

H11-*Camk2a-Grin2b*-polyA Cas9-KI Strategy

Designer: Wenjing Li

Reviewer: Yanhua Shen

Design Date: 2020-12-25

Project Overview

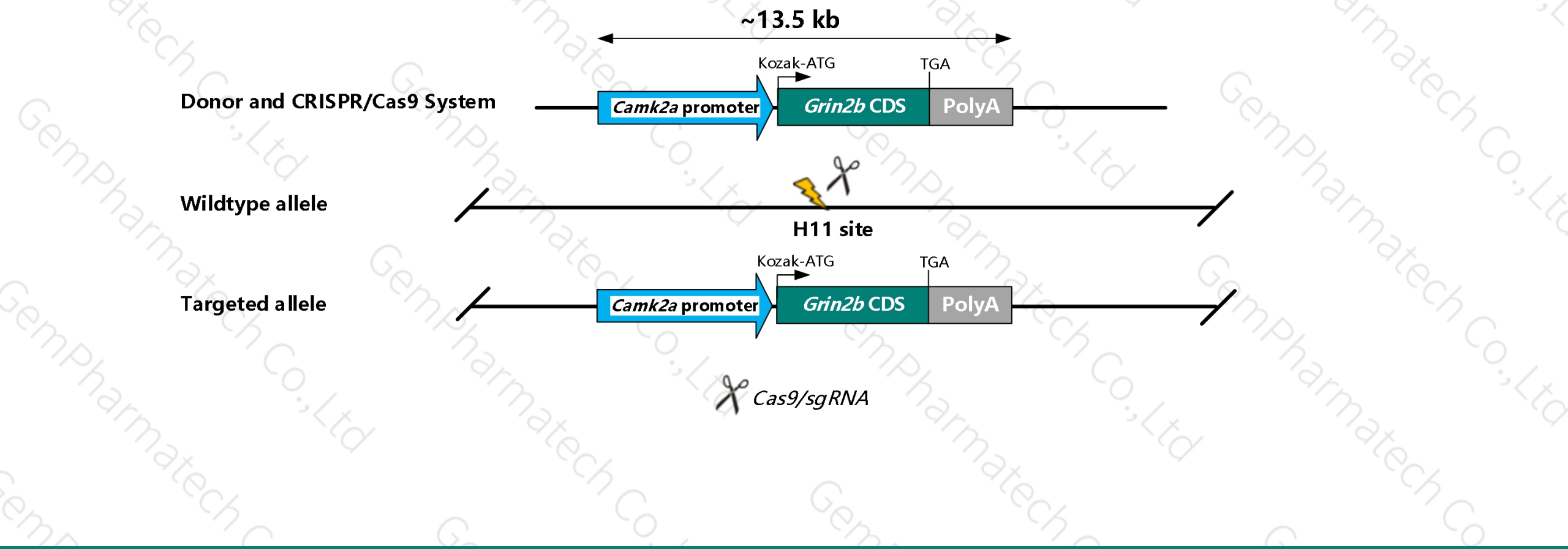
Project Name	H11-Camk2a-Grin2b-polyA
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Project type	cas9-ki
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Strain background	C57BL/6JGpt
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Knockin strategy

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



- The *Grin2b* gene has 7 transcripts.
- According to the gene structure, the transcript *Grin2b-202* (ENSMUST00000111905.7) is selected for presentation of the recommended strategy.
- The *Camk2a* promoter used in this protocol is a forebrain expression promoter with a length of about 8.5kb, based on references [1][2].
- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion^[3]. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.
- In this study, the *Camk2a-Grin2b*-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/6JGpt mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6JGpt mice by PCR, sequencing, and southern blot, then the stable inheritance of F1 positive mice model was obtained.

- *Camk2a* promoter is based on reference search, please confirm the sequence of *Camk2a* promoter.
- The phenotype of this model is unknown.
- This strategy is based on references, and the phenotype may not be consistent with the reference.
- This model selects the transcript *Grin2b-202* and the CDS needs to be synthesized. Please confirm the sequence.
- *Camk2a* promoter region has CT, AGG, TG and other repeats, and mutations may be introduced during vector construction and model making.
- 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.
- 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.

