Prdm1-P2A-iCre Cas9-KI Strategy

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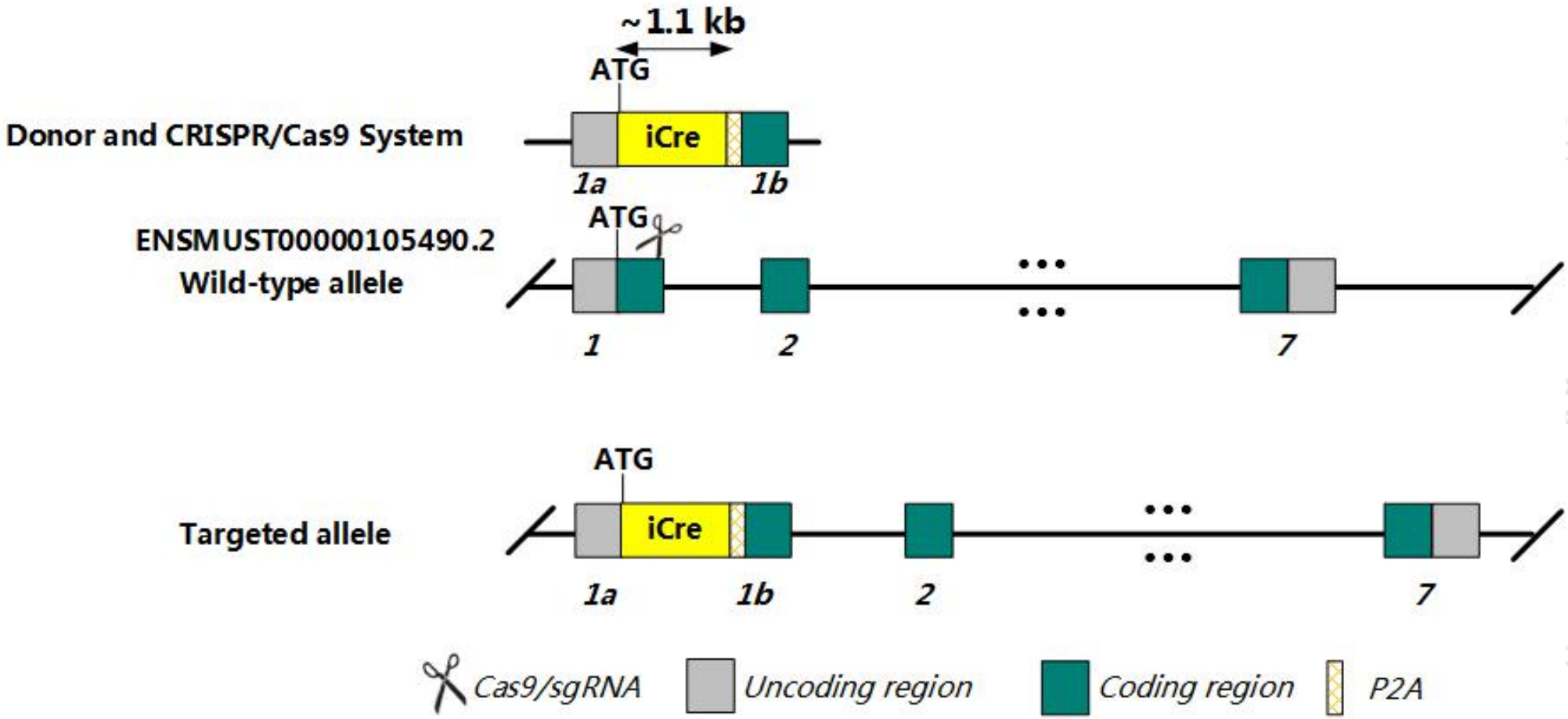
Project Overview

Project Name	***Prdm1-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Prdm1* gene. The schematic diagram is as follows:



Technical routes

- The *Prdm1* gene has 4 transcripts.According to the structure of *Prdm1* gene, *Prdm1-202*(ENSMUST00000105490.2) is selected for presentation of the recommended strategy.

- *Prdm1-202* gene has 7 exons, with the ATG start codon in exon1and TAA stop codon in exon7.

- We make *Prdm1-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start coding(ATG) of Prdm1 gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in iCre-P2A after start coding(ATG) of Prdm1 gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, Homozygous null mice display embryonic lethality and impaired primordial germ cell development, while heterozygotes display a decreased numbers of primordial germ cells but normal migration. Conditional mutants display impaired plasma cell and pre-plasmamemory B cell development.
- According to the existing JAX data, Cre-mediated recombination is detected in 55-76% of primordial germ cells when this strain is crossed with Gt(ROSA)26-GFP reporter mice. Expression is also seen in plasma cells.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of iCre may affect the regulation of the 5' end of the *Prdm1* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon1 of *Prdm1* gene in this strategy.
- The *Prdm1* gene is located on the Chr10. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- 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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Prdm1 PR domain containing 1, with ZNF domain [*Mus musculus* (house mouse)]

