

Cd8a-iCre-PolyA BAC-TG Strategy

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Reviewer:

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Design Date:

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Project Overview

Project Name

Cd8a-iCre-PolyA

Project type

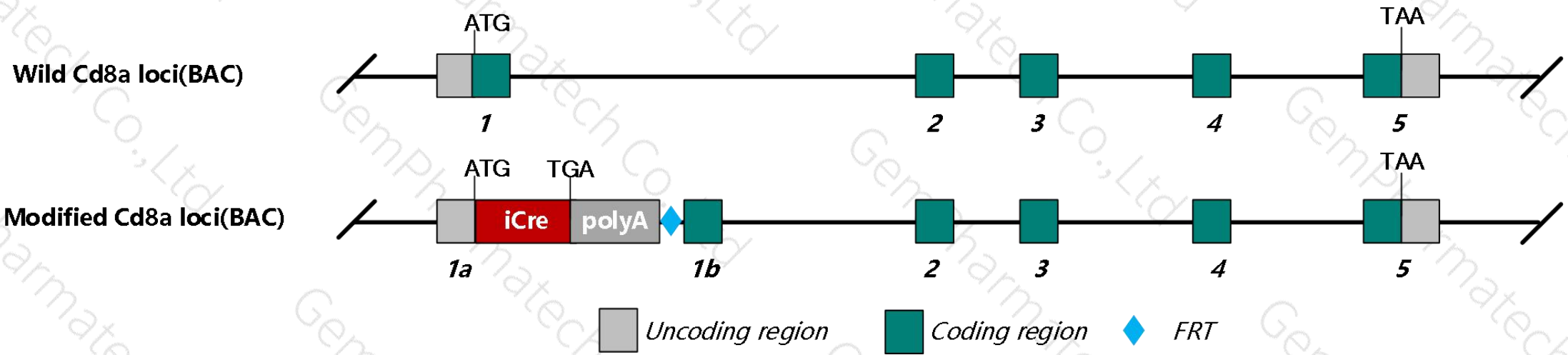
BAC-TG

Strain background

C57BL/6JGpt

BAC-TG strategy

This mice model is made by transgenic technology, and the schematic diagram is as follows:



Targeted Progress (from Jax)

➡ *Cd8a*^{tm1.1(cre)Koni}

Allele Symbol: *Cd8a*^{tm1.1(cre)Koni} 

Allele Name	targeted mutation 1.1, Pandelakis A Koni
Allele Type	Targeted (Recombinase-expressing)
Allele Synonym(s)	Cd8acre
Gene Symbol and Name	<i>Cd8a</i>  , CD8 antigen, alpha chain
Gene Synonym(s)	BB154331; CD8; Leu2; Ly-2; Ly-2; Ly-35; Ly-35; Ly-B; Lyt-2; Lyt-2; MAL; T-lymphocyte antigen 2; expressed sequence BB154331; lymphocyte antigen 2; lymphocyte antigen 35; p32

➡ Development

A targeting vector was designed to insert a Cre-recombinase coding sequence, a neomycin resistance (neo) cassette, and an internal ribosome entry site (IRES) into the start codon of the CD8 antigen alpha chain (*Cd8a*) gene. The construct was electroporated into C57BL/6NHsd-derived LK-1 embryonic stem (ES) cells. Correctly targeted ES cells were injected into blastocysts and the resulting chimeric males were bred to C57BL/6 females. Offspring were then bred to B6.Cg-Tg(ACTFLPe)9205Dym/J mice (Stock No. 005703) to delete the neo cassette. The resulting mice were backcrossed to C57BL/6J for at least 3 generations to generate a colony of *Cd8a*^{cre} mice. Upon arrival at The Jackson Laboratory, mice were bred to C57BL/6J (Stock No. 000664) for at least one generation.

<https://www.jax.org/strain/017562>

Technical routes

- Transcript *Cd8a-201*(ENSMUST00000066747.13) is selected for presentation of the recommended strategy.
- *Cd8a-201* gene has 5 exons, with the ATG start codon in exon1 and TAA stop codon in exon5.
- RP23-139M18(~181kb) or RP23-229N15 (~200kb) of C57BL/6JGpt mouse bacterial artificial chromosome (BAC) containing the entire *Cd8a* locus (and other genes), was modified by targeting a *iCre-polyA* sequence after the ATG translation stop codon of the *Cd8a* locus, so the *iCre* is expressed from the endogenous promoter/enhancer elements of *Cd8a*.
- In this study, the transgenic vector was constructed in vitro, and transgenic fragments containing *Cd8a-iCre-polyA* were micro-injected into the fertilized eggs of C57BL/6JGpt mice, and pcr-positive F0 generation (i.e., founder) mice were obtained.