Reference model information^[2]

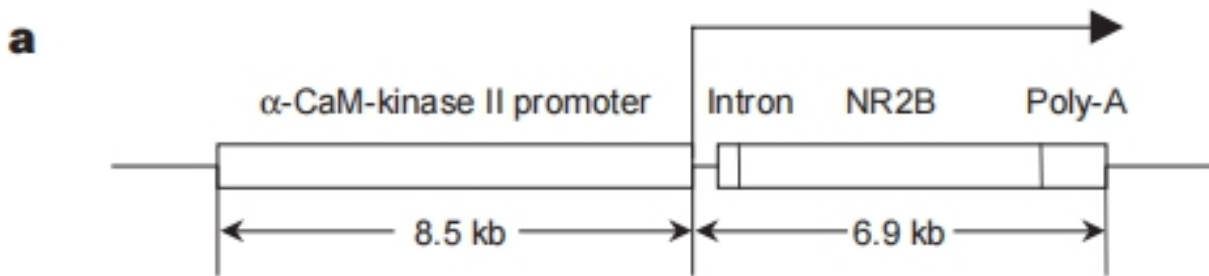


Figure 1 Construction and biochemical characterization of transgenic NR2B mice. **a**, The construct pJT-NR2B for production of NR2B transgenic mice. kb, kilobases.

b, Expression of NR2B transgene mRNA in transgenic mice. Lane 1, cortex/striatum/amygdala; lane 2, hippocampus; lane 3, brain stem and thalamus; lane 4, cerebellum.

c, Synaptic NMDA-receptor protein in hippocampus (HP) and cortex (CTX) in both transgenic lines (Tg-1 and Tg-2) and wild-type (WT). The same immunoblot was used for blotting with antibodies against NR1 (relative molecular mass 120K), NR2A (170K) and NR2B (180K). **d**, Forebrain-specific expression of NR2B transgene revealed by *in situ* hybridization. CTX, cortex; STM, striatum; HP, hippocampus; AMG, amygdala. **e**, Normal brain morphology in transgenic mice (Nissl staining). **f**, Higher magnification of the Nissl-stained transgenic hippocampus. DG, dentate gyrus; CA1 and CA3 are marked. **g**, Golgi staining of the dendritic spines of CA1 cells from wild-type (left) and transgenic mice (right). Scale bar, 5 μ m.

To test whether the NR2B subunit is crucial for implementing Hebb's rule and gating synaptic plasticity and memory, we over-expressed the NR2B subunit postnatally in the mouse forebrain using the CaM-kinase-II promoter^{15,16} (Fig. 1a). Of seven lines produced, we report here results from two independent lines (Tg-1 and Tg-2) that we have systematically analysed. They show similar expression patterns and levels of NR2B, and nearly identical electrophysiological and behavioural phenotypes.

Production and basic characterization of transgenic mice.

The transgenic founders were produced by pronuclear injection of the linearized DNA into C57B/6 inbred zygotes as described¹⁶, and then intercrossed with B6/CBF1 for various analyses. F2 wild-type mice on this hybrid background consistently showed excellent learning. This mating strategy, therefore, sets a high standard for our behavioural enhancement experiments. For detailed procedures for genotyping, northern blot, western blot and *in situ* hybridization, see Supplementary Information.

