

***H11-Cd4-iCre-PolyA* Cas9-KI Strategy**

Designer:

Xiaojing Li

Design Date:

2019-8-19

Reviewer

JiaYu

Project Overview

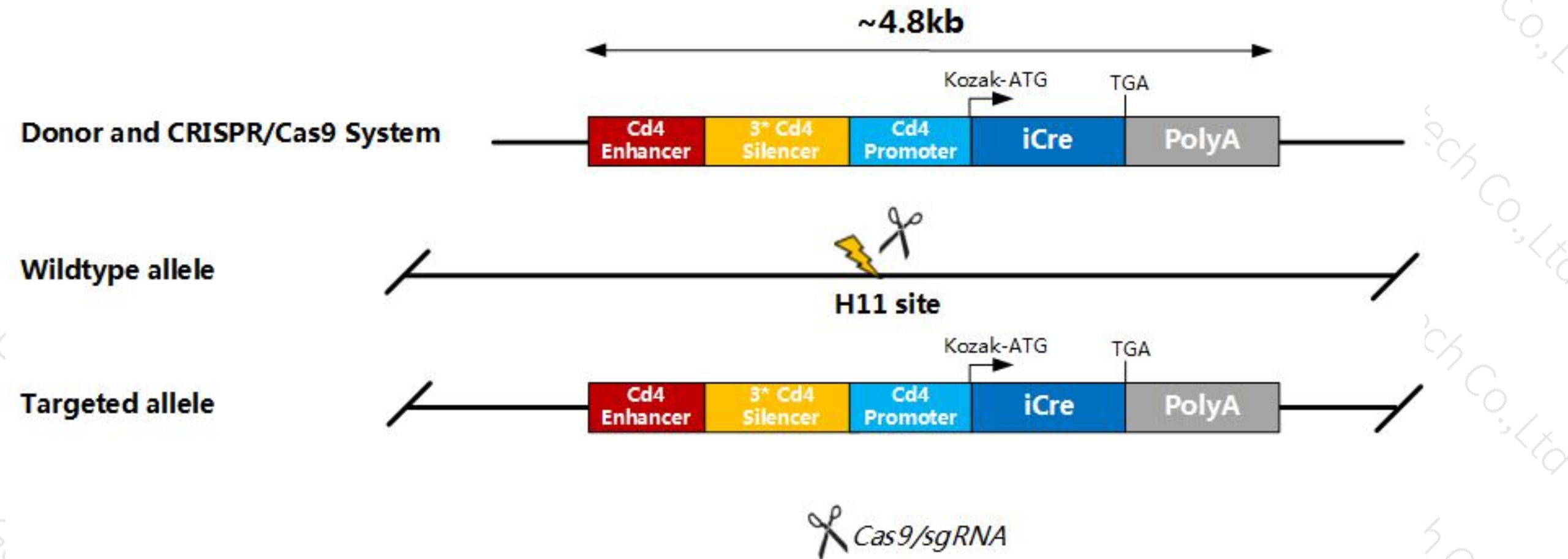
Project Name	<i>H11-Cd4-iCre-PolyA</i>
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Project type	Cas9-KI
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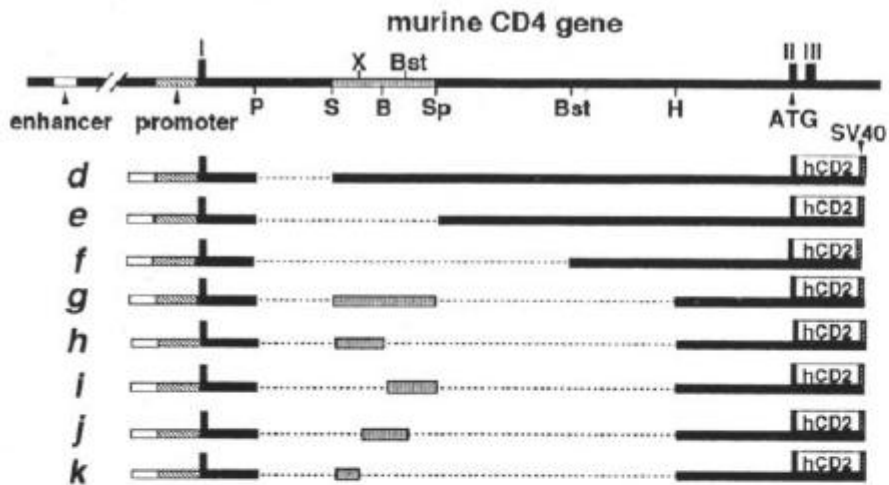
Strain background	C57BL/6J
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Knockin strategy

