H11-C1ql2-iCre-ployA Cas9-KI Strategy

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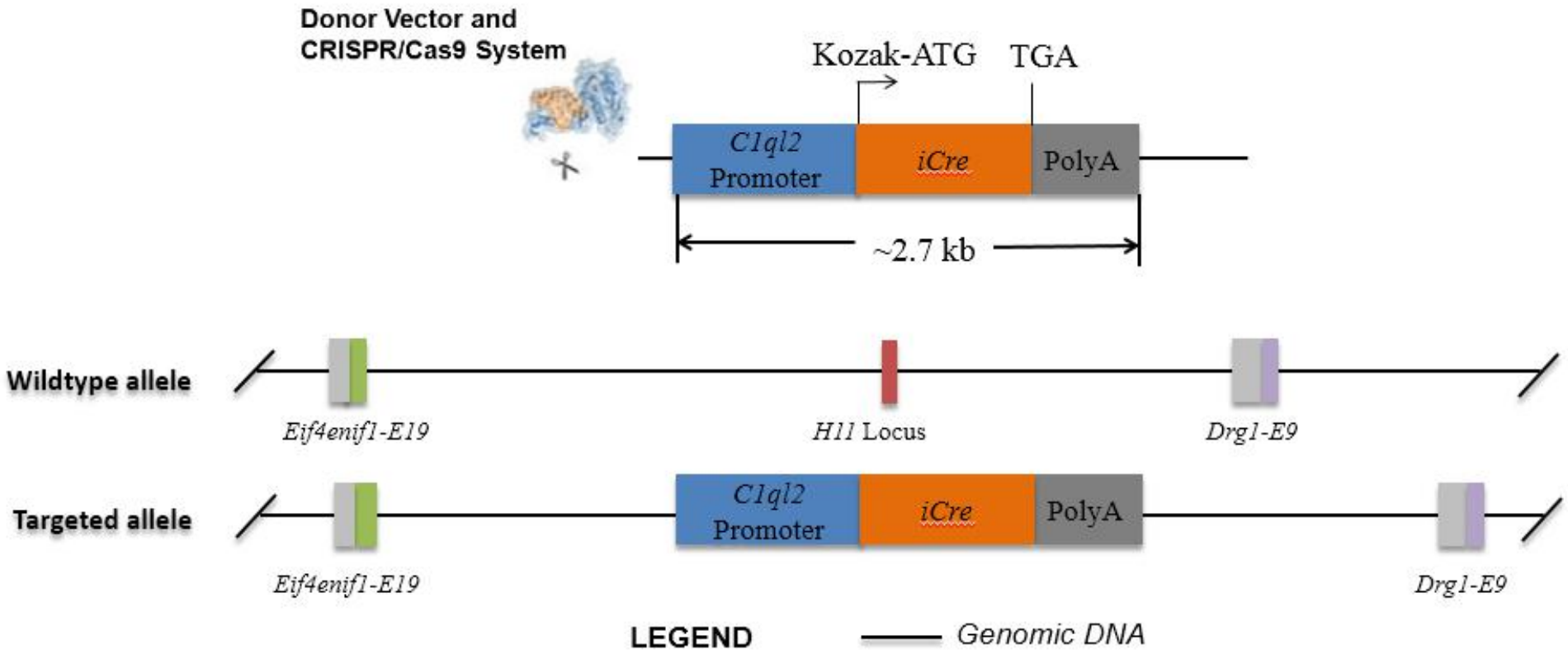
Design Date:	**2018/9/5**

Project Overview

Project Name	***H11-C1ql2-iCre-ployA***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

The *C1ql2-iCre-ployA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



Summary of *C1ql2* promoter [1]

64 expression of the C1ql2 gene is largely restricted to the DG in the hippocampus[19]. We cloned
65 a sequence of the promoter rich in CpG islands which includes a 1 kb fragment upstream of
66 the transcriptional start site and part of the 5' untranslated region of the mRNA sequence to
67 create a C1ql2 minimal promoter (**Supplementary Fig. 1a**). The minimal promoter was used

535 promoter has been exchanged with the C1ql2 minimal promoter. This sequence was cloned
536 from mouse genomic DNA using the following primers EcoRV/C1ql2-F:
537 ATATATGATATCagcacccacatagcagc; BamH1/C1ql2-R: ATATATGGATCCgctctggaactgat
538 ctg. The 1.3kb sequence was then inserted between EcoRV and BamH1 restriction sites of
539 the lentiviral backbone. After the promoter, the following cDNA sequences were inserted in the

Promoter Sequence of Mouse *C1ql2*(1315bp) [1]

