Foxg1-iCre-P2A Cas9-KI Strategy

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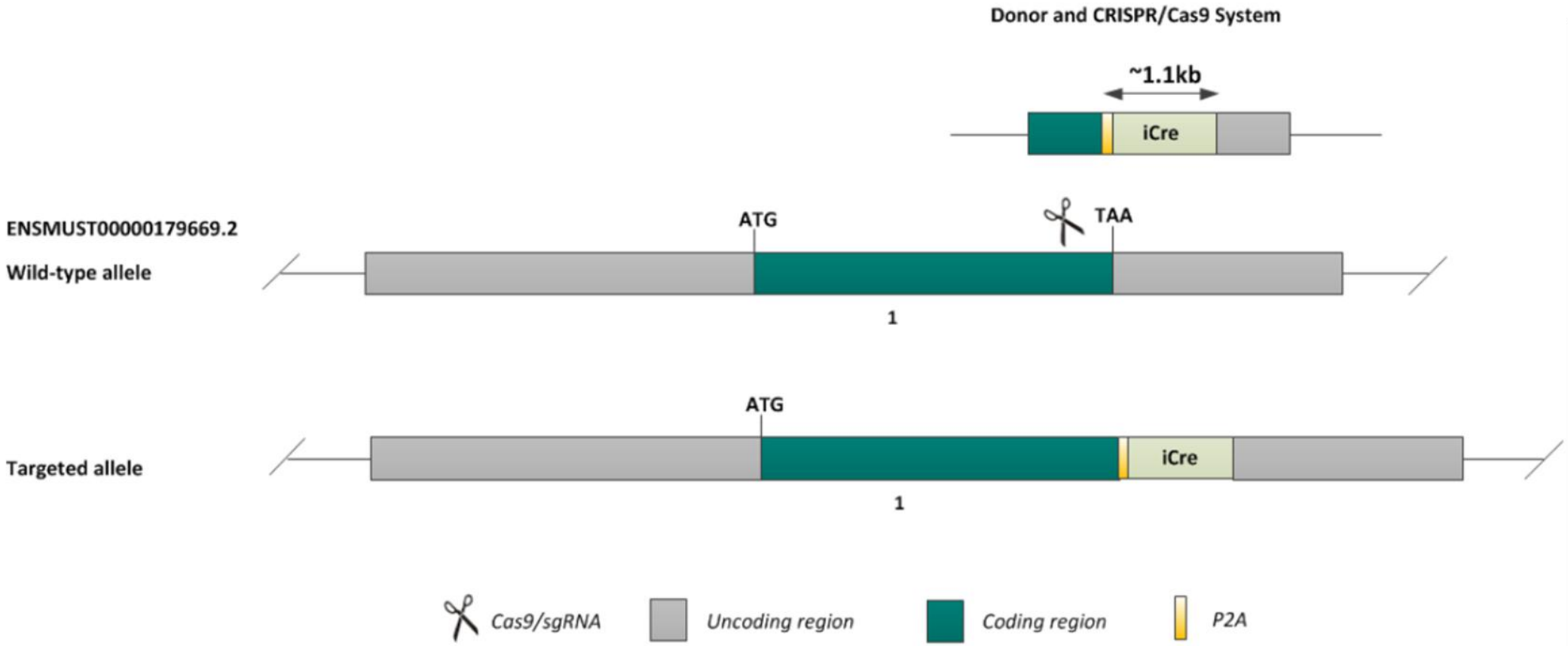
Project Overview

Project Name	***Foxg1-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Foxg1* gene. The schematic diagram is as follows:



Technical routes

- The *Foxg1* gene has 4 transcripts. According to the structure of *Foxg1* gene, *Foxg1-204*(ENSMUST00000179669.2) is selected for presentation of the recommended strategy.

- *Foxg1-204* gene has 1 exons, with the ATG start codon and TAA stop codon in exon1.

