

***Foxp3-iCre-PloyA* BAC-TG Strategy**

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Reviewer

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Design Date:

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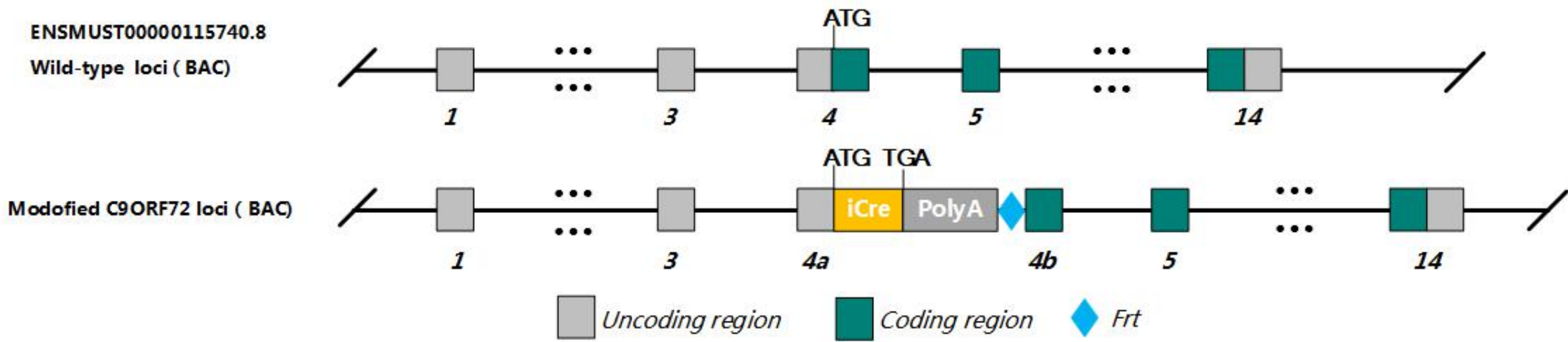
Project Overview

Project Name	<i>Foxp3-iCre-PloyA</i>
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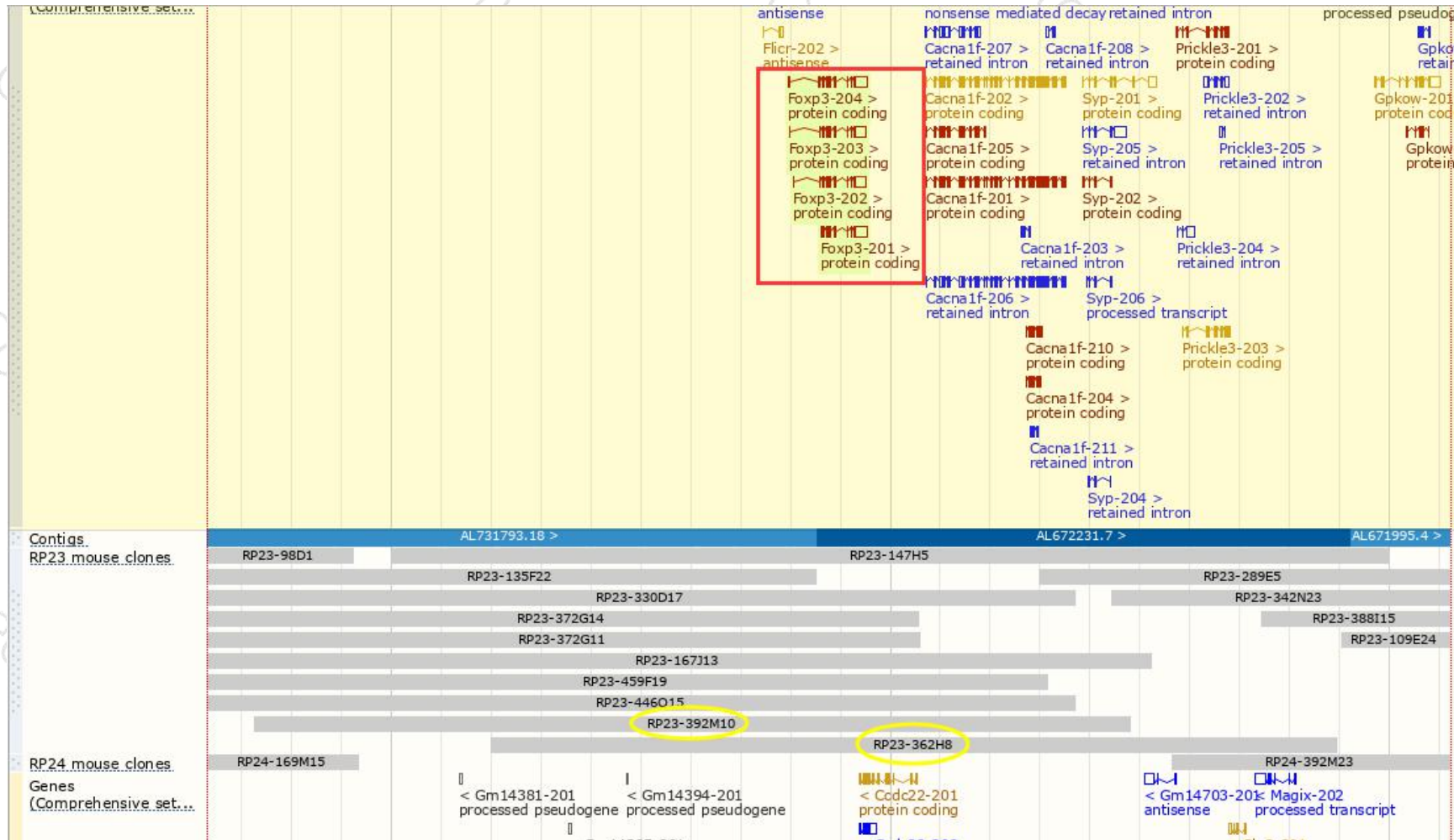
Project type	BAC-TG
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Strain background	C57BL/6J
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This mice model is made by transgenic technology, and the schematic diagram is as follows:

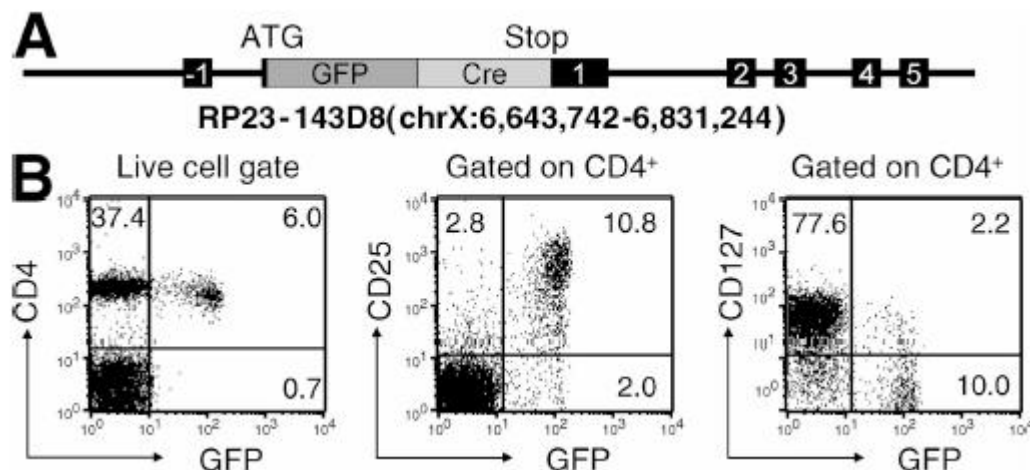


Mouse *Foxp3* BAC Clone



RP23-362H8 (~169kb) or *RP23-392M10* (~175kb) of C57BL/6J mouse bacterial artificial chromosome (BAC) preferred for making mice model.

Knock in Site by Reference



MATERIALS AND METHODS

Generation of T reg cell-specific Dicer conditional KO mice. The GFP-hCre cDNA fragment was inserted immediately downstream of the FoxP3 ATG translational start site by homologous recombination into a 188-kb mouse BAC (from the C57BL/6 genome; clone RP23-143D8) carrying the intact FoxP3 gene, as previously described (29). We used a codon-optimized “humanized” Cre (hCre) to improve translational efficiency in eukaryotic cells. An EGFP-hcre-Frt-Neo-Frt cassette was provided by N. Killeen (UCSF, San Francisco, CA). The modified BAC was purified using cesium chloride gradient ultracentrifugation and microinjected by the UCSF Transgenic/Targeted Mutagenesis Core Facility into the pronuclei of nonobese diabetic mouse embryos to generate FoxP3-GFP-hCre BAC Tg mice. Mice carrying