Gene ID: 12142, updated on 6-Jul-2019

Summary

Official Symbol	Prdm1 provided by MGI
Official Full Name	PR domain containing 1, with ZNF domain provided by MGI
Primary source	MGI:MGI:99655
See related	Ensembl:ENSMUSG00000038151
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	Blimp1; Blimp-1; PRDI-BF1; ZNFPR1A1; b2b1765Clo
Expression	Broad expression in placenta adult (RPKM 3.0), subcutaneous fat pad adult (RPKM 1.7) and 20 other tissues See more
Orthologs	human all

Genomic context

Location: 10 B2; 10 23.24 cM

See Prdm1 in Genome Data Viewer

Exon count: 12

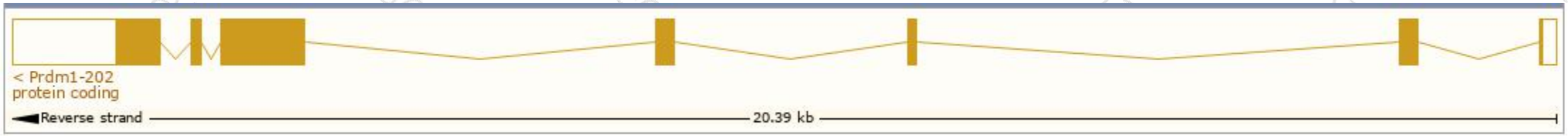
Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	10	NC_000076.6 (44437174..44528560, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	10	NC_000076.5 (44156981..44178493, complement)

Transcript information （Ensembl）

The gene has 4 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Prdm1-202	ENSMUST00000105490.2	4025	823aa	Protein coding	CCDS23825	Q60636	TSL:1 GENCODE basic APPRIS P2
Prdm1-201	ENSMUST00000039174.10	5281	856aa	Protein coding	-	Q60636	TSL:2 GENCODE basic APPRIS ALT2
Prdm1-204	ENSMUST00000218369.1	2517	838aa	Protein coding	-	Q60636	TSL:5 GENCODE basic APPRIS ALT2
Prdm1-203	ENSMUST00000167340.1	830	No protein	Retained intron	-	-	TSL:1

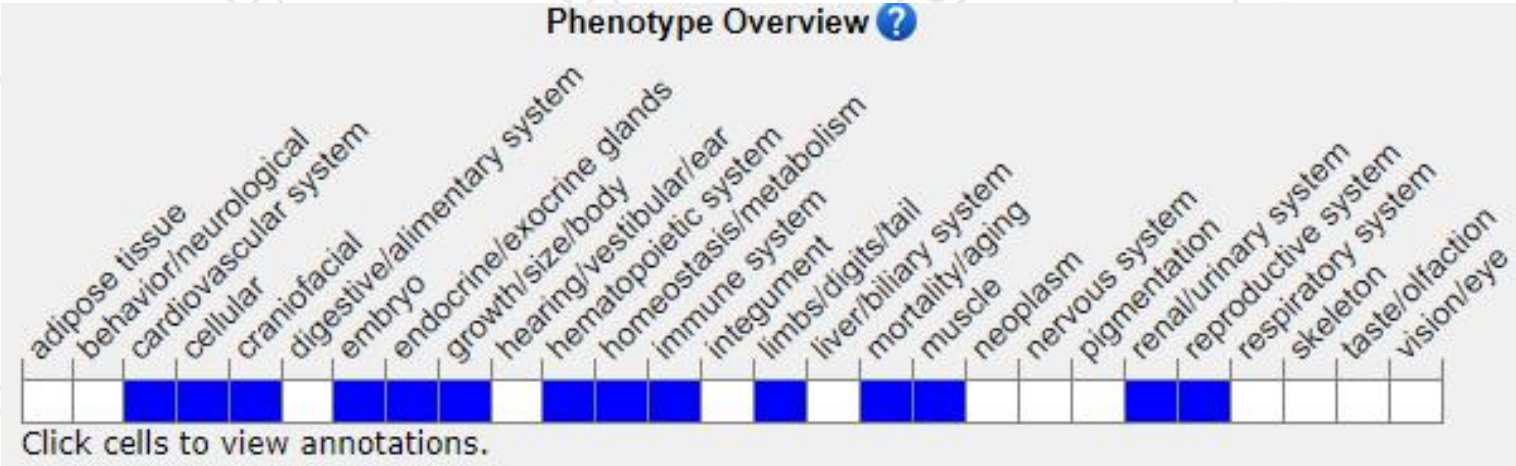
The strategy is based on the design of *Prdm1-202* transcript,The transcription is shown below



Genomic location distribution

Protein domain

Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/marker/MGI:99655) .

Homozygous null mice display embryonic lethality and impaired primordial germ cell development, while heterozygotes display a decreased numbers of primordial germ cells but normal migration. Conditional mutants display impaired plasma cell and pre-plasmamemory B cell development.

