Trex1-D18N Cas9-KI(PM) Mouse Model Strategy

CRISPR-Cas9 Technology

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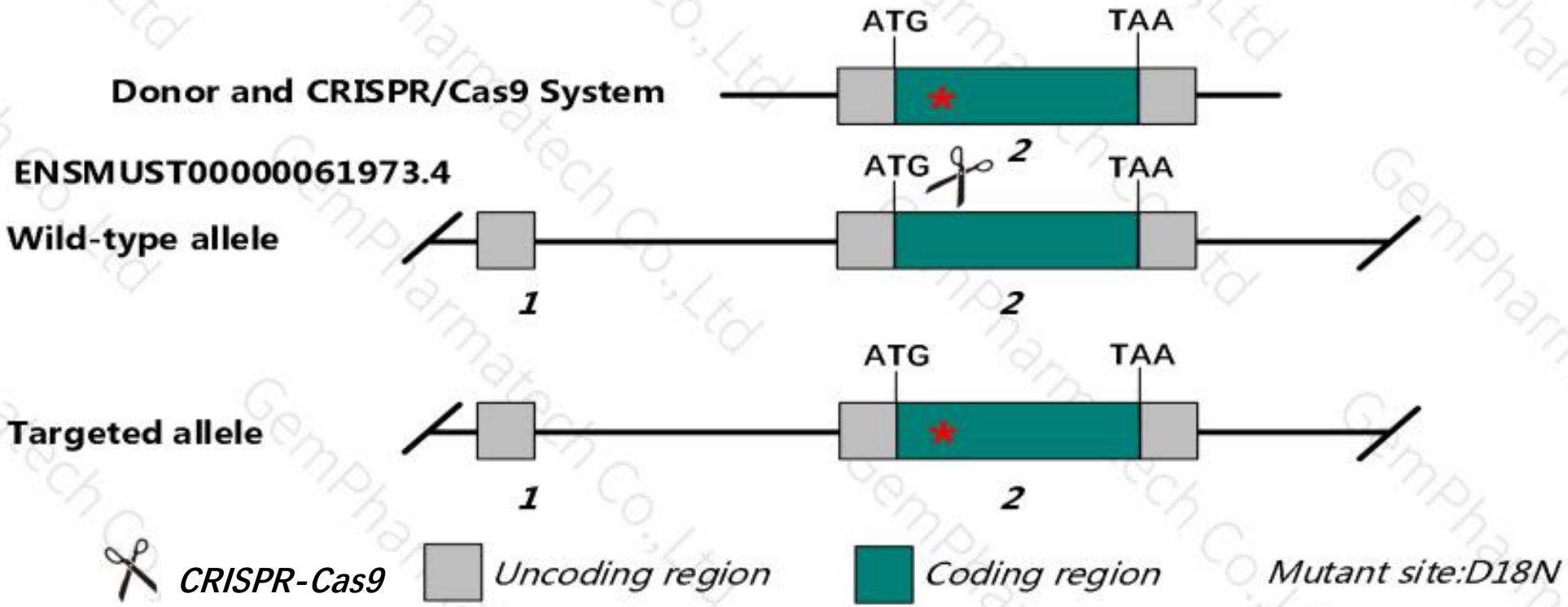
Project Overview

Project Name	*Trex1-D18N*
Project Type	Cas9-KI(PM)
Background	C57BL/6JGpt
Project Timeline	5-8 months

Strategy

This model uses CRISPR-Cas9 technology to edit the *Trex1* gene and the schematic diagram is as follows:



Technical Description

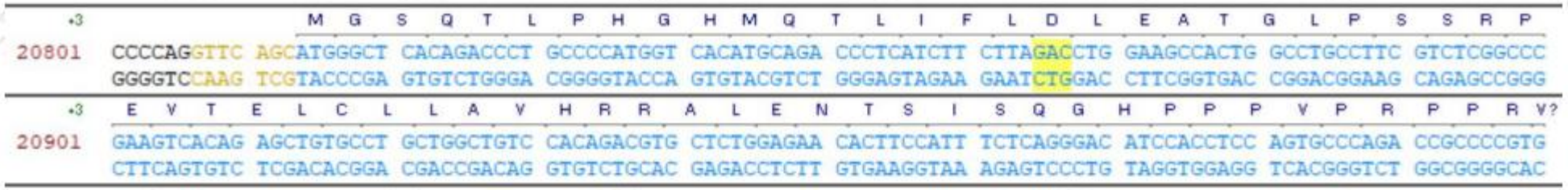
- Based on the data from Ensembl, mouse *Trex1* has 2 transcripts. The *Trex1*-201 (ENSMUST00000061973.4) transcript will be targeted in this strategy, which will carry the D18N mutation in exon 2.
- *Trex1*-201 (ENSMUST00000061973.4) transcript has 2 exons, with both the translation start codon ATG and the stop codon TAA located in exon 2, encoding a polypeptide of 314 amino acids.
- In this project, *Trex1* gene will be modified by CRISPR-Cas9 technology. The brief process is as follows: the CRISPR-Cas9 and donor vector will be microinjected into the fertilized eggs of C57BL/6JGpt mice, to obtain positive F0 mice. The F0 positive mice will be bred with C57BL/6JGpt mice to generate F1 pups, which will be genotyped by PCR, followed by on-target amplicon sequencing analysis.

