

Resf1-3 ×flag-P2A-icre cas9-ki Strategy

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Reviewer

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Project Overview

Project Name

Resf1-3 ×flag-P2A-icre

Project type

Cas9-KI

Strain background

C57BL/6JGpt

- The *Resf1* gene has 11 transcripts. According to the reference and structure of *Resf1* gene, *Resf1-201*(ENSMUST00000046689.13) is selected for presentation of the recommended strategy.
- *Resf1-201* gene has 5 exons, with the ATG start codon in exon3 and TAG stop codon in exon5.
- In this project, *Resf1* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, gRNA and donor vectors were constructed. Cas9, gRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR, sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

Coding Sequence of Codon-Optimized *Cre* Gene ^[1](*iCre* :1056 bp)

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGC
CACCTCTGATGAAGTCAGGAAGAACCTGATGGACATGTTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAG
ATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAACAACAGGAAATGGTTCCCTGCTGAACCTGA
GGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTGGGGCCAG
CTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATC
AGAAAGGAGAATGTGGATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCA
GATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAGGAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTG
CTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGAATGCTGATCCACAT
TGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGGTTACCAAGCTGGTGGAG
AGATGGATCTCTGTGTCTGGTGTGGCTGATGACCCCAACAACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCT
GCCCCTTCTGCCACCTCCCAACTGTCCACCCGGGGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGT
GCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGAGTGGGTGCTGCCAGGGACATGGC
CAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCAG
AAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (EGFP-P2A-iCre) expression in the mouse. *Genesis*.2002 Jan.32(1):19-26.

Knockin strategy

This model will use CRISPR-Cas9 technology to edit the *Resf1* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Resf1-201
(ENSMUST00000046689.13)
Wild-type allele

Targeted allele



CRISPR-Cas9



Uncoding region



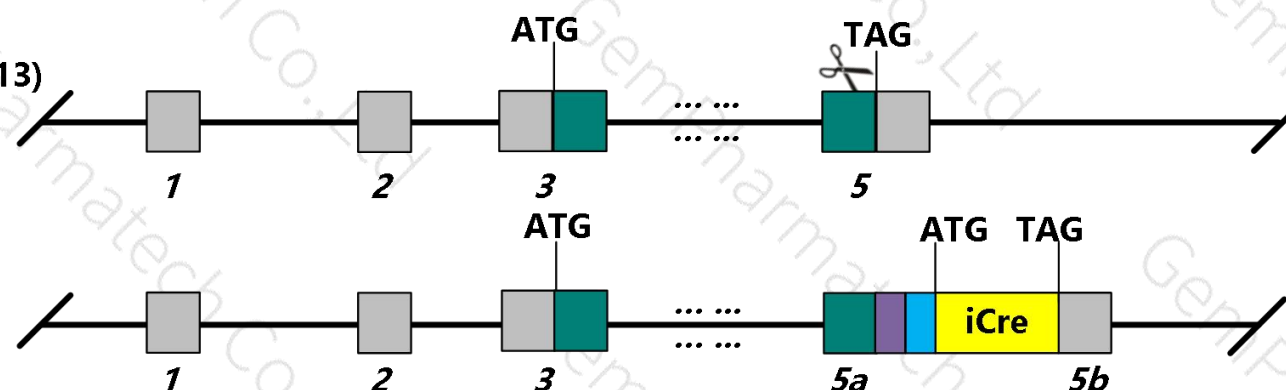
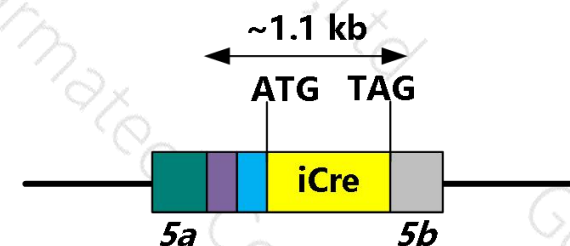
Coding region



P2A



3×flag



- The two genes are linked by P2A, which will be transcribed and translated together but spliced into two protein molecules, with the front protein carrying the polypeptide translated from the P2A sequence.
- Insertion of 3×flag-P2A-iCre may affect the regulation of the 3' end of the *Resf1* gene.
- There may be 1 to 2 amino acid synonymous mutation in exon5 of *Resf1* gene in this strategy.
- The effect of this strategy on transcripts *Resf1*-203,204,206,207,210 is unknown
- The *Resf1* gene is located on the Chr6. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene after the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

