En1-P2A-iCre cas9-ki Mouse Model Strategy

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Reviewer: Ruirui Zhang

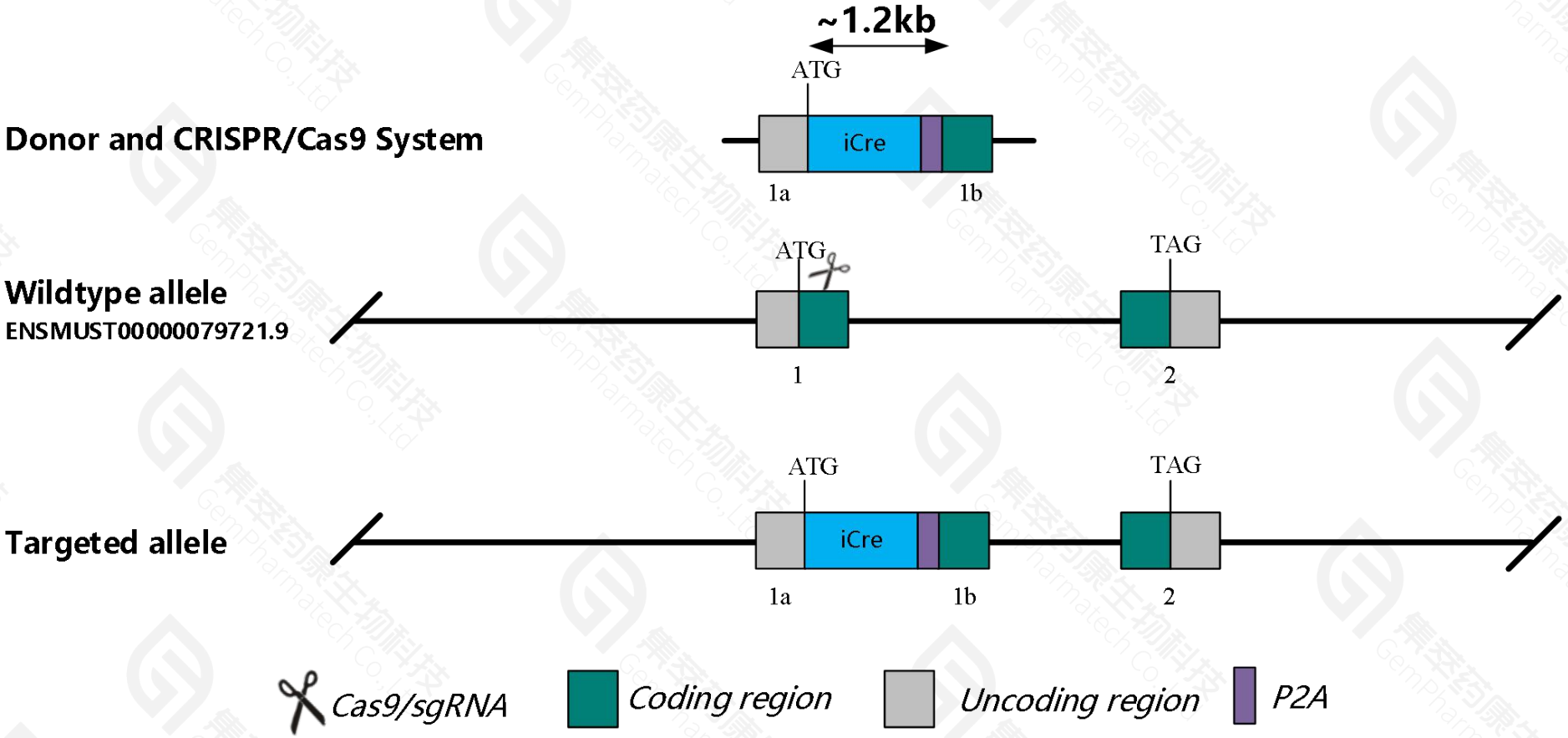
Design Date: 2021-5-26

Project Overview

Project Name	**En1-P2A-iCre**
Project type	**cas9-ki**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *En1* gene. The schematic diagram is as follows:



Technical routes

➢ According to the data of Ensembl, mouse *En1* gene has 1 transcript. According to the requirements of customer and gene structure, the targeting construct contained *iCre-P2A* element will be inserted into the translation start codon of *En1* gene(*En1*-201 ENSMUST00000079721.9), and the expression of foreign *iCre* was depend on the regulation of endogenous *En1*.

➢ The mouse *En1*-201 transcript contains 2 exons. The translation initiation site ATG is located at exon1, and the translation termination site TAG is located at exon2, encoding 401aa.

