

***Cd4-IRES-iCre* Mouse Model Strategy -CRISPR/Cas9 technology**

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Project Overview



Project Name

Cd4-IRES-iCre

Project type

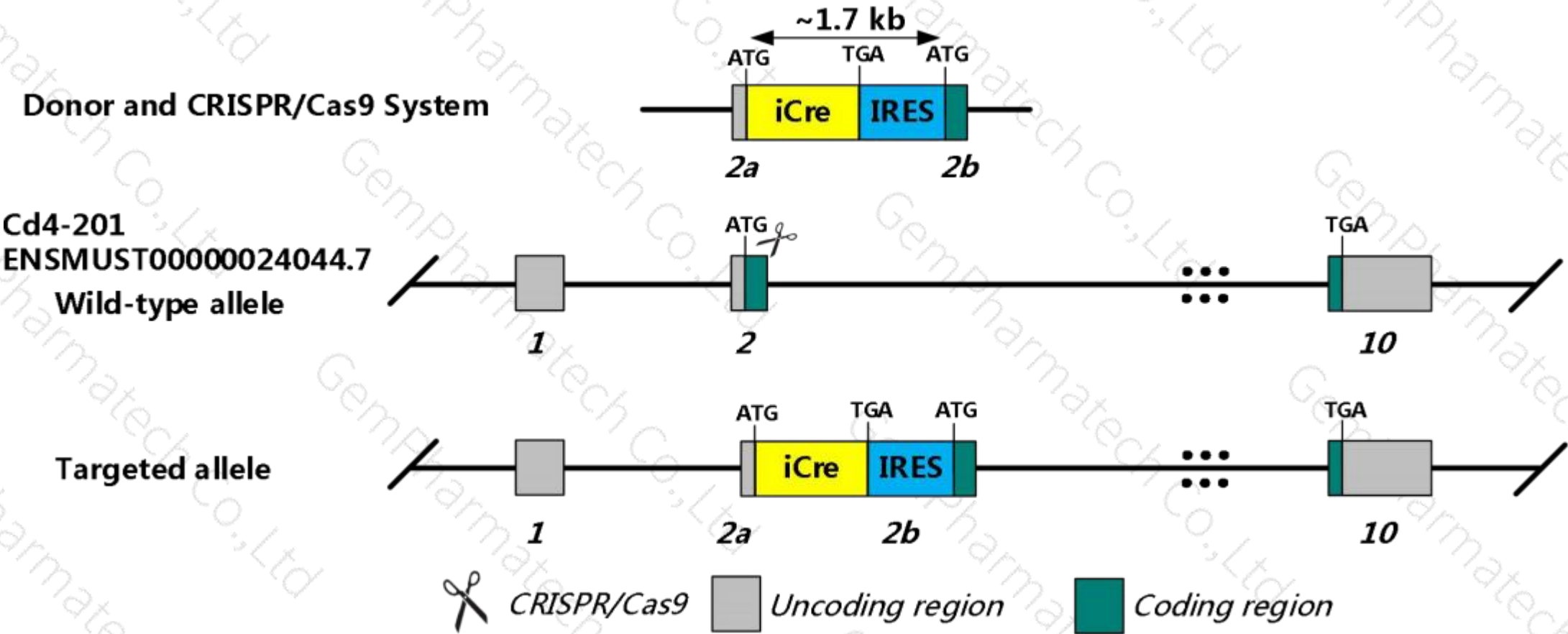
Cas9-KI

Strain background

C57BL/6JGpt

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Cd4* gene. The schematic diagram is as follows:



Technical routes

- The mouse *Cd4* gene has 5 transcripts. According to the structure of *Cd4* gene, *Cd4-201* (ENSMUST00000024044.7) transcript is selected for this strategy. The transcript of *Cd4-201* contains 10 exons, codes 457aa, the ATG is located in exon2, and the TGA is located in exon10.
- We constructed CRISPR/Cas9 system targeting mouse *Cd4* gene and donor vector, iCre-IRES will be introduced to near the ATG of mouse *Cd4* gene. The iCre will be expressed under the direction of endogenous regulatory mechanism.
- The project will use CRISPR/Cas9 technology to modify *Cd4* gene. The brief process is as follows: CRISPR/Cas9 system and donor were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

