

***H11-Gfap-iCre-PolyA* Cas9-KI Strategy**

Designer:

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Design Date:

2019-8-19

Reviewer

JiaYu

Project Overview

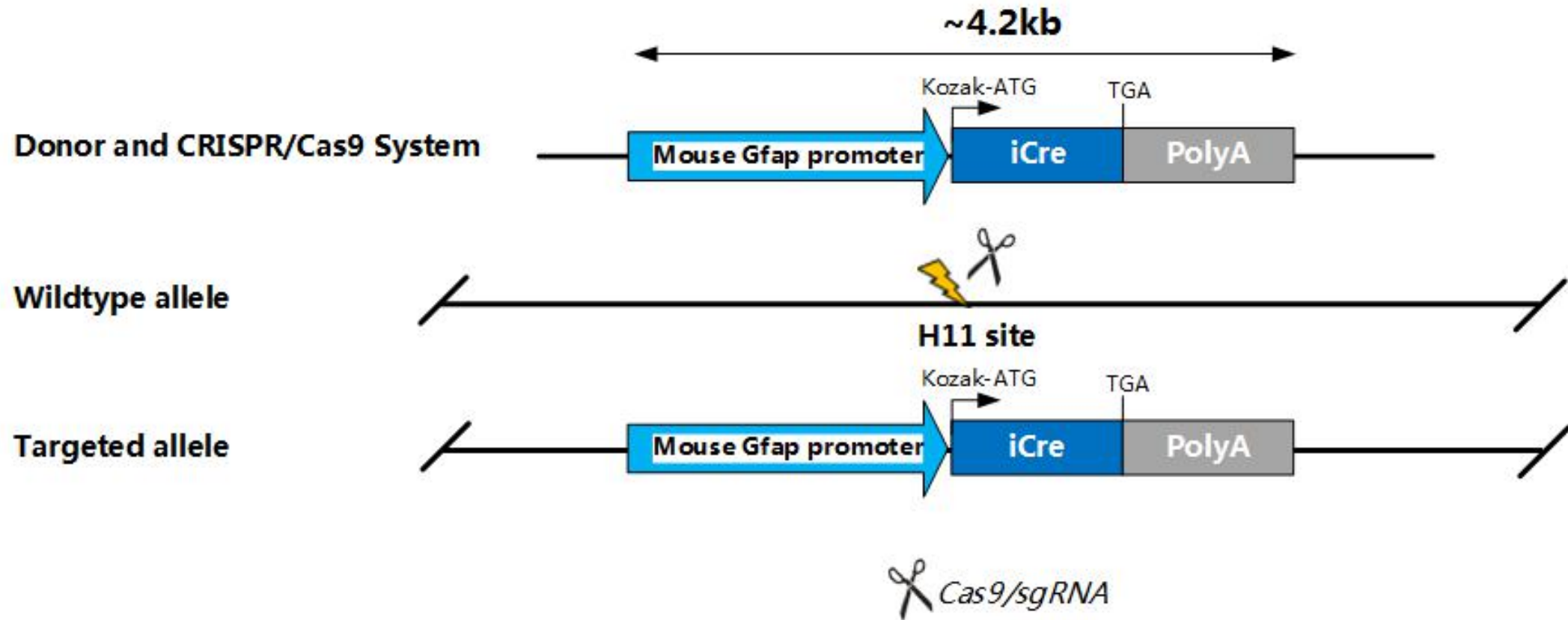
Project Name	<i>H11-Gfap-iCre-PolyA</i>
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Project type	Cas9-KI
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Strain background	C57BL/6J
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Knockin strategy

The *Gfap-iCre-PolyA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:





AAAGCTTAGACCCAAGCAAGGTAGTACATGCCTTTAATCCCAGAAGACTCGGCAAGGAGATCTCTGAGTTCAAGGTCAGCCTGGGACGAAGCAAGTCCTAGATCCAGGT
GTGCCAGCACATCTGTTGGAACCTACTTCTACAGAAGACCAGCTGAAACAAGTAGCCTCGTGGGTGGGACTGAGCAACTACTAGATCCTTGGTCTTCCCATCCACAGCTG
CTCATTGTTGGGTAGTTGGACTACAGACTGTACATCATCACAATAAAGTCCCTTAATCTATAGAGGCGTTCCATAAATTCTGTGACTAGAGAACCCTGACTAACACAGGAG
GGATTCTCCAGCGTTGCTGACAAACCTTTCCCTACCCCTCGTCATATCTTTTTCCTTCAGAACCCTCTGTTTATACCTGGAAAGATGGGCCACTCCTATTCTGTGAGATGTGC
CTGAGTTAAAGGCTAATCTCTAGTAAGGCTCTTCCTTTAAAGTCCCCTACTCTTTACCTTCCCAACTTATAGAAGTATGAAAAGTCACCAGAGGCAGAAAGGTGGTCTCACA
ATCTTTAATCCCCAAAGGGTAGTTGGCTTTCTGTGAATTCCATACCTGCTTGATCCACATATGAACTACAGGGGGACATGATGAGGTCCAGTCTAAAGGTCACTGGCAACCT
CTCTCAAGATCTCCCTCACTATGCCATTATTCAGGATTGGGGAAGATGTGGCTGGAGCCTAAGGGGCTCTTCCCTTCCCTATGGTGGGACTCATTAGGAGAACCTCAGCAA
GCAGCCCACTGTAAGCTCAAACAATAACCATGTCGCTGGTATGGAGTATAGGCTGTTGCTATGACAGGAACTCAGGGGTCTTAAGTGGCTTGAGCGCTGGGAGGGGGCA
AGCAGCCAGGCCTTGTCTGTAAGCTGAAGACCTGGCAGTGCTGAGCTGGTCAGCCCCCAGGACCTCCTTTTGTGCCACGAGTGACTCACCTTGGCATAGACATAATGGT
CAGGGGTGGGCACGCAGCCTGCTTCCCGCTGTGCTCCAGGCCTCCTTCGATGCTTTCCGAGAAGTCTATTGAGCTGGGAGCTTGTACTGCACCCGGGGCTGACATCCTGG
CATCCTGGGATAAAAGCAGCCACGGGGCTGCCCTTGCCATATGCCTCACTGGCGGCAGAGAACAAGGCTCTATTGAGCGAGTACCCTGGAGTAGACACCAGAAGCCC
AAGCATGGGCAGAGGAAGGCAGGGGTTGGGGGGAGCAGAGCTGTCTGTGTTCCAGAAGCCCAAGGACACAGATGGCTAAGGCGCCTGGGAGAGGGGACCTGAGTGG
AAGAGATAGATGGGCCTGAAGTCTCAAGCAGCAACAGCCTCCTCCCCGCCATTGGTGAGGGTGGGGTTTGGTTTCCCGGACCTACATATCCCTCAGAGGCCTGGTGTGTA
GGAATTTAAAGGGGGTAAATCTCCTGAGAGAATGAGGGGTACCCAGGAAGACGGGGTGTTACAGAAAGAAAGACTCCAGCATGCACAGCCAACCTATTCAAACTACT
CTGTCAGGGGCTGCCAGGGGCCAGGCTCGGGGTGGGGGTGGGGGGCAACGAGAAGCTGGATCAGGGAGAAATGGCCCACTAGGCTGGATAAGAGGCCACAGAGG
GGCTCAGGAATGAAGCCTGCTGTCTTACCCTATTAGGATCTGCGTGCATACCTTCTGCCGTGCACTCTAAACACACAGCCAGAGGCTCAAGTTGACCCTGGAGTCACAGA
GAGGGCTCCAACCTTAGCCCTCCACTCCTGAACTCCAGGAATGAGAAGATAGAGTTGGAGAGATTCAGGGGAGAGGACTCTGTTGAGAATGGGGGTACAGGAACTG
TAATATAGGTTGATCCCGGAGGAAGGGAATAGGTTCTTCAAGTTCCTAGCATCTCACAGGCCCCCAGAGAAGGACAGAGTTGGGGTGGTCTGGCTTACAGGCTCTAAG
AACTGGAAGCTGATTACCCACCGAGCTGTGCACTCTCTGTCTCTGTCTGTGTGCGCTCGTGCACACTTATCACACAAATGTTTCATGTGTGTGCACATACATGTGTTGA
GACCAGAGGTCAACCTCAGGCACTGTTGCCTTGGTTTTCTGAGAGAGCATTCTCTCTGGATCTGGAACCTCGCCAATTAGTGAGAGCCAGGAAGTCTGCTGATTTTCACT
GCCCAGCACTGGAGTTTACAAGTATGCACTGTCAACCCAGGCCTTTTGTATTATTCTGCACTAGAACTTGGGTGGGTCTTCATGCTTGACAGGCAAGCAATTTATGGAC
TAAGCTGTTCCCTCGGCCCTCTCTTGACCCATTTACCAGAAAGGGGGTTCCTTGATCAATGGCGAAGCCAGGCTGGTGTTCCTCAAGAAAGCCTTGACTCTGGGTACAGTG
ACCTCAGTGGGGTGAGAGGAGTTCTCCCCCTAGCTGGGCTGGGGCCCAGCTCCACCCCTCAGGCTATTCAATGGGGGTGCTTCCAGGAAGTCAGGGGCAGATTTAGTC
CAACCCGTTCCCTCCATAAAGGCCCTGACATCCCAGGAGCCAGCAGAGGCAGGGCAGG

