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Nfe2l1 Cas9-CKO Strategy

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Reviewer: Huan Wang

Design Date: 2020-5-26

Project Overview

Project Name	***Nfe2l1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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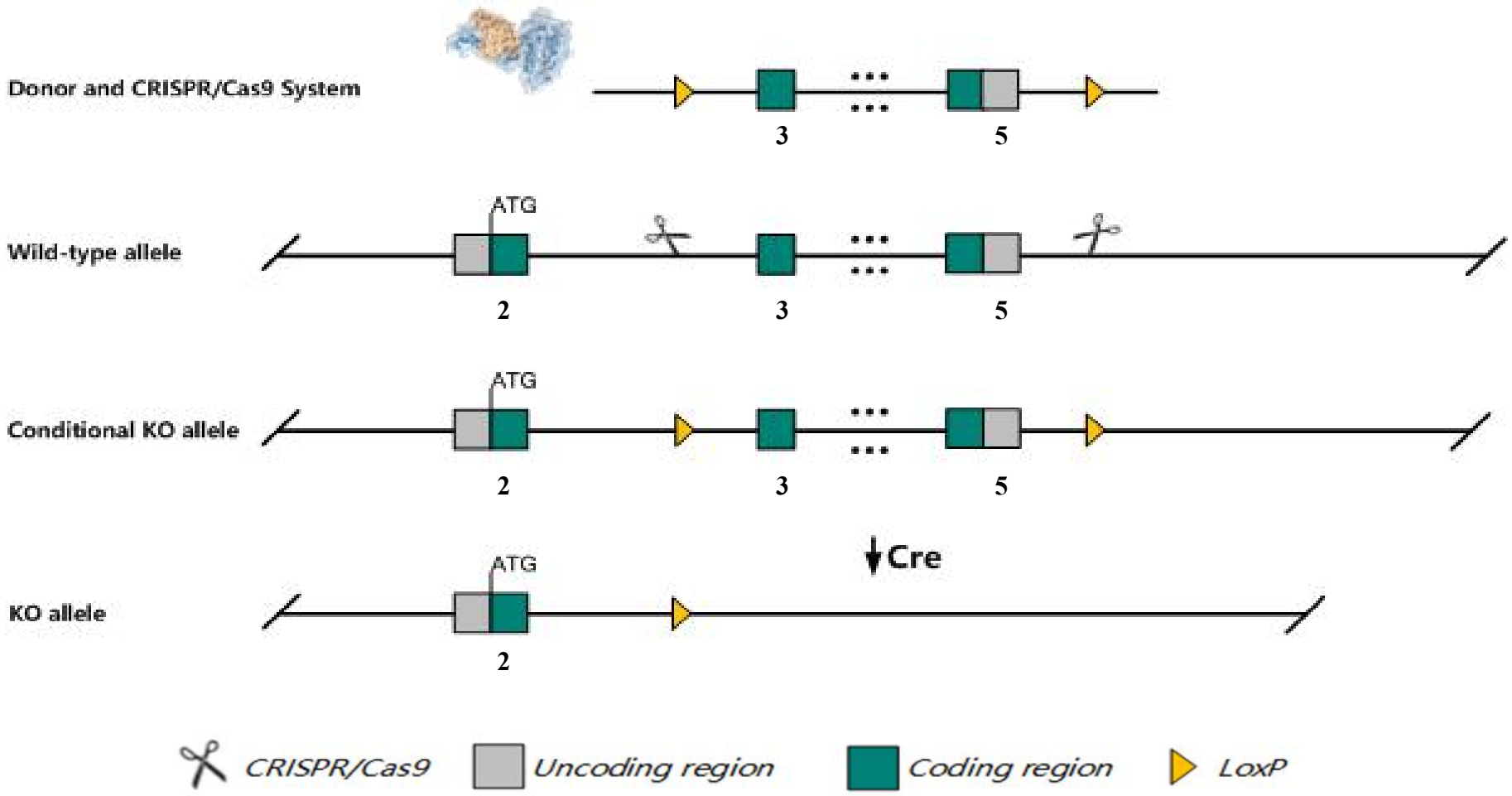
This model will use CRISPR/Cas9 technology to edit the *Nfe2l1* gene. The schematic diagram is as follows:



Technical routes

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The *Nfe2l1* gene has 12 transcripts. According to the structure of *Nfe2l1* gene, exon3-exon5 of *Nfe2l1-201* (ENSMUST00000081775.11) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Nfe2l1* gene. The brief process is as follows:CRISPR/Cas9 system 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.