- According to the existing MGI data, mice homozygous for knock-out alleles exhibit abnormal immune system morphology and physiology.
- The IRES-linked genes will be transcribed together and then be translated two protein separately, but the downstream protein is lower than the upstream protein.
- There may be 1 to 2 amino acid synonymous mutation in exon2 of *Cd4* gene in this strategy.
- After customer verification, mice homozygous for knock-in alleles appear the CD4-KO phenotype, the expression of heterozygous for knock-in alleles is halved, and the homozygous could not detect CD4⁺ cells.
- The *Cd4* gene is located on the Chr6. 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, it is impossible to predict all of them at the existing technology level.

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

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGC
CACCTCTGATGAAGTCAGGAAGAACCTGATGGACATGTTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAG
ATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAACAACAGGAAATGGTTCCCTGCTGAACCTGA
GGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTGGGGCCAG
CTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATC
AGAAAGGAGAATGTGGATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCA
GATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAGGAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTG
CTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGAATGCTGATCCACAT
TGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGGTTACCAAGCTGGTGGAG
AGATGGATCTCTGTGTCTGGTGTGGCTGATGACCCCAACAACCTACCTGTTCTGCCGGGGTCAGAAAGAATGGTGTGGCT
GCCCCTTCTGCCACCTCCCAACTGTCCACCCGGGGCCCTGGAAGGGATCTTTGAGGGCCACCCACCGCCTGATCTATGGT
GCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGAGTGGGTGCTGCCAGGGACATGGC
CAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCAG
AAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

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

Existing model information

<https://www.informatics.jax.org/allele/summary?markerId=MGI:88335>

Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems
Cd4^{del} CD4 antigen; deletion	6		Spontaneous	hematopoietic, immune
Cd4^{em1(IMPC)H} CD4 antigen; endonuclease-mediated mutation 1, Harwell	6		Endonuclease-mediated (Null/knockout)	
Cd4^{em1Doi} CD4 antigen; endonuclease-mediated mutation 1, Christophe Benoist and Diane Mathis	6	Cd4 ^{tdtomato}	Endonuclease-mediated (Epitope tag, Reporter)	
Cd4^{em1Gpt} CD4 antigen; endonuclease-mediated mutation 1, GemPharmatech Co., Ltd	6	Cd4 ^{em1Cflox}	Endonuclease-mediated (Conditional ready, No functional change)	
Cd4^{em1Litt} CD4 antigen; endonuclease-mediated mutation 1, Dan R Littman	6	Cd4 ^{E4mdelta} , Rr95 ^{em1Litt}	Endonuclease-mediated (Modified regulatory region)	no abnormal phenotype observed
Cd4^{em1Smoc} CD4 antigen; endonuclease-mediated mutation 1, Shanghai Model Organisms Center	6	Cd4 ^{em1(flox)Smoc}	Endonuclease-mediated (Conditional ready, No functional change)	
Cd4^{em2(CD4)Smoc} CD4 antigen; endonuclease-mediated mutation 2, Shanghai Model Organisms Center	6	Cd4 ^{em1(hCD4)Smoc}	Endonuclease-mediated (Inserted expressed sequence)	
Cd4^{em2(IMPC)H} CD4 antigen; endonuclease-mediated mutation 2, Harwell	6		Endonuclease-mediated (Null/knockout)	
Cd4^{em2Gpt} CD4 antigen; endonuclease-mediated mutation 2, GemPharmatech Co., Ltd	6	Cd4 ^{em1Cd}	Endonuclease-mediated (Null/knockout)	
Cd4^{em2Litt} CD4 antigen; endonuclease-mediated mutation 2, Dan R Littman	6	Cd4 ^{E4mdelta} , Rr95 ^{em2Litt}	Endonuclease-mediated (Modified regulatory region)	
Cd4^{em3(dre/ERT2)Smoc} CD4 antigen; endonuclease-mediated mutation 3, Shanghai Model Organisms Center	6	Cd4 ^{em3(2A-DreERT2)Smoc}	Endonuclease-mediated (Inducible, Recombinase)	
Cd4^{em3(IMPC)H} CD4 antigen; endonuclease-mediated mutation 3, Harwell	6		Endonuclease-mediated (Null/knockout)	
Cd4^{em3Smoc} CD4 antigen; endonuclease-mediated mutation 3, Shanghai Model Organisms Center	6		Endonuclease-mediated (Null/knockout)	
Cd4^{em4(CD4)Smoc} CD4 antigen; endonuclease-mediated mutation 4, Shanghai Model Organisms Center	6	Cd4 ^{em3(hCD4)/Smoc}	Endonuclease-mediated (Inserted expressed sequence)	