The *Cd4-iCre-PolyA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



Promoter Sequence of Mouse *Cd4*^[1]



The 339 bp minimal CD4 enhancer fragment (BstXI-AvaI), the XbaI-XhoI fragment(487 bp) of the CD4 promoter region, which contains the transcription initiation site and 70 bp of the untranslated first exon; the SacI-BglII CD4 silencer fragment (774 bp).

Construct	Founder (no. of copies)	%hCD2/CD13P	%hCD2/CD85P
d	7574 (1)	30	0
	7581 (3)	54	1
	7586 (9)	43	1
e	10122 (8)	26	26
	10129 (8)	34	27
	10135 (14)	0	0
f	10162 (16)	84	91
	10176 (12)	4	10
	10178 (9)	13	6
	10179 (15)	95	90
	10181 (7)	44	44
	10187 (7)	17	18
g	10189 (5)	13	12
	6858 (4)	97	1
h	6861 (2)	5	0
	7085 (3)	93	4
	7087 (18)	94	5
	7088 (4)	3	0
k	7095 (7)	60	0

Reference:

A Lineage-Specific Transcriptional Silencer Regulates CD4 Gene Expression during T Lymphocyte Development.

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Cell, Vol. 77, 917-929, June 17, 1994, Copyright © 1994 by Cell Press

A Lineage-

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CCAAGGTGTTGGGGTTCAAATTTGAGCCCCAGCTGTTAGCCCTCTGCAAAGAAAAAAAAAAAAAAAAAAGAACAAAGGGCCTAGATTTCCTTCTGAGCCCCACCCTAAGATGAAGCCTCTTCT
TTCAAGGGAGTGGGGTTGGGGTGGAGGCGGATCCTGTCAGCTTTGCTCTCTCTGTGGCTGGCAGTTTCTCAAAGGGTAACAGGTGTCAGCTGGCTGAGCCTAGGCTGAACCCTGAGACATGCT
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GAGCTCCCACCCACTGCCACCCACCCGCTGCGTGTAGGCACCCGAGGCAAAGGAGAGGGTGGCAGAGGTTGCAGCCACTGAACCACAAGGGTCGCTTAGAGGGTCACATCTCTAGGAAGTTTAT
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TTGAGGCTTCTGTGGTTGGTTTGTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTTCCAGAAAACATGTCCCCTCTCCCTCAACTCTGCAGCCAATTCCCTCTCACATCCACCCTTCCGGTCC
AGCCTATACTCCACAGCTTCCTGTCAGATCAAAGCTCCTGGAAGACCTACCAGAAGCCAGGCTACTTGTTTACAGGGTAGAAGGAACCAGAGATCT

Promoter Sequence of Mouse *Cd4* (492bp)^{[1][2][3]}

TCTAGAACAGCCGACTTAGTGGATCTGTTGGGCAACGTAATCTAAATTTTCCATTGATTAAATTGCTAACTTTTTTTTTTTTAAAACATGGCA
TTACTGCATAGGCAGCCTAGTCTGGCCTTGAGCTTGTGATTTTTCTGCCTAAATCACCCAGTGCTGGGGTTACAGTTACATGCCCCGTGCCC
GTGCCCCGTGCCTGTAAGCCTTGCTCACGTTGACCTAACCAGGCTGTTTCAGCTTTTTACGGCACCAACAATAGTGACATTCCAGACTCC
AGCTTGATTCTGCTCAGGCATTTTCAGTGACTTTGGCATTTTTAATTTTTCAACTTCCCCAACAACCTGGGGGTGGGAGGGAGGGACTCCTG
AGGGCTGGCTTACGTCCGCCGTGCAGAGGAGCCTCACGACCAGGCTTCCTGTCTTTTCATTTACGAACATCTGTGAAGGCAAAGCAAGACT
CTCTTCTTCACTAGGTACCTGTTTGCAAAGTCTCGAG

- The Mouse *Cd4* gene has 5 transcripts. According to the structure of *Cd4* gene, *Cd4-201*(ENSMUST00000024044.6) is selected for presentation of the recommended strategy.
- The Mouse *Cd4* promoter is from reference, the length is about 0.5kb.
- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.
- In this study, the *Cd4-iCre-PolyA* gene fragment was inserted into H11 site of mice by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and sgRNA were constructed in vitro, Cas9, donor and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6J mice by PCR, sequencing, and southern blot, then the stable inheritance of F1 positive mice model was obtained.