AGCACCCACATAGCAGCTCACAAATGTCTGAAACTCCAATTCTTGGGAATCTGACACGATCACACATGCAGGCAAAATACC
AATGTACATGAATTAAAAAAAAAAAAAAACAACCTTTAAAAGAAACAAGGGTTCAGTACCACTACTGACATCTTGTTTCCCC
AGAGGCCTTACTTTAATTATTTATTGTTTCCACTTAGTTGCTCAATTAATTAATTTAGAGGTTTTTTTCTTCCTTTCTTTTTCTTT
TTTCTTTCTCTCTTTTTTTTCTTCTTAAGACAGGGTTTCTCTGTGTAGCTCAGGCTATCCTGGAACTCACTCTGTAGACCAGG
CTGGCCTTGTACTCAAAGATCTGCCTGCCTCTGCCTCCCCAGTGCTGGGATTAAAGACATGCACCATCACTGCCCTGCTTTC
CTCTTTTTATTTTGAAAATTGTTCATCAACAGTTACTAAACGTGTTCGAATTCCAAGAGCTGACTAGACATATAAGACCATTC
AGCCTTCTGAATAAGATGTAGGTGTGCCCCTCCTCTTACTCCTCTATTTGGAAGTTGGTTACTTTCTGTATGTAGTATGCGAAT
CCCCCTCTGCCACCCCGCTTTCTGTTTTAAAACAGAAAAGGCTGCAACATACAGTGTGTGCTTCTGTTCTTGAACTGGAAG
CTTAGGCTGTCCTGGACTTGGGTTGAGACCTGGGCTCATCCAGATAGGAAATGGATTTGGTGACCCCGCCAGGACTTCGCA
GGCACCACATCGTGGTCGTGTGTGGGTGCTGTATGCACCCACTGATTGCGCGCGTGGGTTCCAGAGCTTGGTGGTCTGCGA
GAGGAGAGTGGGCAAGAGTGGGTGTGTCTGTGGAGCCCCAGCTAGGGGCTGCTGCCCGCTGCTCCCACTTGTGGCTCCTG
GGCGCCGCCAGCAGGCACATCTCCGGAGGACGCCGCGGGATGGGAGCTGATGACAGGAGAGCGCCGTCTCCCGAGTGATG
GCAGCGCACGCTGCTGCCTCGCCGCCTCCGCCGCTCAGTCCTGATCTTACGTTAGGGTAGCTGGGTACCCCCTCCGCCCGG
GAACCAGCTAGTAGAGGGAGAACAGAGCAGAGCGTGCGGCAGAGCCGATCCCGCGTCCCGCCGAACCCTGCCAAGCCCC
GCCAATCCCAGCAGAGCAGGAACCAGCGCAGCTGAGCCAACACCGGACGCCGCACTGAGACCCAGCATTCCCCAGCCGC
CACTACCCGGTCCCCGCCGGGGTGCCGGGCTCGTCCTGTGAGCCCCTCGTCATGCGTGTCGGGCTCTTCGACTCTCCAGAT
CAGTTCCAGAGC

Technical routes

- 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 *C1ql2-iCre-ployA* 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 and sequencing, then the stable inheritance of F1 positive mice model was obtained.

Notice

- 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)

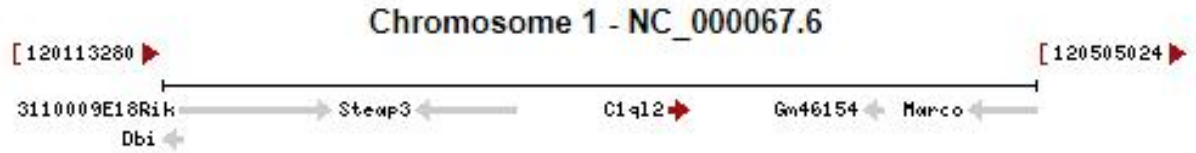
集萃药康 GemPharmatech

C1ql2 complement component 1, q subcomponent-like 2 [*Mus musculus* (house mouse)]

Gene ID: 226359, updated on 12-Aug-2019

Summary

Official Symbol	C1ql2 provided by MGI
Official Full Name	complement component 1, q subcomponent-like 2 provided by MGI
Primary source	MGI:MGI:3032521
See related	Ensembl:ENSMUSG00000036907
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	Adii; CTRP10; BC040774
Expression	Biased expression in cortex adult (RPKM 1.9), CNS E18 (RPKM 1.8) and 8 other tissues See more
Orthologs	human all

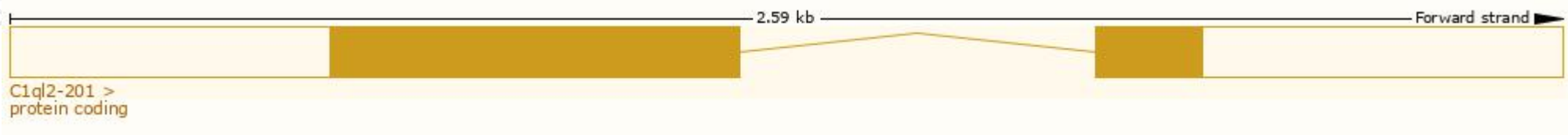


Transcript information （Ensembl）

The gene has 1 transcript, and the transcript is shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
C1ql2-201	ENSMUST00000037286.9	2000	287aa	Protein coding	CCDS15233	A0A3B0J351 Q8CFR0	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *C1ql2-201* transcript,The transcription is shown below



Reference

[1] Barthet et al. (2018) Presenilin-mediated cleavage of APP regulates synaptotagmin-7 and presynaptic plasticity. bioRxiv February 1, 2018.

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

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