Existing model information

<https://www.informatics.jax.org/allele/summary?markerId=MGI:88335>

Cd4^{em5(Hbegf)Smoc} CD4 antigen; endonuclease-mediated mutation 5, Shanghai Model Organisms Center	6	Cd4 ^{em5(IRES-DTREGFP)Smoc}	Endonuclease-mediated (Inserted expressed sequence, Reporter)	
Cd4^{m1} CD4 antigen; mutation 1	6	Cd4 ^{fs}	Spontaneous (Null/knockout)	
Cd4^{m1Btlr} CD4 antigen; mutation 1, Bruce Beutler	6	craw	Chemically induced (ENU)	hematopoietic, immune
Cd4^{maat} CD4 antigen; maat	6		Chemically induced (ENU)	hematopoietic, immune
Cd4^{mo} CD4 antigen; deletion 1	6		Spontaneous	
Cd4^{sest} CD4 antigen; sest	6		Chemically induced (ENU)	hematopoietic, immune
Cd4^{thb} CD4 antigen; thebes	6		Chemically induced (ENU)	
Cd4^{tht} CD4 antigen; thoth	6		Chemically induced (ENU)	hematopoietic, immune
Cd4^{tm1(Cd8b1,Cd8a)Asin} CD4 antigen; targeted mutation 1, Alfred Singer	6	8in4, Cd4(CD8)	Targeted (Inserted expressed sequence, Null/knockout)	
Cd4^{tm1(cre/ERT2)Thbu} CD4 antigen; targeted mutation 1, Thorsten Buch	6	CD4-CreER ^{t2}	Targeted (Inducible, Recombinase)	
Cd4^{tm1(Hbegf)Aox} CD4 antigen; targeted mutation 1, Annette Oxenius	6		Targeted (Conditional ready, Inserted expressed sequence)	
Cd4^{tm1.1Arah} CD4 antigen; targeted mutation 1.1, Amin Rahemtulla	6	CD4deltasil, Cd4Sil ^f	Targeted (Constitutively active)	
Cd4^{tm1Dim} CD4 antigen; targeted mutation 1, Diane Mathis	6	CD4 ^L	Targeted (Null/knockout, Reporter)	
Cd4^{tm1Doi} CD4 antigen; targeted mutation 1, Christophe Benoist and Diane Mathis	6	LAG ⁰ CD4 ⁰	Targeted (Null/knockout)	
Cd4^{tm1Jrp} CD4 antigen; targeted mutation 1, Jane R Parnes	6	Cd4 ^{low}	Targeted (Hypomorph)	hematopoietic, immune
Cd4^{tm1Knw} CD4 antigen; targeted mutation 1, Barbara B Knowles	6	CD4 ⁻ , CD4D	Targeted (Null/knockout)	hematopoietic, immune
Cd4^{tm1Litt} CD4 antigen; targeted mutation 1, Dan R Littman	6	CD4 ⁻ , Cd4 ^{tm1Drl}	Targeted (Null/knockout)	cellular, hematopoietic, immune

