

***H11-Itgax-iCre-ployA* Cas9-KI Strategy**

Designer:

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Design Date:

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Reviewer

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Project Overview

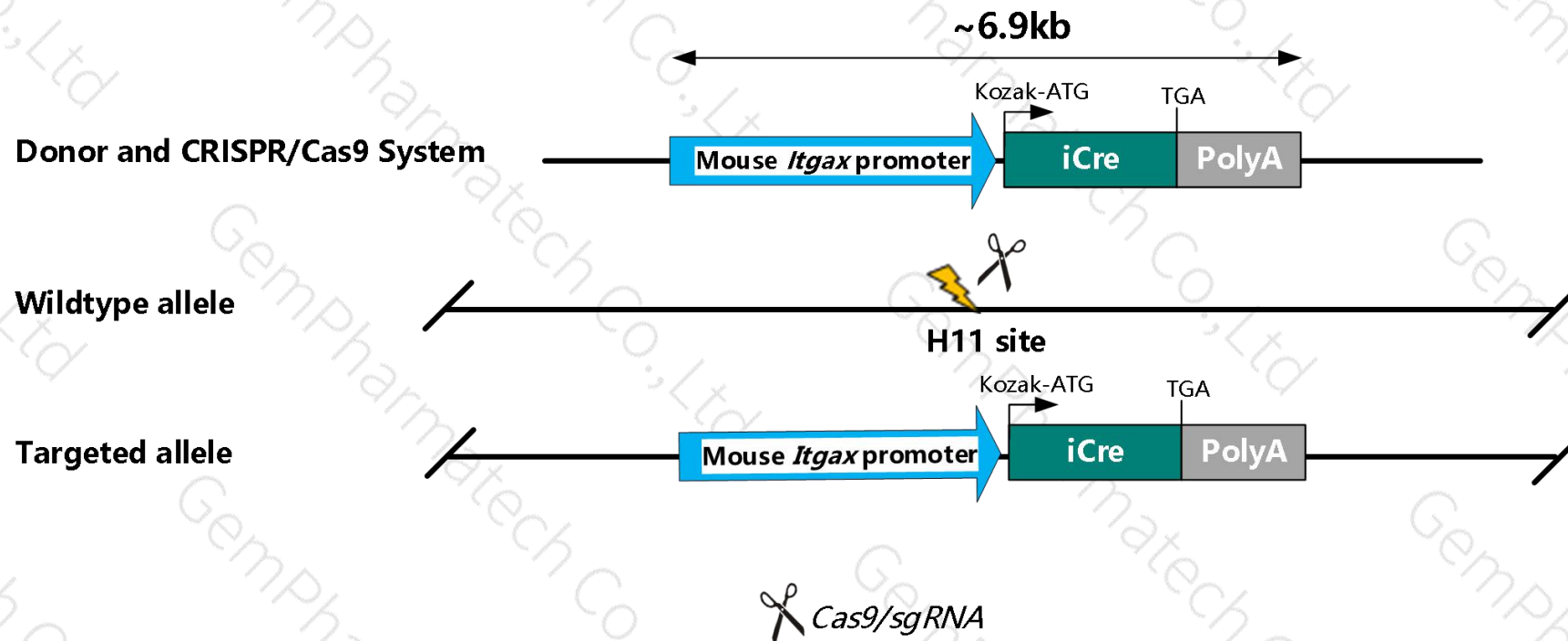
Project Name	<i>H11-Itgax-iCre-ployA</i>
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Project type	Cas9-KI
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Strain background	C57BL/6JGpt
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Knockin strategy

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



Summary of mouse *Itgax* promoter^[1,2]

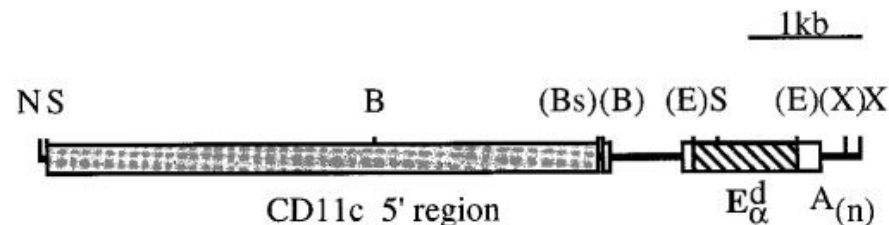


Figure 1. Restriction map of the transgenic construct CD11c-E α^d . MHC class II I-E α^d cDNA (striped box) was placed under the control of a mouse CD11c promoter-containing DNA segment (gray box). The rabbit β -globin gene fragment providing the cDNA with an intron and a polyadenylation signal ($A_{(n)}$) is displayed as a white box. B, BamHI; Bs, BspHI; E, EcoRI; N, NotI; S, SstI; X, XhoI; restriction sites in parentheses have been destroyed by blunt-end cloning.

