



Models to
Accelerate Innovation



***H11-Thy1-CreERT2* Cas9-KI Mouse Model Strategy**

CRISPR-Cas9 Technology

Designer

Shanshan Liu

Reviewer

Xueting Zhang

Date

2025-02-10



Project Overview

Project Name *H11-Thy1-CreERT2*

Project Type **Cas9-KI**

Background **C57BL/6JGpt**

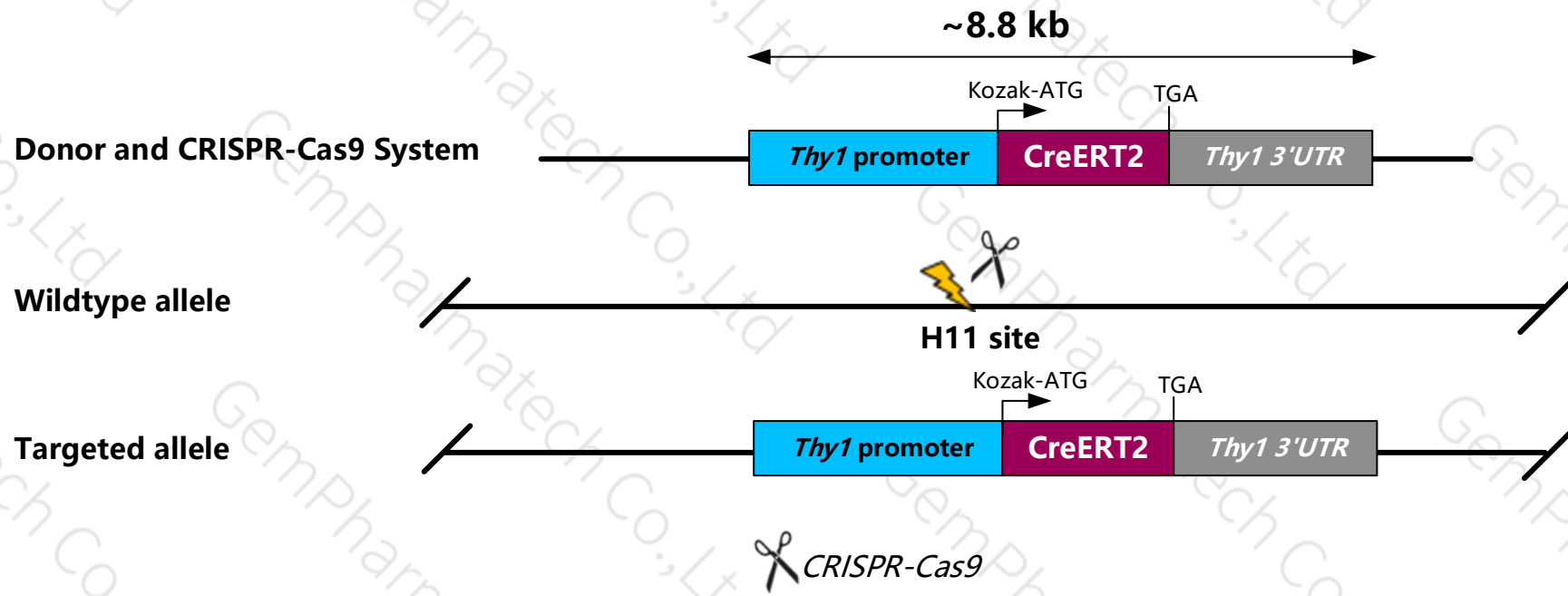
Timeline **5-8 Months**

Deliverable **3~5 F1 Heterozygous Mice**



Strategy

The *Thy1-CreERT2* fragment was inserted into H11 site of mice and the schematic diagram is as follows:





Technical Description

- The Mouse *Thy1* gene has 6 transcripts. According to the structure of *Thy1* gene, *Thy1-201*(ENSMUST00000114840.2) is selected for this strategy. It has 4 exons and codes 152 aa. The ATG is located in exon 2, and the TGA is located in exon 4.
- The mouse *Thy1* promoter^[1-3] is from reference, the length is about 4.2 k.
- 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 *Thy1-CreERT2* 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.