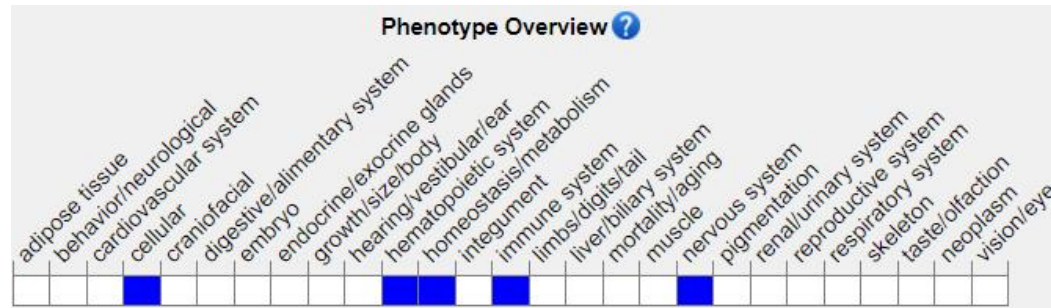
Existing model information

<https://www.informatics.jax.org/allele/summary?markerId=MGI:88335>

Cd4^{tm1Mak} CD4 antigen; targeted mutation 1, Tak Mak	6	CD4 ⁻ , CD4 ⁰ , CD4 KO	Targeted (Null/knockout)	hematopoietic, homeostasis, immune, nervous system
Cd4^{tm1Nik} CD4 antigen; targeted mutation 1, Nigel Kileen	6	Cd4 ^{lox}	Targeted (Conditional ready, No functional change)	hematopoietic, immune
Cd4^{tm2(EGFP/cre/ERT2)Wtsi} CD4 antigen; targeted mutation 2, Wellcome Trust Sanger Institute	6	Cd4 ^{tm2(EGFP/cre/ERT2)Hmgu}	Targeted (Inducible, Recombinase, Reporter)	
Tg(Cd4-CADM1)1Kmori transgene insertion 1, Kazuhiro Morishita; transgene insertion 1, Kazuhiro Morishita	UN	Tg(Cd4-CADM1)#Kmori	Transgenic (Inserted expressed sequence)	
Cd4^{Gt(402B1)Cmhd} CD4 antigen; gene trap 402B1, Centre for Modeling Human Disease	6		Gene trapped (Cell Line)	
Cd4^{Gt(OST77829)Lex} CD4 antigen; gene trap OST77829, Lexicon Genetics	6		Gene trapped (Cell Line)	
Cd4^{Gt(OST92301)Lex} CD4 antigen; gene trap OST92301, Lexicon Genetics	6		Gene trapped (Cell Line)	
Cd4^{Gt(OST213770)Lex} CD4 antigen; gene trap OST213770, Lexicon Genetics	6		Gene trapped (Cell Line)	
Cd4^{Gt(OST264015)Lex} CD4 antigen; gene trap OST264015, Lexicon Genetics	6		Gene trapped (Cell Line)	
Cd4^{tm2e(EUCOMM)Hmgu} CD4 antigen; targeted mutation 2e, Helmholtz Zentrum Muenchen GmbH	6		Targeted (Null/knockout, Reporter) (Cell Line)	

Mouse phenotype description(MGI)

<https://www.informatics.jax.org/marker/MGI:88335>



Mice homozygous for knock-out alleles exhibit abnormal immune system morphology and physiology.

Gene information (NCBI)



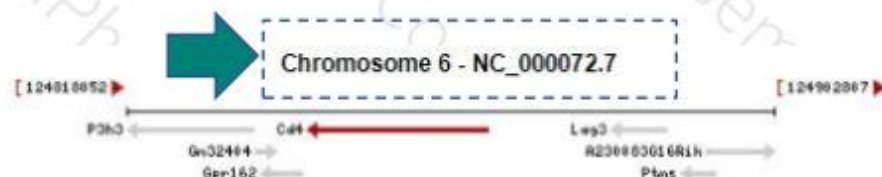
Cd4 CD4 antigen [*Mus musculus* (house mouse)]

Gene ID: 12504, updated on 17-Aug-2021

 Download Datasets

 Summary  

Official Symbol Cd4 provided by [MGI](#)
Official Full Name CD4 antigen provided by [MGI](#)
Primary source [MGI:MGI:88335](#)
See related [Ensembl:ENSMUSG00000023274](#)
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 L3T; Ly-; L3T4; Ly-4
Expression Biased expression in thymus adult (RPKM 167.0), spleen adult (RPKM 15.0) and 1 other tissue [See more](#)
Orthologs [human](#) [all](#)

