Prox1-CreERT2-polyA BAC-TG Strategy

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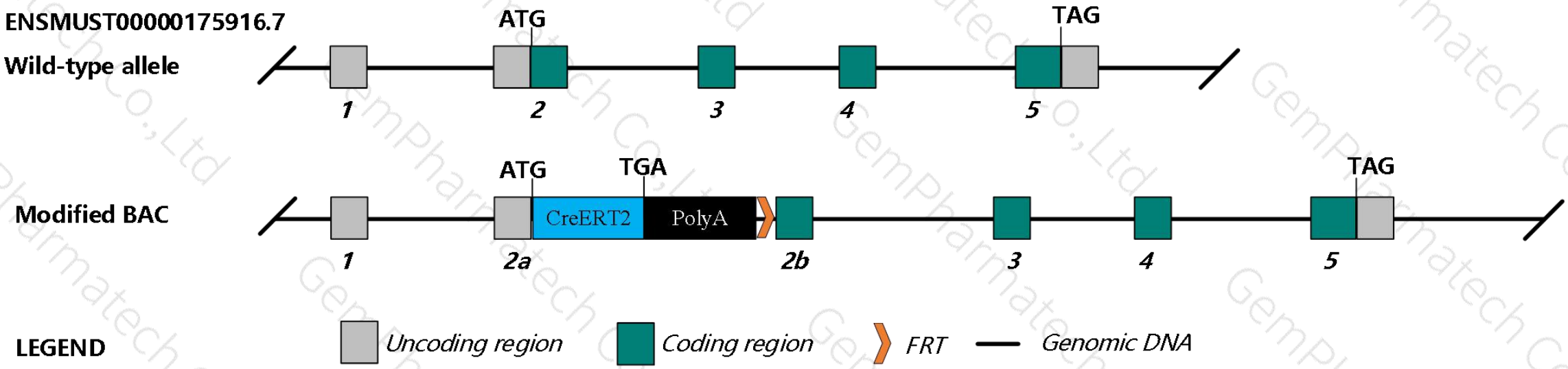
Project Overview

Project Name	***Prox1-CreERT2-polyA***
Project type	**BAC-TG**
Strain background	**C57BL/6J**

Knockin strategy

This model will use pronucleus injection technology to obtain the *Prox1-CreERT2-polyA* model. The schematic diagram is as follows:



Technical routes

- The Prox1-202 ENSMUST00000175916.7 is selected to describe the strategy. Prox1 gene has 5 exons, with the ATG start codon in exon2 and TAG stop codon in exon5.
- RP23-360I16 (~196kb) or RP23-385H16 (~215kb) of C57BL/6J mouse bacterial artificial chromosome (BAC) containing the entire Prox1 locus (and other genes), was modified by targeting CreERT2-polyA sequence to the exon2 near the translation start codon of the Prox1 locus, ensuring CreERT2 is expressed from the endogenous promoter/enhancer elements of Prox1.
- The pups will be genotyped by PCR analysis.

Notice

- According to the existing references, Cre-ERT2 fusion protein is expressed from *Prox1* promoter/enhancer elements during the development of lymphatic endothelial cells*, and* also highly expressed in lens and retina.

- Transgene fragment will be injected into the fertilized eggs, and randomly integrated into the genome, by the influence of insertion site and copy number, expression level of the transgenic mice may be different.

- This strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

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Prox1 prospero homeobox 1 [*Mus musculus* (house mouse)]

Gene ID: 19130, updated on 14-Aug-2019

Summary

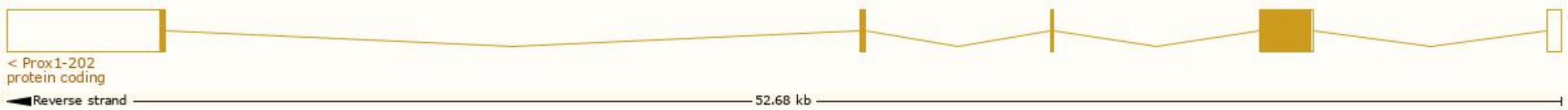
Official Symbol	Prox1 provided by MGI
Official Full Name	prospero homeobox 1 provided by MGI
Primary source	MGI:MGI:97772
See related	Ensembl:ENSMUSG00000010175
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	PROX-1; A230003G05Rik
Expression	Broad expression in liver adult (RPKM 6.6), heart adult (RPKM 5.0) and 19 other tissues See more
Orthologs	human all