Hopital Necker, Paris, France) and were bred to OT-1 transgenic mice. To produce mice with Cre expressed under control of the CD11c promoter, the Cre recombinase gene from pBS185 plasmid (Gibco BRL) was introduced into the pIRES2-EGFP plasmid (BD Biosciences Clontech, Palo Alto, CA), excised as a Cre-IRES-GFP fragment and subcloned into a plasmid containing the 5.3 Kb genomic CD11c promoter/enhancer fragment (Brocker et al., 1997), (a generous gift from Klaus Karjalainen, Institute for Research in Biomedicine, Bellinzona, Switzerland). All mice were genotyped by PCR with primers amplifying the Cre transgene (5'-tgatgaggttcgaagaacc-3' and 5'-ccatgagtgaacgaacctgg-3'); and Fas (5'-gctgtgtctatcagtct-3' and 5'-agagaccacaccttaggtag-3') generating 320bp wild-type and 400bp "floxed" allele

1. Stranges PB; Watson J; Cooper CJ; Choisy-Rossi CM; Stonebraker AC; Beighton RA; Hartig H; Sundberg JP; Servick S; Kaufmann G; Fink PJ; Chervonsky AV. 2007. Elimination of antigen-presenting cells and autoreactive T cells by Fas contributes to prevention of autoimmunity. *Immunity* 26(5):629-41.
2. Brocker T, Riedinger M, Karjalainen K. Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo. *J Exp Med*. 1997;185:541-550.

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- The *Itgax* gene has 4 transcripts. According to the structure of *Itgax* gene, *Itgax-201*(ENSMUST00000033053.7) is selected for presentation of the recommended strategy.
- *Itgax-201* gene has 30 exons, with the ATG start codon in exon1 and TGA stop codon in exon30.
- The *Itgax* promoter is from article, the length is about 5.3kb.
- 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 *Itgax-iCre-ployA* 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/6JGpt mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6JGpt 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)

Itgax integrin alpha X [*Mus musculus* (house mouse)]

Gene ID: 16411, updated on 12-Aug-2019

Summary

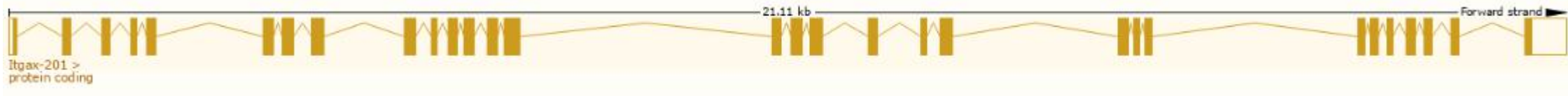
Official Symbol	Itgax provided by MGI
Official Full Name	integrin alpha X provided by MGI
Primary source	MGI:MGI:96609
See related	Ensembl:ENSMUSG00000030789
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cr4; N418; Cd11c; AI449405
Expression	Biased expression in spleen adult (RPKM 12.4), lung adult (RPKM 9.2) and 10 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

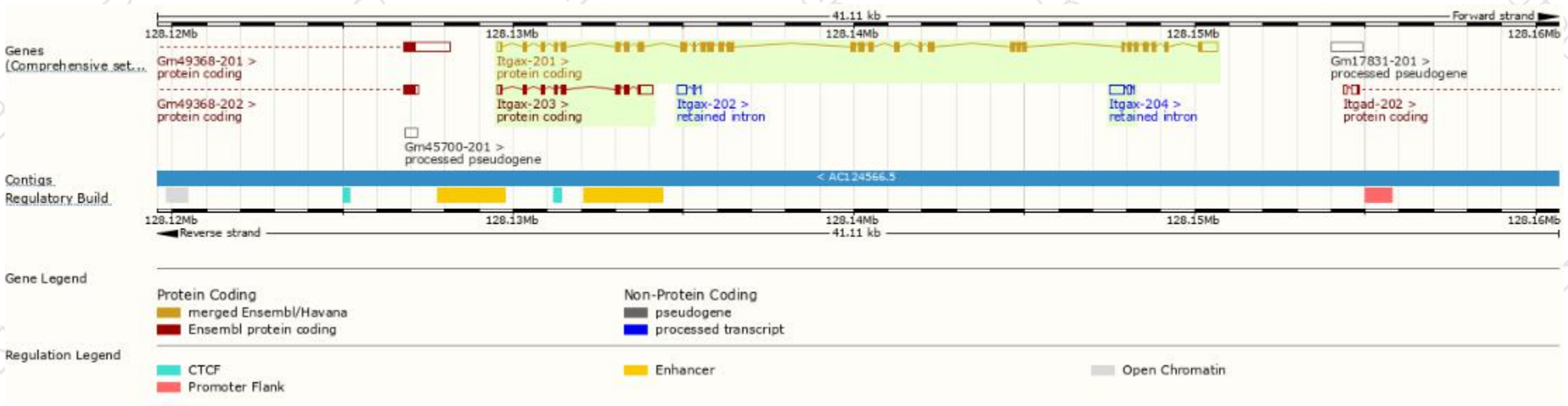
The gene has 4 transcripts, and the transcript is shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Itgax-201	ENSMUST00000033053.7	4046	1169aa	Protein coding	CCDS40150	Q9QXH4	TSL:1 GENCODE basic APPRIS P1
Itgax-203	ENSMUST00000205460.1	1215	257aa	Protein coding	-	A0A0U1RNJ3	TSL:1 GENCODE basic
Itgax-204	ENSMUST00000206396.1	544	No protein	Retained intron	-	-	TSL:3
Itgax-202	ENSMUST00000205408.1	352	No protein	Retained intron	-	-	TSL:3

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



Genomic location distribution



Protein domain



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