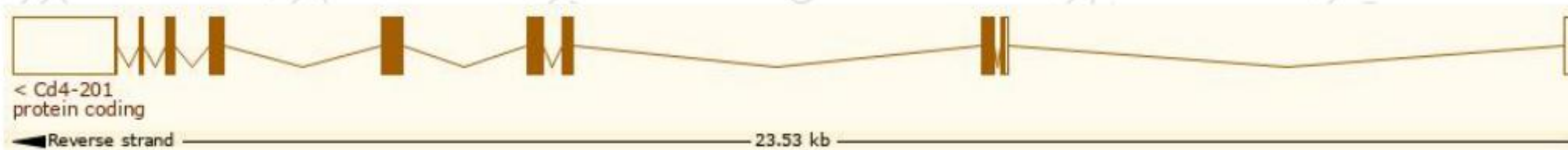


Transcript information (Ensembl)

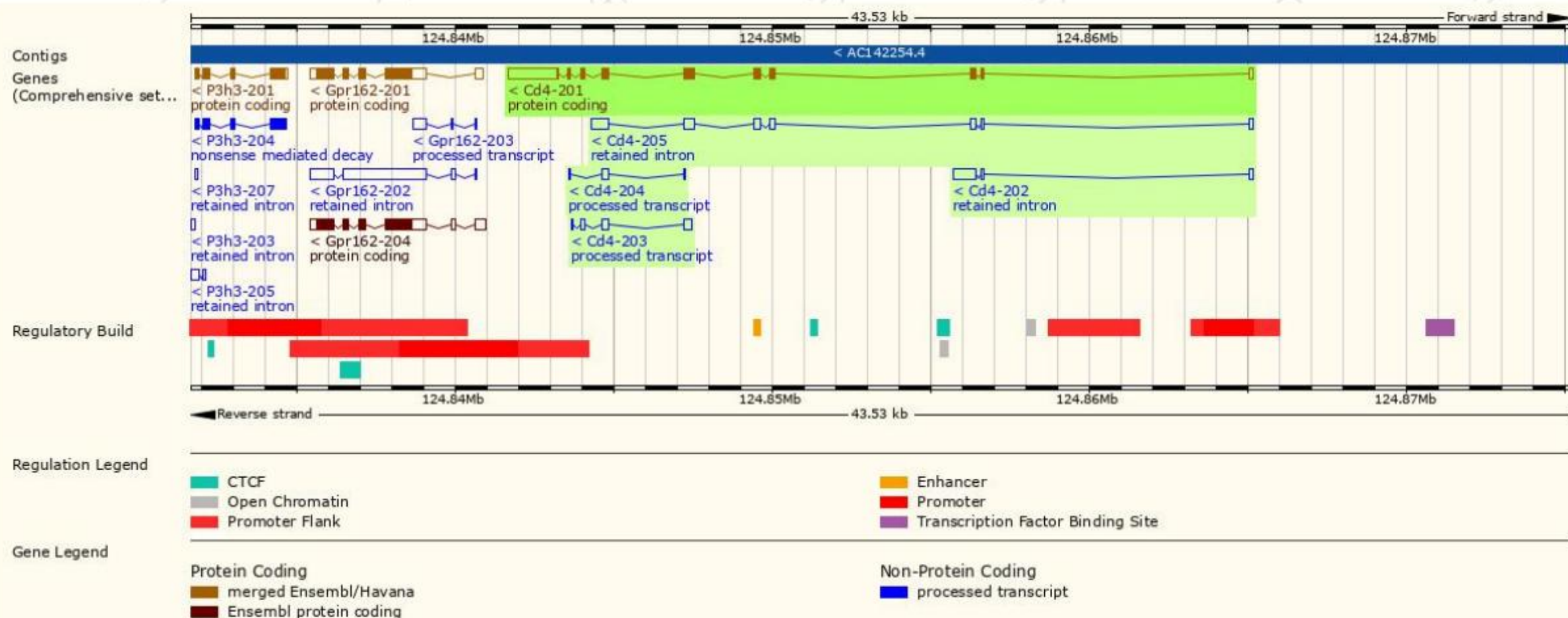
The gene has 5 transcripts, all transcriptional information is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Cd4-201	ENSMUST00000024044.7	3108	457aa	Protein coding	CCDS20535	P06332-1	GENCODE basic APPRIS P1 TSL:1
Cd4-203	ENSMUST00000145818.2	591	No protein	Processed transcript	-	-	TSL:5
Cd4-204	ENSMUST00000145977.8	273	No protein	Processed transcript	-	-	TSL:1
Cd4-205	ENSMUST00000151594.8	1647	No protein	Retained intron	-	-	TSL:1
Cd4-202	ENSMUST00000130378.2	946	No protein	Retained intron	-	-	TSL:1

