

***H11-hS100A8(hMRP8)-iCre-ployA* Cas9-KI Strategy**

Designer:

Jia Yu

Design Date:

2019-8-15

Reviewer

Yanhua Shen

Project Overview

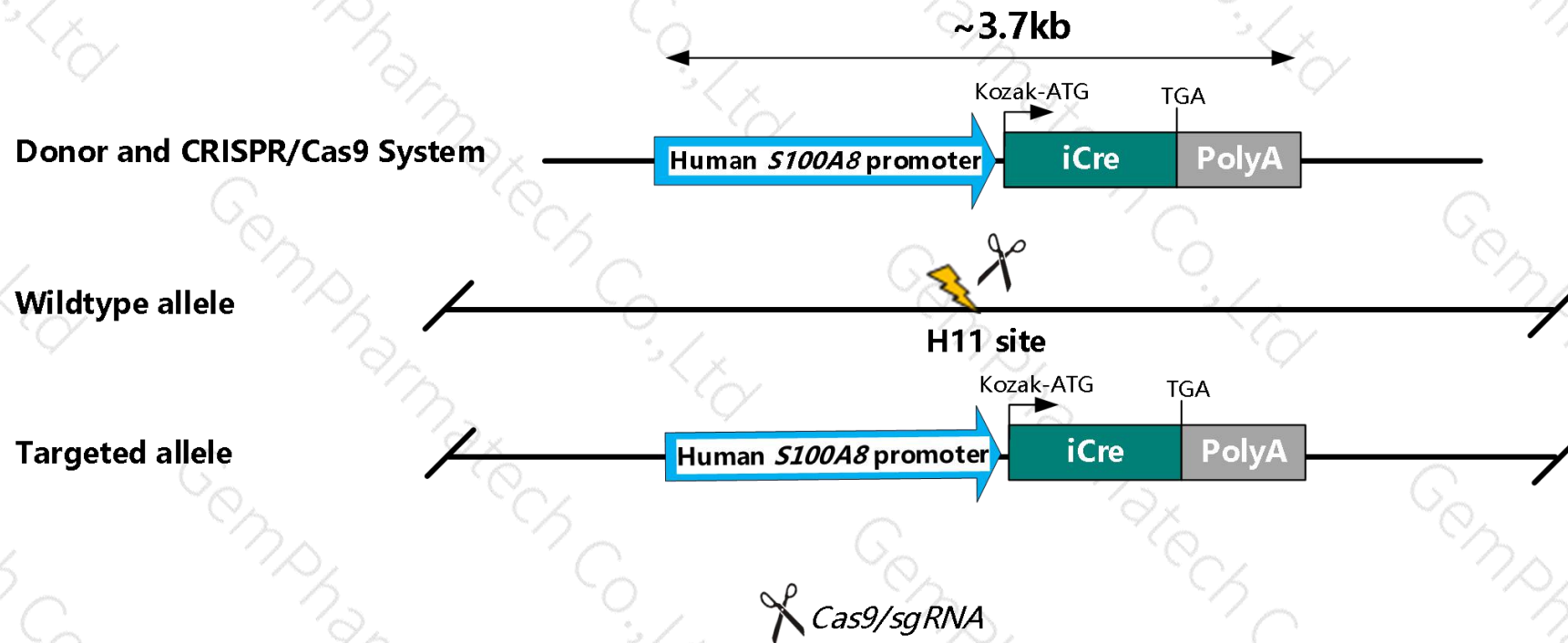
Project Name *H11-hS100A8(hMRP8)-iCre-ployA*

Project type **Cas9-KI**

Strain background **C57BL/6J**

Knockin strategy

The *hS100A8-iCre-loyA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



Summary of Human *S100A8* promoter [1,2]

