Kdr-iCre-P2A Cas9-KI Strategy

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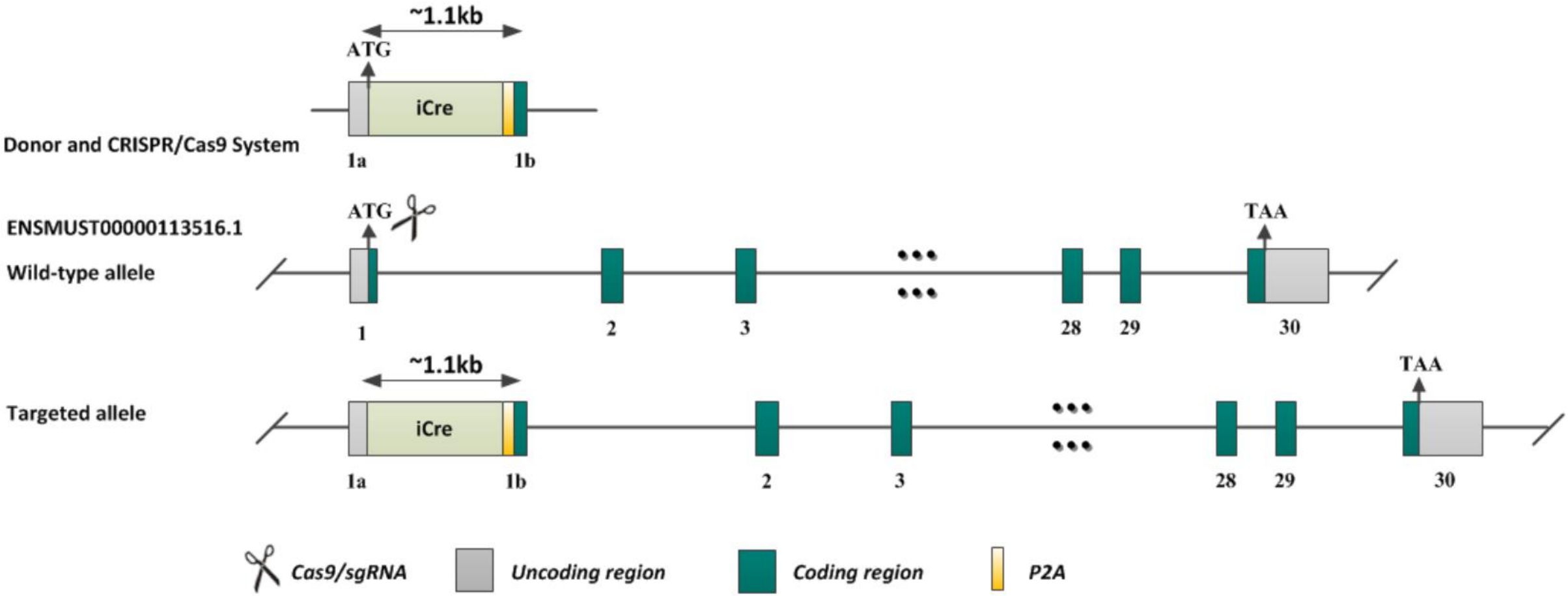
Project Overview

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

Knockin strategy

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



Technical routes

- The *Kdr* gene has 3 transcripts.According to the structure of *Kdr* gene, *Kdr-201*(ENSMUST00000113516.1) is selected for presentation of the recommended strategy.

- *Kdr-201* gene has 30 exons, with the ATG start codon in exon1 and TAA stop codon in exon30.

