Exoc2 Cas9-CKO Strategy

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Project Overview

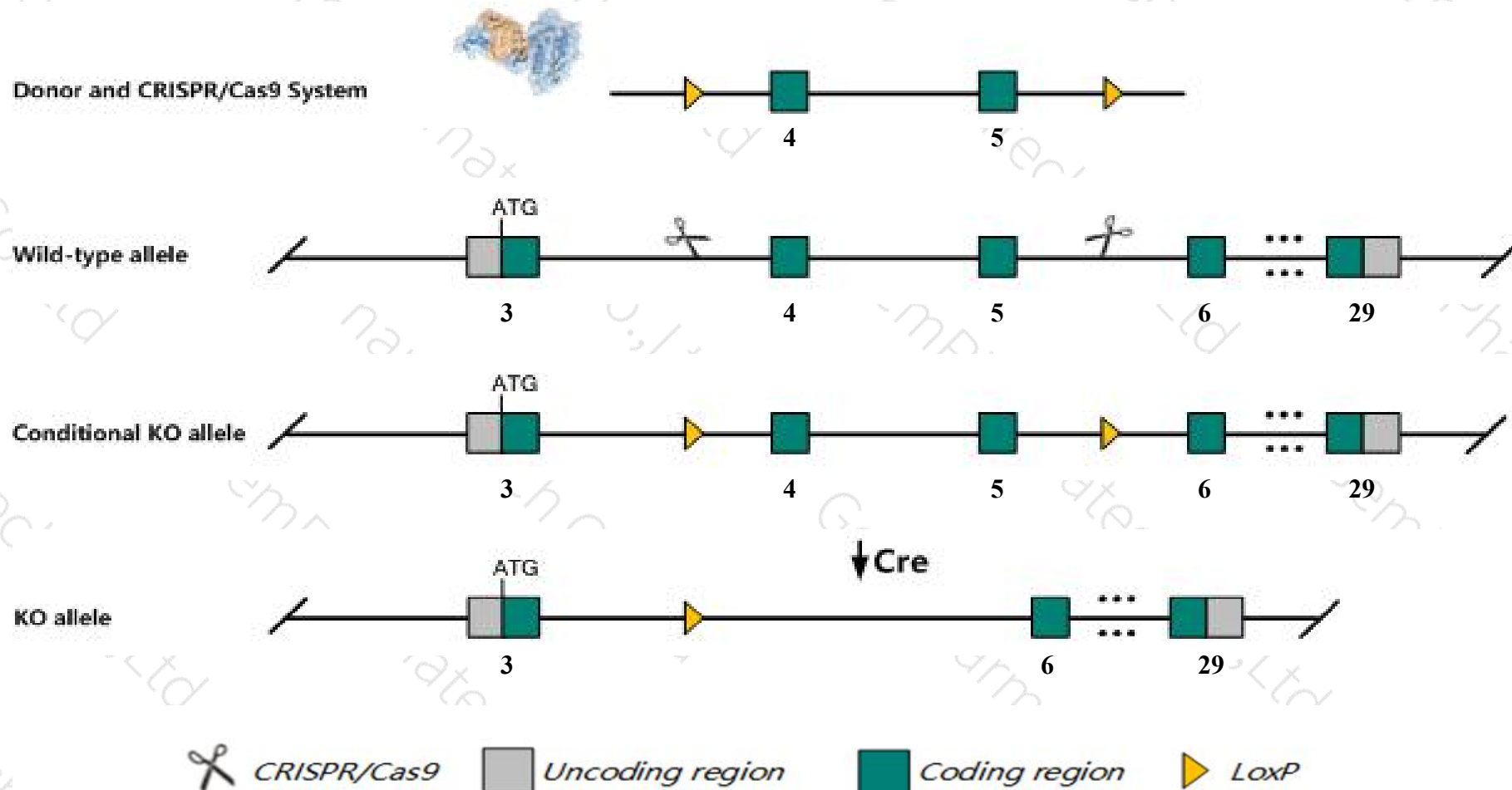|	
Project Name	*Exoc2*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Exoc2* gene. The schematic diagram is as follows:

Technical routes

➤ The *Exoc2* gene has 7 transcripts. According to the structure of *Exoc2* gene, exon4-exon5 of *Exoc2-202* (ENSMUST00000102946.7) transcript is recommended as the knockout region.The region contains Ens 304bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Exoc2* 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,mice homozygous for a knock-out allele show complete embryonic lethality between implantation and somite formation and failure of blastocysts to hatch from the zona pellucida with increased cell death during outgrowth culture.