Transcript information （Ensembl）

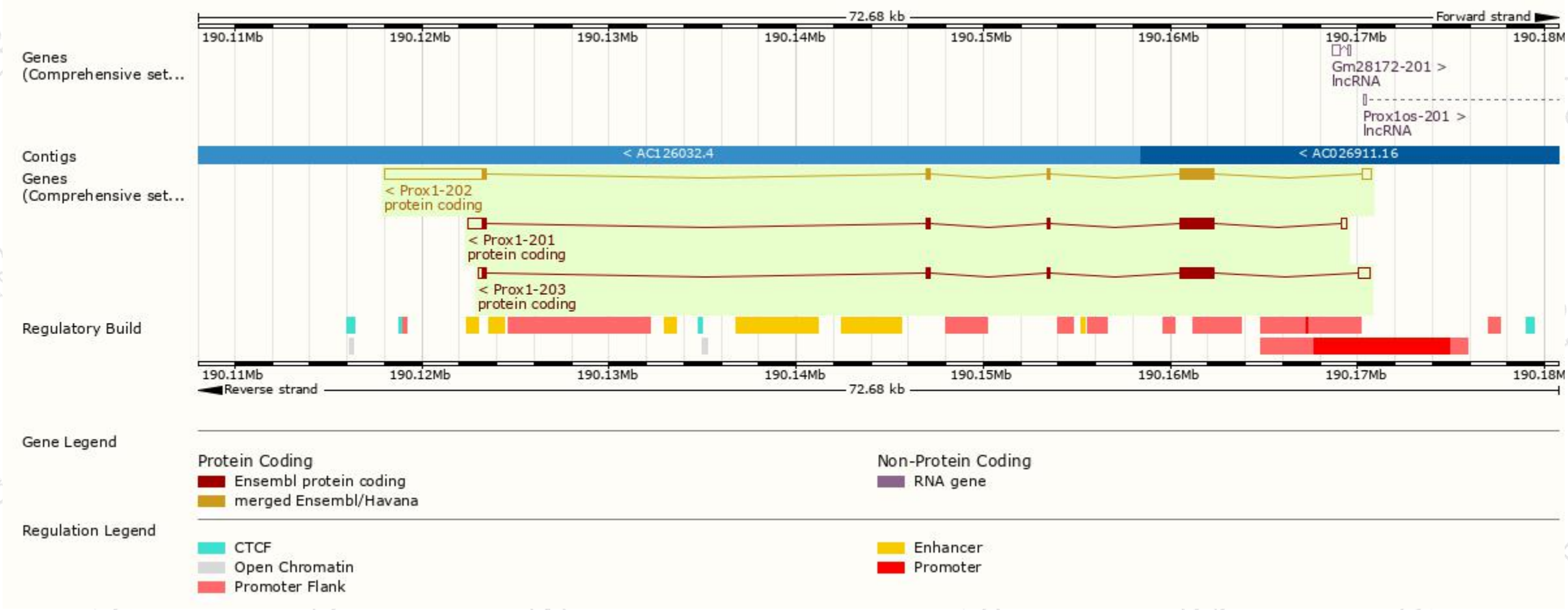
The gene has 3 transcripts, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Prox1-202	ENSMUST00000175916.7	7913	737aa	Protein coding	CCDS35822	P48437	TSL:1 GENCODE basic APPRIS P1
Prox1-201	ENSMUST00000010319.13	3285	737aa	Protein coding	CCDS35822	P48437	TSL:1 GENCODE basic APPRIS P1
Prox1-203	ENSMUST00000177288.3	3144	737aa	Protein coding	CCDS35822	P48437	TSL:5 GENCODE basic APPRIS P1