- We make *Kdr-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 *Kdr* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre after start coding(ATG) of *Kdr* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, Homozygous mice die at early embryonic stages due to failure of blood vessel formation.
- According to to the existing references, Cre-mediated recombination is expressed in blood and vascular endothelial cells ,which can be detected at 8.5 days of embryonic stage.
- Insertion of iCre may affect the regulation of the 5' end of the *Kdr* gene.
- 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.
- There will be 1 to 2 amino acid synonymous mutation in exon1 of *Kdr* gene in this strategy.
- Downstream of insertion site exists CCCTT repeat structure sequence, mutations base may occur during vector construction.
- The *Kdr* gene is located on the Chr5. 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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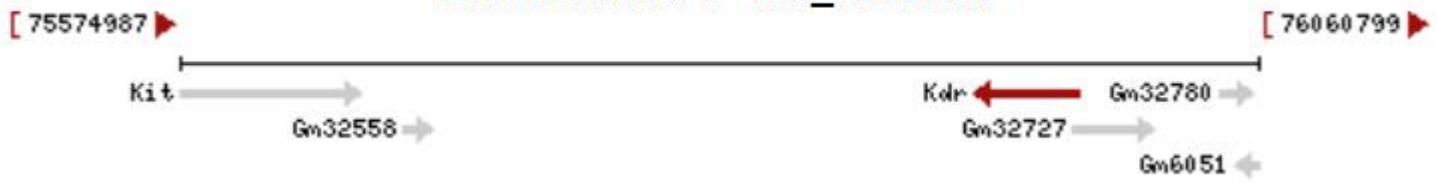
Kdr kinase insert domain protein receptor [*Mus musculus* (house mouse)]

Gene ID: 16542, updated on 13-Aug-2019

Summary

Official Symbol	Kdr provided by MGI
Official Full Name	kinase insert domain protein receptor provided by MGI
Primary source	MGI:MGI:96683
See related	Ensembl:ENSMUSG00000062960
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	orv; Flk1; Ly73; Flk-1; Krd-1; VEGFR2; VEGFR-2; sVEGFR-2; 6130401C07
Expression	Broad expression in lung adult (RPKM 42.4), heart adult (RPKM 19.3) and 22 other tissues See more
Orthologs	human all

Chromosome 5 - NC_000071.6

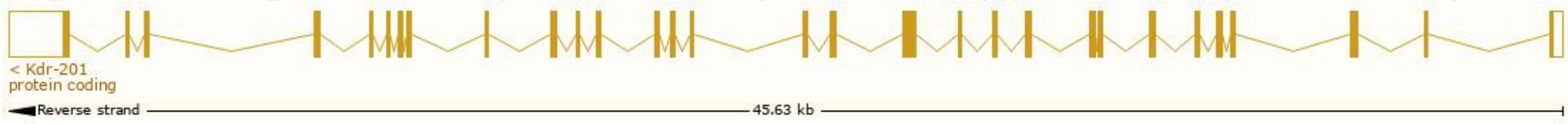


Transcript information （Ensembl）

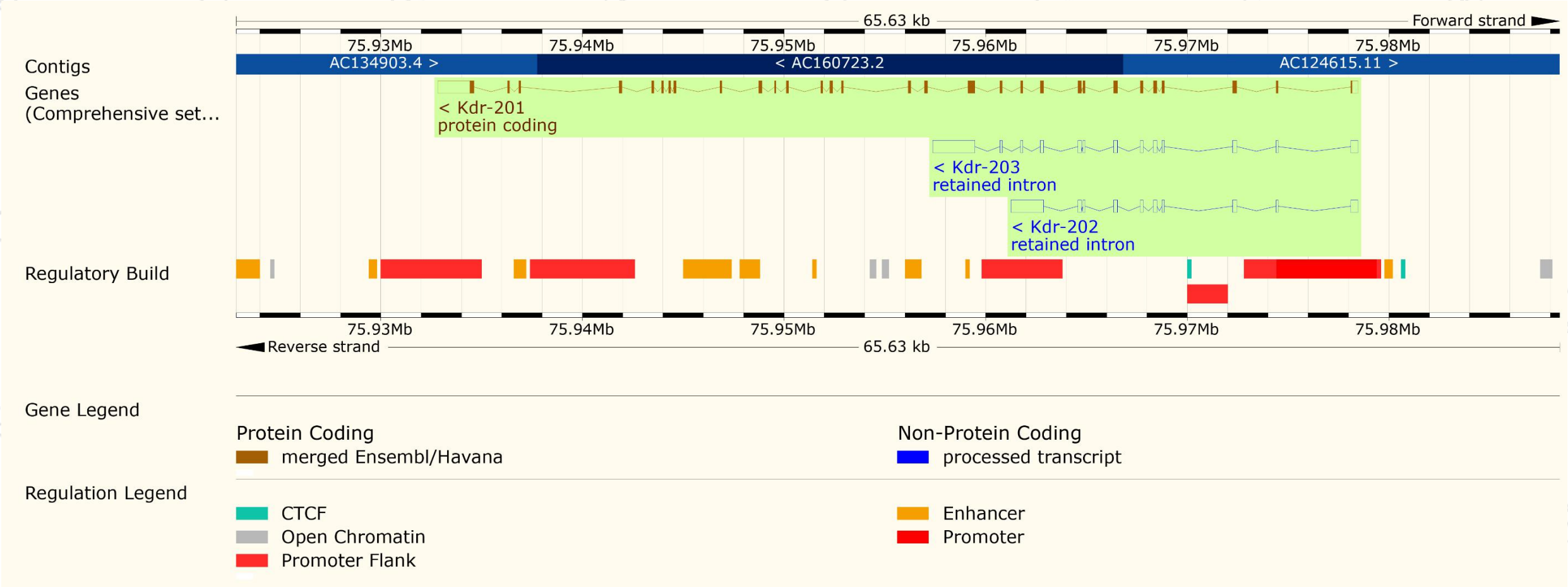
The gene has 3 transcripts, and all transcripts are shown below:

Name ▲	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Kdr-201	ENSMUST00000113516.1	5924	1345aa	ENSMUSP00000109144.1	Protein coding	CCDS39114	Q8VCD0	TSL:1 GENCODE basic APPRIS P1
Kdr-202	ENSMUST00000149573.1	3174	No protein	-	Retained intron	-	-	TSL:1
Kdr-203	ENSMUST00000202473.3	4023	No protein	-	Retained intron	-	-	TSL:1

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

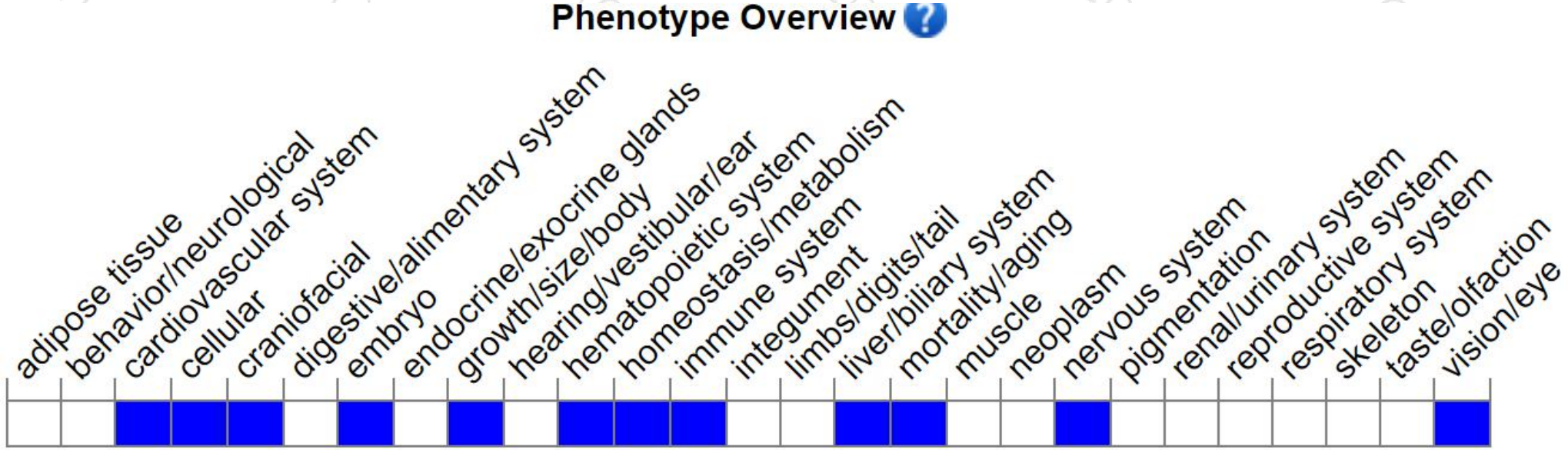


Genomic location distribution



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: 96683) .