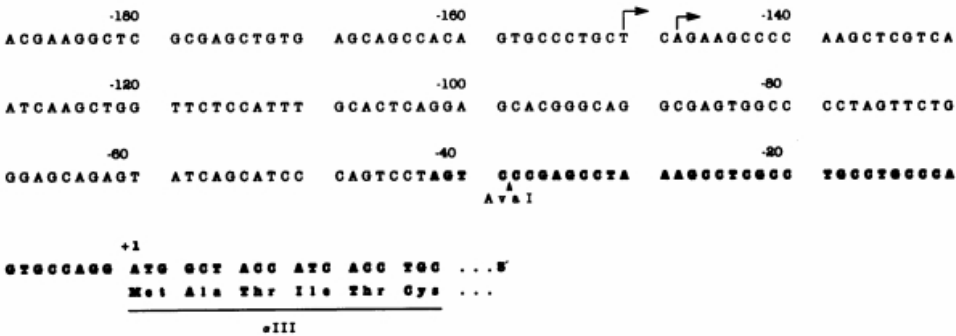
According to the reference, a mouse model was made by TG, and the *Camk2a* promoter was used to drive *Grin2b* overexpression. This strategy is made by referring to the literature, but it is inserted into the H11 site. The **Intron** sequence and information in the reference are not clear. **The intron is not added in this strategy. Please confirm the plan.**

Camk2a Promoter information^{[2][4][5]}

Reference 2

To test whether the NR2B subunit is crucial for implementing Hebb's rule and gating synaptic plasticity and memory, we over-expressed the NR2B subunit postnatally in the mouse forebrain using the CaM-kinase-II promoter^{15,16} (Fig. 1a). Of seven lines produced, we report here results from two independent lines (Tg-1 and Tg-2) that we have systematically analysed. They show similar expression patterns and levels of NR2B, and nearly identical electrophysiological and behavioural phenotypes.

Reference 5



Reference 4

Results

Generation of Mice Carrying a Transgene with a Thr-286→Asp Mutation in CaMKII

The α subunit of CaMKII is the most abundantly expressed isoform in forebrain structures such as hippocampus (McGuinness et al., 1985; Miller and Kennedy, 1985), and gene targeting experiments show that it is critical for the induction of LTP (Silva et al., 1992a). We therefore used the α subunit gene of CaMKII for the generation of transgenic mice. To limit the transgene to only specific target regions of the forebrain, we expressed the transgene under the control of its own promoter. We obtained 8.5 kb upstream of the CaMKII α transcription initiation site (Sunyer and Sahyoun, 1990) as well as a 5' portion of the coding region from a mouse genomic library. We then

According to the references, select the promoter sequence from 147bp upstream of ATG and 8.5kb upwards. Please confirm the sequence.

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CDS sequence of *Grin2b* gene (4449bp)

<https://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi?REQUEST=CCDS&DATA=CCDS20648>

ATGAAGCCAGCGCAGAGTGCTGTTCTCCCAAGTTCTGGTTGGTGTGGCCGTATCGGGCAGCAAAGCTCGTTCCCAAAAGAGCGCCCCAGCATCGGCATCGCTGTATCCTCGTGGGCACTTCCGACGAAGTGGCC
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GCAATGGACATGTTTATGAGAACTTTCTAGTATTGAGTCTGATGTCTGA

This sequence is based on the transcript *Grin2b-202*. **Please confirm the sequence**

Gene information of promoter (NCBI)

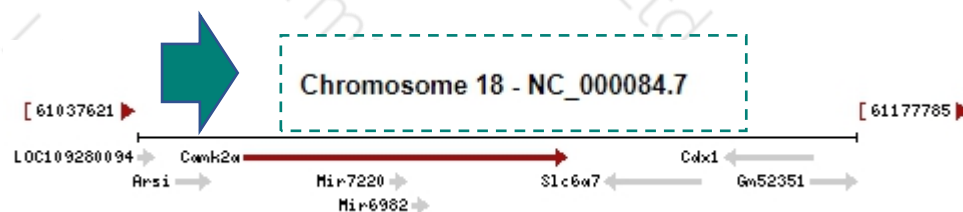


Camk2a calcium/calmodulin-dependent protein kinase II alpha [*Mus musculus* (house mouse)]

Gene ID: 12322, updated on 22-Dec-2020

Summary

Official Symbol Camk2a provided by [MGI](#)
Official Full Name calcium/calmodulin-dependent protein kinase II alpha provided by [MGI](#)
Primary source [MGI:MGI:88256](#)
See related [Ensembl:ENSMUSG00000024617](#)
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 CaMKII; R74975; mKIAA0968
Expression Biased expression in cortex adult (RPKM 365.9) and frontal lobe adult (RPKM 310.4) [See more](#)
Orthologs [human](#) [all](#)