The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

According to the existing MGI data,homozygotes for targeted null mutations fail to form a primitive streak, lack mesoderm, show a non-cell autonomous defect in definitive erythropoiesis, and die around embryonic day 7.5.

The *Nfe2l1* gene is located on the Chr11. If the knockout 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 biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information NCBI

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Nfe2l1 nuclear factor, erythroid derived 2,-like 1 [Mus musculus (house mouse)]

Gene ID: 18023, updated on 13-Mar-2020

Summary

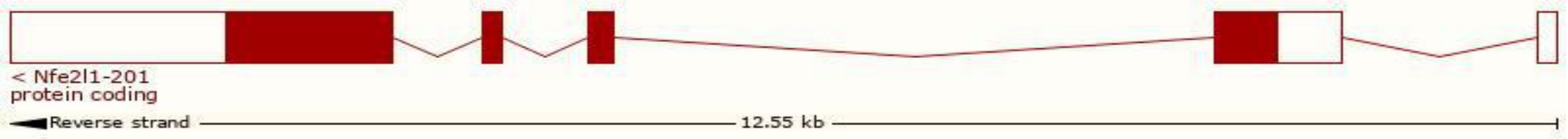
Official Symbol	Nfe2l1 provided by MGI
Official Full Name	nuclear factor, erythroid derived 2,-like 1 provided byMGI
Primary source	MGI:MGI:99421
See related	Ensembl:ENSMUSG00000038615
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA408798, AW212678, LCR-F1, Lcrf1, NRF1, TCF-11, TCF11
Summary	This gene encodes a protein that is involved in globin gene expression in erythrocytes. Confusion has occurred in bibliographic databases from the use of NRF1 for this gene, NFE2L1, and for "nuclear respiratory factor 1" which has an official symbol of NRF1. [provided by RefSeq, Jul 2008]
Expression	Ubiquitous expression in adrenal adult (RPKM 102.3), heart adult (RPKM 69.1) and 28 other tissuesSee more
Orthologs	human all