➢ The *Exoc2* gene is located on the Chr13. 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）

Exoc2 exocyst complex component 2 [Mus musculus (house mouse)]

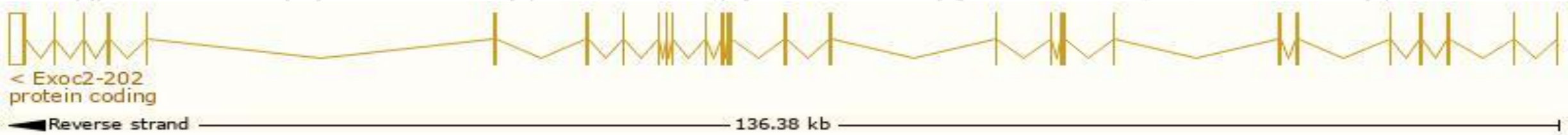Gene ID: 66482, updated on 13-Mar-2020

Summary

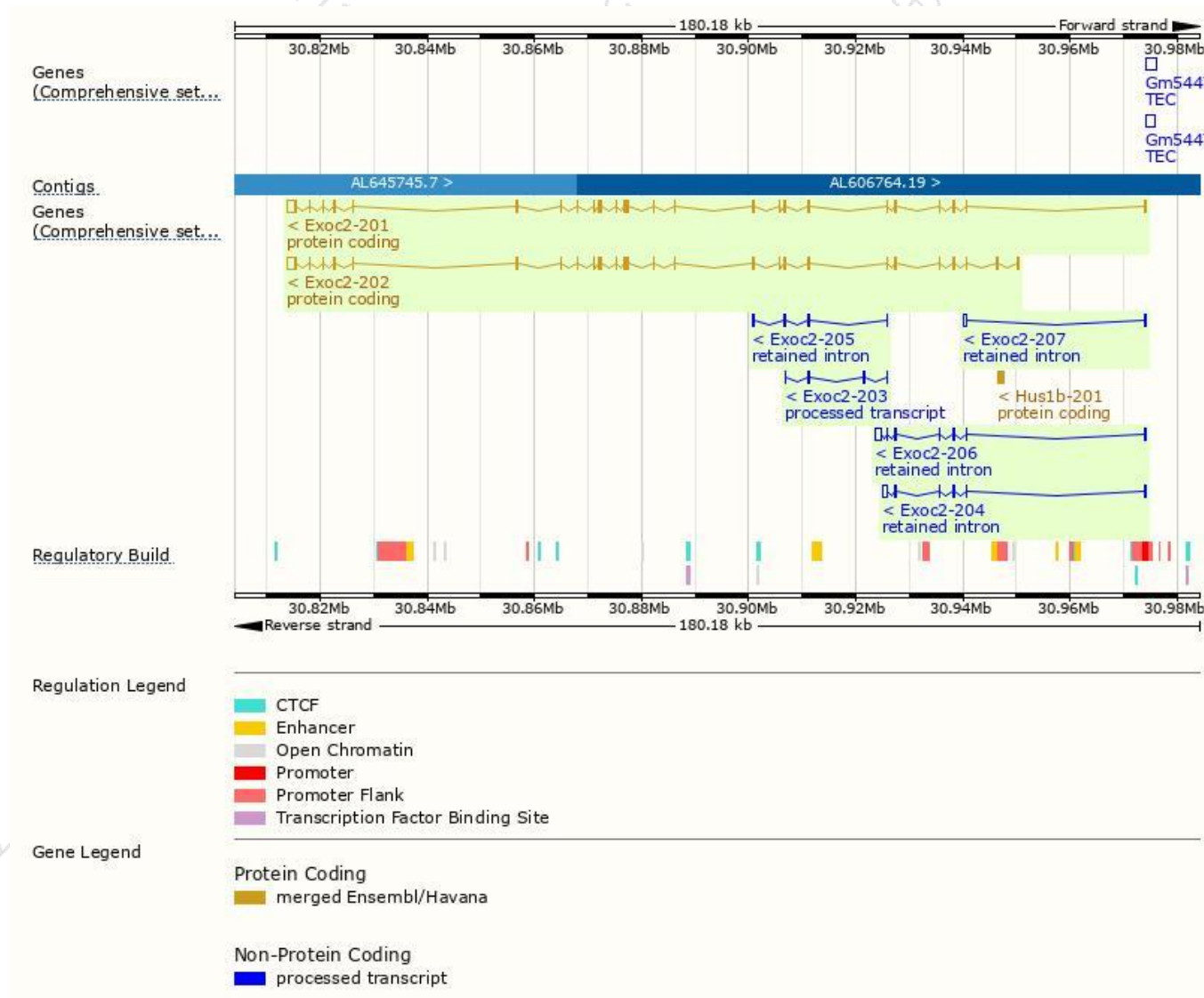|	
Official Symbol	Exoc2 provided by MGI
Official Full Name	exocyst complex component 2 provided by MGI
Primary source	MGI:MGI:1913732
See related	Ensembl:ENSMUSG00000021357
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2410030I24Rik, AI648199, Sec5, Sec5l1
Expression	Ubiquitous expression in CNS E18 (RPKM 9.5), CNS E14 (RPKM 7.9) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Exoc2-202	ENSMUST00000102946.7	4375	924aa	Protein coding	CCDS26420	Q9D4H1	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 P1