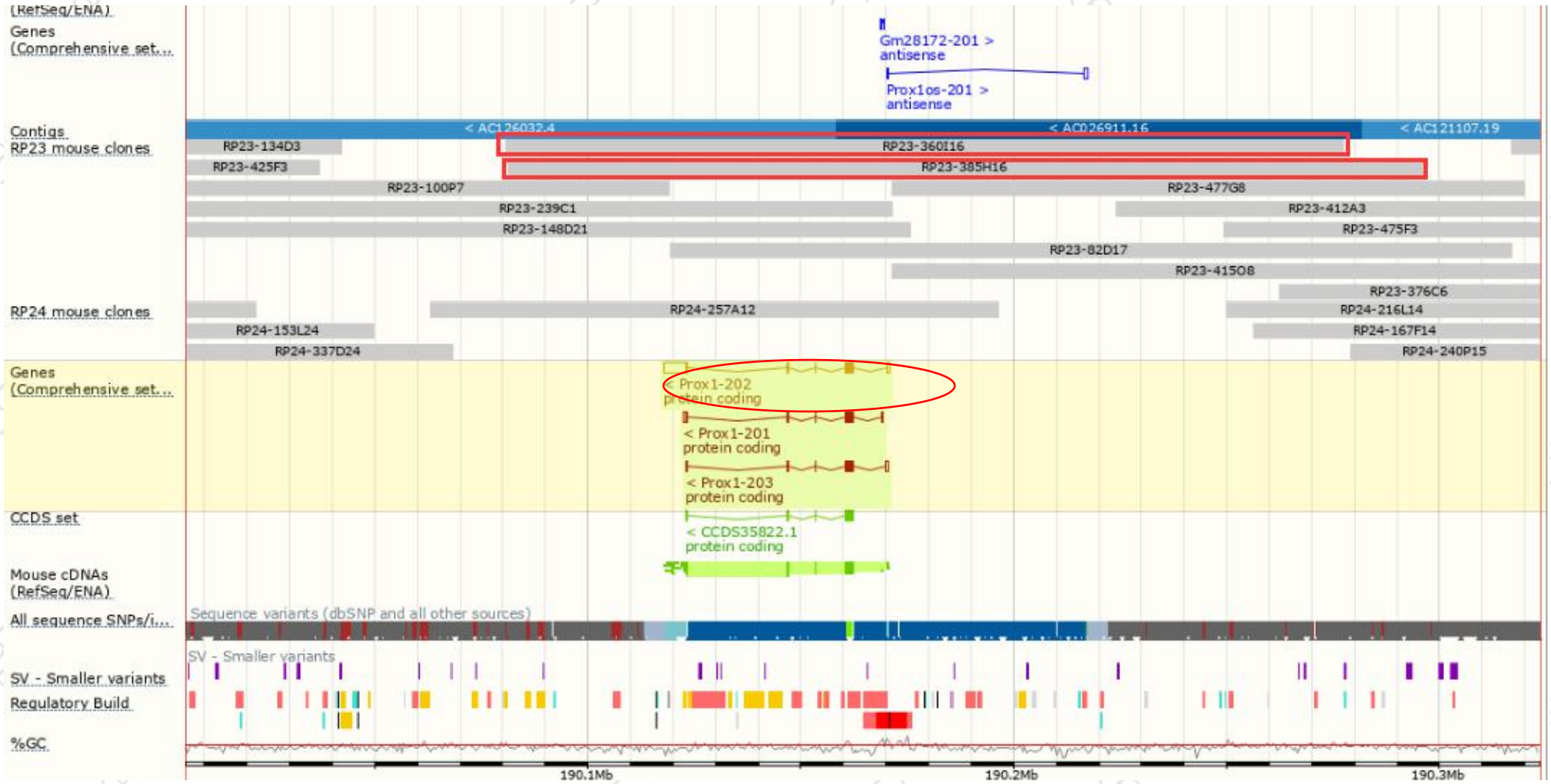
The strategy is based on the design of *Prox1-202* transcript,The transcription is shown below:



Genomic location distribution

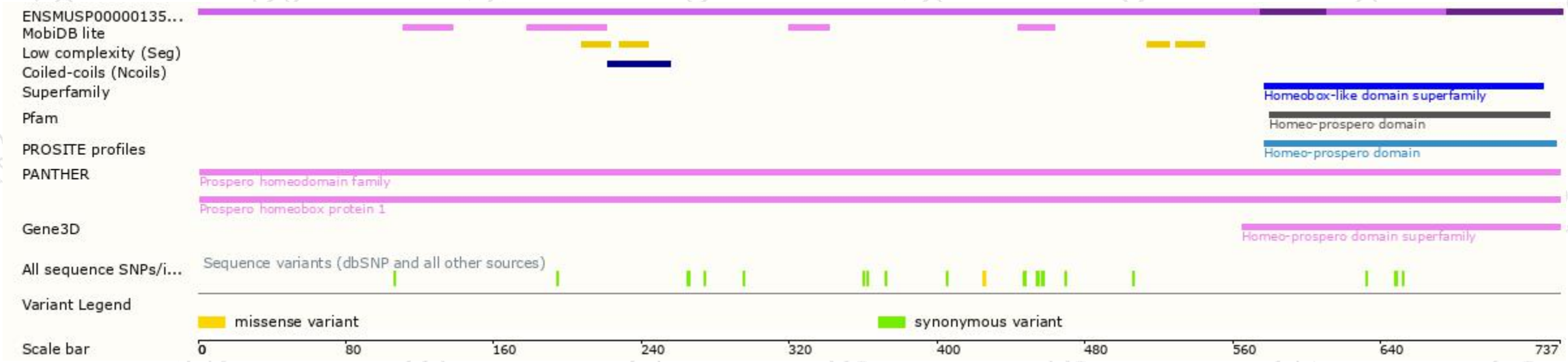


BAC Clone Selection



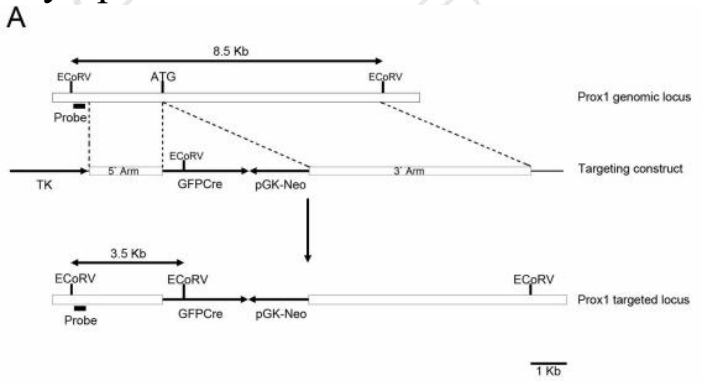
BAC克隆*RP23-360I16*、*RP23-385H16*，可用于该转基因小鼠的注射。

Protein domain



Reference

1. Srinivasan RS, Geng X, et al. The nuclear hormone receptor Coup-TFII is required for the initiation and early maintenance of Prox1 expression in lymphatic endothelial cells. Genes Dev. 2010 Apr 1;24(7):696-707.



Supplementary Figure 1. Generation of the *Prox1*$^{+/GFPCre}$ knock-in strain.
(A) A *GFPCre* expression cassette was inserted into the transcription start site (ATG) of the *Prox1* genomic locus with a neomycin (pGK-Neo) positive-selection cassette. A thymidine kinase (TK) negative-selection cassette was also included. This targeting construct was used to electroporate W9.5 mouse ES cells. After selection, DNA from targeted ES cells was digested with EcoRV restriction endonuclease and analyzed by Southern blot analysis using the indicated probe (B). Following selection and screening

2. Bazigou E, Lyons OT, et al. Genes regulating lymphangiogenesis control venous valve formation and maintenance in mice. J Clin Invest. 2011 Aug 1; 121(8): 2984–2992.

Mutation details: The BAC clone RP23-190F21 is used to generate a transgene in which a cre/ERT2 fusion was inserted at the Prox1 start codon. Two founders were generated and the one with complete recombination in all Prox1-expressing tissues was used for further studies. The pound symbol (#a) is used to represent this specific unnamed line. (*J:218590*)

3. http://www.gensat.org/.

Mutation details: A cre-expression cassette, followed by a polyadenylation sequence, was inserted into BAC clone RP23-360I16 at the initiating ATG codon of the first coding exon of the Prox1 gene so that cre expression is driven by the regulatory sequences of the BAC gene. The resulting modified BAC (BX3057) was used to generate this transgene. (*J:100256*)

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

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