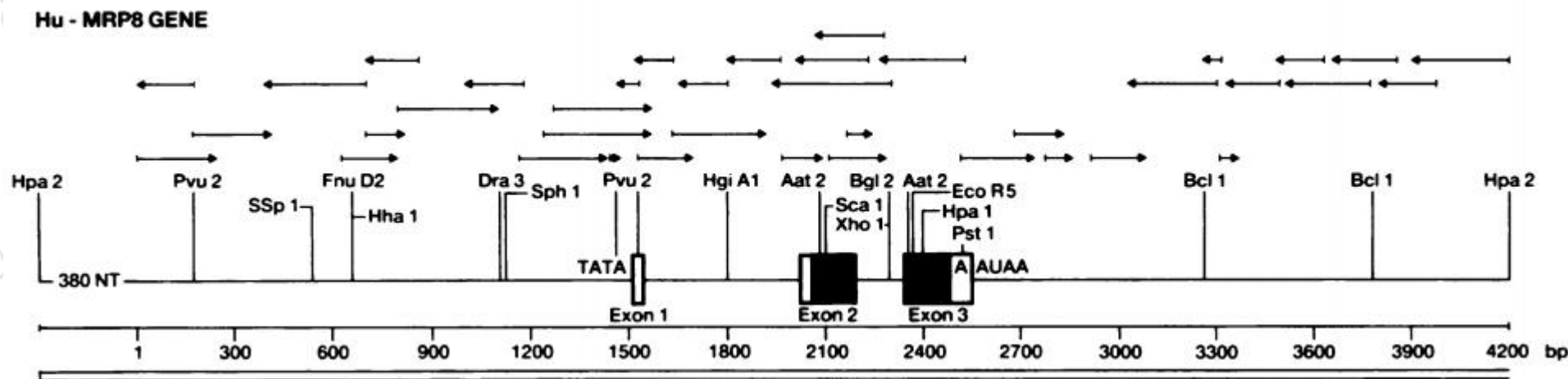


FIG. 3. Genomic organization and DNA-sequencing strategy of the human MRP8 and MRP14 genes. The black areas stand for the coding regions, and the boxed-in regions show the untranslated parts of the genes. The arrows point to the sequencing direction and end when the sequence is not clearly readable.

with MRP8 and MRP14 cDNA probes. Several overlapping clones, as determined by restriction analysis (data not shown), were isolated. For sequence analysis, a 5-kb *HpaII* DNA fragment containing the MRP8 gene and a 4.2-kb *PstI* DNA fragment encompassing the MRP14 gene were subcloned (Fig. 3). The entire sequences of the MRP8 and MRP14 genes were determined (Fig. 3). The entire sequences of the MRP8 and MRP14 genes were determined (Fig. 3).

hMRP8-AML1-ETO transgene includes 1.5 kb of human *MRP8* gene upstream regulatory element, a 0.5 kb of human *MRP8* gene sequence (including exon 1, intron 1, and part of exon 2) upstream of the AML1-ETO cDNA, and 0.6 kb of exon 3 and the downstream flanking sequence of the human *MRP8* gene downstream of the AML1-ETO cDNA. The transgene was released from the AML1-ETO cDNA. The transgene was released from the AML1-ETO cDNA.

Eric Lagasse, Roger G. Clerc.(1988). "Cloning and Expression of Two Human Genes Encoding Calcium-Binding Proteins That Are Regulated during Myeloid Differentiation." Mol. Cell. Biol. 8(6):2402-2410.

Youzhong Yuan, Liming Zhou,et al.(2001). "AML1-ETO expression is directly involved in the development of acute myeloid leukemia in the presence of additional mutations." PNAS.98(18):10398-10403.