- We make *Foxg1-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop codon(TAA) of *Foxg1* gene, and create a DSB (double-strand break). Such breaks will be repaired, and result in P2A-iCre before stop codon(TAA) of *Foxg1* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, Homozygous mutants exhibit dramatically reduced cerebral hemispheres, missing ventral telencephalic structures, impaired migration of efferent thalamocortical axons, and multiple eye defects. Mutants die at birth from respiratory failure.
- Insertion of iCre may affect the regulation of the 3' end of the *Foxg1* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon1 of *Foxg1* gene in this strategy.
- The insertion site is approximately 3.7 kb from the *310039M20Rik* gene and approximately 4 kb from the *Gm34304* gene, which may affect the 5-terminal regulation of these genes. The insertion site is located in the intron of the *Gm43517* gene and may affect the normal splicing of the *Gm43517* gene.
- The *Foxg1* gene is located on the Chr12. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

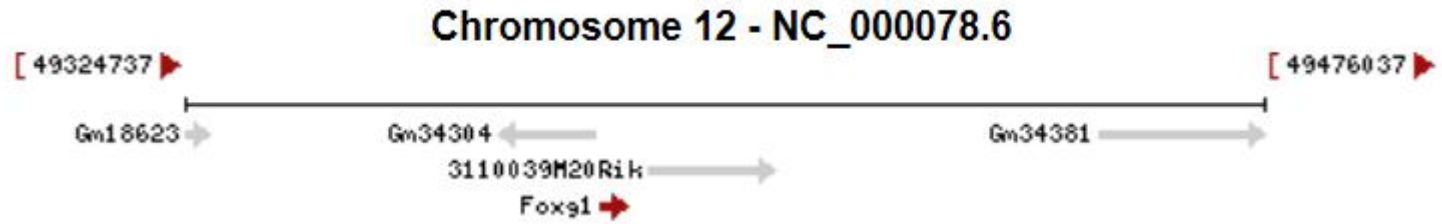
集萃药康 GemPharmatech

Foxg1 forkhead box G1 [*Mus musculus* (house mouse)]

Gene ID: 15228, updated on 12-Aug-2019

Summary

Official Symbol	Foxg1 provided by MGI
Official Full Name	forkhead box G1 provided by MGI
Primary source	MGI:MGI:1347464
See related	Ensembl:ENSMUSG00000020950
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	Bf1; BF-1; Hfh9; Hfhbf1; 2900064B05Rik
Expression	Biased expression in CNS E14 (RPKM 45.4), whole brain E14.5 (RPKM 34.5) and 4 other tissues See more
Orthologs	human all