➢ In this project we use CRISPR/Cas9 technology to modify *En1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

Notice

➢ According to the existing MGI data, mutant homozygotes usually die within 24 hours of birth. Mutants exhibit nervous system defects, including a lack of most of the colliculi, cerebellum, and the third and fourth cranial nerves in some lines. Skeletal anomalies have also been described.

➢ It is necessary to introduce 1-2 synonymous mutation in exon1.

➢ There are repeats sequence near the insertion site, and mutation may occur in the process of mouse model making.

➢ The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.

➢ The *En1* gene is located on the Chr1. Please take the loci in consideration when breeding this knockin mice with other gene modified strains, if the other gene is also on Chr1, it may be extremely hard to get double gene positive homozygotes.

➢ The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 5'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complexity of biological processes, it cannot be predicted completely at the present technology level.

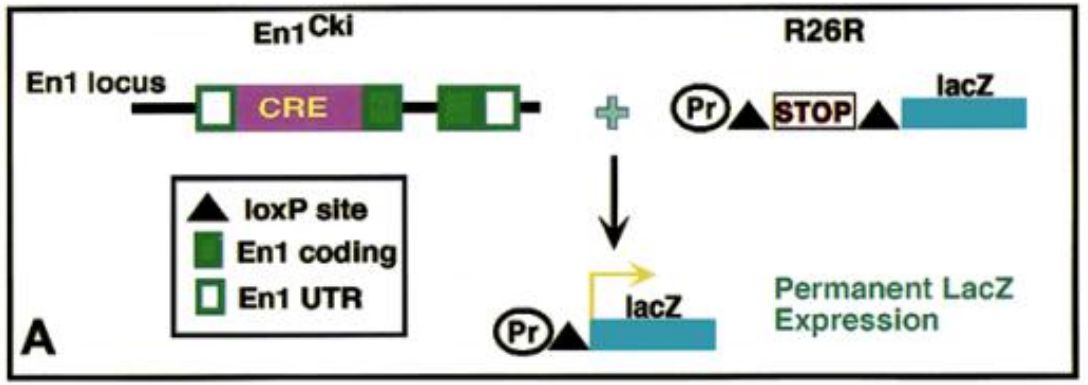
References

Mice

Transgenic mouse strains: En-1^{Cre} (En1$^{tm2(cre)Wrst}$/J), En-1$^{Cre-ERT}$ (En1$^{tm7(cre/ESR1)Alj}$/J), R26^{mTmG} (Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo}$/J), Ai6 (B6.Cg-*Gt(ROSA)26Sor*$^{tm6(CAG-ZsGreen1)Hze}$/J), R26^{iDTR} (C57BL/6-*Gt(ROSA)26Sor*$^{tm1(HBEGF)Awai}$/J, and YAP$^{fl/fl}$ (YAP1$^{tm1.1Dupa}$/J).

Mice were bred and maintained at the Stanford University Comparative Medicine Pavilion in accordance with Stanford APLAC guidelines (APLAC-11048). Mice were housed and bred under the care of the Department of Comparative Medicine in the Veterinary Service Center (VSC). All transgenic mouse strains were obtained from Jackson Laboratories. *En1*^{Cre} and *En1*$^{Cre-ERT}$ mice were crossed with Ai6 and mT/mG reporter mice to trace all EPFs and postnatal EPFs, respectively, as defined *in vivo* by their GFP positivity.

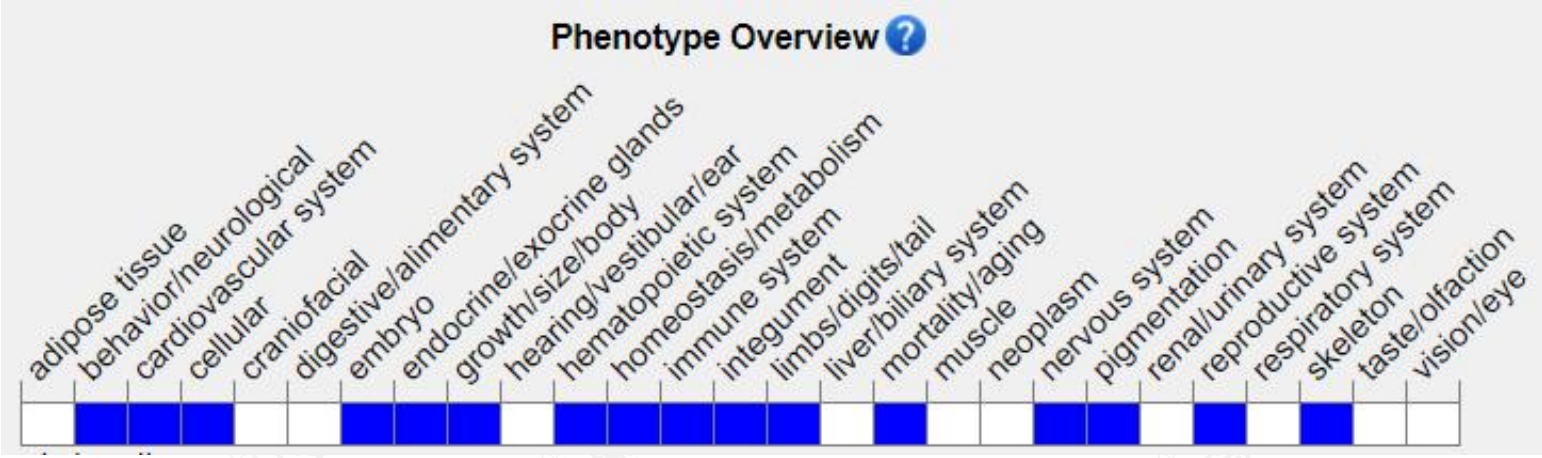
Mascharak S , Desjardins-Park H E , Davitt M F , et al. Preventing Engrailed-1 activation in fibroblasts yields wound regeneration without scarring[J]. Science, 372.



