Gpx1 Cas9-CKO Strategy

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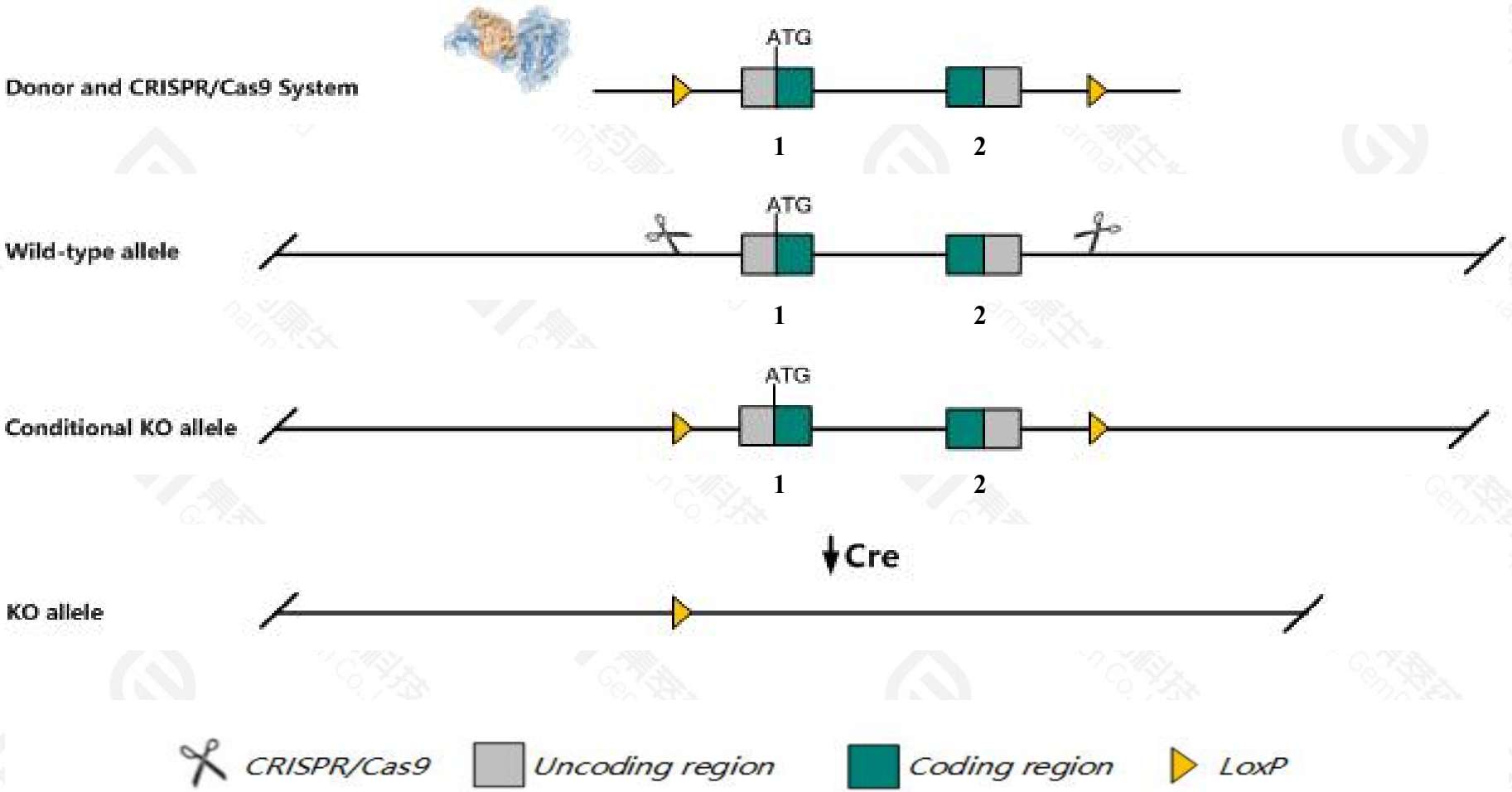
Project Overview

Project Name	***Gpx1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gpx1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Gpx1* gene has 4 transcripts. According to the structure of *Gpx1* gene, exon1-exon2 of *Gpx1-201*(ENSMUST00000082429.8) transcript is recommended as the knockout region.The region contains all 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 *Gpx1* 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 show increased sensitivity to the oxidative stress agents paraquat and hydrogen peroxide and to ischemia/reperfusion and cold-induced brain injury. Mutants also show paradoxical bradykinin-induced vasoconstriction.