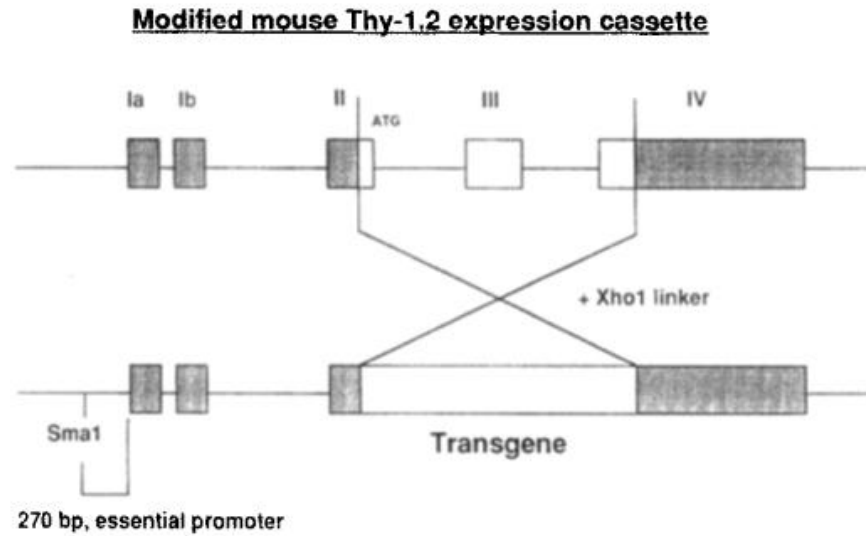


Note

- 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.



Reference



Thy-1 is a GPI-linked cell surface glycoprotein expressed in a variety of tissues and cell types, including the thymus, the nervous system, and connective tissue fibroblasts (Gordon et al., 1987; Morris, 1992). A schematic representation of the Thy-1 expression cassette used in these studies is shown in Fig. 1. The deletion encompasses the entire coding sequence of the Thy-1 protein, including its translation initiation site. The construct still contains parts of the 3'-untranslated sequence, including the Thy-1 mRNA polyadenylation site. Therefore, transgenes must include their own translation initiation and Kozak consensus sites, but can be devoid of most untranslated sequences. Transgenes are cloned into the *XhoI* site, and the expression construct is recovered as an *EcoRI-PvuI* fragment of approx. 6.5 kb, plus transgene sequences. One important feature of this particular expression cassette is that the deletion eliminates expression of the transgene in the thymus (Vidal et al., 1990). In fact, outside the nervous system we only detected weak expression in the lung. Due to the marked context sensitivity of the promoter and enhancer elements, major modifications of the construct are not advisable, as they are likely to affect expression properties severely (Kelley et al., 1995).

PMID: 9125370



Mouse *Thy1* 3'UTR (2640 bp)

CTCGAGGTCCTTCCTCTGCAGAGGTCTTGCTTCTCCCGGTCAGCTGACTCCCTCCCCAAGTCCTTCAAATATCTCAGAACATGGGGAGAAACGGGGAC
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TCCCAGTCTTCTGCCTTTGCGCCAGCCTCCTGTCTGGCCATGCCTGAAGAAGGCTGGAGAAGCCACCCACCTCAGGCCATGACACTGCCAGCCACTT
GGCAGGTGCAGCCAAACCTGAGCTGTCCCAGAAAGGGACATTCTCAAGACCCAGGCACCCTGATCAGCACTGACTTGGAGCTACAAGTGTGATGCCA
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TAAGCTCCCCACCCACCACTCGTTCCTACTGTCCCTTATTCTCTCTCTACCTTCAGCCACTTAGTTTCCCTACCTTAAGTCCTAGAATT



Target Gene

Gene name	mouse <i>Thy1</i>
Gene ID (NCBI)	21838
Gene link (NCBI)	https://www.ncbi.nlm.nih.gov/gene/21838
Gene link (Ensembl)	http://useast.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000032011;r=9:43954681-43959876
Chromosome location	Chr 9



Gene Information (NCBI)



Thy1 thymus cell antigen 1, theta [*Mus musculus* (house mouse)]

Gene ID: 21838, updated on 14-Jan-2025

[Download Datasets](#)