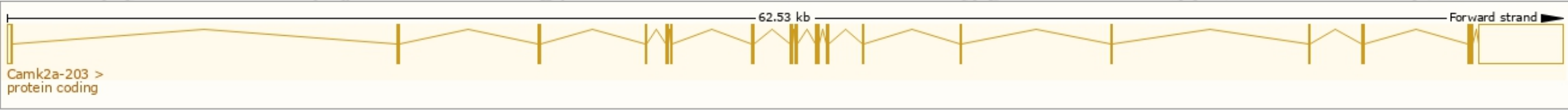


Transcript information (Ensembl)

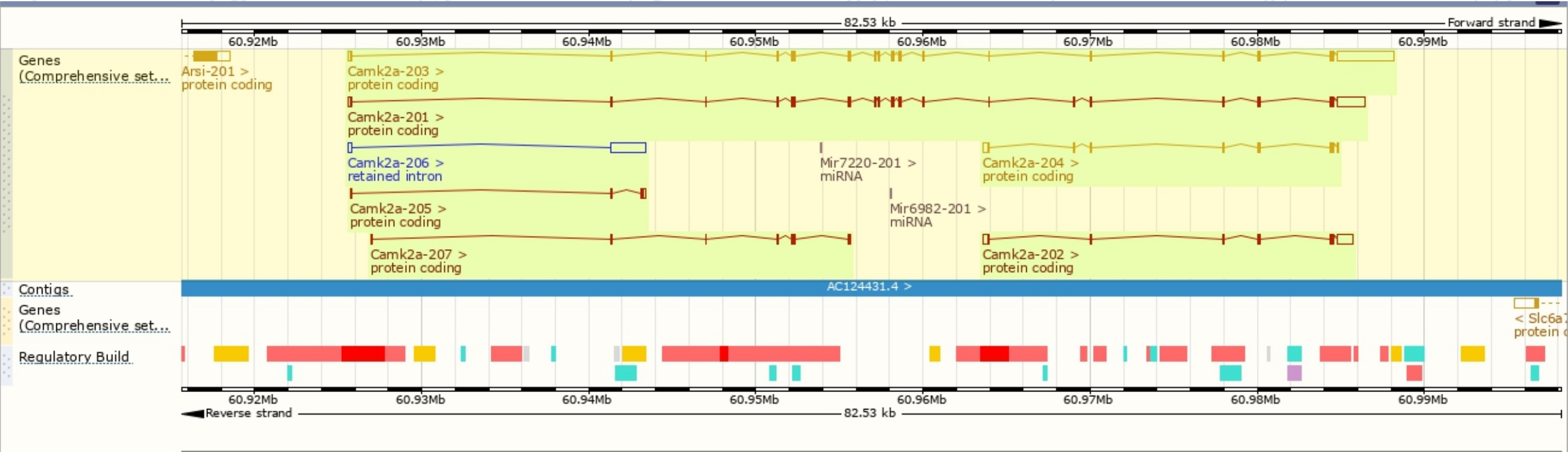
The gene has 7 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Camk2a-203	ENSMUST00000102888.9	4970	478aa	Protein coding	CCDS29276	P11798	TSL:1 GENCODE basic APPRIS P2
Camk2a-202	ENSMUST00000039904.6	1734	189aa	Protein coding	CCDS70898	F8WHB5	TSL:5 GENCODE basic
Camk2a-204	ENSMUST00000115295.8	979	200aa	Protein coding	CCDS29277	P11798	TSL:1 GENCODE basic
Camk2a-201	ENSMUST00000025519.10	3244	489aa	Protein coding	-	F8WIS9	TSL:5 GENCODE basic APPRIS ALT1
Camk2a-207	ENSMUST00000137805.2	530	177aa	Protein coding	-	F6WHR9	CDS 5' and 3' incomplete TSL:3
Camk2a-205	ENSMUST00000115297.7	499	108aa	Protein coding	-	D3Z7K9	TSL:2 GENCODE basic
Camk2a-206	ENSMUST00000134496.1	2301	No protein	Retained intron	-	-	TSL:2