A D/V lineage border corresponds to the distal limit of En1 expression. (*A*) Schematic showing the fate-mapping approach used with Cre/LoxP. In *En1*^{Cki} (*top left*), Cre recombinase replaces part of the *En1* coding sequence. The R26R reporter allele (*top right*) consists of a loxP–stop–loxP cassette inserted upstream of the *lacZ* gene in the Rosa26 locus. In *En1*-expressing cells of mice heterozygous for both *En1*^{Cki} and R26R, the Cre excises the stop sequence, resulting in constitutive *lacZ* production. (*B–E*) Whole-mount RNA insitu

Kimmel R A , Turnbull D H , Blanquet V , et al. Two lineage boundaries coordinate vertebrate apical ectodermal ridge formation[J]. Genes & Development, 2000, 14(11):1377-1389.

Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/) .

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Gene information (NCBI)

集萃药康 GemPharmatech

En1 engrailed 1 [*Mus musculus* (house mouse)]

Download Datasets

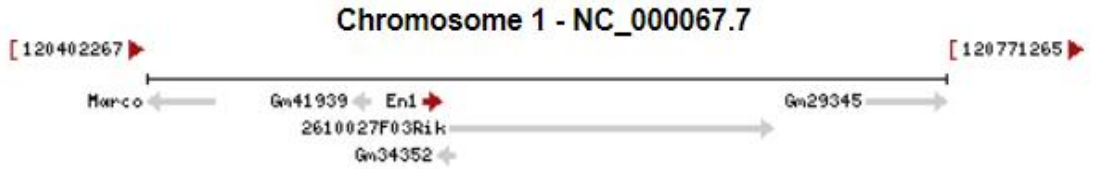
Gene ID: 13798, updated on 9-May-2021

Summary

Official Symbol	En1 provided by MGI
Official Full Name	engrailed 1 provided by MGI
Primary source	MGI:MGI:95389
See related	Ensembl:ENSMUSG00000058665
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	En-; En-1; Mo-en; engrai; Mo-en.1; engrailed-1
Expression	Biased expression in CNS E11.5 (RPKM 5.5), whole brain E14.5 (RPKM 3.8) and 5 other tissues See more
Orthologs	human all