- H11 is located on Chr11. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., iCre) strains, if the other gene is also on Chr11, it may be extremely hard to get double gene positive homozygotes.
- 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)

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

Gene ID: 12504, updated on 12-Aug-2019

Summary

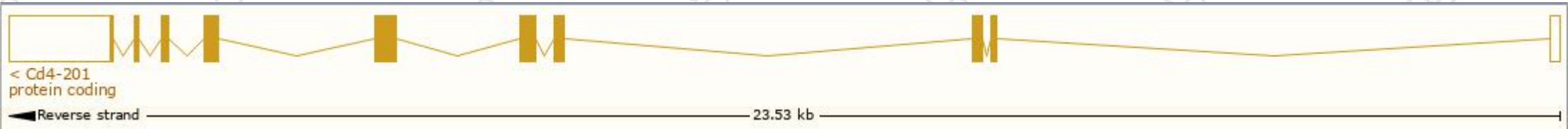
Official Symbol	Cd4 provided by MGI
Official Full Name	CD4 antigen provided by MGI
Primary source	MGI:MGI:88335
See related	Ensembl:ENSMUSG00000023274
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	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)

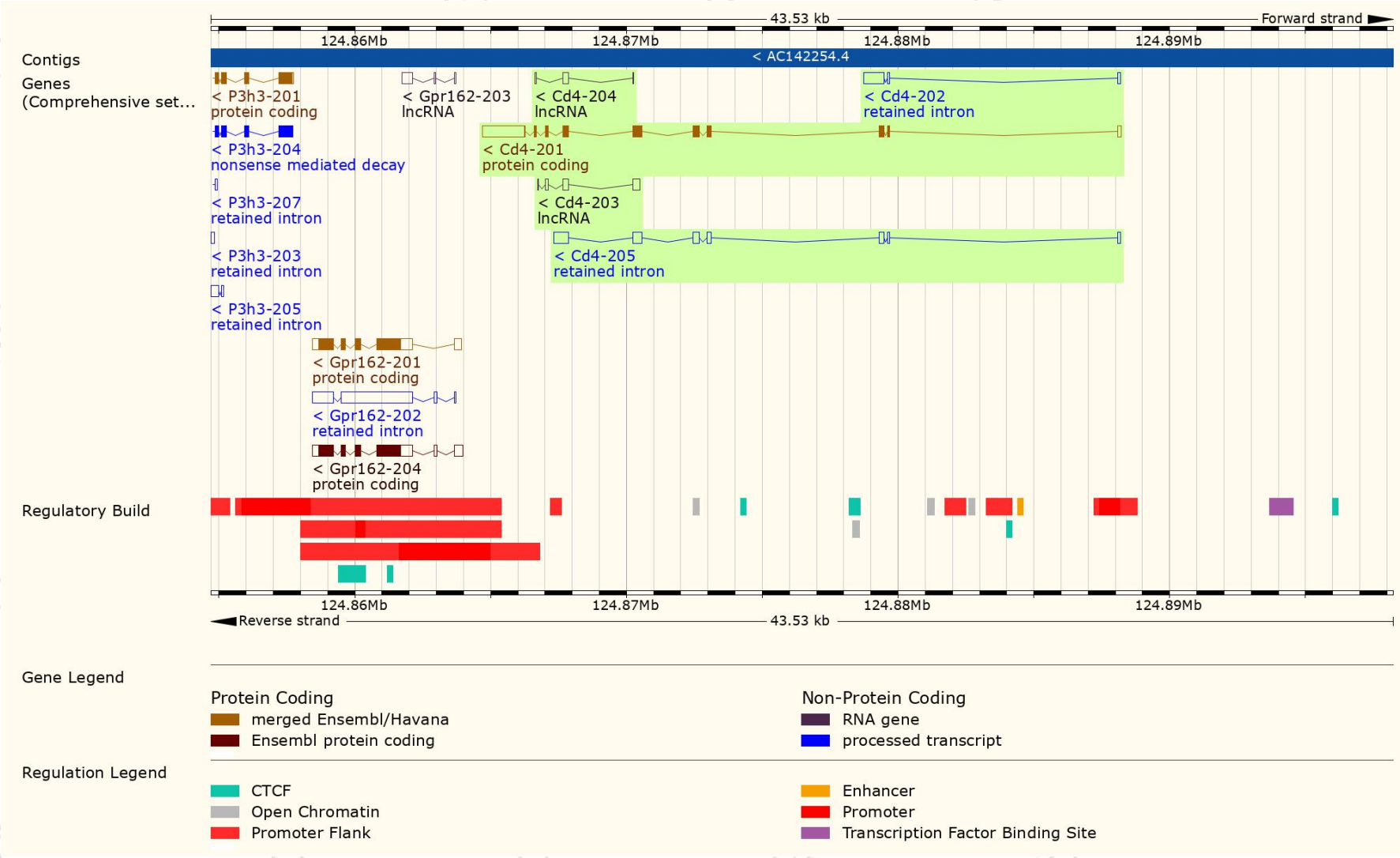
The gene has 5 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cd4-201	ENSMUST00000024044.6	3108	457aa	Protein coding	CCDS20535	P06332 Q3TSV7	TSL:1 Gencode basic APPRIS P1
Cd4-202	ENSMUST00000130378.1	946	No protein	Retained intron	-	-	TSL:1
Cd4-203	ENSMUST00000145818.1	591	No protein	lncRNA	-	-	TSL:5
Cd4-204	ENSMUST00000145977.7	273	No protein	lncRNA	-	-	TSL:1
Cd4-205	ENSMUST00000151594.7	1647	No protein	Retained intron	-	-	TSL:1

