

H11-CAG-LSL-mutant Capn2-3*flag-P2A-CFP **Cas9-KI(H11) Strategy**

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Reviewer: Zihe Cui

Design Date: 2021-8-13

Project Overview

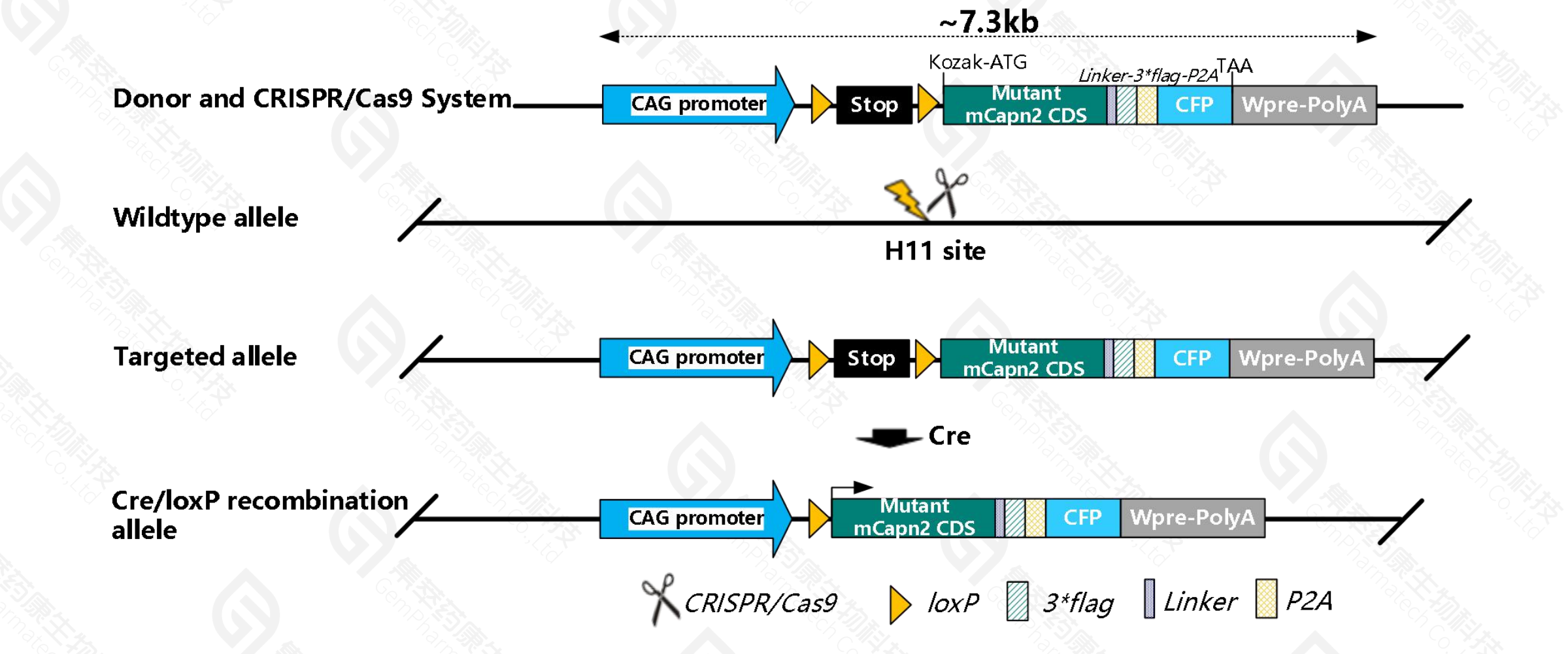
Project Name	H11-CAG-LSL-mutant Capn2-3*flag-P2A-CFP
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Project type	cas9-ki(H11)
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Strain background	C57BL/6JGpt
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Knockin strategy