Targeted Progress (from Jax)

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Detailed Description

https://www.jax.org/strain/008827

These transgenic mice express Cre recombinase under the control of the mouse *Prdm1* (PR domain containing 1, with ZNF domain; Blimp1) promoter. Cre-mediated recombination is detected in 55-76% of primordial germ cells when this strain is crossed with Gt(ROSA)26-GFP reporter mice. Expression is also seen in plasma cells. These mice may be useful for generating tissue-specific targeted mutants for studies of development and germ cell fate.

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The Jackson Laboratory

MOUSE STRAIN DATASHEET - 008827

B6.Cg-Tg(Prdm1-cre)1Masu/J

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Typically mice are recovered in 10-14 weeks. Contact Customer Service to place an order or for more information.

OVERVIEW | DETAILS | GENETICS | DISEASE/PHENOTYPE | TECHNICAL SUPPORT | PRICING & AVAILABILITY | TERMS OF USE | RELATED STRAINS

Allele Name	transgene insertion 1, M Azim Surani
Allele Type	Transgenic (Recombinase-expressing)
Allele Synonym(s)	Blimp1-Cre; Blimp1Cre
Gene Symbol and Name	Tg(Prdm1-cre)1Masu MGI, transgene insertion 1, M Azim Surani
Gene Synonym(s)	Blimp1-Cre; Blimp1Cre
Promoter	*Prdm1*, PR domain containing 1, with ZNF domain, mouse, laboratory
Expressed Gene	*cre*, cre recombinase, bacteriophage P1
Site of Expression	Cre-mediated recombination is detected in 55-76% of primordial germ cells when this mice carryint this transgene are crossed with Gt(ROSA)26-GFP reporter mice.
Strain of Origin	(C57BL/6 x CBA)F1
Chromosome	UN
Molecular Note	The coding region in exon 1 and 2 were replaced by an NLS and Cre cDNA sequence, followed by a transcriptional STOP cassette.
Mutations Made By	M. Azim Surani, Gurdon Institute,University of Cambridge

References

1. Arne Mould, Marc A.J. Morgan, Li Li, Elizabeth K. Bikoff, and Elizabeth J. Robertson. Blimp1/Prdm1 governs terminal differentiation of endovascular trophoblast giant cells and defines multipotent progenitors in the developing placenta. *GENES & DEVELOPMENT.* 2012 26:2063–2074
2. Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse. *Genesis.*2002 Jan.32(1):19-26

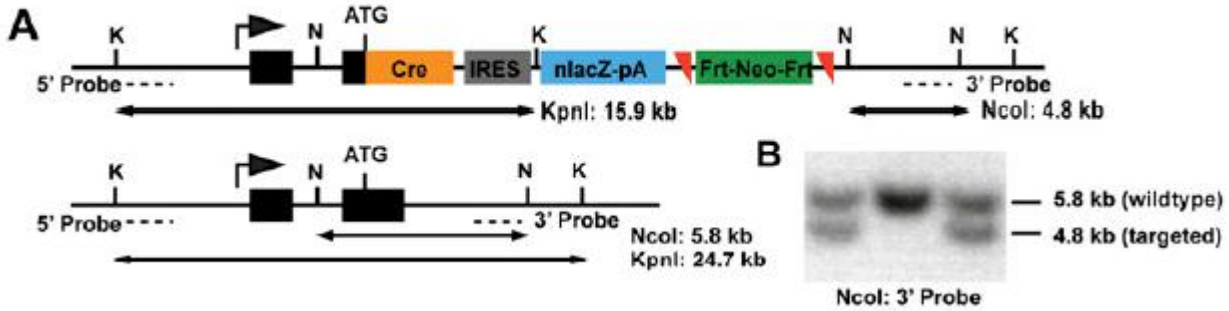

Figure 5. Generation and validation of a dual-purpose Prdm1.Cre-LacZ reporter allele. (*A*) Targeting strategy for generating the *Prdm1-Cre-IRES-nlacZ* allele. Mutant (*top*) and wild-type (*bottom*) alleles with Southern blotting restriction enzyme sites, fragment sizes, and location of probes indicated. (K) KpnI; (N) NcoI; (ATG) Prdm1 translation initiation methionine codon. (*B*) Southern blot screening

Animals

Prdm1^{BEH} (Vincent et al. 2005), *Blimp1*^{gfp} (Kallies et al. 2004), *Flk.LacZ* (Shalaby et al. 1995), and *R26R* reporter (Soriano 1999) mouse strains have been described. *Prdm1*$^{BEH/+}$ animals were intercrossed to generate null placental tissue. The *Prdm1*$^{Cre\text{-}LacZ}$ allele was generated by introducing a cassette containing a codon-optimized *Cre* (Shimshek et al. 2002) upstream of IRES-nlacZ followed by a FRT-flanked *neo* cassette (iCre-IRES-nLacZ-FRT-neo-FRT) into the ATG translational site in exon 3 via homologous recombination in embryonic stem (ES) cells. Correctly targeted ES cell clones were transiently transfected with a FLP expression construct. Drug-excised subclones were injected into blastocysts to generate germline chimeras. All experiments using mice were performed in accordance with Home Office regulations.

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

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