- Other genes on the *Cd8a* BAC are also expressed together.
- The BAC plasmid is large, and the BAC backbone may affect the expression of the gene of interest.
- Transgenic fragments injected into the prokaryotes will be randomly integrated into the mouse genome. Affected by the insertion site and copy number of transgenic fragments, the expression level of transgenic mice may be different.
- 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.

Gene information (NCBI)

Cd8a CD8 antigen, alpha chain [*Mus musculus* (house mouse)]

Gene ID: 12525, updated on 12-Aug-2019

Summary

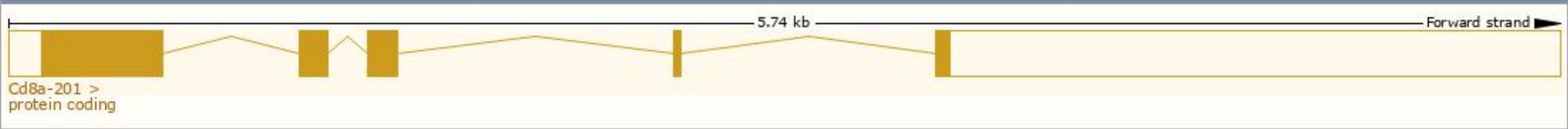
Official Symbol	Cd8a provided by MGI
Official Full Name	CD8 antigen, alpha chain provided by MGI
Primary source	MGI:MGI:88346
See related	Ensembl:ENSMUSG00000053977
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Ly-2; Ly-B; Ly-35; Lyt-2; BB154331
Expression	Restricted expression toward thymus adult (RPKM 213.1) 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
Cd8a-201	ENSMUST00000066747.13	3128	247aa	Protein coding	CCDS39507	P01731	TSL:1 GENCODE basic APPRIS P3
Cd8a-202	ENSMUST00000172321.2	2975	222aa	Protein coding	CCDS51806	Q8CAX3	TSL:1 GENCODE basic APPRIS ALT2
Cd8a-203	ENSMUST00000205054.1	1950	No protein	Retained intron	-	-	TSL:1

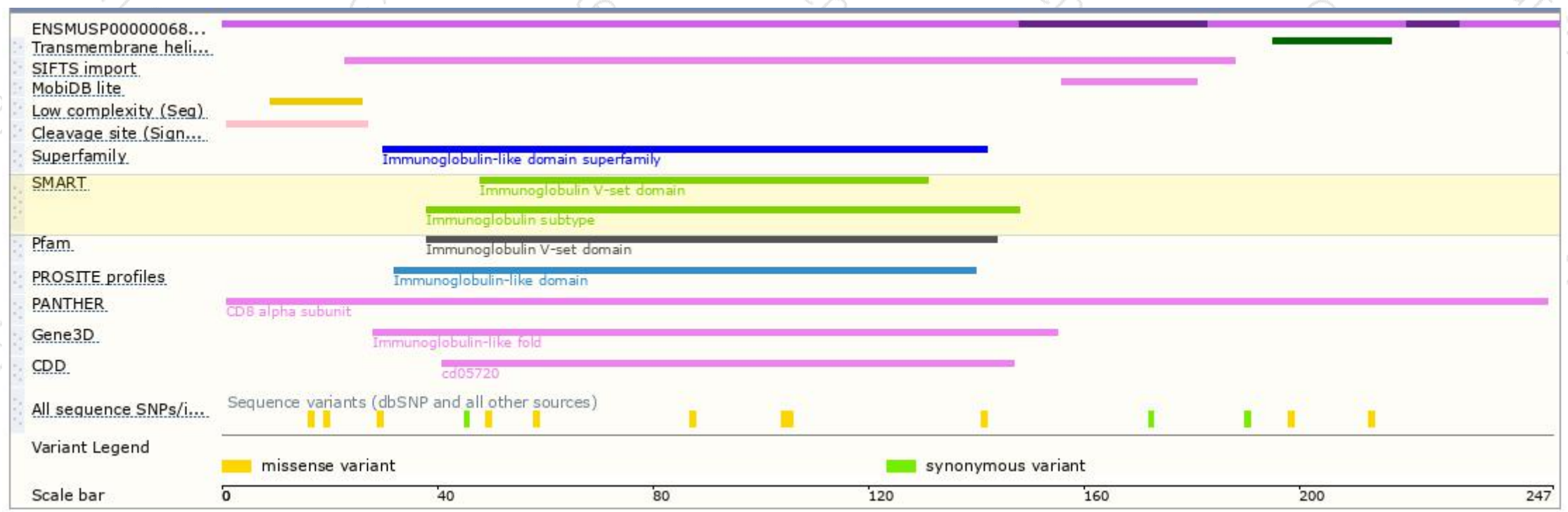
The strategy is based on the design of *Cd8a-201* transcript, The transcription is shown below



Genomic location distribution



Protein domain



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