

# ***H11-Syn1-iCre-ployA* Cas9-KI Strategy**

**Designer:**

**Jia Yu**

**Design Date:**

**2019-8-15**

**Reviewer**

**Yanhua Shen**

# Project Overview

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| <b>Project Name</b> | <b><i>H11-Syn1-iCre-ployA</i></b> |
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|---------------------|----------------|
| <b>Project type</b> | <b>Cas9-KI</b> |
|---------------------|----------------|

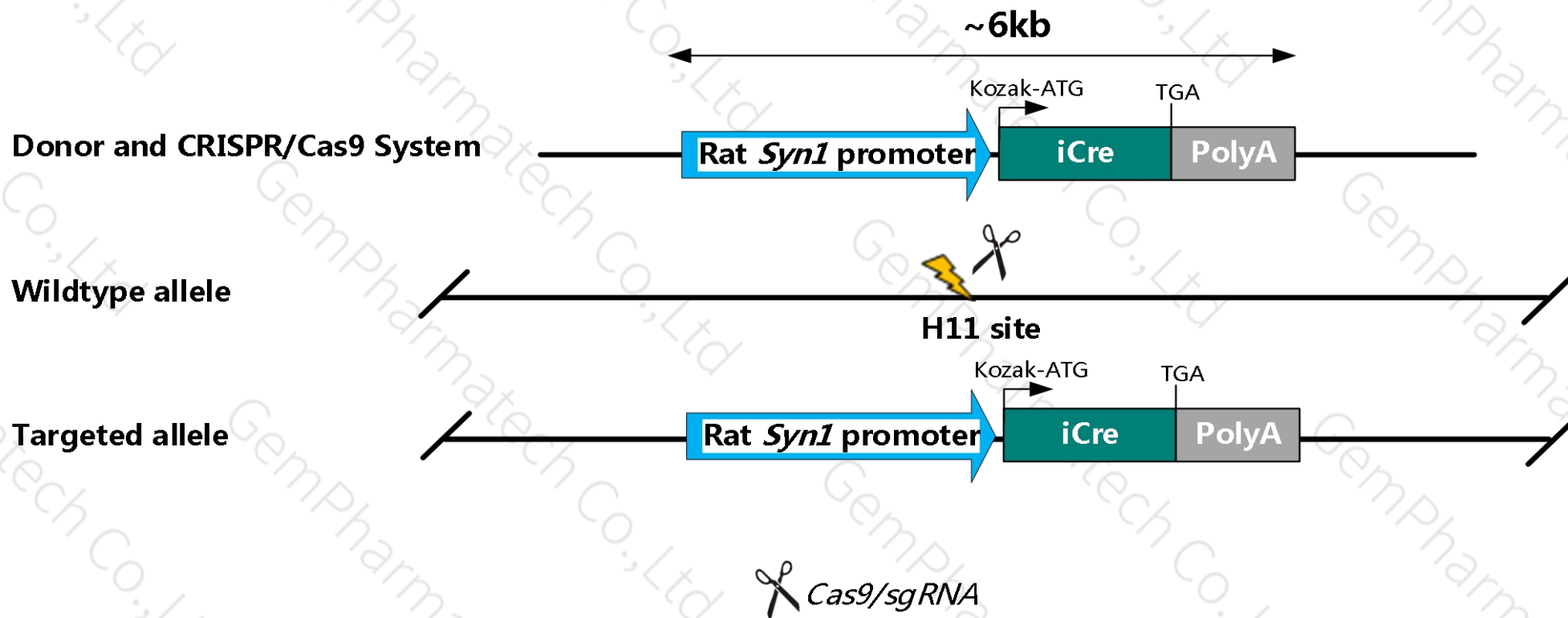
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|                          |                 |
|--------------------------|-----------------|
| <b>Strain background</b> | <b>C57BL/6J</b> |
|--------------------------|-----------------|

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# Knockin strategy

The *Syn1-iCre-ployA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



# Summary of Rat *Syn1* promoter from JAX



## MOUSE STRAIN DATASHEET - 003966

B6.Cg-Tg(Syn1-cre)671Jxm/J

POPULAR

### ➡ Detailed Description

These transgenic mice express Cre recombinase under the direction of a synapsin promoter. Mice that are homozygous for the transgenic insert are viable, fertile, normal in size and do not display any gross physical or behavioral abnormalities. Recombinase activity is detected in neuronal cells by embryonic day 12.5.