RESULTS AND DISCUSSION

Generation of bacterial artificial chromosome (BAC)

Tg mice that enable GFP and Cre recombinase expression in FoxP3⁺ cells

We developed a Foxp3 promoter-driven BAC Tg mouse strain that carries a fusion protein containing GFP fused to the N-terminus of a codon-optimized hCre (see Materials and methods; Fig. 1 A). Phenotypic analyses of the Tg mice showed that GFP⁺ cells were uniformly CD4⁺, with the majority CD25^{bright} and CD127^{low} (Fig. 1 B), as previously described for the T reg cell subset (15). The GFP⁺ T cells were

Reference:

Zhou X; Jeker LT; Fife BT; Zhu S; Anderson MS; McManus MT; Bluestone JA. Selective miRNA disruption in T reg cells leads to uncontrolled autoimmunity. J Exp Med, 2008;205 (9) 1983-91.

Technical routes

- Transcript *Foxp3-204*(ENSMUST00000115740.8) is selected for presentation of the recommended strategy, which has 14 exons, with the ATG start codon at exon4 and TGA stop codon at exon14.
- *RP23-362H8* (~169kb) or *RP23-392M10* (~175kb) of C57BL/6J mouse bacterial artificial chromosome (BAC) containing the entire *Foxp3* locus (and other genes), was modified by targeting a *iCre-PolyA* sequence to the exon4 near the translation start codon of the *Foxp3* locus, ensuring *iCre* is expressed from the endogenous promoter/enhancer elements of *Foxp3*.
- The pups will be genotyped by PCR analysis.

- In this strategy, while expressing iCre, other genes co-existing on BAC will also be expressed.
- BAC plasmid is large, and BAC skeleton may affect the expression of target gene.
- 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)

Foxp3 forkhead box P3 [*Mus musculus* (house mouse)]

Gene ID: 20371, updated on 6-Aug-2019

Summary

- Official Symbol
- Foxp3 provided by MGI
- Official Full Name
- forkhead box P3 provided by MGI
- Primary source
- [MGI:MGI:1891436](#)
- See related
- [Ensembl:ENSMUSG00000039521](#)
- Gene type
- protein coding
- RefSeq status
- REVIEWED
- Organism
- [Mus musculus](#)
- Lineage
- Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
- Also known as
- sf; JM2; scurfin
- Summary
- The protein encoded by this gene is a member of the forkhead/winged-helix family of transcriptional regulators. Defects in this gene result in the scurfy phenotype (sf). Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2015]
- Expression
- Ubiquitous expression in spleen adult (RPKM 3.0), thymus adult (RPKM 2.4) and 28 other tissues [See more](#)
- Orthologs
- [human](#) [all](#)

Genomic context

Location: X A1.1; X 3.41 cM

Exon count: 15

See Foxp3 in [Genome Data Viewer](#)

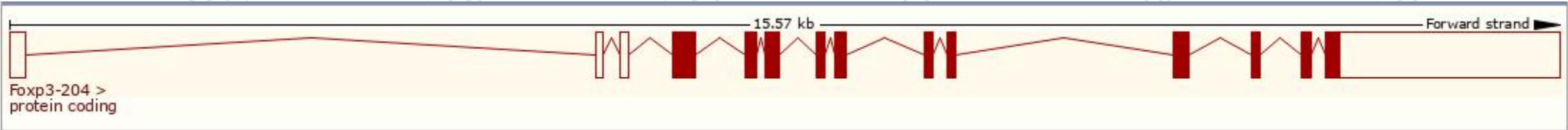
Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	X	NC_000086.7 (7579676..7595243)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	X	NC_000086.6 (7156819..7172360)

Transcript information (Ensembl)