Resf1 retroelement silencing factor 1 [Mus musculus (house mouse)]

Gene ID: 67246, updated on 17-Dec-2020

Summary



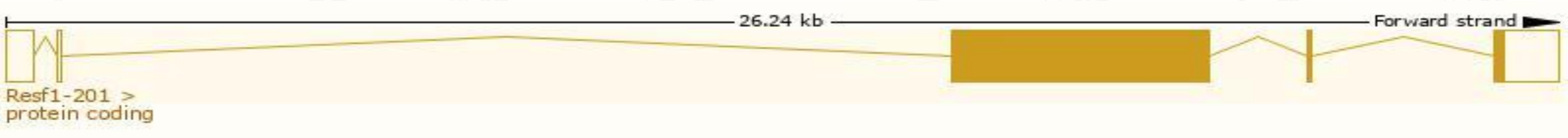
Official Symbol	Resf1 provided by MGI
Official Full Name	retroelement silencing factor 1 provided by MGI
Primary source	MGI:MGI:1914496
See related	Ensembl:ENSMUSG00000032712
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810474O19Rik, 6720435I21Rik, AA536804, AU020969, GET
Expression	Ubiquitous expression in bladder adult (RPKM 4.3), ovary adult (RPKM 3.6) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

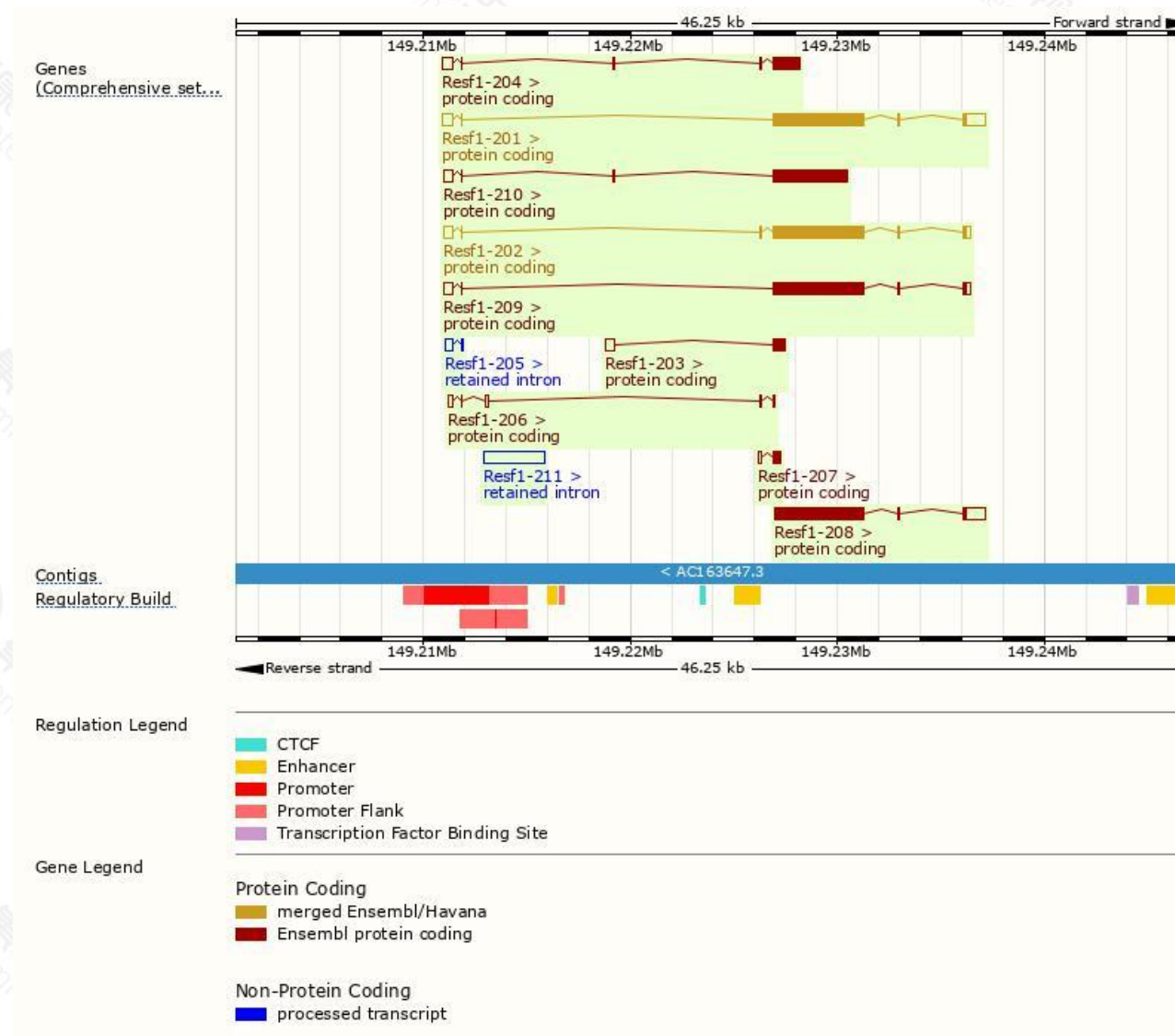
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Resf1-201	ENSMUST00000046689.13	6066	1521aa	Protein coding	CCDS20717		TSL:1 , GENCODE basic , APPRIS P2 ,
Resf1-202	ENSMUST00000100765.11	5356	1521aa	Protein coding	CCDS20717		TSL:1 , GENCODE basic , APPRIS P2 ,
Resf1-209	ENSMUST00000189932.7	5269	1521aa	Protein coding	CCDS20717		TSL:1 , GENCODE basic , APPRIS P2 ,
Resf1-208	ENSMUST00000189837.2	5469	1519aa	Protein coding	-		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Resf1-210	ENSMUST00000190785.7	4152	1173aa	Protein coding	-		CDS 3' incomplete , TSL:1 ,
Resf1-204	ENSMUST00000130664.8	1957	404aa	Protein coding	-		CDS 3' incomplete , TSL:1 ,
Resf1-203	ENSMUST00000127680.2	938	170aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Resf1-207	ENSMUST00000187881.2	514	105aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Resf1-206	ENSMUST00000185930.2	511	10aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Resf1-211	ENSMUST00000203348.2	2951	No protein	Retained intron	-		TSL:NA ,
Resf1-205	ENSMUST00000137983.2	466	No protein	Retained intron	-		TSL:2 ,