The strategy is based on *Cd4-201* transcript design, it contains 10 exons, the length of transcript is 3108bps, and encodes 457 amino acids.



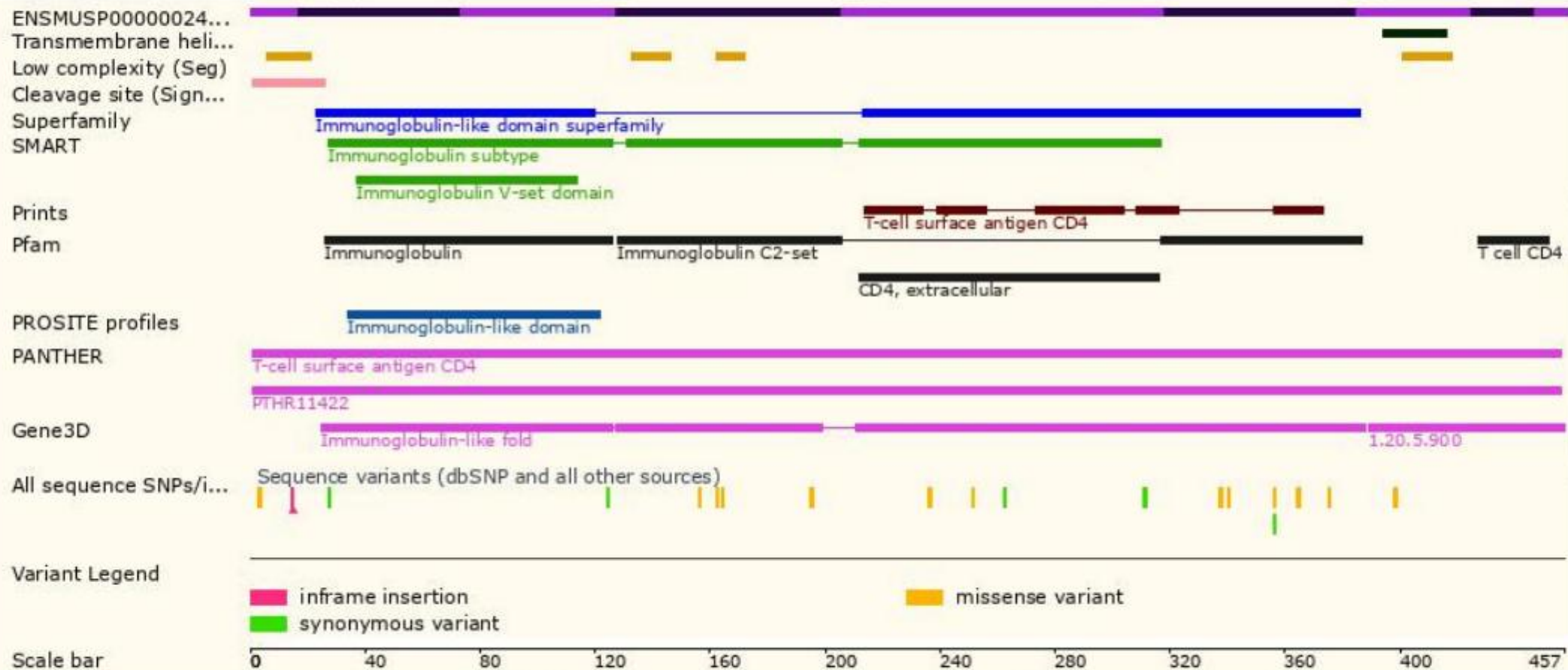
Genomic location distribution



Protein domain



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