- The Mouse *Gfap* gene has 3 transcripts. According to the structure of *Gfap* gene, *Gfap-201*(ENSMUST00000067444.9) is selected for presentation of the recommended strategy.
- The Mouse *Gfap* promoter is from reference, the length is about 2.6kb.
- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.
- In this study, the *Gfap-iCre-PolyA* gene fragment was inserted into H11 site of mice by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and sgRNA were constructed in vitro, Cas9, donor and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6J mice by PCR, sequencing, and southern blot, then the stable inheritance of F1 positive mice model was obtained.

- H11 is located on Chr11. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., iCre) strains, if the other gene is also on Chr11, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. 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)]

Gene ID: 14580, updated on 13-Aug-2019

Summary

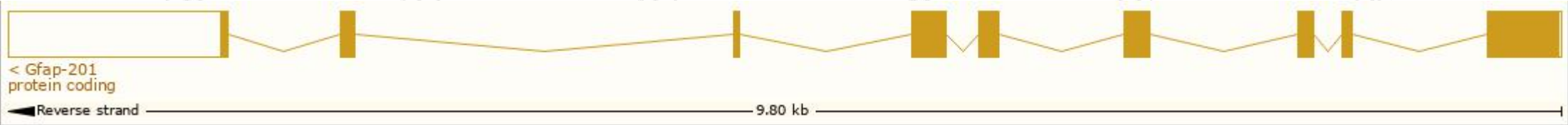
Official Symbol	Gfap provided by MGI
Official Full Name	glial fibrillary acidic protein provided by MGI
Primary source	MGI:MGI:95697
See related	Ensembl:ENSMUSG00000020932
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata;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

Transcript information (Ensembl)