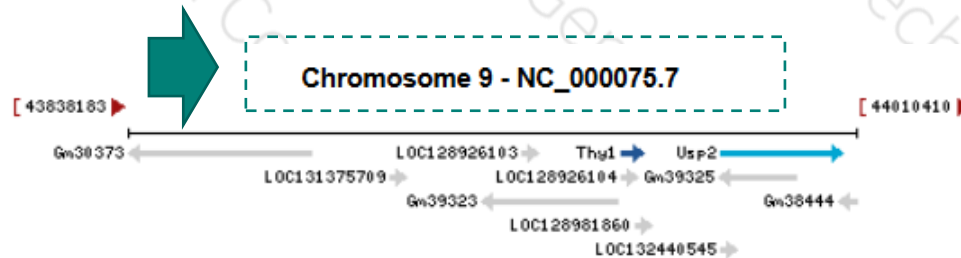
Summary

- Official Symbol** Thy1 provided by [MGI](#)
- Official Full Name** thymus cell antigen 1, theta provided by [MGI](#)
- Primary source** [MGI:MGI:98747](#)
- See related** [Ensembl:ENSMUSG00000032011](#) [AllianceGenome:MGI:98747](#)
- Gene type** protein coding
- RefSeq status** REVIEWED
- Organism** [Mus musculus](#)
- Lineage** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus T25; CD90; Thy-1; Thy1.1; Thy1.2; Thy1.2
- Also known as** T25; CD90; Thy-1; Thy1.1; Thy1.2; Thy1.2
- Summary** This gene encodes a glycoprotein that is anchored to the cell surface of thymocytes, neuronal and other cells through a glycosyl-phosphatidylinositol moiety. A soluble form of the encoded protein has also been detected in serum and cerebrospinal fluid. The encoded protein undergoes further processing to generate the mature protein which mediates cell-cell interactions to trigger downstream signaling pathways. [provided by RefSeq, Jul 2015]
- Expression** Biased expression in thymus adult (RPKM 668.7), cortex adult (RPKM 206.5) and 6 other tissues [See more](#)
- Orthologs** [human](#) [all](#)

NEW

Try the new [Gene table](#)

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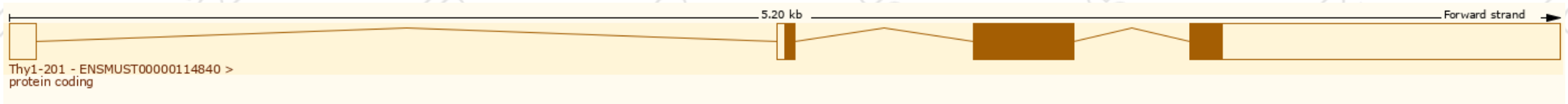


Transcript Information (Ensembl)

The gene has 6 transcripts, as shown below:

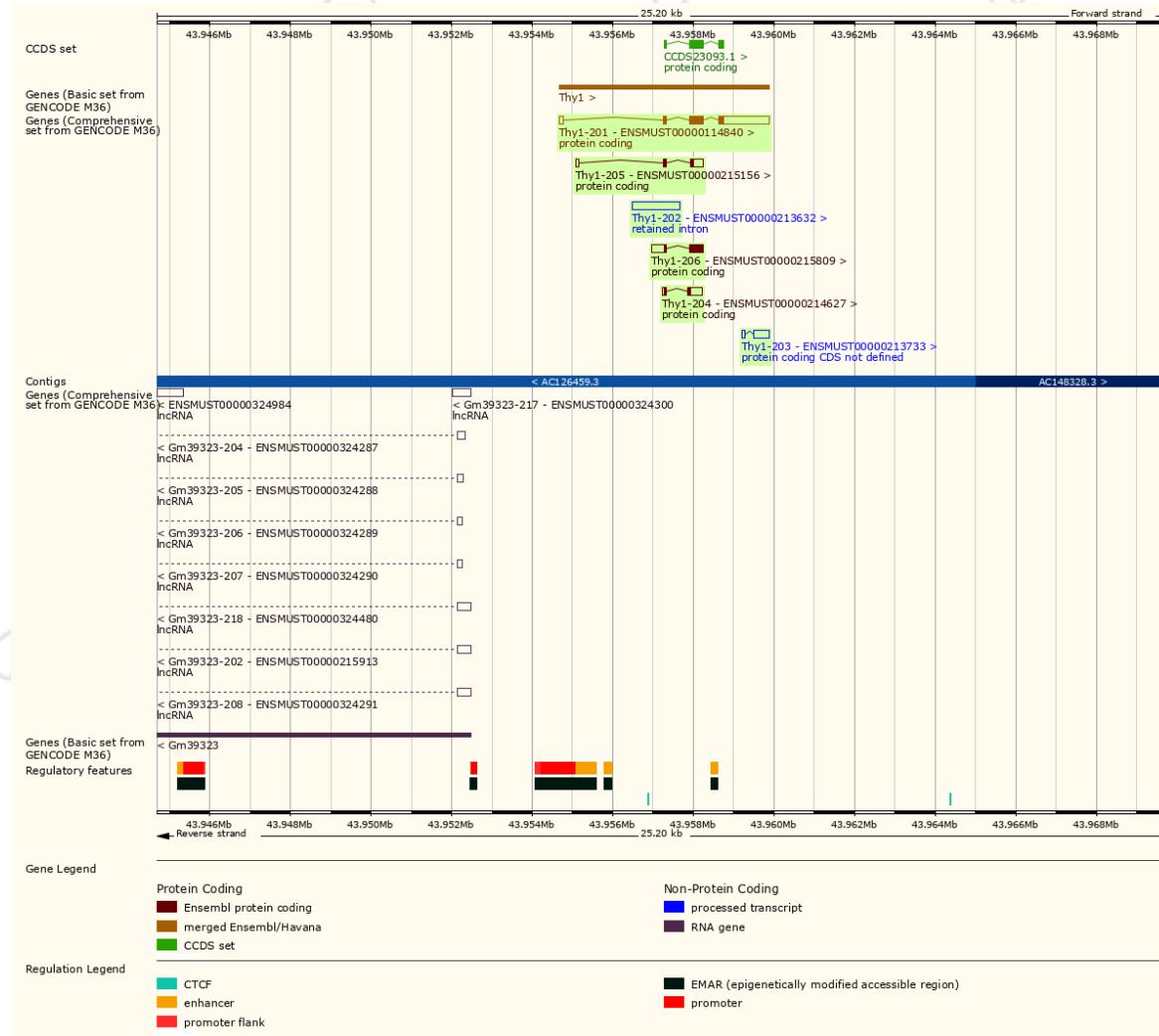
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000114840.2	Thy1-201	1735	162aa	Protein coding	CCDS23093	P01831	Ensembl Canonical Gencode Basic APPRIS P1 TSL:2
ENSMUST00000215809.2	Thy1-206	685	125aa	Protein coding		A0A1L1SUX8	TSL:2 CDS 3' incomplete
ENSMUST00000215156.2	Thy1-205	460	37aa	Protein coding		A0A1L1ST40	Gencode Basic TSL:5
ENSMUST00000214627.2	Thy1-204	446	40aa	Protein coding		A0A1L1SRS7	Gencode Basic TSL:3
ENSMUST00000213733.2	Thy1-203	449	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000213632.2	Thy1-202	1192	No protein	Retained intron		-	TSL:NA

The strategy is based on *Thy1*-201 transcript, which contains 4 exons, is 1735 bps long, and encodes 162 amino acids.