➢ This strategy may affect the regulation of the three terminus of *Rhoa* gene.

➢ This strategy may affect the 5-terminal regulation of target genes.

➢ This strategy may disrupt the *Gm37401* gene.

➢ The *Gpx1* gene is located on the Chr9. 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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Gpx1 glutathione peroxidase 1 [Mus musculus (house mouse)]

Gene ID: 14775, updated on 19-Jan-2021

Summary

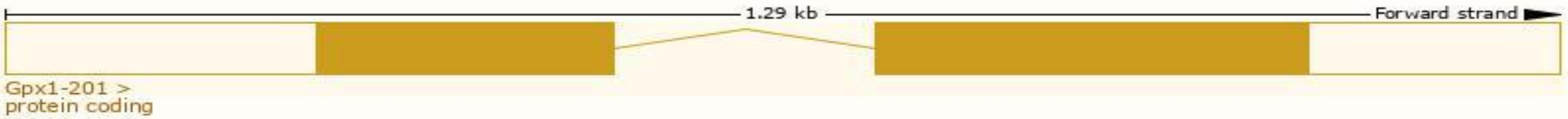
Official Symbol	Gpx1 provided by MGI
Official Full Name	glutathione peroxidase 1 provided by MGI
Primary source	MGI:MGI:104887
See related	Ensembl:ENSMUSG00000063856
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI195024, AL033363, CGP, CGPx, GPx-, GPx-1, GSHPx, GSHPx-1, Gp, Gpx
Summary	The protein encoded by this gene belongs to the glutathione peroxidase family, members of which catalyze the reduction of organic hydroperoxides and hydrogen peroxide (H2O2) by glutathione, and thereby protect cells against oxidative damage. Knockout mice lacking this gene are highly sensitive to oxidants, and develop mature cataracts due to damage to the eye lens nucleus. Other studies indicate that H2O2 is also essential for growth-factor mediated signal transduction, mitochondrial function, and maintenance of thiol redox-balance; therefore, by limiting H2O2 accumulation, glutathione peroxidases are also involved in modulating these processes. Several isozymes of this gene family exist in vertebrates, which vary in cellular location and substrate specificity. This isozyme is the most abundant, is ubiquitously expressed and localized in the cytoplasm, and whose preferred substrate is hydrogen peroxide. It is also a selenoprotein, containing the rare amino acid selenocysteine (Sec) at its active site. Sec is encoded by the UGA codon, which normally signals translation termination. The 3' UTRs of selenoprotein mRNAs contain a conserved stem-loop structure, designated the Sec insertion sequence (SECIS) element, that is necessary for the recognition of UGA as a Sec codon, rather than as a stop signal. Alternatively spliced transcript variants have been found for this gene. [provided by RefSeq, Jul 2016]
Expression	Broad expression in liver adult (RPKM 2481.2), liver E14.5 (RPKM 1548.2) and 19 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