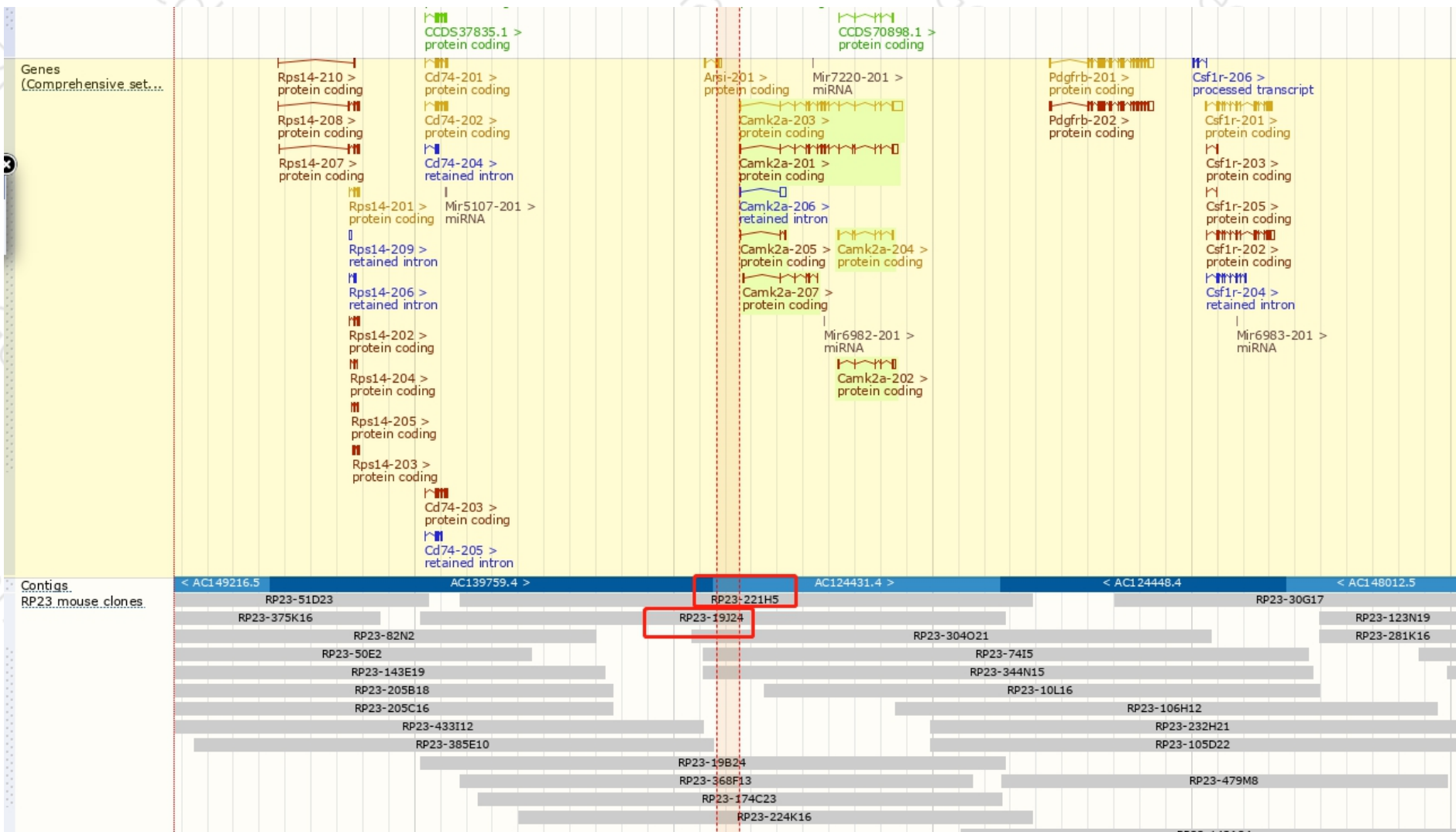
The strategy is based on the design of *Camk2a-203* transcript, The transcription is shown below