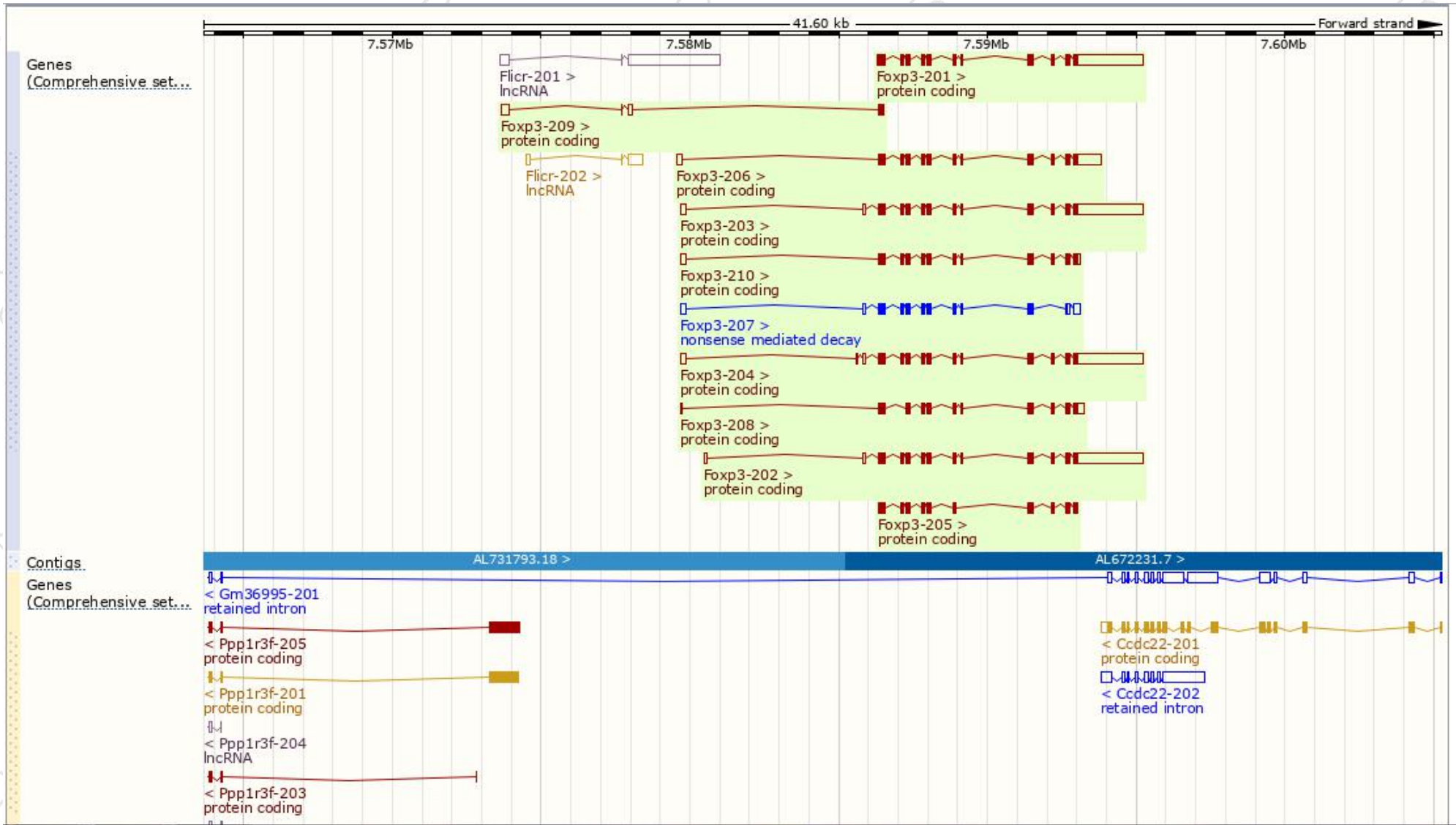
The gene has 10 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Foxp3-201	ENSMUST00000045566.6	3572	429aa	Protein coding	CCDS29965	Q53Z59 Q99JB6	TSL:5 GENCODE basic APPRIS P1
Foxp3-202	ENSMUST00000115738.7	3681	429aa	Protein coding	CCDS29965	Q53Z59 Q99JB6	TSL:1 GENCODE basic APPRIS P1
Foxp3-203	ENSMUST00000115739.8	3763	429aa	Protein coding	CCDS29965	Q53Z59 Q99JB6	TSL:1 GENCODE basic APPRIS P1
Foxp3-204	ENSMUST00000115740.8	3834	429aa	Protein coding	CCDS29965	Q53Z59 Q99JB6	TSL:1 GENCODE basic APPRIS P1
Foxp3-205	ENSMUST00000234015.1	1214	402aa	Protein coding	-	-	GENCODE basic
Foxp3-206	ENSMUST00000234363.1	2226	429aa	Protein coding	CCDS29965	Q53Z59	GENCODE basic APPRIS P1
Foxp3-207	ENSMUST00000234479.1	1557	335aa	Nonsense mediated decay	-	-	-
Foxp3-208	ENSMUST00000234574.1	1477	394aa	Protein coding	-	-	GENCODE basic
Foxp3-209	ENSMUST00000234896.1	662	54aa	Protein coding	-	-	CDS 3' incomplete
Foxp3-210	ENSMUST00000235116.1	1551	429aa	Protein coding	CCDS29965	Q53Z59	GENCODE basic APPRIS P1