Transcript information Ensembl

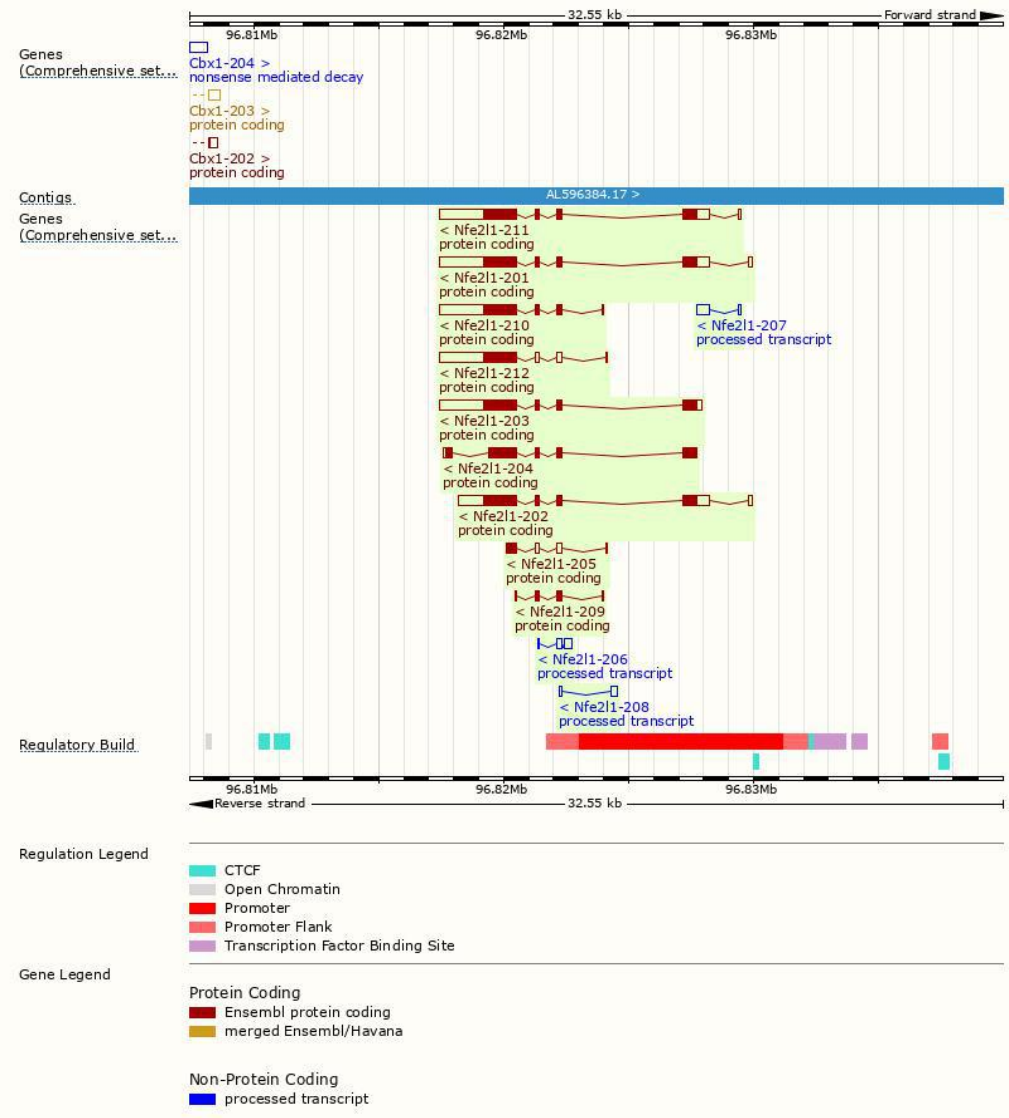
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nfe2l1-201	ENSMUST00000081775.11	4654	741aa	Protein coding	CCDS25304	Q61985 Q6GTN8	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Nfe2l1-211	ENSMUST00000167149.7	4596	741aa	Protein coding	CCDS25304	Q61985 Q6GTN8	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Nfe2l1-203	ENSMUST00000107658.7	4219	741aa	Protein coding	CCDS25304	Q61985 Q6GTN8	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Nfe2l1-202	ENSMUST00000107657.7	3869	741aa	Protein coding	CCDS25304	Q61985 Q6GTN8	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Nfe2l1-210	ENSMUST00000167110.7	3547	583aa	Protein coding	CCDS48894	Q61985	TSL:2 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Nfe2l1-212	ENSMUST00000169828.7	3530	453aa	Protein coding	CCDS48893	Q3V3M1	TSL:2 GENCODE basic
Nfe2l1-204	ENSMUST00000107659.7	2351	742aa	Protein coding	-	A2A6D2	TSL:1 GENCODE basic
Nfe2l1-205	ENSMUST00000126949.7	851	148aa	Protein coding	-	A2A6D0	CDS 3' incomplete TSL:2
Nfe2l1-209	ENSMUST00000142065.1	486	152aa	Protein coding	-	A2A6D1	CDS 3' incomplete TSL:2
Nfe2l1-206	ENSMUST00000134951.1	597	No protein	Processed transcript	-	-	TSL:2
Nfe2l1-207	ENSMUST00000135836.1	582	No protein	Processed transcript	-	-	TSL:1
Nfe2l1-208	ENSMUST00000140138.1	354	No protein	Processed transcript	-	-	TSL:2