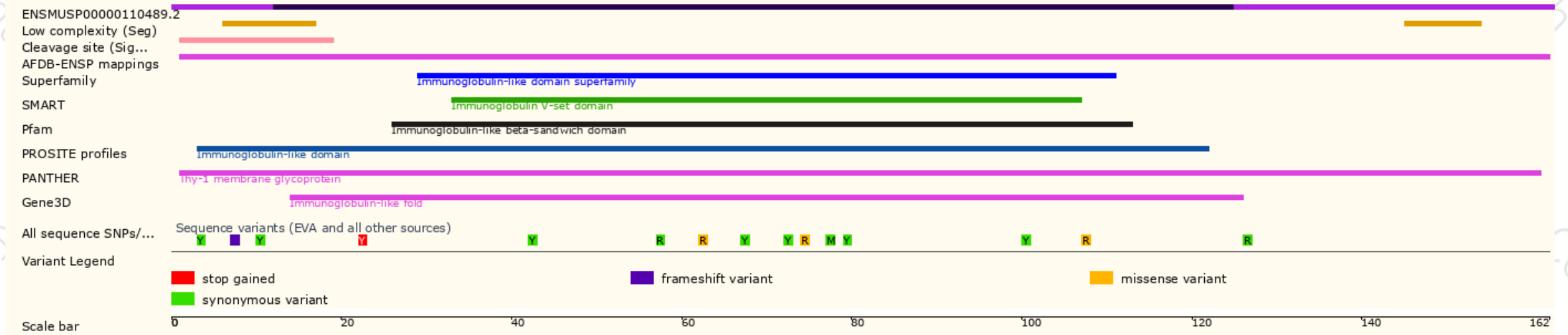


Genomic Information



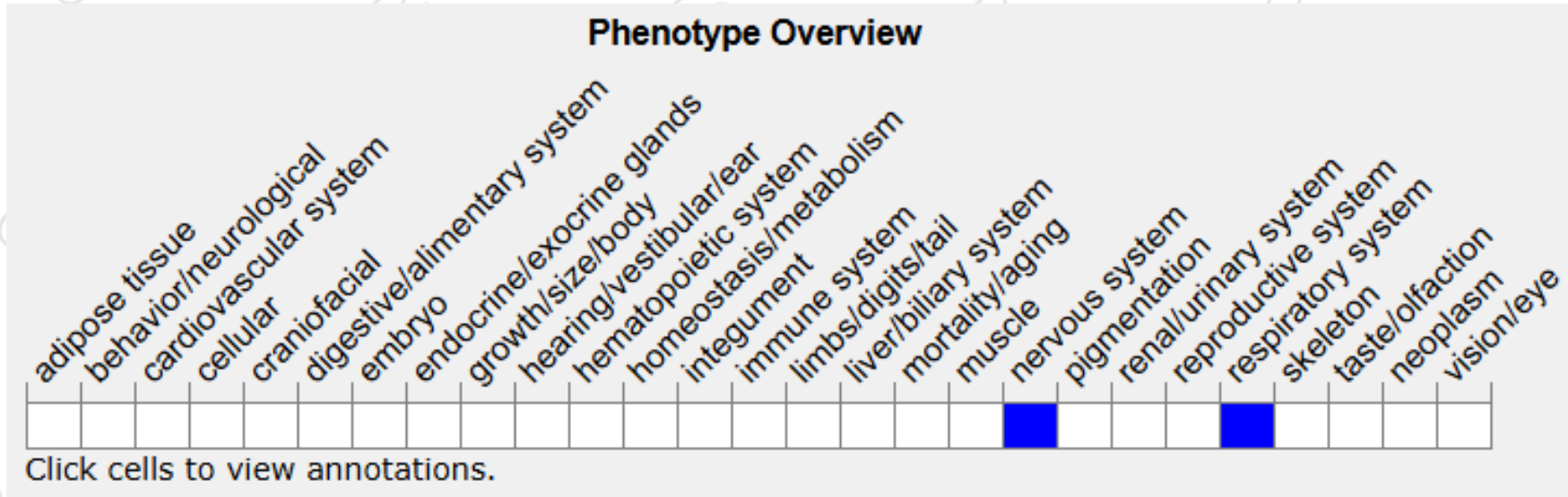


Protein Information





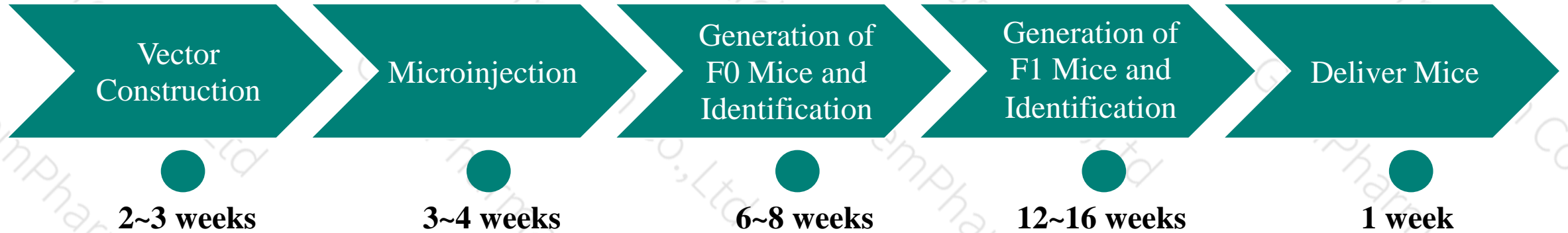
MGI Information



Homozygous null mice are viable, fertile, and display no abnormalities in the brain and spinal cord, have normal axonal development and regeneration and no behavioral abnormalities. Long term potentiation is inhibited in the dentate gyrus.



Work Flow





Reference

1. Vidal M, Morris R, Grosveld F, Spanopoulou E. Tissue-specific control elements of the Thy-1 gene. *EMBO J*. 1990 Mar;9(3):833-40.
2. Caroni P. Overexpression of growth-associated proteins in the neurons of adult transgenic mice. *J Neurosci Methods*. 1997 Jan;71(1):3-9.
3. Campsall KD, Mazerolle CJ, De Repentingy Y, Kothary R, Wallace VA. Characterization of transgene expression and Cre recombinase activity in a panel of Thy-1 promoter-Cre transgenic mice. *Dev Dyn*. 2002 Jun;224(2):135-43.



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025-58641508

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globalservice@gempharmatech.com
<https://en.gempharmatech.com/>