The promoter Sequence of Human *S100A8*(2041bp)

ttccaccttttggtctttaaataatgctgctatgaacatgaatgtacaaacatctgttgaatccctgcattcaattctttgcatatataaccaggagcagaatgatggatcatatggtaatt
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gaatcctctgcgtactgcctggccagatgcaaattccctgccatgggattccccagaaggttctgttttccaggtgggggcaagtccgtgggcac

- The human *S100A8* gene has 3 transcript. According to the structure of *S100A8* gene, *S100A8-202*(ENST00000368733.4) is selected for presentation of the recommended strategy.
- *S100A8-202* gene has 3 exons, with the ATG start codon in exon2 and TAG stop codon in exon3.
- The *hS100A8* promoter is from article, the length is about 2.1kb.
- 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 *hS100A8-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/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)

S100A8 S100 calcium binding protein A8 [*Homo sapiens* (human)]

Gene ID: 6279, updated on 7-Jul-2019

Summary

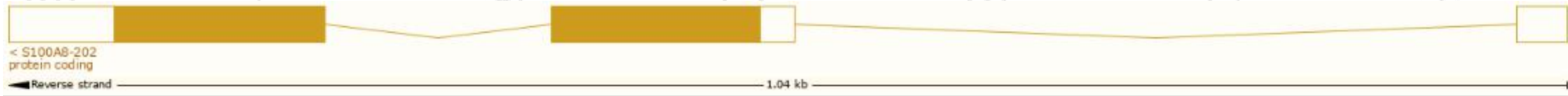
Official Symbol	S100A8 provided by HGNC
Official Full Name	S100 calcium binding protein A8 provided by HGNC
Primary source	HGNC:HGNC:10498
See related	Ensembl:ENSG00000143546 MIM:123885
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Homo sapiens
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as	P8; MIF; NIF; CAGA; CFAG; CGLA; L1Ag; MRP8; CP-10; MA387; 60B8AG
Summary	The protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. S100 genes include at least 13 members which are located as a cluster on chromosome 1q21. This protein may function in the inhibition of casein kinase and as a cytokine. Altered expression of this protein is associated with the disease cystic fibrosis. Multiple transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jan 2016]
Expression	Biased expression in bone marrow (RPKM 3778.8), esophagus (RPKM 2115.4) and 1 other tissue See more
Orthologs	mouse all

Transcript information (Ensembl)

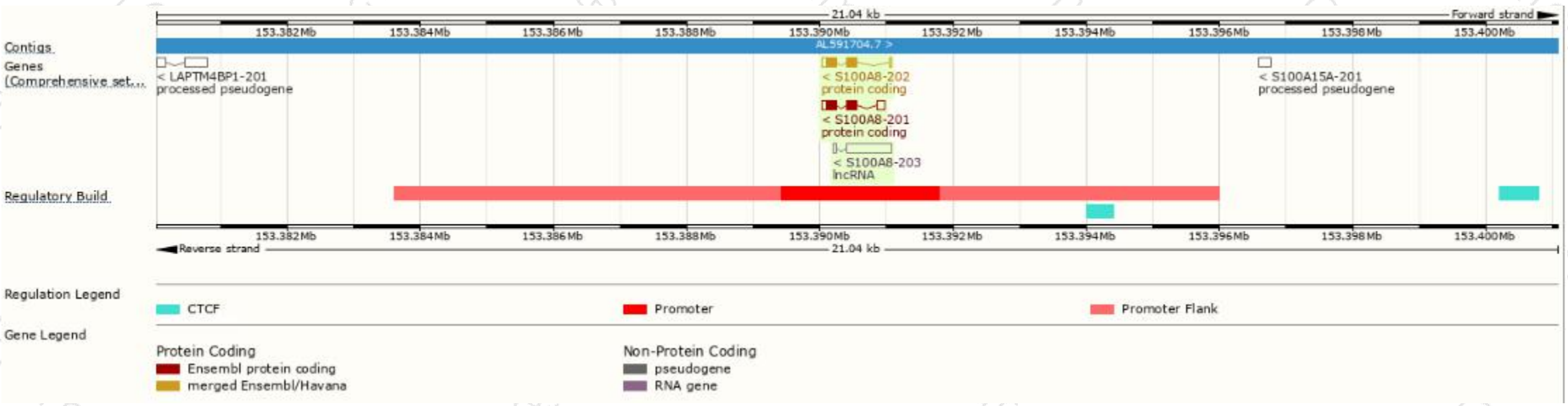
The gene has 3 transcripts, and the transcript is shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq Match	Flags
S100A8-201	ENST00000368732.5	495	93aa	Protein coding	CCDS1038	P05109	-	TSL:3 Gencode basic APPRIS P1
S100A8-202	ENST00000368733.4	408	93aa	Protein coding	CCDS1038	P05109	NM_002964.5	TSL:1 Gencode basic APPRIS P1 MANE Select v0.5
S100A8-203	ENST00000477801.1	717	No protein	lncRNA	-	-	-	TSL:1