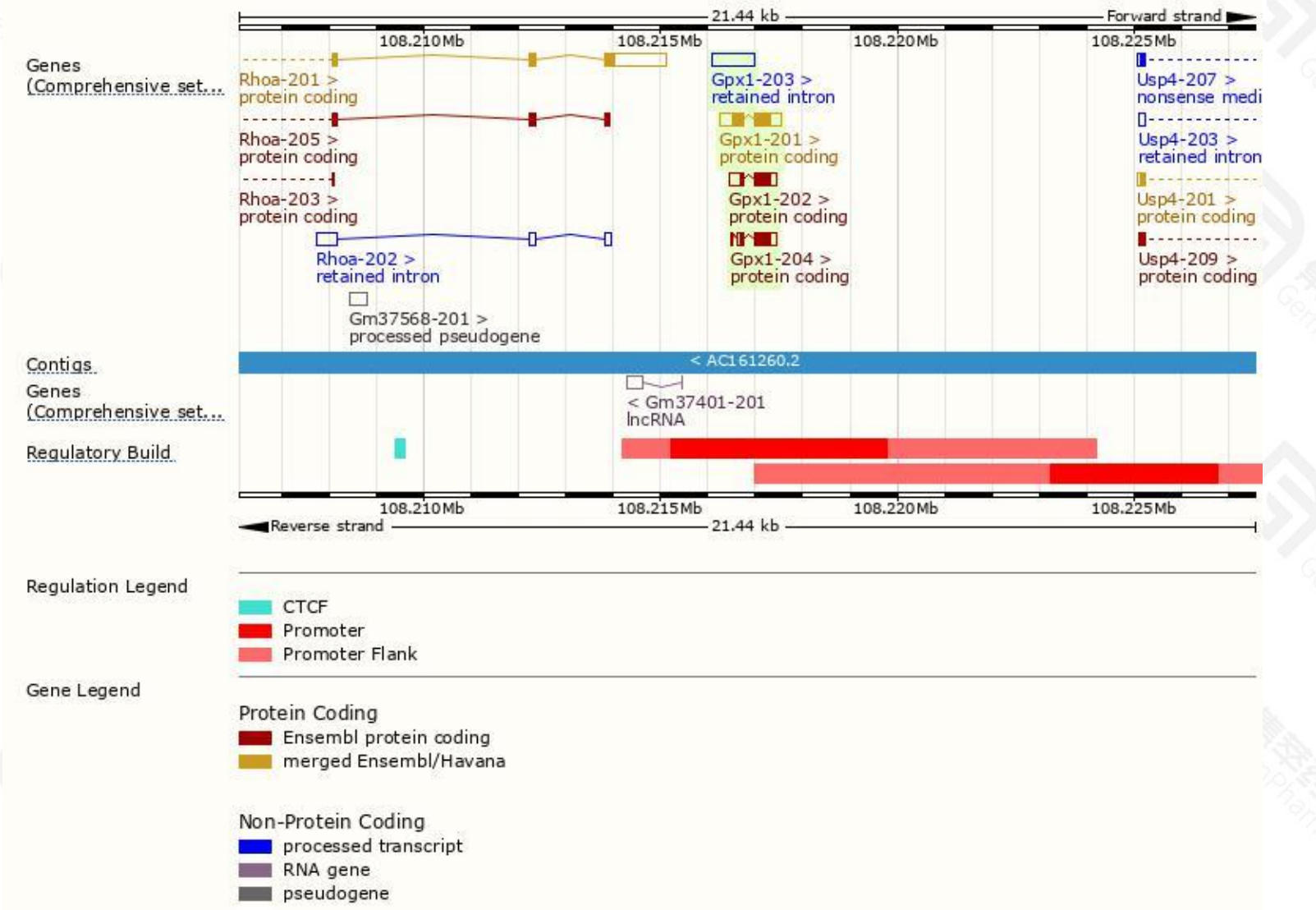
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gpx1-201	ENSMUST00000082429.8	1072	201aa	Protein coding	CCDS23522		TSL:1 , GENCODE basic , APPRIS P1 ,
Gpx1-202	ENSMUST00000191997.2	787	144aa	Protein coding	-		TSL:2 , GENCODE basic ,
Gpx1-204	ENSMUST00000193987.2	653	145aa	Protein coding	-		TSL:3 , GENCODE basic ,
Gpx1-203	ENSMUST00000192401.2	900	No protein	Retained intron	-		TSL:NA ,