Exoc2-201	ENSMUST00000021785.7	4256	924aa	Protein coding	CCDS26420	Q9D4H1	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 P1
Exoc2-203	ENSMUST00000220490.1	543	No protein	Processed transcript	-	-	TSL:3
Exoc2-206	ENSMUST00000222133.1	1787	No protein	Retained intron	-	-	TSL:1
Exoc2-204	ENSMUST00000220532.1	1441	No protein	Retained intron	-	-	TSL:1
Exoc2-205	ENSMUST00000221678.1	688	No protein	Retained intron	-	-	TSL:3
Exoc2-207	ENSMUST00000223216.1	639	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Exoc2-202* transcript,The transcription is shown below
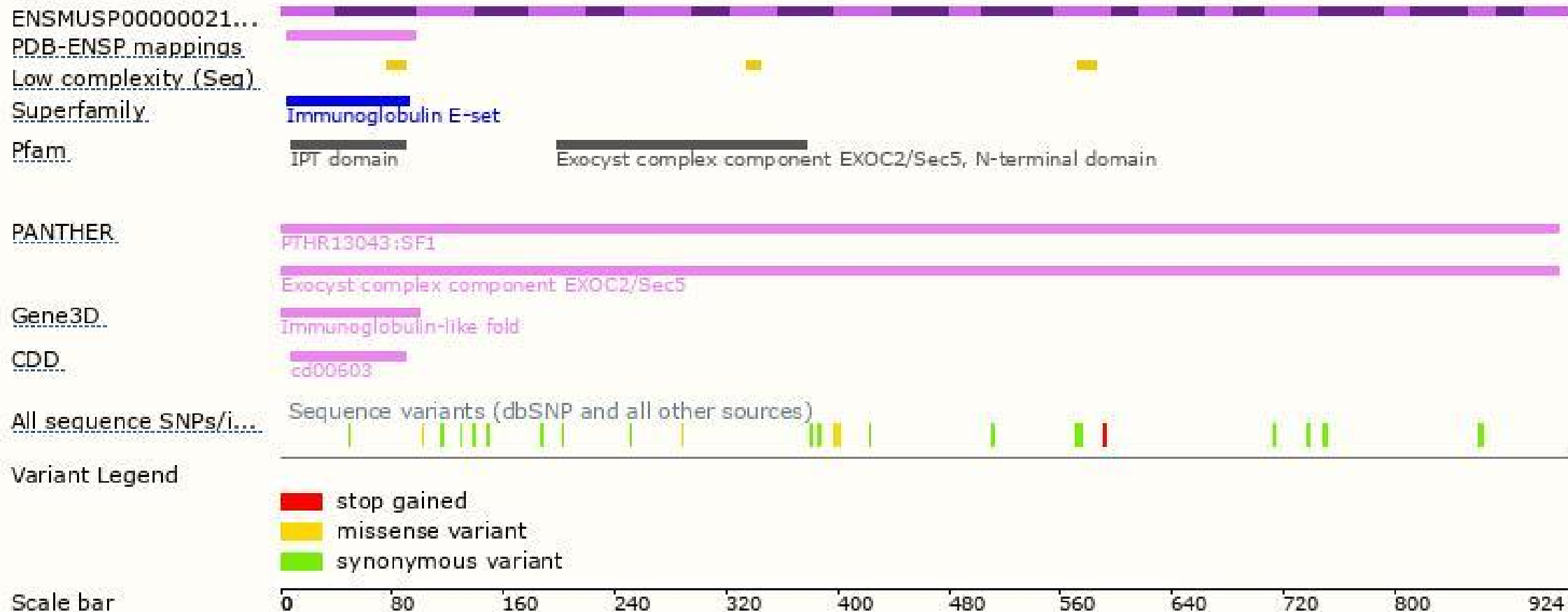