Note

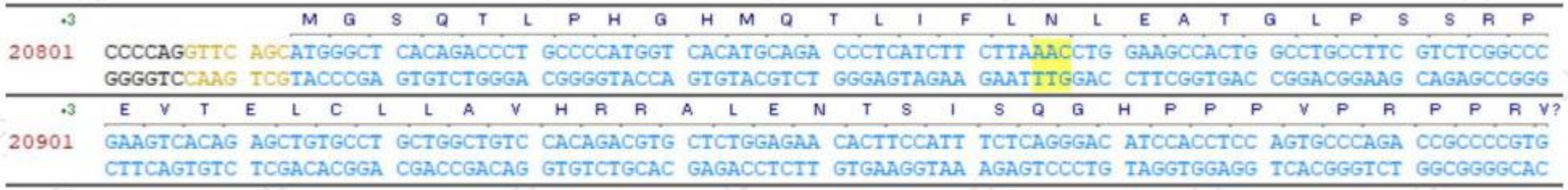
- During the process of CRISPR-Cas9 mediated gene editing, 1-2 synonymous mutations may be introduced into exon 2.
- Acroding to the data from MGI, homozygous mutant mice display premature death, cardiomyopathy, myocarditis, atrial thrombosis, and altered spleen morphology. Homozygotes for the D18N allele develop lupus-like disease with systemic inflammation, lymphoid hyperplasia, vasculitis, production of autoantibodies to dsDNA, and renal disease.
- This strategy may cause mutations in the overlapping regions between the target gene and the transcripts *Atrip*-202, 204 and 205, the effect of which is unknown.
- The point mutation site is near the C-terminus of *Shisa5*, which may affect the C-terminal function of *Shisa*5.
- Mouse *Trex1* is located on Chr9. Please take this into consideration when breeding this knockin mouse with other genetically engineered strains, if the other gene is on the same chromosome. It may be extremely hard to obtain double homozygous mutant mice.
- This strategy is designed according to the genomic information from existing databases. Due to the complexity of gene expression regulation, the effect of this strategy on gene expression cannot be predicted completely at the present technology level.

Trex1 Point Mutation

Before mutagenesis



After mutagenesis



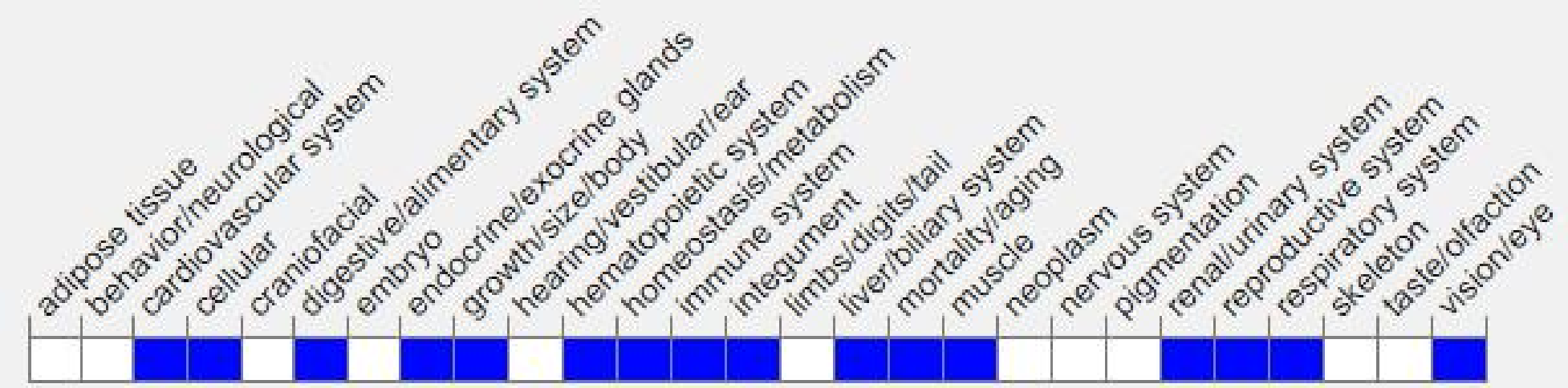
The point mutation site is highlighted in yellow. The codon for the 18th amino acid was mutated from GAC to AAC. Consequently, the amino acid was mutated from D to N.