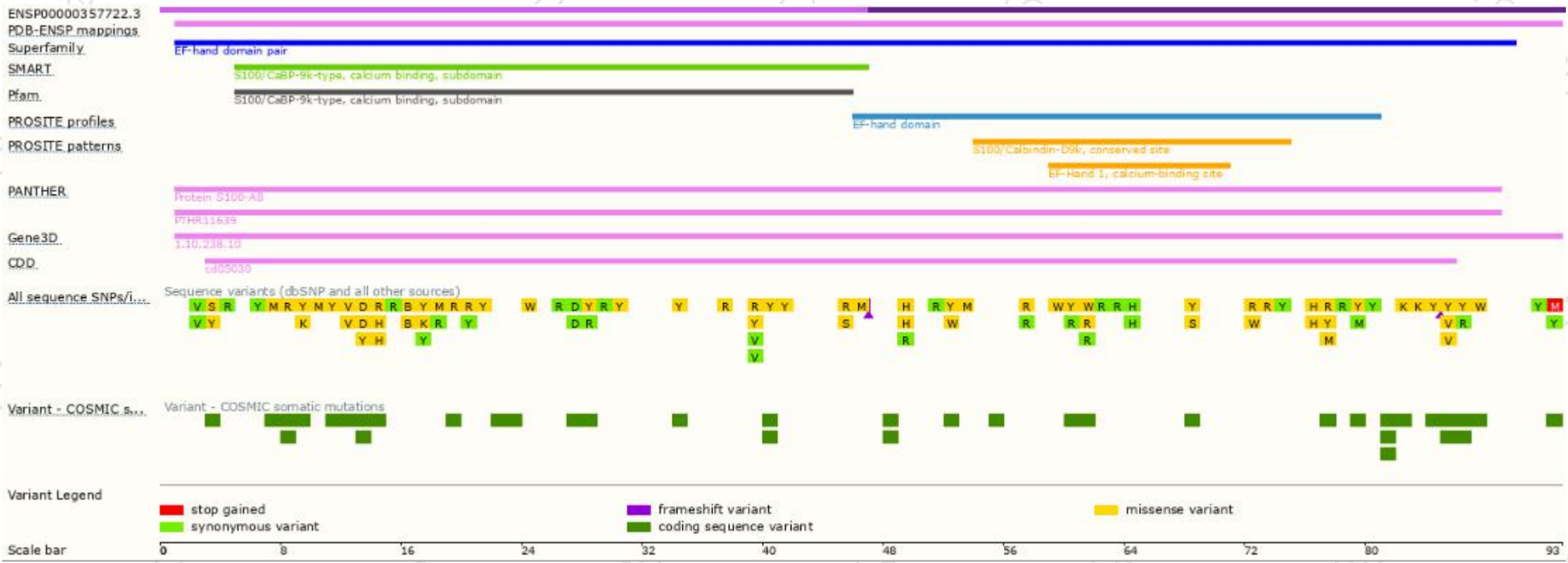
The strategy is based on the design of *S100A8-202* transcript, The transcription is shown below



Genomic location distribution



Protein domain



If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534



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