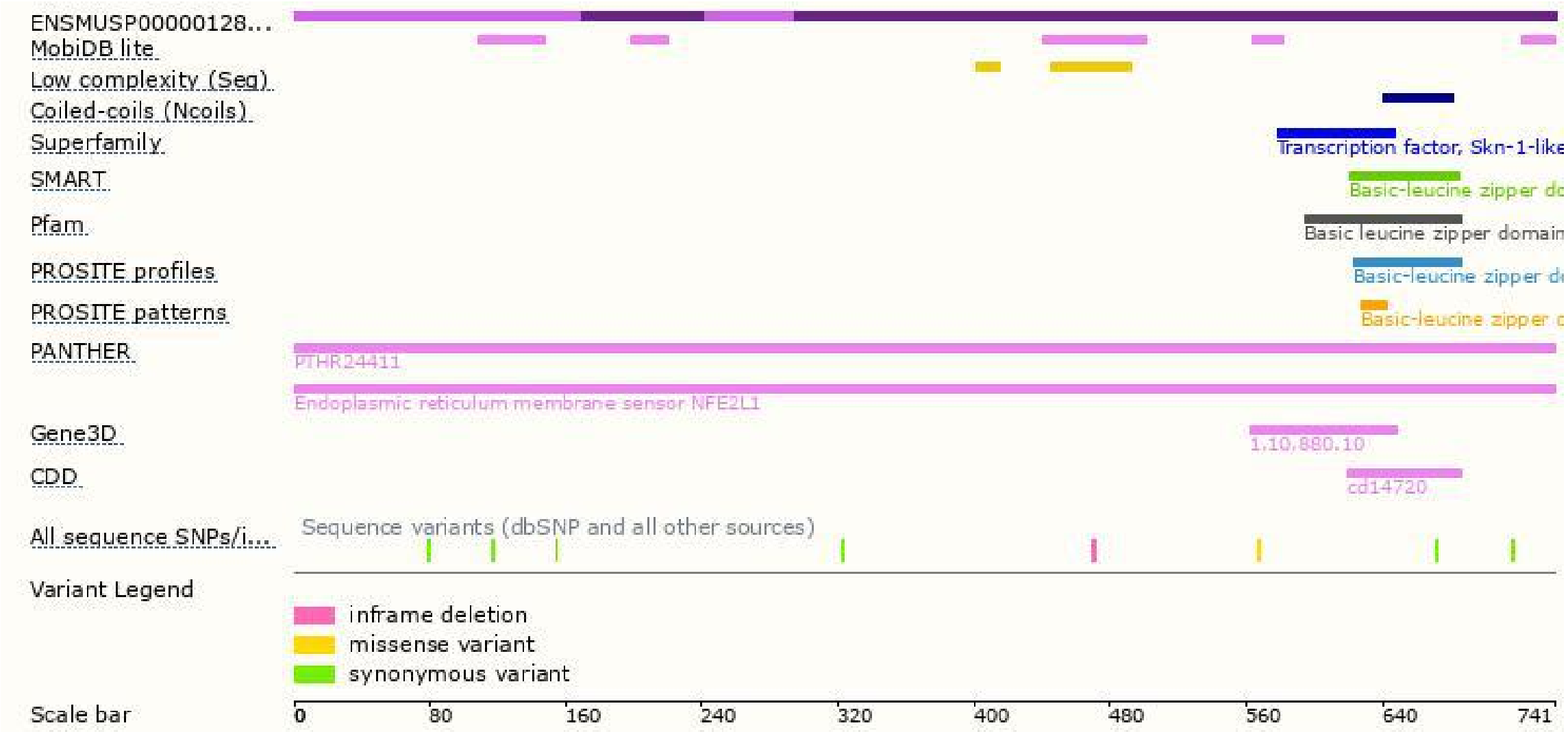
The strategy is based on the design of *Nfe2l1-201* transcript,the transcription is shown below:



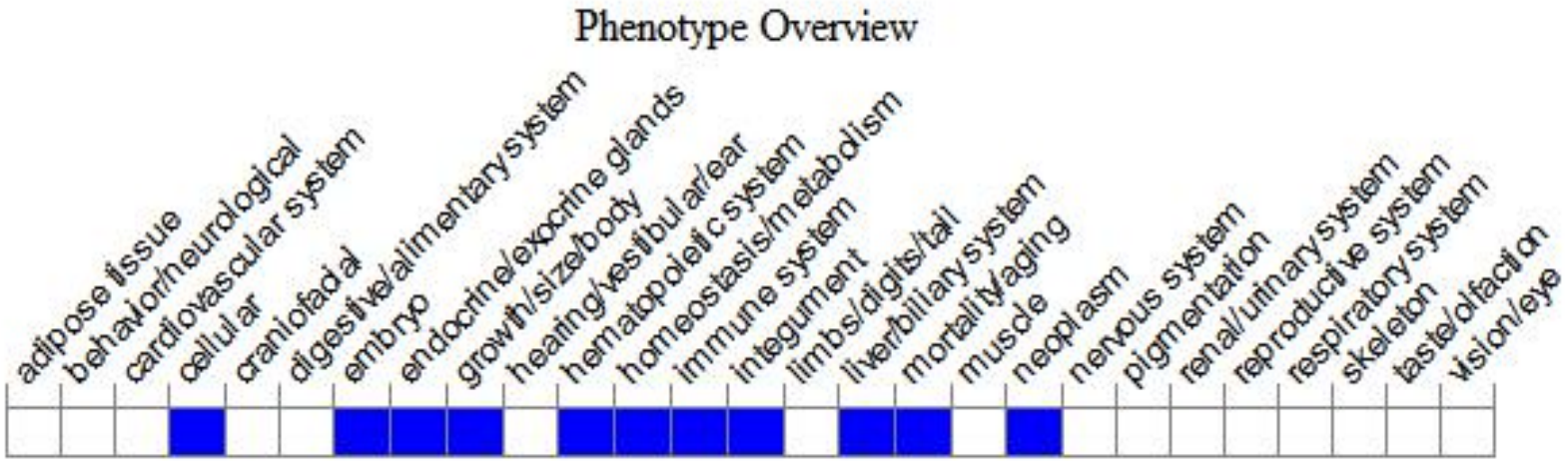
Genomic location distribution



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/).

According to the existing MGI data,homozygotes for targeted null mutations fail to form a primitive streak, lack mesoderm, show a non-cell autonomous defect in definitive erythropoiesis, and die around embryonic day 7.5.

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

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