Mouse Phenotype Information (MGI)

http://www.informatics.jax.org/allele/summary?markerId=MGI:1328317&alleleType=Targeted

Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems	Human Disease Models
Trex1$^{tm1(KOMP)Wtsi}$ three prime repair exonuclease 1; targeted mutation 1, Wellcome Trust Sanger Institute	9		Targeted (Null/knockout, Reporter)		
Trex1$^{tm1.1Beh}$ three prime repair exonuclease 1; targeted mutation 1.1, Rayk Behrendt	9	Trex1^{F1}Dresden	Targeted (Conditional ready)		
Trex1$^{tm1.1Fwpe}$ three prime repair exonuclease 1; targeted mutation 1.1, Fred W Perrino	9	TREX1^{D18N}	Targeted (Dominant negative)	cardiovascular, digestive/alimentary, endocrine/exocrine, hematopoietic, homeostasis, immune, liver/biliary, mortality/aging, renal/urinary, reproductive, respiratory, vision/eye	
Trex1$^{tm1.1Kpk}$ three prime repair exonuclease 1; targeted mutation 1.1, Klaus-Peter Knobeloch	9	Trex1^{F1}Freiburg	Targeted (Conditional ready)		
Trex1$^{tm1.1Nyan}$ three prime repair exonuclease 1; targeted mutation 1.1, Nan Yan	9	TREX1-V235fs	Targeted (Humanized sequence)		
Trex1^{tm1Tld} three prime repair exonuclease 1; targeted mutation 1, Tomas Lindahl	9	Trex1^{-}	Targeted (Null/knockout)	cardiovascular, digestive/alimentary, endocrine/exocrine, hematopoietic, homeostasis, immune, integument, liver/biliary, mortality/aging, muscle, renal/urinary	Aicardi-Goutieres syndrome (IDs) cutaneous lupus erythematosus (IDs)
Trex1$^{tm2.1Fwpe}$ three prime repair exonuclease 1; targeted mutation 2.1, Fred W Perrino	9	Trex1 R114H	Targeted (Not Specified)		

Phenotype Overview (MGI)



http://www.informatics.jax.org/marker/MGI:1328317

Homozygous mutant mice display premature death, cardiomyopathy, myocarditis, atrial thrombosis, and altered spleen morphology. Homozygotes for the D18N allele develop lupus-like disease with systemic inflammation, lymphoid hyperplasia, vasculitis, production of autoantibodies to dsDNA, and renal disease.

Basic Information of *Trex1* Gene

Target Gene	*Trex1*
Gene ID	22040
Link(NCBI)	https://www.ncbi.nlm.nih.gov/gene/22040
Link(Ensembl)	http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000049734;r=9:108887001-108888802
Location	Chr 9

Trex1 Gene (NCBI)

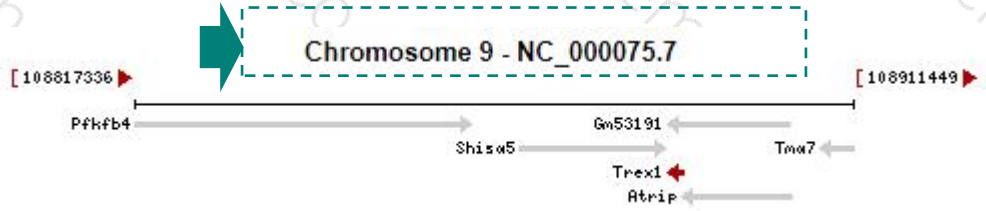
Trex1 three prime repair exonuclease 1 [*Mus musculus* (house mouse)]