### ➡ Expression Data

Expressed Gene

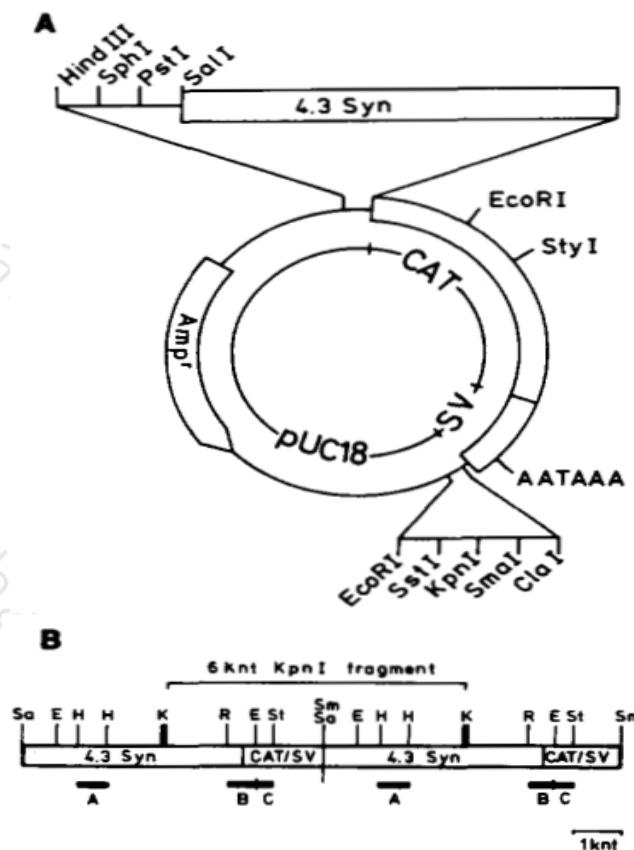
cre, cre recombinase, bacteriophage P1

Site of Expression

neuronal cells by embryonic day 12.5

<https://www.jax.org/strain/003966>

# Summary of Rat *Syn1* Promoter [1,2]



**FIG. 1. Transgene construction.** *Panel A*, plan of the pBL4.3Syn-CAT expression plasmid from which the transgene was excised. The plasmid is based on pUC18 and contains, in its multiple cloning site, ~4,300 nucleotides of 5'-flanking sequence of the rat synapsin I gene including its transcription start site and 105 nucleotides of 5'-untranslated sequence (Sauerwald *et al.*, 1990), the *Escherichia coli* CAT gene, and the SV40 splice and polyadenylation signal sequences (SV) from pSV2-cat (Gorman *et al.*, 1982). *Panel B*, plan of the predicted transgene structure in tandem orientation and location of hybridization probes within the transgene. Approximate positions of restriction sites are indicated: E, *EcoRI*; H, *HindIII*; K, *KpnI*; R, *RsaI*; Sa, *Sall*; Sm, *SmaI*; St, *StyI*.

## Production and characterization of *SynI-Cre* Tg mice

The *SynI-Cre* transgene was constructed using the *Sall-XhoI* 4.4-kb fragment of pBL4.3Syn-CAT, kindly provided by Dr. Manfred Kiliman (Hoesche *et al.* 1993), containing the rat *Syn-*

apsin I promoter and 100 bp of 5' untranslated CAT sequence. This was placed immediately 5' of a modified bacteriophage P1 Cre recombinase gene that included translational consensus and nuclear localization signals. The human growth hormone gene was placed 3' of Cre to provide transcription termination and polyadenylation signals. The transgene was isolated from

1. Zhu Y; Romero MI; Ghosh P; Ye Z; Charnay P; Rushing EJ; Marth JD; Parada LF. 2001. Ablation of NF1 function in neurons induces abnormal development of cerebral cortex and reactive gliosis in the brain. *Genes Dev* 15(7):859-76.
2. Hoesche C, Sauerwald A, Veh RW, Krippel B, Kilimann MW. The 5'-flanking region of the rat synapsin I gene directs neuron-specific and developmentally regulated reporter gene expression in transgenic mice. *J Biol Chem*. 1993 Dec 15; 268(35):26494-502.