According to the existing MGI data, Homozygous mice die at early embryonic stages due to failure of blood vessel formation.

iCre Sequence[1] (1056bp)

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGCCACCTCTGATGAAGTCAGGAAGA
ACCTGATGGACATGTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAGATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAA
CAACAGGAAATGGTTCCCTGCTGAACCTGAGGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTG
GGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATCAGAAAGGAGAATGTGG
ATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCAGATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAG
GAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGA
ATGCTGATCCACATTGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGTTACCAAGCTGGTGGAGAGATGGATCT
CTGTGTCTGGTGTGGCTGATGACCCCAACAACTACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCTGCCCCTTCTGCCACCTCCCAACTGTCCACCCG
GGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGTGCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGA
GTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCA
GAAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

References

[1] Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse. GeAlbis.2002 Jan.32(1):19-26.

[2] Motoike T, et al. Evidence for novel fate of Flk1+ progenitor: contribution to muscle lineage. Genesis. 2003 Mar;35(3):153-9.

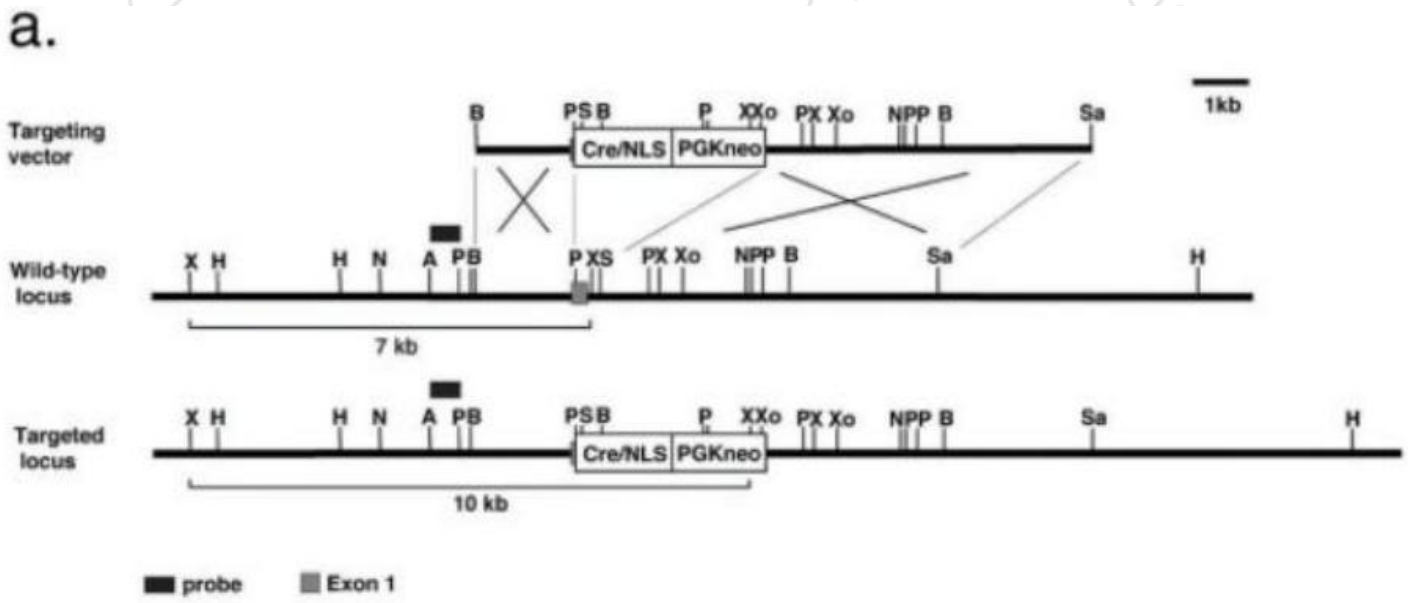


FIG. 1. Fate-mapping of Flk1^{+} cells by Cre-knock-in line. **a:** Cre was knocked-in the *Flk1* locus to generate the Flk1::Cre knock-in line. The targeting vector was designed to replace the first coding exon. In the targeting vector, the

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

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