Gene ID: 22040, updated on 15-Feb-2022

Download Datasets

Summary

Official Symbol	Trex1 provided by MGI
Official Full Name	three prime repair exonuclease 1 provided by MGI
Primary source	MGI:MGI:1328317
See related	Ensembl:ENSMUSG00000049734 AllianceGenome:MGI:1328317
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	AU041952
Summary	Enables several functions, including DNA binding activity; WW domain binding activity; and magnesium ion binding activity. Involved in DNA metabolic process. Acts upstream of or within several processes, including DNA metabolic process; cellular response to cytokine stimulus; and regulation of generation of precursor metabolites and energy. Located in cytosol; endoplasmic reticulum; and nuclear replication fork. Part of oligosaccharyltransferase complex and protein-DNA complex. Used to study Aicardi-Goutieres syndrome and cutaneous lupus erythematosus. Human ortholog(s) of this gene implicated in Aicardi-Goutieres syndrome; Chilblain lupus; retinal vasculopathy with cerebral leukodystrophy; and systemic lupus erythematosus. Orthologous to human TREX1 (three prime repair exonuclease 1). [provided by Alliance of Genome Resources, Nov 2021]
Expression	Ubiquitous expression in spleen adult (RPKM 79.9), mammary gland adult (RPKM 65.5) and 27 other tissues See more
Orthologs	human all

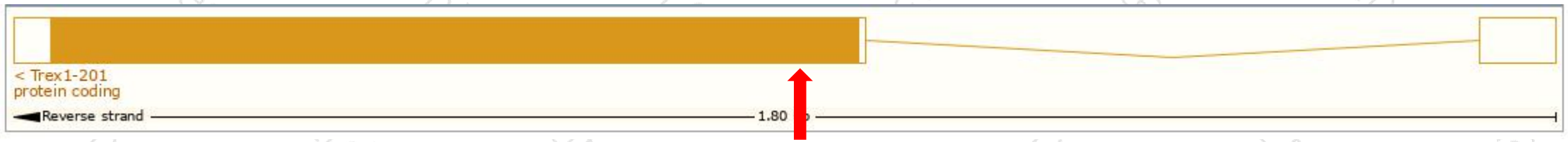


Transcript Information （Ensembl）

The gene has 2 transcripts as shown below:

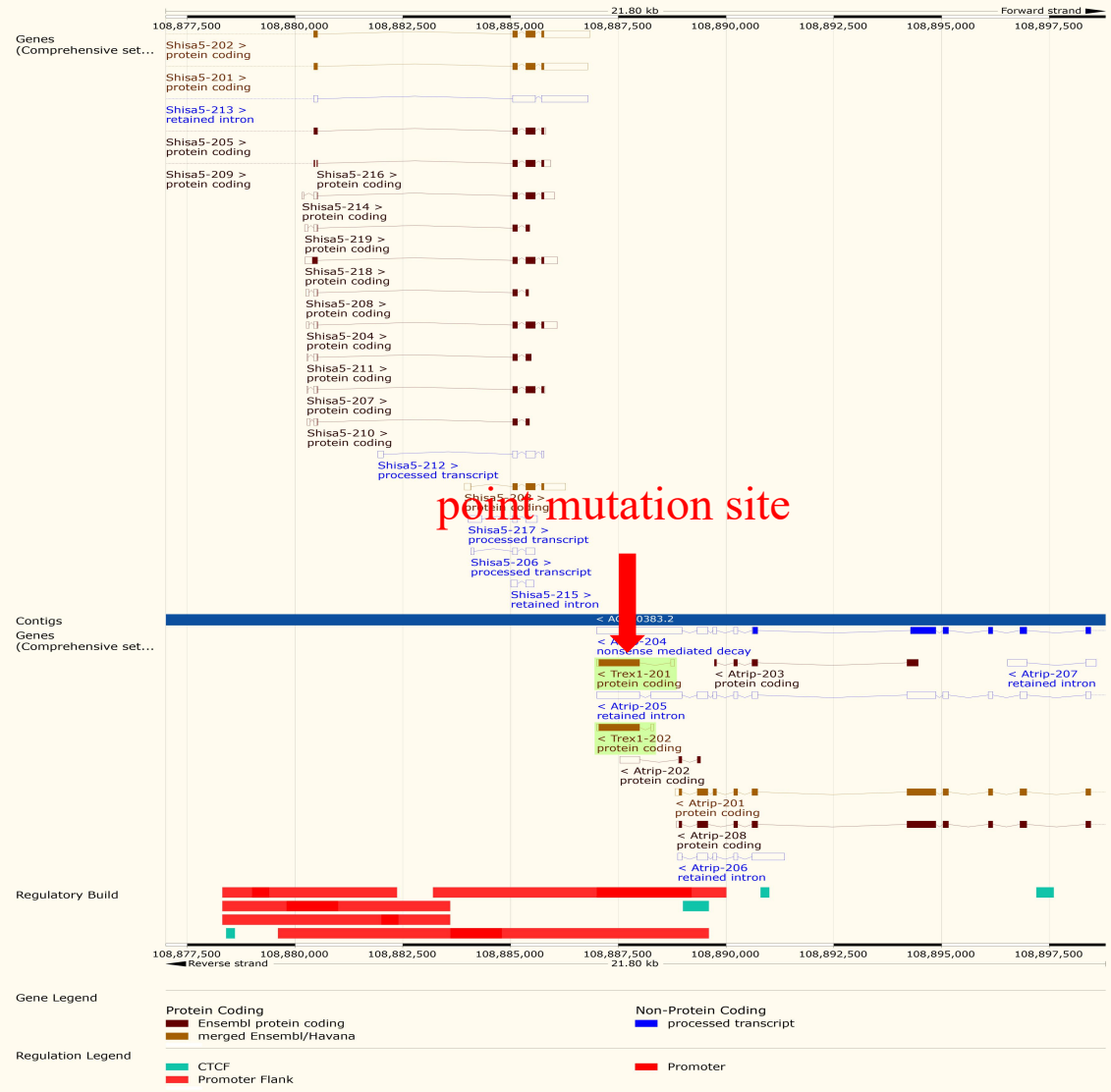
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000061973.5	Trex1-201	1084	314aa	Protein coding	CCDS23544	Q91XB0	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000112053.2	Trex1-202	1054	314aa	Protein coding	CCDS23544	Q91XB0	GENCODE basic APPRIS P1 TSL:1