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

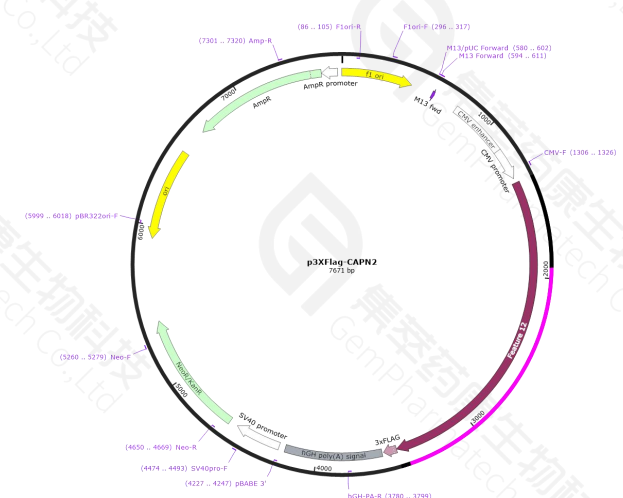


- According to the customer's requirements and gene information, the mutant *Capn2* sequences designed in this strategy was provided by the customer, with a 2103 nt CDS of length and coding of 700aa. CFP and Mutant *Capn2* CDS with 3xflag were expressed by the promoter *CAG*, Kozak^[1] is used to enhance the translation of *Capn2*.
- Before breeding with Cre mice in this model, the expression of intrested gene was turned off. After breeding with Cre mice, the *Stop* element would be deleted to turn on the expression.
- 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^[2].
- In this project we use CRISPR/Cas9 technology to modify H11 localization. The brief process is as follows: CRISPR/Cas9 system and donor vector 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.

- The expression of CFP and Mutant *Capn2* gene were turned off before breeding with Cre mice. After breeding with Cre mice, both genes could be expressed in specific tissues or cells.
- Please confirm the CDS sequence of *Capn2* gene, and the CDS needs to be synthesized.
- Please confirm the the sequence of linker between Mutant *Capn2* and 3xFlag referred to Addgene sequence.
- Mutant *Capn2*-3xFlag and CFP were connected with P2A, and they will be translated together, but can be divided into two independent proteins (non-fusion proteins) , and the protein in front of P2A will retain the peptides translated by 2A.
- The H11 localization is located on the Chr11. 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.

ATGGTGAGCAAGGGCGAGGAGCTGTTACACCGGGGTGGTGCCCATCCTGGTTCGAGCTGGACGGCGACGTAAACGGCCACAGGTTTCAGCGTGTCCGGCGA
GGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGA
CCTGGGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGCGTACC
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CAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACCTACATCAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGG
CCCCTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTG
CCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGG
GATCACTCTCGGCATGGACGAGCTGTACAAGTAA

TGCTCTAGAGGATCCCGGGCT



<https://www.addgene.org/60942/>

Gene information(NCBI)

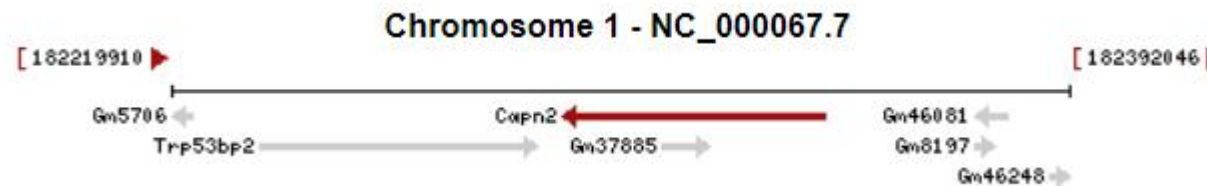
Capn2 calpain 2 [*Mus musculus* (house mouse)]

Gene ID: 12334, updated on 23-Jun-2021

[Download Datasets](#)

Summary

Official Symbol	Capn2 provided by MGI
Official Full Name	calpain 2 provided by MGI
Primary source	MGI:MGI:88264
See related	Ensembl:ENSMUSG000000026509
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	Ca; Cap; m-c; Capa2; CALP80; Capa-2; AI326419; m-calpin; m-calpain
Expression	Ubiquitous expression in bladder adult (RPKM 35.3), subcutaneous fat pad adult (RPKM 29.4) and 27 other tissues See more
Orthologs	human all