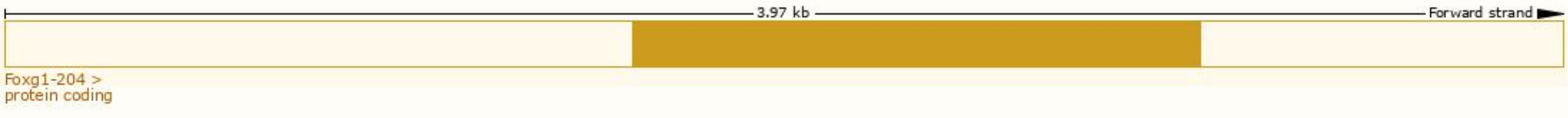


Transcript information （Ensembl）

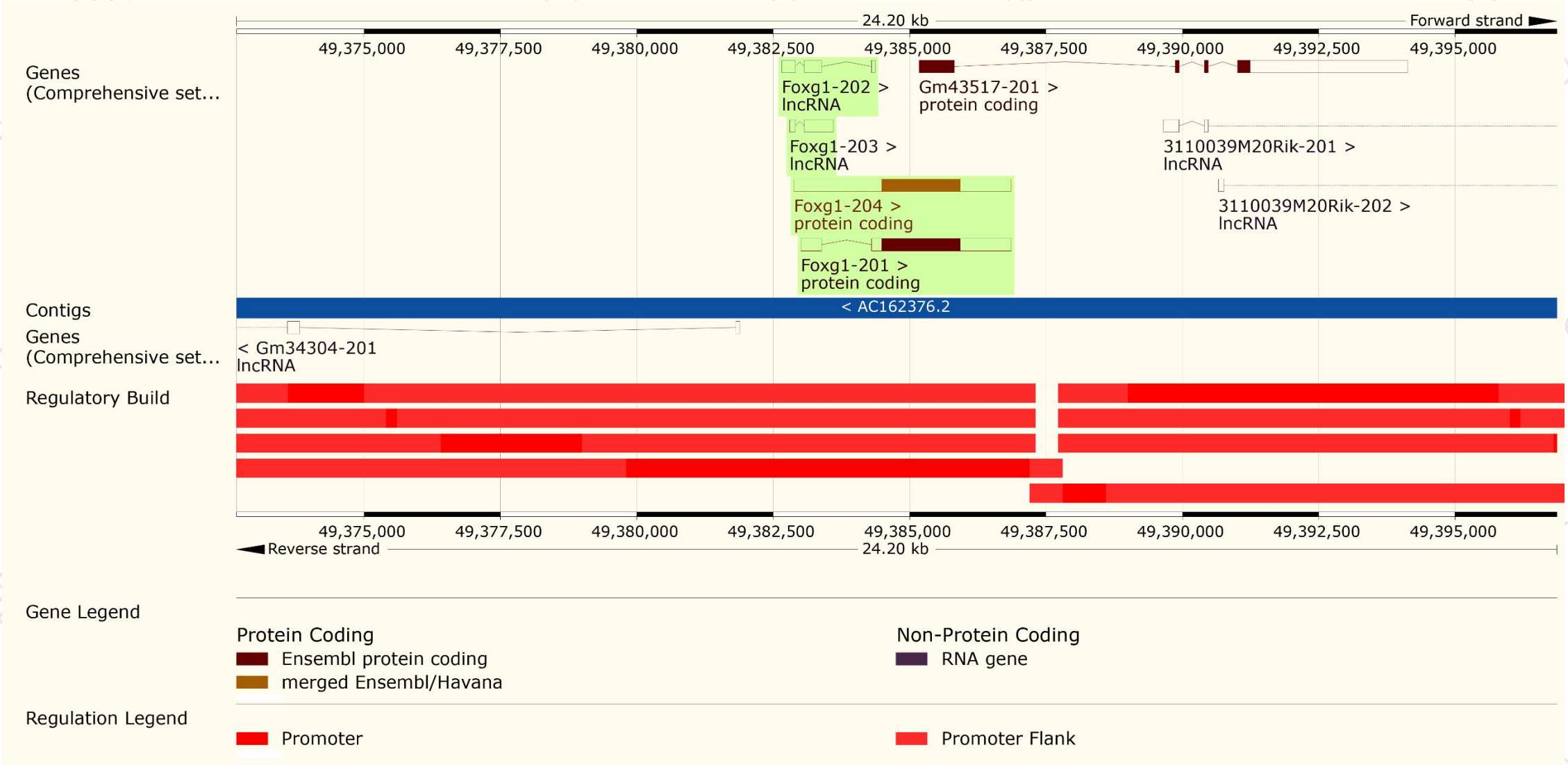
The gene has 4 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Foxg1-201	ENSMUST00000021333.4	2941	481aa	ENSMUSP00000021333.3	Protein coding	CCDS25899	Q3V1Q8 Q60987	TSL:1 GENCODE basic APPRIS P1
Foxg1-202	ENSMUST00000135006.2	623	No protein	-	lncRNA	-	-	TSL:3
Foxg1-203	ENSMUST00000154930.1	633	No protein	-	lncRNA	-	-	TSL:2
Foxg1-204	ENSMUST00000179669.2	3973	481aa	ENSMUSP00000136372.1	Protein coding	CCDS25899	Q3V1Q8 Q60987	TSL:NA GENCODE basic APPRIS P1