The strategy is based on the design of *Resf1-201* transcript,the transcription is shown below:



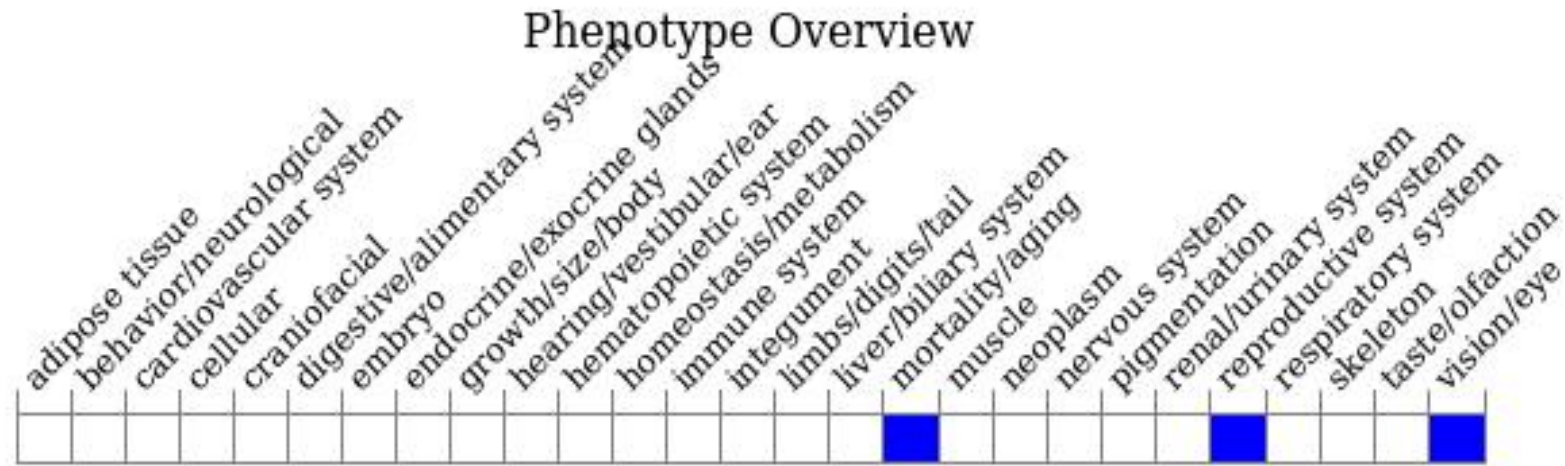
Genomic location distribution



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/>).

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