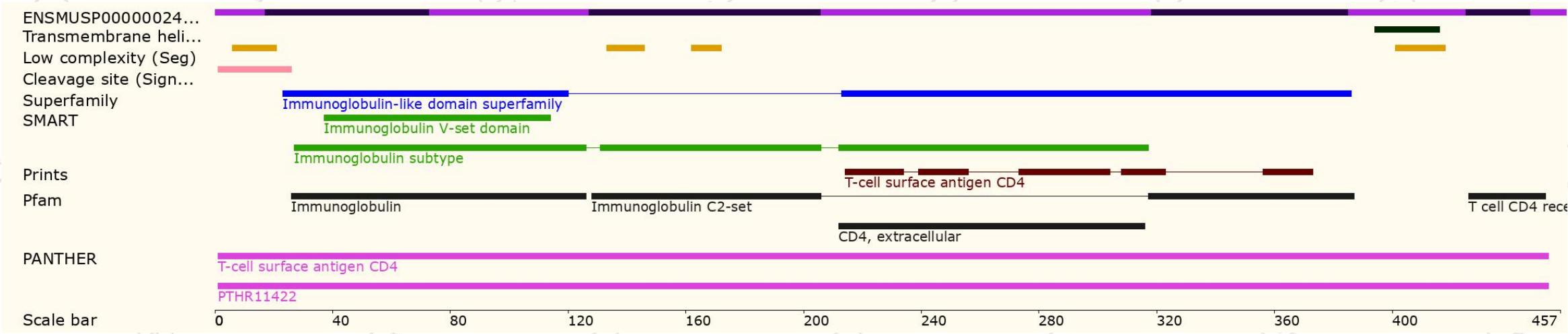
The strategy is based on the design of *Cd4-201* transcript, The transcription is shown below



Genomic location distribution



Protein domain



1. Lee PP; Fitzpatrick DR, et al. A critical role for dnmt1 and DNA methylation in T cell development, function, and survival. *Immunity*. 2001, 15 (5) 763-74
2. Sawada et al. Identification and Characterization of a T-Cell-Specific Enhancer Adjacent to the Murine CD4 Gene. *MOLECULAR AND CELLULAR BIOLOGY*, Nov. 1991, p. 5506-5515
3. Sawada et al. A Lineage-Specific Transcriptional Silencer Regulates CD4 Gene Expression during T Lymphocyte Development. *Cell*, Vol. 77, 917-929, June 17, 1994

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