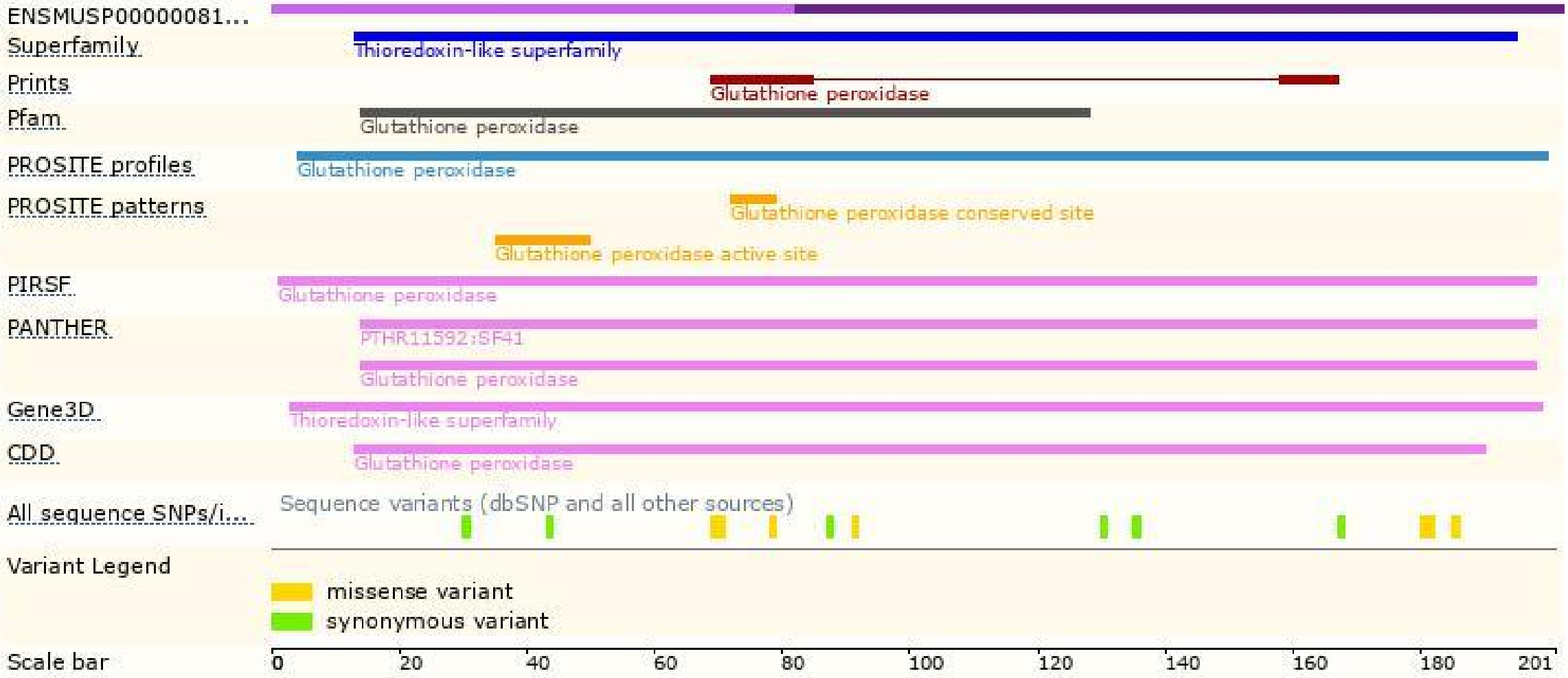
The strategy is based on the design of *Gpx1-201* transcript,the transcription is shown below:



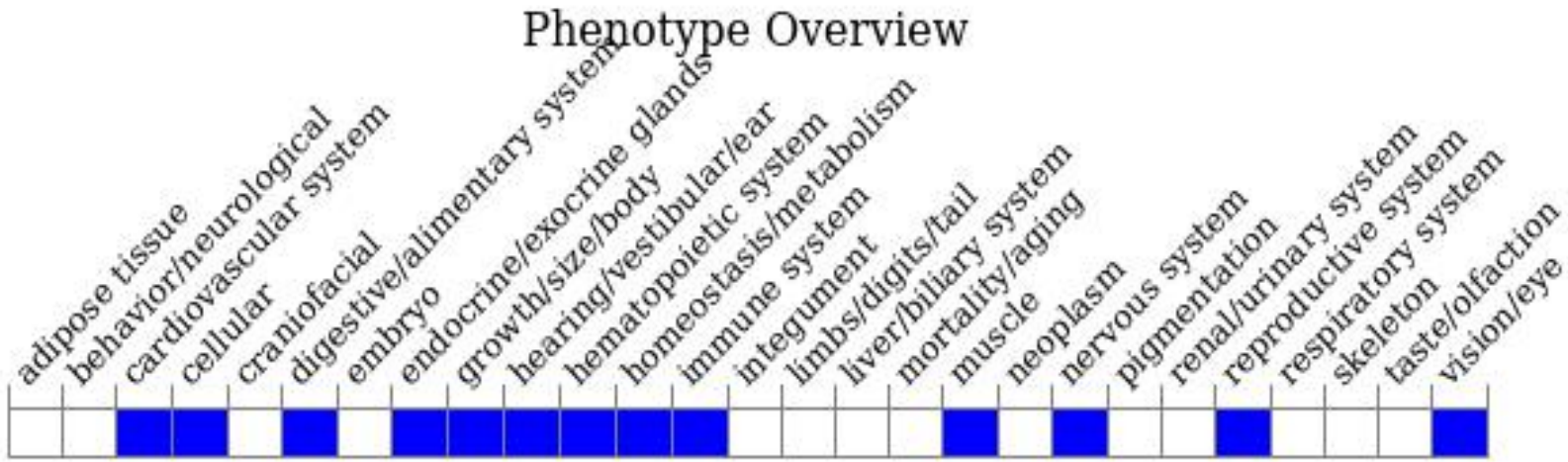
Genomic location distribution



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 show increased sensitivity to the oxidative stress agents paraquat and hydrogen peroxide and to ischemia/reperfusion and cold-induced brain injury. Mutants also show paradoxical bradykinin-induced vasoconstriction.

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