NEW Try the new Gene table

Try the new Transcript table

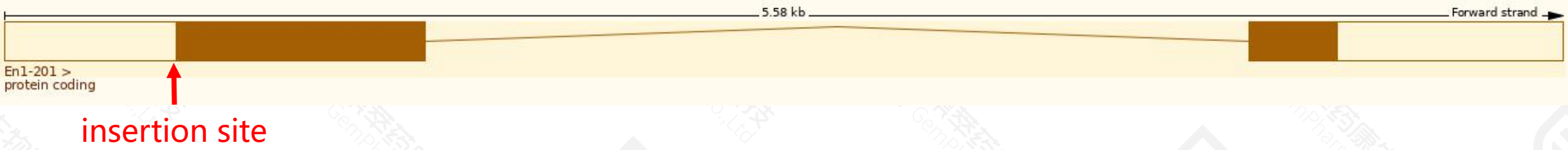


Transcript information （Ensembl）

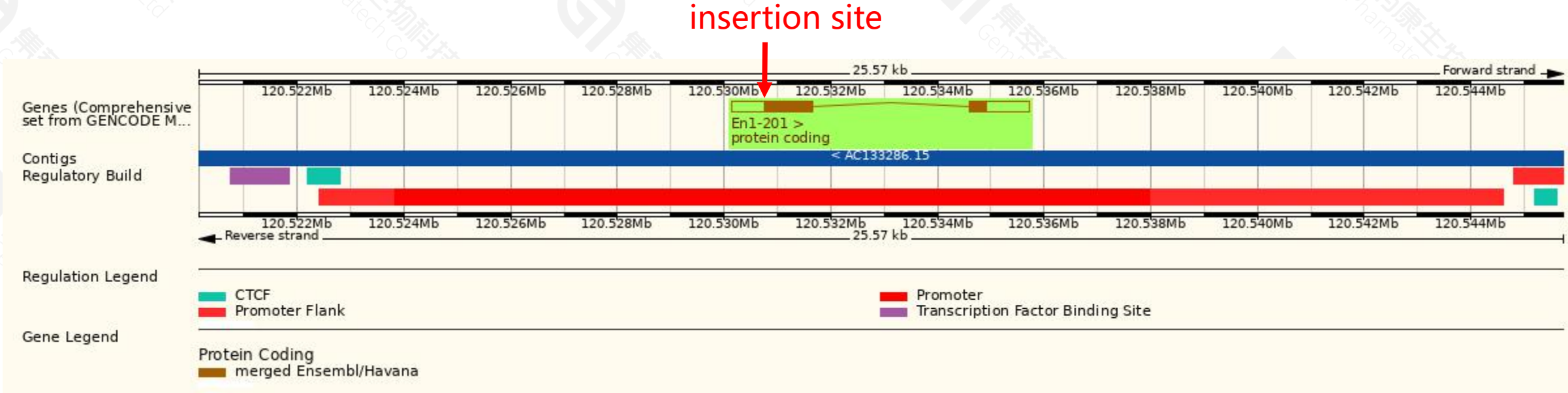
The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
En1-201	ENSMUST00000079721.9	2624	401aa	Protein coding	CCDS15235	P09065	GENCODE basic APPRIS P1 TSL:1

The strategy is based on the design of *En1*-201 transcript,the transcription is shown below:

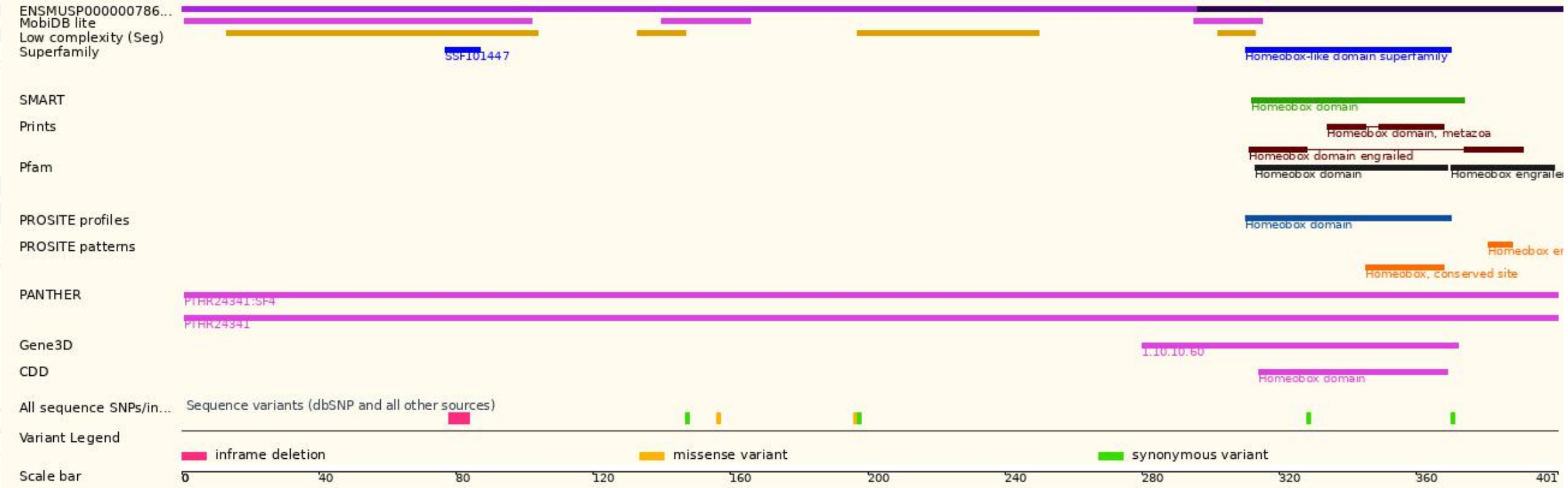


Genomic location distribution



Protein domain

Protein domains for ENSMUSP00000078659.8



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

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