Transcript information(Ensembl)

The gene has 5 transcript, the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Capn2-201	ENSMUST00000068505.10	3225	700aa	Protein coding	CCDS35813	O08529	GENCODE basic APPRIS P1 TSL:1
Capn2-203	ENSMUST00000192483.2	3017	No protein	Retained intron	-	-	TSL:1
Capn2-202	ENSMUST00000192230.6	2366	No protein	Retained intron	-	-	TSL:1
Capn2-205	ENSMUST00000194961.2	728	No protein	Retained intron	-	-	TSL:1
Capn2-204	ENSMUST00000194940.6	580	No protein	Retained intron	-	-	TSL:3

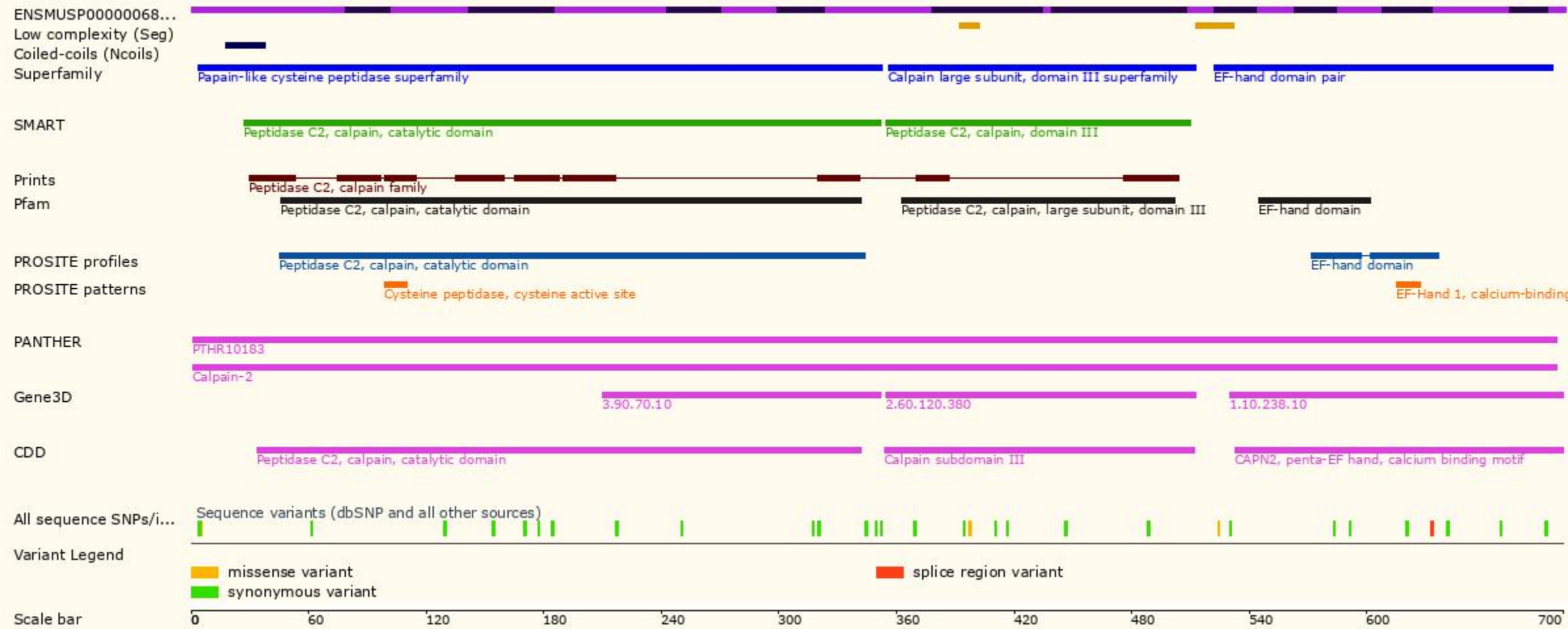
The mutant *Capn2* sequence designed in this strategy was provided by the customer, the detailed sequence were shown below:

Mutant Capn2 CDS (2103 nt)

ATGGCGGGCATCGCGATAAAGCTGGCCAAGGACCGCGAAGCGGCCGAGGGGCTGGGGTCGCATGAGAGAGCCATCAAGTACCTCAACCAGGACTACGAGACGCTGCGGAACGAGTGCCTGGAGGCCGGGGCGCTCTTCCAGGATCCTTCCTTC
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CAGAAGTTGGTGAAAGGACATGCGTACTCTGTCAACCGGAGCCGAGGAGGTTGAAAGTTCAAGGAAGCCTGCAGAAATTGATCCGCATCAGGAATCCCTGGGGACAAGTAGAGTGGAACCGGGAAGTGGAATGACAATTGCCCCAGCTGGAACACG
GTTGACCCAGAAGTAAGGGCAAATCTAACCGAACGGCAGGAGGACGGAGAATTCTGGATGTCTTCAGTGACTTCCTGAGACACTACTCTCGCCTGGAGATCTGCAACCTGACCCCGGACACCCCTCACCTGTGACTCCTATAAGAAGTGAAAA
CTCACCAAGATGGATGGGAACTGGAGGCGAGGCGCCGCGCAGGGGGCTGCAGGAATTACCCAAATACCTTCTGGATGAACCCCTCAGTACCTAATTAAGCTGGAGGAAGAAGACGAAGATGAGGAGGATGGGGAGAGAGGCTGTACCTTCCT
GGTGGGTCTCATCCAGAAGCACCGGCGACGGCAGAGGAAGATGGGCGAGGACATGCACACCATTGGCTTCGGCATCTATGAGGTTCCAGAGGAGCTAACAGGGCAGACCAACATCCACCTCGGCAAAAACTTTTCTCACAACCCGAGCCAG
GGAGCGGTGAGATACCTTCATTAACCTCCGCGAGGTCTCAACCGCTTCAAGCTGCCCCGGGAGAGTATGTCTCGTTCCCTCCACCTTCGAACCCACAAGGATGGCGATTTCTGCATCCGAGTCTTCTCGGAGAAGAAGGCTGACTACCA
AGCTGTTGATGACGAGATCGAGGCCAACATTGAAGAGATTGACGCCAATGAGGAGGACATTGACGATGGATTCCGAAGGCTCTTTGTTTCAGCTGGCTGGAGAGGATGCAGAGATCTCTGCCTTTGAGCTGCAGACCATCTTAAGAAGAGTTCT
AGCCAAACGCCAAGACATCAAGTCAGACGGCTTCAGCATCGAGACCTGTAAGATCATGGTGGACATGCTGGATGAAGATGGGAGTGGCAAGCTTGGCCTGAAGGAGTTCTACATCCTCTGGACGAAGATTGAGAAAATACCAAAAAATCTACCG
GGAAATCGATGTGGACAGGTCTGGAACCATGAATTCTACGAGATGCGGAAAAGCACTGGAAGAAGCAGGTTTCAAGCTGCCCTGTCAACTTCATCAAGTCATCGTTGCCCGGTTTGCAGACGACGAGCTAATCATCGACTTTGACAATTTTGT
GCGGTGTTTGGTCCGTCTGAAAACGCTATTCAAGATATTCAAGCAGCTAGACCCTGAGAACACTGGAACGATACAGCTCAACCTTGCTTCGTGGCTGAGTTTTTCAGTACTCTGA

Protein domain

Protein domains for ENSMUSP00000068895.9



References

[1] Kozak, M. (1987). "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res* 15(20): 8125-8148.

[2] Hippenmeyer, S., et al., Genetic mosaic dissection of *Lis1* and *Ndel1* in neuronal migration. *Neuron*, 2010. 68(4): 695-709.

Additional cycles and costs

Additional itmes	cycle (month)	cost (¥)
Mutant <i>Capn2</i> CDS	1	3155

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