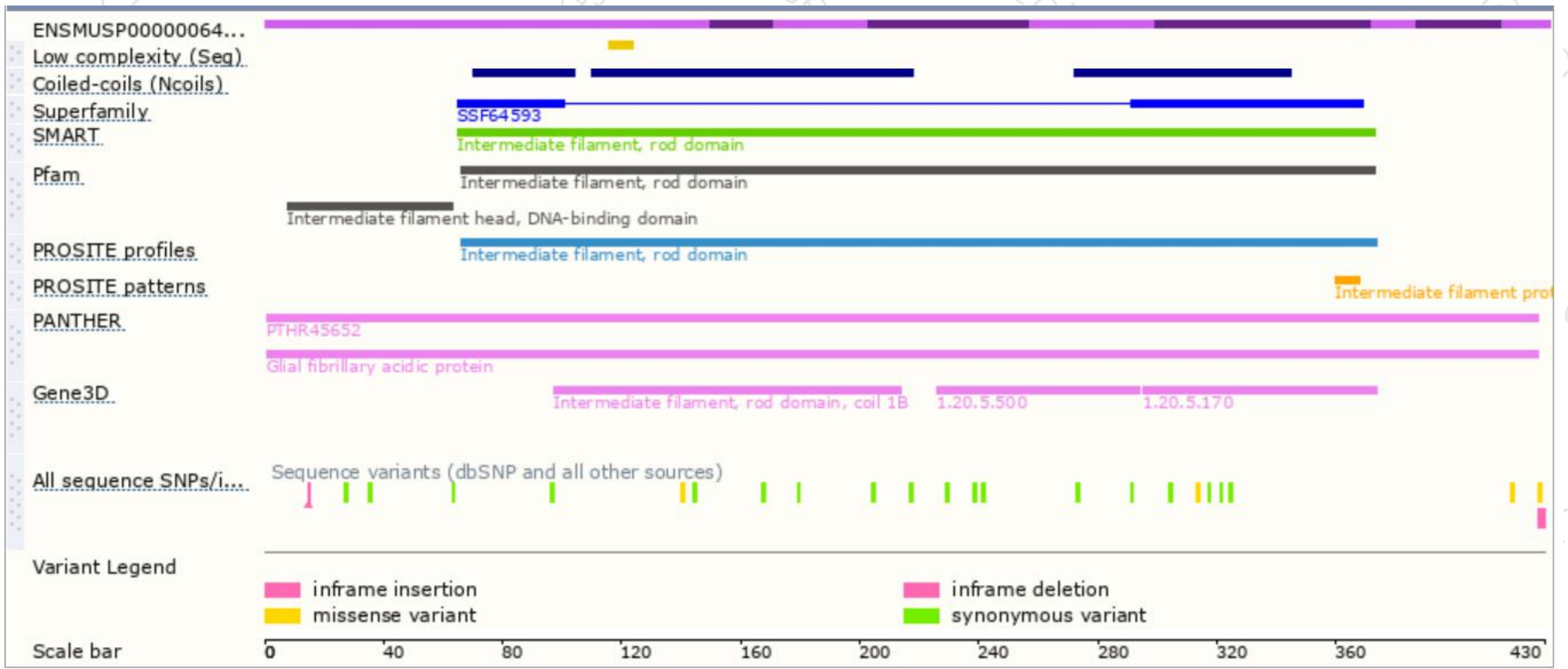
The strategy is based on the design of *Gfap-201* transcript,the transcription is shown below



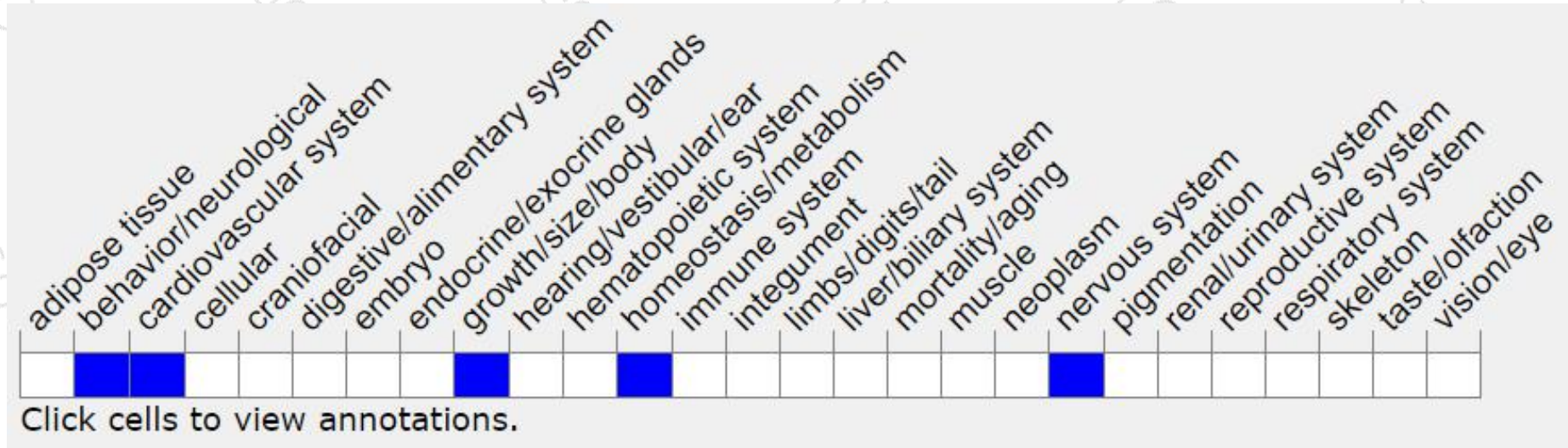
# Genomic location distribution



# 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:95697>) .*

Homozygotes for targeted null mutations show reduced astrocyte-associated intermediate filaments, enhanced long-term potentiation and impaired eye-blink conditioning. Aged mutants may show hydrocephaly, reduced myelination and impaired blood-brain barrier.

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