Genomic location distribution



BAC location information of promoter



It is recommended to order RP23-221H5 (preferred) or RP23-19J24 for promoter sequence amplification

Gene information of *Grin2b* (NCBI)



Grin2b glutamate receptor, ionotropic, NMDA2B (epsilon 2) [*Mus musculus* (house mouse)]
Gene ID: 14812, updated on 13-Dec-2020

Summary

Official Symbol

Grin2b provided by [MGI](#)

Official Full Name

glutamate receptor, ionotropic, NMDA2B (epsilon 2) provided by [MGI](#)

Primary source

[MGI:MGI:95821](#)

See related

[Ensembl:ENSMUSG00000030209](#)

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

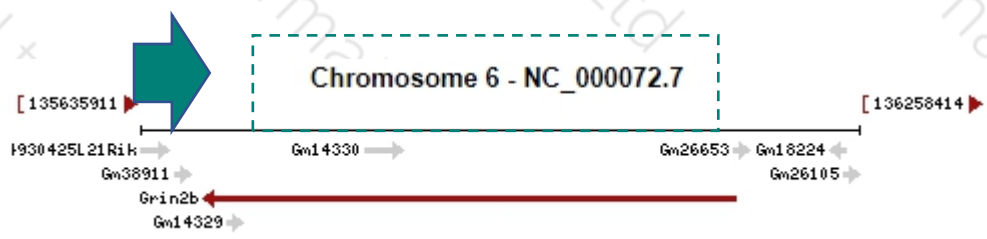
NR; GluN; NMDA NR2B; GluN2B; Nmdar2b; AW490526; GluRepsilon2

Expression

Biased expression in cortex adult (RPKM 5.7), frontal lobe adult (RPKM 5.7) and 5 other tissues [See more](#)

Orthologs

[human](#) [all](#)

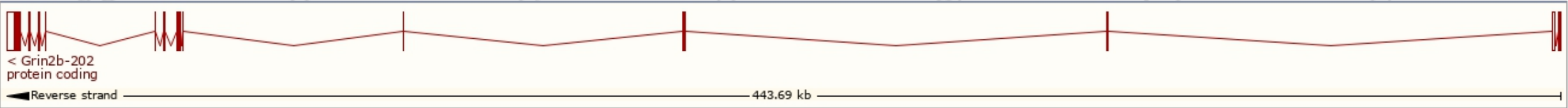


Transcript information (Ensembl)