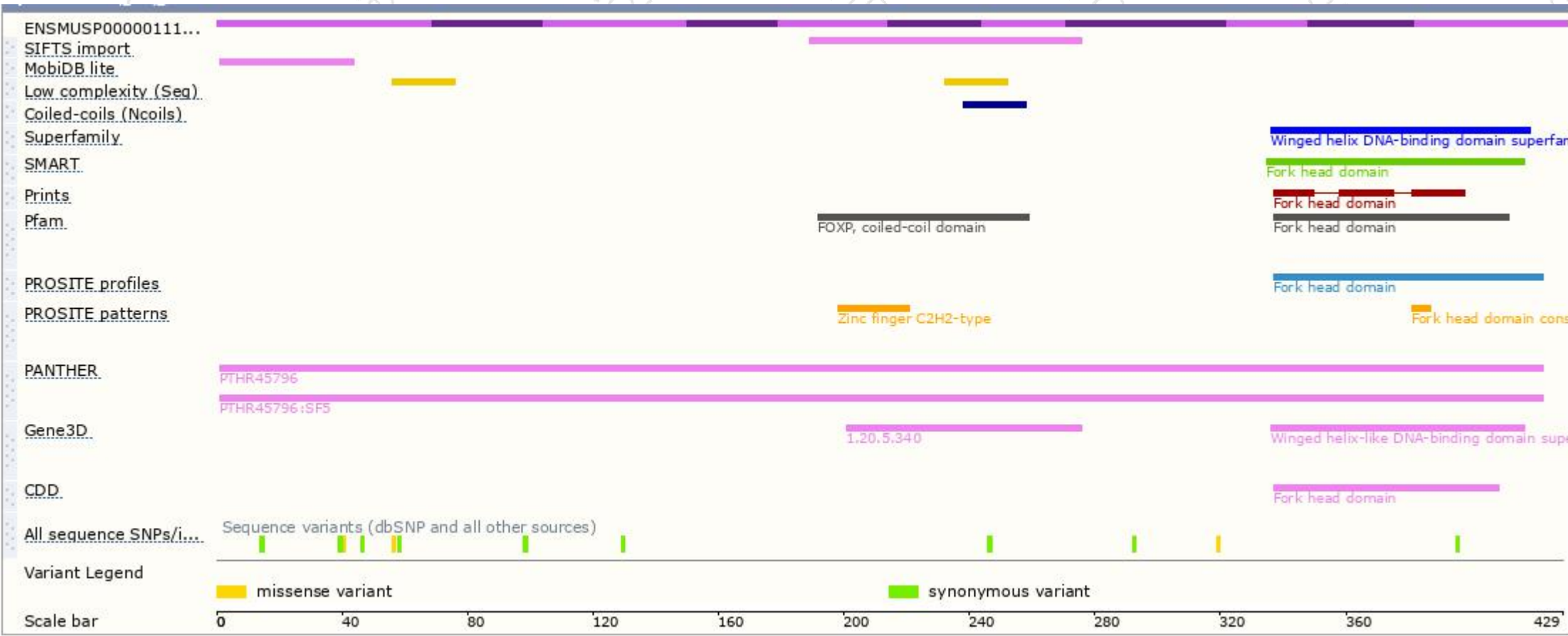
The strategy is based on the design of *Foxp3-204* transcript, The transcription is shown below



Genomic location distribution



Protein domain



如您有任何疑问，欢迎垂询。

Tel: 025-5864 1534



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