This strategy is based on the *Trex1*-201 transcript, which is shown below:

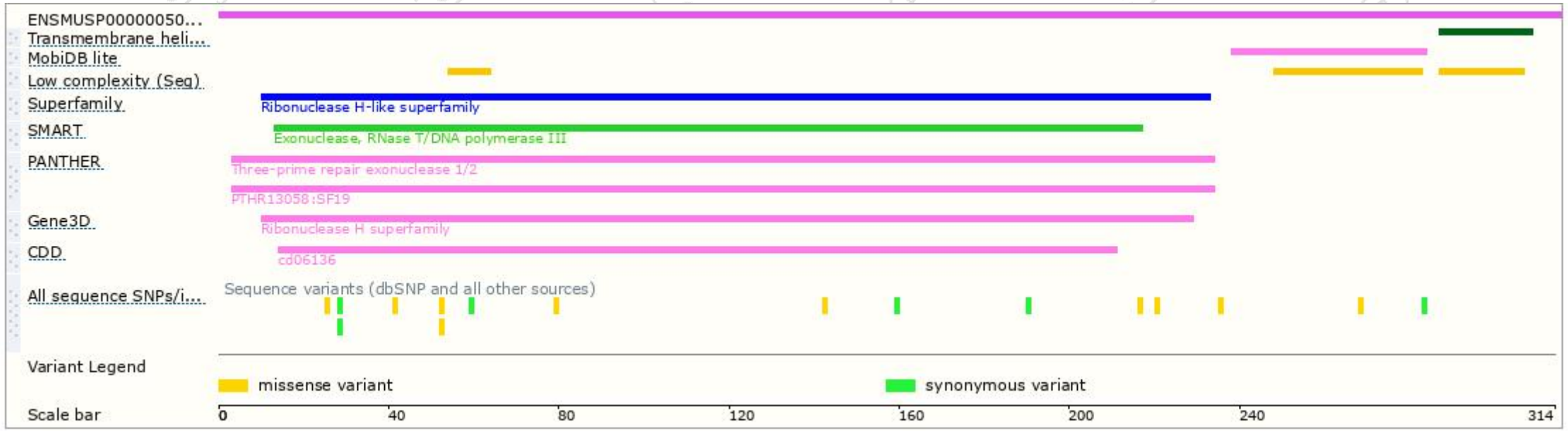


point mutation site

Genomic Information



Protein Information



Workflow


Vector construction
Microinjection
F0 mice generation and identification
F0 breeding, F1 mice generation and identification
Deliver of mice
3~4 weeks
1~3 weeks
6~8 weeks
13~16 weeks
1 week

If you have any questions, please feel free to contact us.
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