# The promoter Sequence of Rat *Syn1*(4400bp)

GCATCTCAGCGAGCAGATCTGCTACAGTGATGAATTGGGTGGGATGTGACCAGAGTTGGCACCCAGCTGAGAAAGCACCTGCACCCACCCACTGAAAAACAGCCTTAAGTTCAACACCACAATTACCAACA  
ACAAATAGCTGGGGGGTTAGGGGAAGTGAAGAGGTGATTATAATGCAGTCTCAGTTAGGGCAAGGGTAAAGAGAGGCTAGAGTTAGCATTGGCCTGGAGGACCCCCAAAAACAAACCCAGATGCACCTTTT  
TGTATGTGTTTCATTTAACACTTTAAAGTCTACTGTATGCTAGAGATAAGTCTGAGCATCTGGGCTGGGGGAGGACTGGTAGAAGGTGAGAACATCTGATAGCCAGTGAGCAGACTCAAAATAGGCATTCT  
TTTGTTCAAACATGTATTCAATGTTTAACACCTACTGTATACTAACAATAGTGCTGGAAATGGAGACACAAGGAAGGAATCAGCTGCCTATGTCTCCACAGTAAAGGGGAACAAACCCAGACACCAGTGTC  
TTAGAATTTGCTCATTCTTTTCCATTCACTGAATGCTGAGCTCCTTCATGATGGAATCACAAGGACTCATCATAATCAGGGTAATAGCTAACACTTAAAAAGCAATTTACTGAAAGCCCAATAATGCCTT  
AAGTCTTCCATAGTGTGAATCCATTTAATACACAATGAATTCAGAGGGTCGATATTATCCCCATTTTACAAAGTACGAAATTAGCACACAACAGTAATGAGCTGGATATTTTGAAGCACCGCACATAGT  
GATGCATATTAATTATTAGGAGCAGAGTTTGCAACTTTGTACAGTGGGTGAAAGGGACCTTCTGTGTAGGAGTGTTCAGTAGGAGATTGTGTAAAACCAGTCTGTGCAGAACATGTAATATGTCCCACTC  
GATCGATAGACGTGGAGACAAGTCAATGAGGATTTAGTCATCTTGGGAAGACTGAGTCAAAGTGAGGGTTTTAGTTGCTACTACAGGGTGTAAGAGATACTTGCTTGGAAGAAAGTAGAAGCTTGACTGT  
GTAAGTATACATTGTGAGGTACACATCTCTGTAATGTAGACTTTTACAGAGCTGGGCAAGAATTAGGACTGTATTGGATGTGTGTGATATATGAGTTTATAACAAAACCATAAGGGGTGTGTGTTCACTCCCAC  
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ATGGTGGCAGACACTGGCTTGATGCTGGAGACATAGCTGAGAGCTGCATGCTGATCCACAGGCAACACAGACAGACAGACAGACAGACAGACTGACTGACTAACTGACTGACTCTGGGCTTG  
GCGTGGGTTTTTGAACCCCCAAAGCCACCCCATAGACATGCTTCTTCCAACAAGGTCACATATCCTAATCCTTTTAATCATTCCCAACAGTGCCACTCCCTTGTGACTAAGCATTCAAATATATGAACCTC  
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CTGTGATGTACATATACCCTGTGTCATTCTTGTGTTTTCTATTTGTGTTATTCCATGTGTTTCTTACAGGCTCTCACTACCCAAGTGTCCACCTCCGCTGTCTGGTGATGTTTACGCTACCCCGTGTCTTTTTCTT  
TGCCTGACAGTGTGTGCTGGAAGACATCTCGCCAGGAACACTGCAGTAAGGAGAATTTCTAGTTTTATGTTCCCTCCGAGTATGCTTCTATCCCGACCTCAACCCCAAAATGCCTTCAGAGGTGAAAAAT  
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ACTCCCCAAATTGCGCATTCCCTCCCCCATCAGAGGGGGAGGGGAAGAGGATGCAGCGCGGCGCGGCGCGTGCAGCTGTCGGATTTAGTACCGCGGACAGAGCCTTCGCCCCGCTGCCGGCGCGCGCCA  
CCACCTCCCCAGCACAAAGGCGGGCTGACGTCACCTCTCCAGCCCTCCCCAACTCCCCCTACCTCACCAGCCTTGGTCGCGTCCGTGCAGCGGTGAGTCCAGTCCGGGCCGACCACAAGAGGTGCAAGATAG  
GGGGGTGCAGGCGCGACCATACGCTCTGCGGCGGCAGAGCCTCAGCGCTGCCTCAGTCTGCAGCGGGCAGCAGAGGAGTCGCGTCTGCCAGAGAGCGCCGCGCTGCTCCTGAGCCCCCTTGCCTCCGCC  
CCGCGGCCACCGACCCACTGCCCTTGGATCCCCTGCCCAAGCTGCCACC