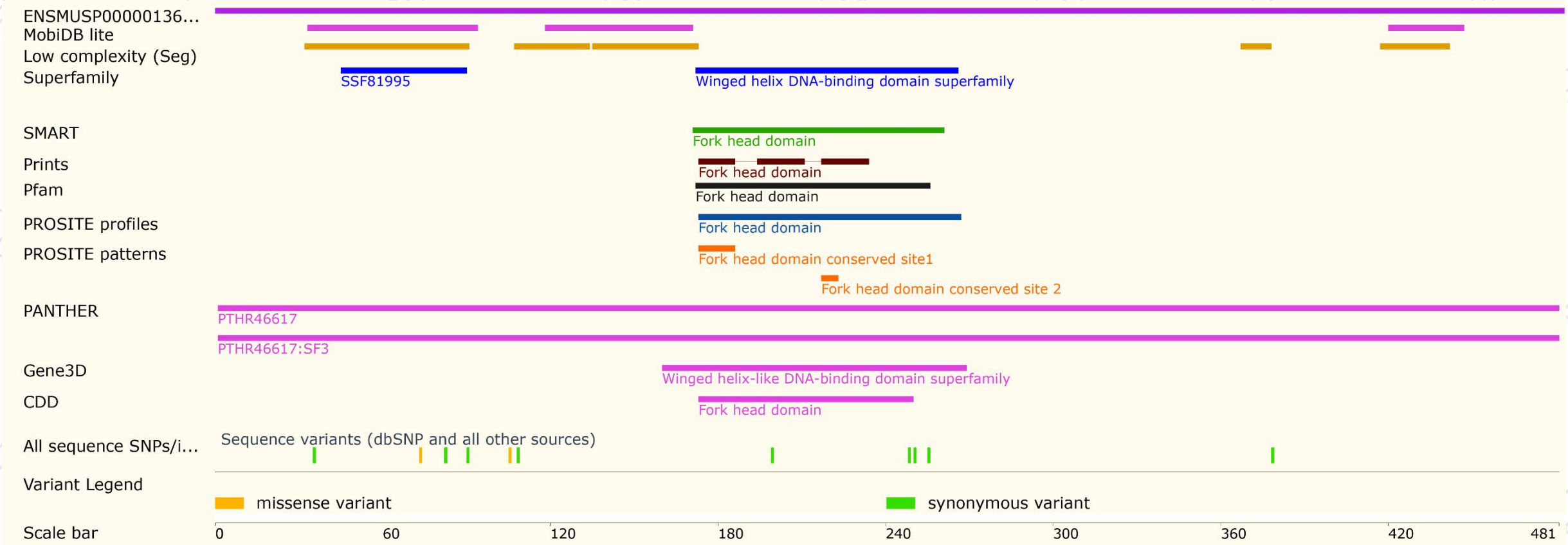
The strategy is based on the design of *Foxg1-204* transcript,The transcription is shown below



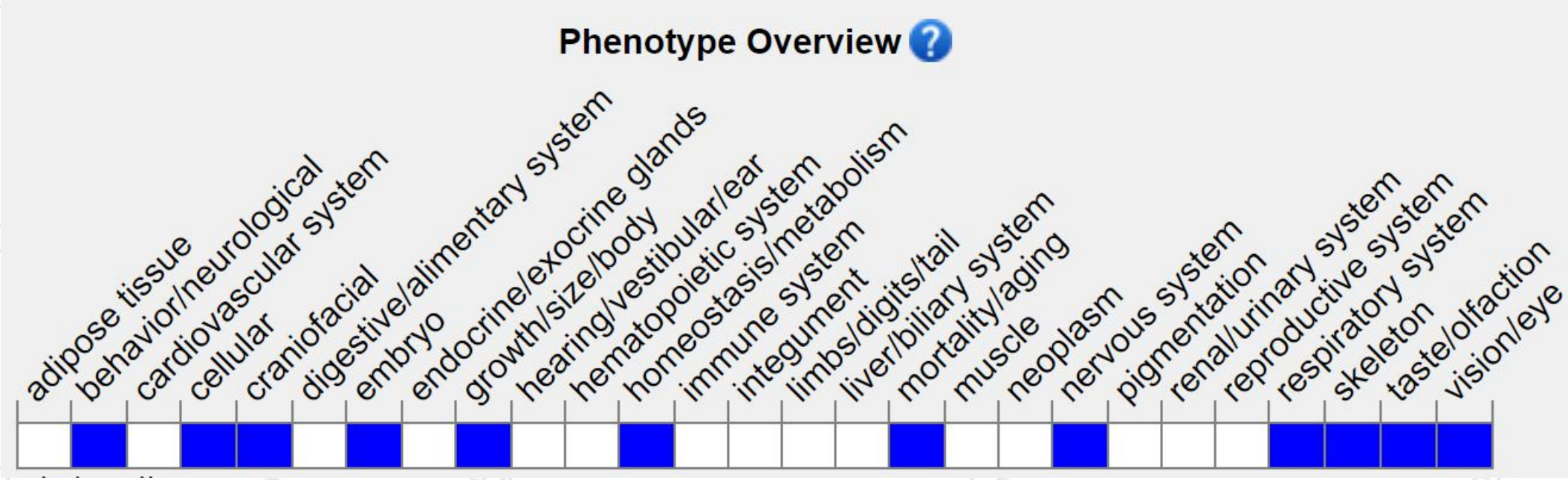
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/marker/MGI:1347464) .