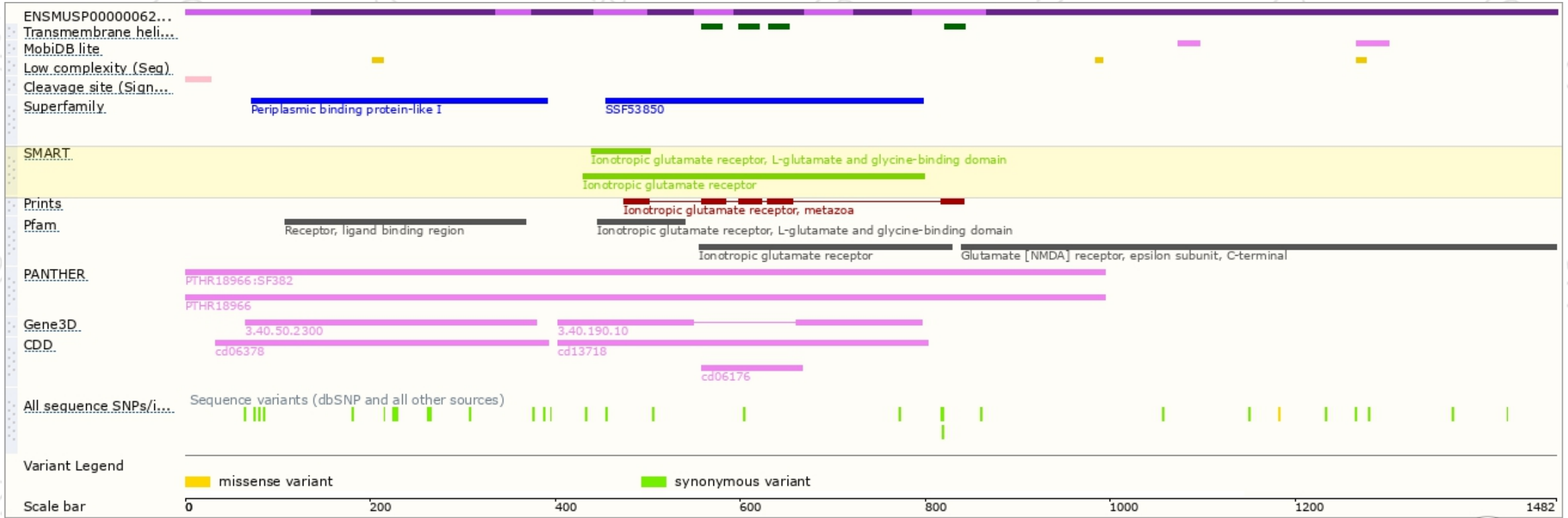
The gene has 7 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Grin2b-201	ENSMUST00000053880.12	24060	1482aa	Protein coding	CCDS20648	G3X9V4	TSL:5 GENCODE basic APPRIS P1
Grin2b-202	ENSMUST00000111905.7	7484	1482aa	Protein coding	CCDS20648	G3X9V4	TSL:5 GENCODE basic APPRIS P1
Grin2b-205	ENSMUST00000152012.7	2461	337aa	Protein coding	-	A0A0G2JEA7	TSL:1 GENCODE basic
Grin2b-204	ENSMUST00000143943.7	703	35aa	Protein coding	-	Q8CG69	CDS 3' incomplete TSL:1
Grin2b-203	ENSMUST00000125905.1	630	35aa	Protein coding	-	Q8CG69	CDS 3' incomplete TSL:1
Grin2b-206	ENSMUST00000188999.2	601	36aa	Protein coding	-	A0A087WR33	CDS 3' incomplete TSL:1
Grin2b-207	ENSMUST00000198283.1	241	No protein	Processed transcript	-	-	TSL:5

The strategy is based on the design of *Grin2b-202* transcript, The transcription is shown below



Protein domain



- [1] Tsien JZ, Chen DF, Gerber D, Tom C, Mercer EH, Anderson DJ, Mayford M, Kandel ER, Tonegawa S. Subregion- and cell type-restricted gene knockout in mouse brain. *Cell*. 1996 Dec 27;87(7):1317-26.
- [2] Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ. Genetic enhancement of learning and memory in mice. *Nature*. 1999 Sep 2;401(6748):63-9. doi: 10.1038/43432. PMID: 10485705.
- [3] Hippenmeyer, S., et al., Genetic mosaic dissection of *Lis1* and *Ndel1* in neuronal migration. *Neuron*, 2010. 68(4): p.695-709.
- [4] Mayford M, Wang J, Kandel ER, O'Dell TJ. CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. *Cell*. 1995 Jun 16;81(6):891-904.
- [5] Sunyer T, Sahyoun N. Sequence analysis and DNA-protein interactions within the 5' flanking region of the *Ca2+*/calmodulin-dependent protein kinase II alpha-subunit gene. *Proc Natl Acad Sci U S A*. 1990 Jan;87(1):278-82.

Additional cycles and costs

Additional itmes	Cycle (month)	Cost (¥)
BAC purchase for <i>Camk2a</i> promoter	1-1.5	3400
<i>Grin2b</i> synthesis	1	6674
Vector construction	1	/

CDS synthesis and BAC ordering is not included in the agreement cycle. If the customer can provide it, please inform in advance, and the cost and cycle will not be calculated.

If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534