- The Rat *Syn1* gene has 1 transcript. According to the structure of *Syn1* gene, *Syn1-201*(ENSRNOT00000014250.6) is selected for presentation of the recommended strategy.
- *Syn1-201* gene has 13 exons, with the ATG start codon in exon1 and TGA stop codon in exon13.
- The Rat *Syn1* promoter is from JAX, the length is about 4.4kb.
- 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 *Syn1-iCre-psyA* 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 )

## Syn1 synapsin I [ *Rattus norvegicus* (Norway rat) ]

Gene ID: 24949, updated on 13-Aug-2019

Summary

|                    |                                                                                                                                                                                               |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Official Symbol    | Syn1 provided by <a href="#">RGD</a>                                                                                                                                                          |
| Official Full Name | synapsin I provided by <a href="#">RGD</a>                                                                                                                                                    |
| Primary source     | <a href="#">RGD:3797</a>                                                                                                                                                                      |
| See related        | <a href="#">Ensembl:ENSRNOG00000010365</a>                                                                                                                                                    |
| Gene type          | protein coding                                                                                                                                                                                |
| RefSeq status      | VALIDATED                                                                                                                                                                                     |
| Organism           | <a href="#">Rattus norvegicus</a>                                                                                                                                                             |
| Lineage            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Rattus                       |
| Summary            | may act as an anchoring protein by linking the vesicle phospholipid layer and the cytoskeleton in neuronal presynaptic terminals; may play a role in neurotransmitter release [RGD, Feb 2006] |
| Expression         | Biased expression in Brain (RPKM 151.4), Spleen (RPKM 21.6) and 5 other tissues <a href="#">See more</a>                                                                                      |
| Orthologs          | <a href="#">human</a> <a href="#">mouse</a> <a href="#">all</a>                                                                                                                               |

Genomic context

Location: Xq11  
Exon count: 13

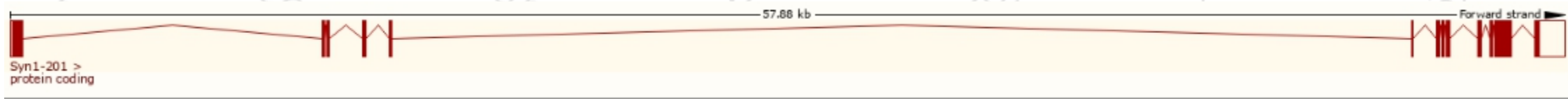
See Syn1 in [Genome Data Viewer](#)

# Transcript information ( Ensembl )

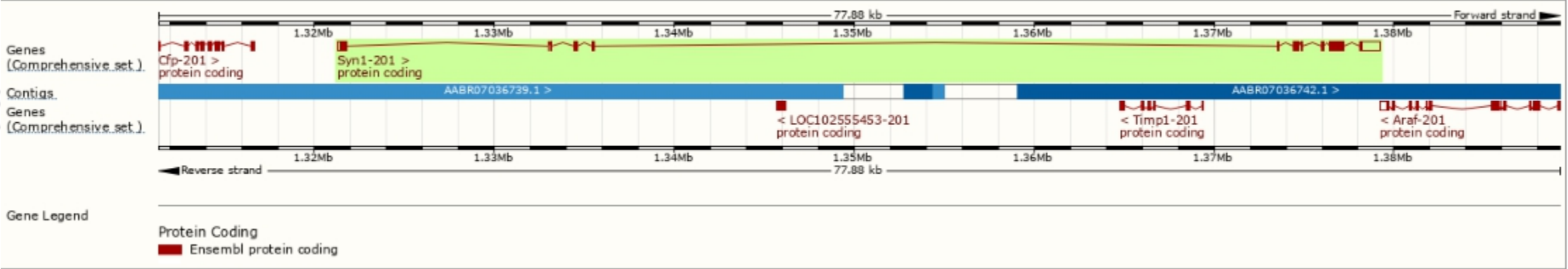
The gene has 1 transcript, and the transcript is shown below :

| Name     | Transcript ID                        | bp   | Protein               | Biotype        | UniProt                | Flags     |
|----------|--------------------------------------|------|-----------------------|----------------|------------------------|-----------|
| Syn1-201 | <a href="#">ENSRNOT00000014250.6</a> | 3166 | <a href="#">704aa</a> | Protein coding | <a href="#">P09951</a> | APPRIS P1 |

The strategy is based on the design of *Syn1-201* transcript, The transcription is shown below



# Genomic location distribution



# Protein domain



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



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