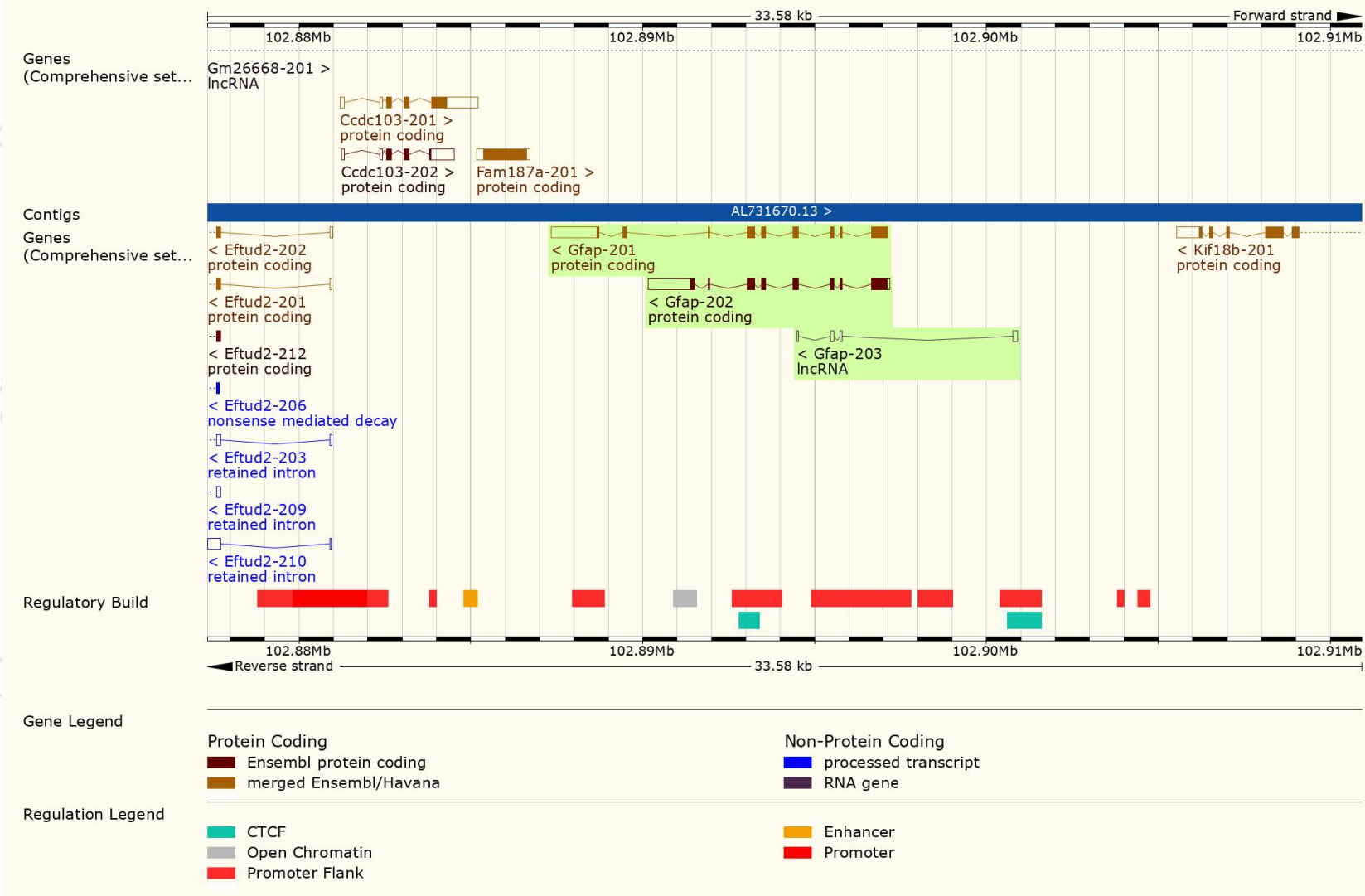
The gene has 3 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gfap-201	ENSMUST00000067444.9	2648	430aa	Protein coding	CCDS25507	P03995	TSL:1 GENCODE basic APPRIS P1
Gfap-202	ENSMUST00000077902.4	2600	428aa	Protein coding	CCDS48950	P03995	TSL:1 GENCODE basic
Gfap-203	ENSMUST00000127909.1	356	No protein	lncRNA	-	-	TSL:3

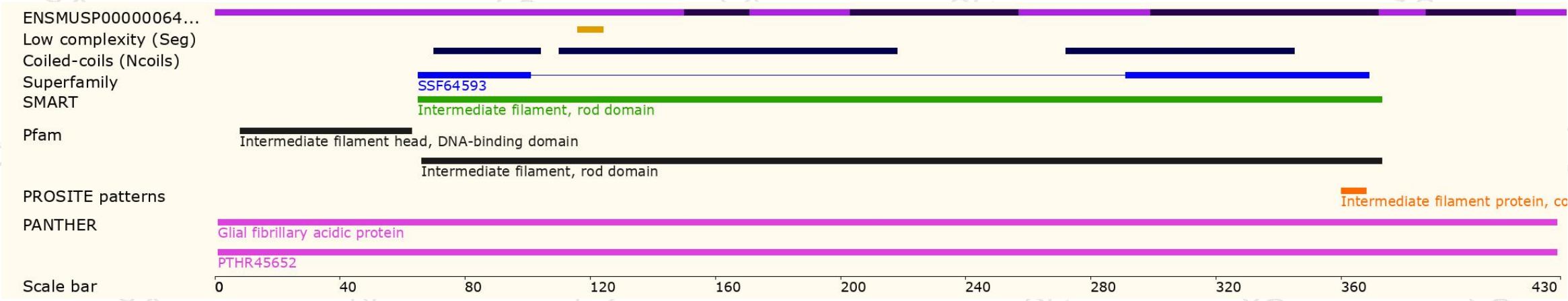
The strategy is based on the design of *Gfap-201* transcript, The transcription is shown below



Genomic location distribution



Protein domain



[1]Miura M, Tamura T, Mikoshiba K. Cell-specific expression of the mouse glial fibrillary acidic protein gene: identification of the cis- and trans-acting promoter elements for astrocyte-specific expression. J Neurochem. 1990 Oct;55(4):1180-8.

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