According to the existing MGI data, Homozygous mutants exhibit dramatically reduced cerebral hemispheres, missing ventral telencephalic structures, impaired migration of efferent thalamocortical axons, and multiple eye defects. Mutants die at birth from respiratory failure.

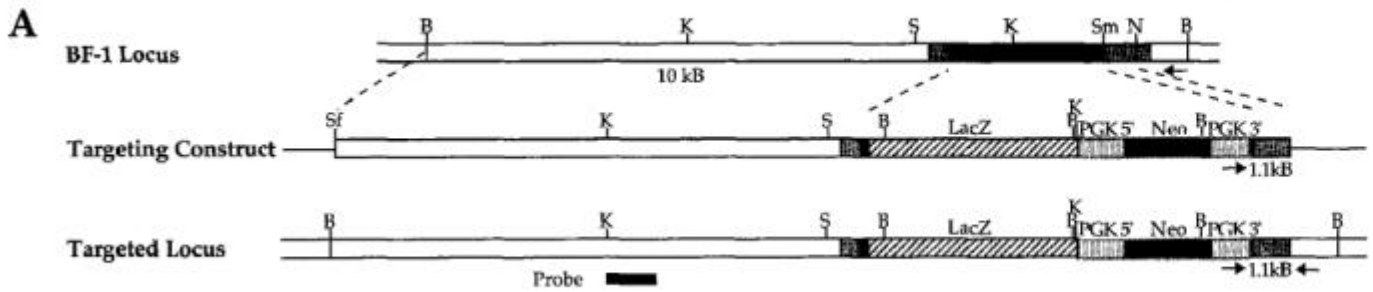
Coding Sequence of Codon-Optimized Cre Gene[1]

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGCCACCTCTGATGAAGTCAGGAAGA
ACCTGATGGACATGTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAGATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAA
CAACAGGAAATGGTTCCCTGCTGAACCTGAGGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTG
GGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATCAGAAAGGAGAATGTGG
ATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCAGATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAG
GAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGA
ATGCTGATCCACATTGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGTTACCAAGCTGGTGGAGAGATGGATCT
CTGTGTCTGGTGTGGCTGATGACCCCAACAACTACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCTGCCCCTTCTGCCACCTCCCAACTGTCCACCCG
GGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGTGCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGA
GTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCA
GAAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

References

[1] Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse. GeAlbis.2002 Jan.32(1):19-26.

[2] Xuan S, et al. Winged helix transcription factor BF-1 is essential for the development of the cerebral hemispheres. Neuron.1995 Jun;14(6):1141-52.



of the coding sequence of BF-1, which is contained in a single exon, with a lacZ and neomycin cassette (Figure 1A). The β-galactosidase (β-gal) sequence containing a nuclear localization signal was fused in-frame to the first 13 amino acids of BF-1, with the goal of placing the expression of this enzyme under the control of the BF-1 promoter and the BF-1 translation initiation site. Electroporated em-

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

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