< Exoc2-202
protein coding

◄ Reverse strand ─────────────────── 136.38 kb ───────────────

Genomic location distribution

Protein domain

ENSMUSP00000021...	
PDB-ENSP mappings	
Low complexity (Seg)	
Superfamily	Immunoglobulin E-set
Pfam	IPT domain Exocyst complex component EXOC2/Sec5, N-terminal domain
PANTHER	PTHR13043:SF1
	Exocyst complex component EXOC2/Sec5
Gene3D	Immunoglobulin-like fold
CDD	cd00603
All sequence SNPs/i...	Sequence variants (dbSNP and all other sources)
Variant Legend	

Variant Legend

- ▇ stop gained
- ▇ missense variant
- ▇ synonymous variant

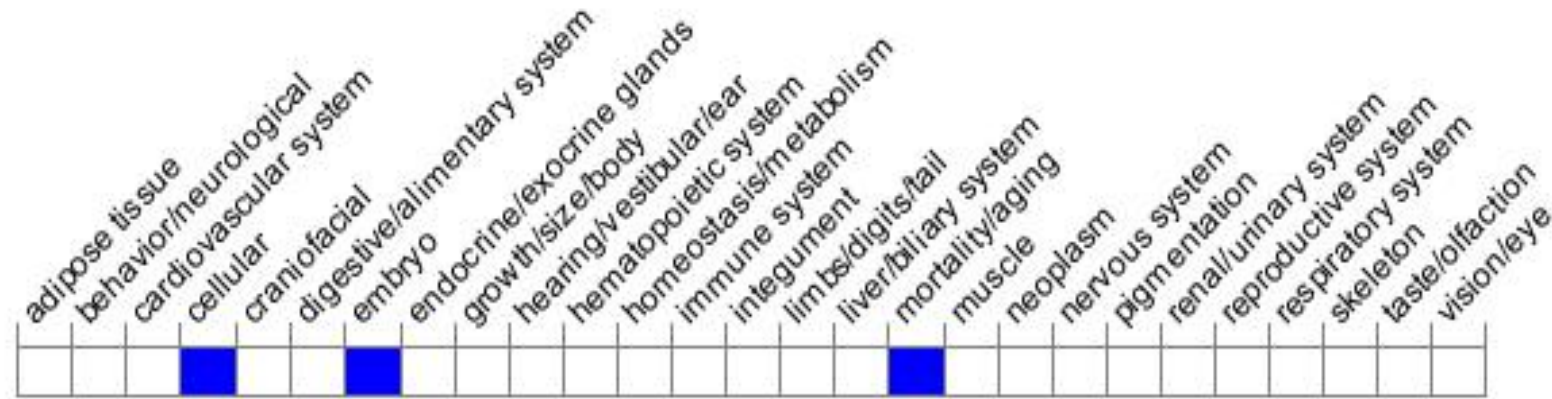Scale bar   0   80   160   240   320   400   480   560   640   720   800   924

Mouse phenotype description(MGI)

Phenotype Overview

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,Mice homozygous for a knock-out allele show complete embryonic lethality between implantation and somite formation and failure of blastocysts to hatch from the zona pellucida with increased cell death during outgrowth culture.

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