

Pum2 Cas9-CKO Strategy

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Overview

Target Gene Name

- *Pum2*

Project Type

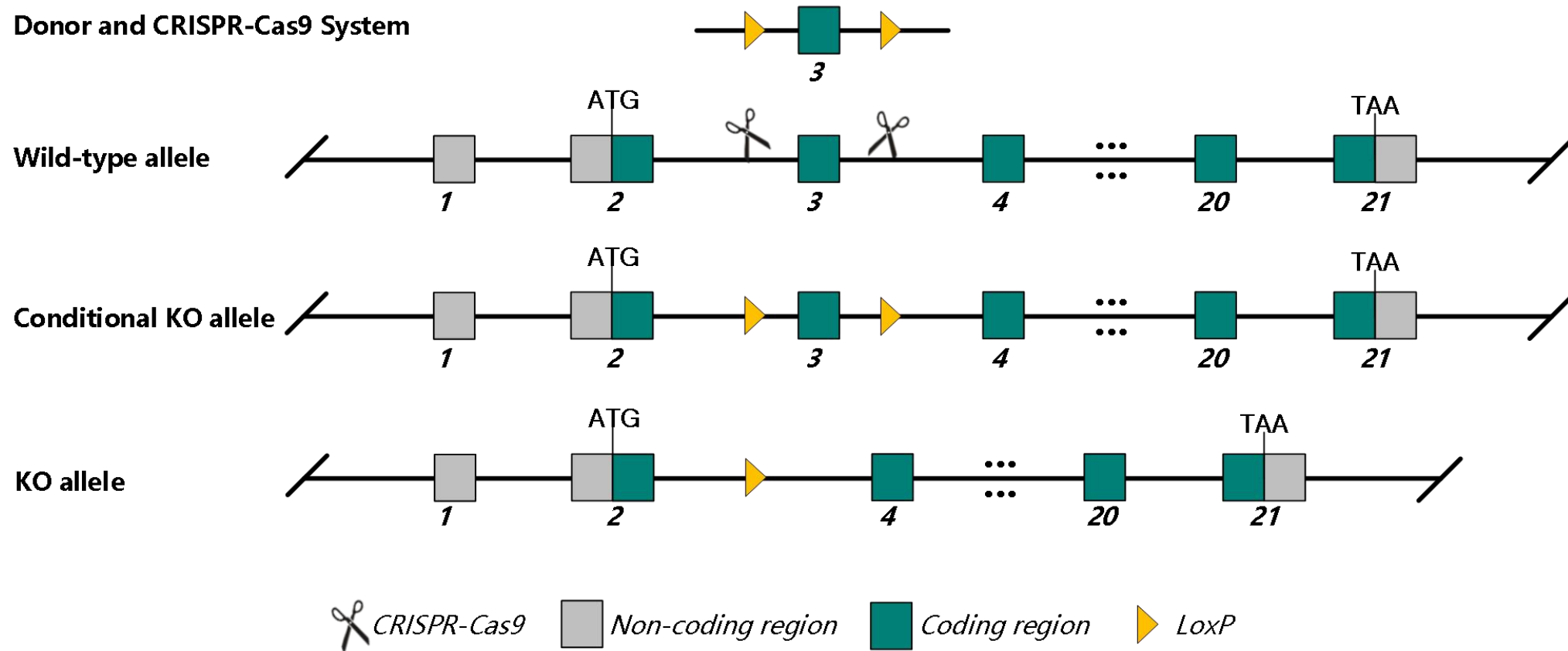
- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy

Donor and CRISPR-Cas9 System



Schematic representation of CRISPR-Cas9 engineering used to edit the *Pum2* gene.

Technical Information

- The *Pum2* gene has 16 transcripts. According to the structure of *Pum2* gene, exon 3 of *Pum2*-204 (ENSMUST00000163569.8) is recommended as the knockout region. The region contains 109 bp of coding sequence. Knocking out the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Pum2* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

Gene Information

Pum2 pumilio RNA-binding family member 2 [*Mus musculus* (house mouse)]

[Download Datasets](#)

Gene ID: 80913, updated on 21-Jun-2023

Summary

Official Symbol Pum2 provided by [MGI](#)

Official Full Name pumilio RNA-binding family member 2 provided by [MGI](#)

Primary source [MGI:MGI:1931751](#)

See related [Ensembl:ENSMUSG00000020594](#) [AllianceGenome:MGI:1931751](#)

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 Pumm2; 5730503J23Rik

Summary Enables RNA binding activity. Acts upstream of or within posttranscriptional regulation of gene expression and stress granule assembly. Located in cytoplasmic stress granule. Is expressed in several structures, including adipose tissue; alimentary system; immune system; nervous system; and reproductive system. Orthologous to human PUM2 (pumilio RNA binding family member 2). [provided by Alliance of Genome Resources, Apr 2022]

Expression Ubiquitous expression in thymus adult (RPKM 23.4), whole brain E14.5 (RPKM 23.1) and 28 other tissues [See more](#)

Orthologs [human](#) [all](#)

NEW

Try the new [Gene table](#)
Try the new [Transcript table](#)

Genomic context

Location: 12; 12 A1.1

Exon count: 27

See Pum2 in [Genome Data Viewer](#)

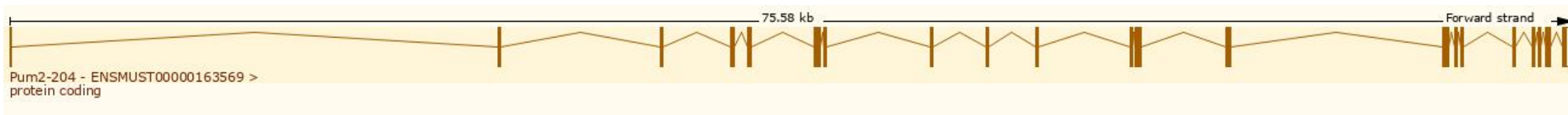
<https://www.ncbi.nlm.nih.gov/gene/80913>

Transcript Information

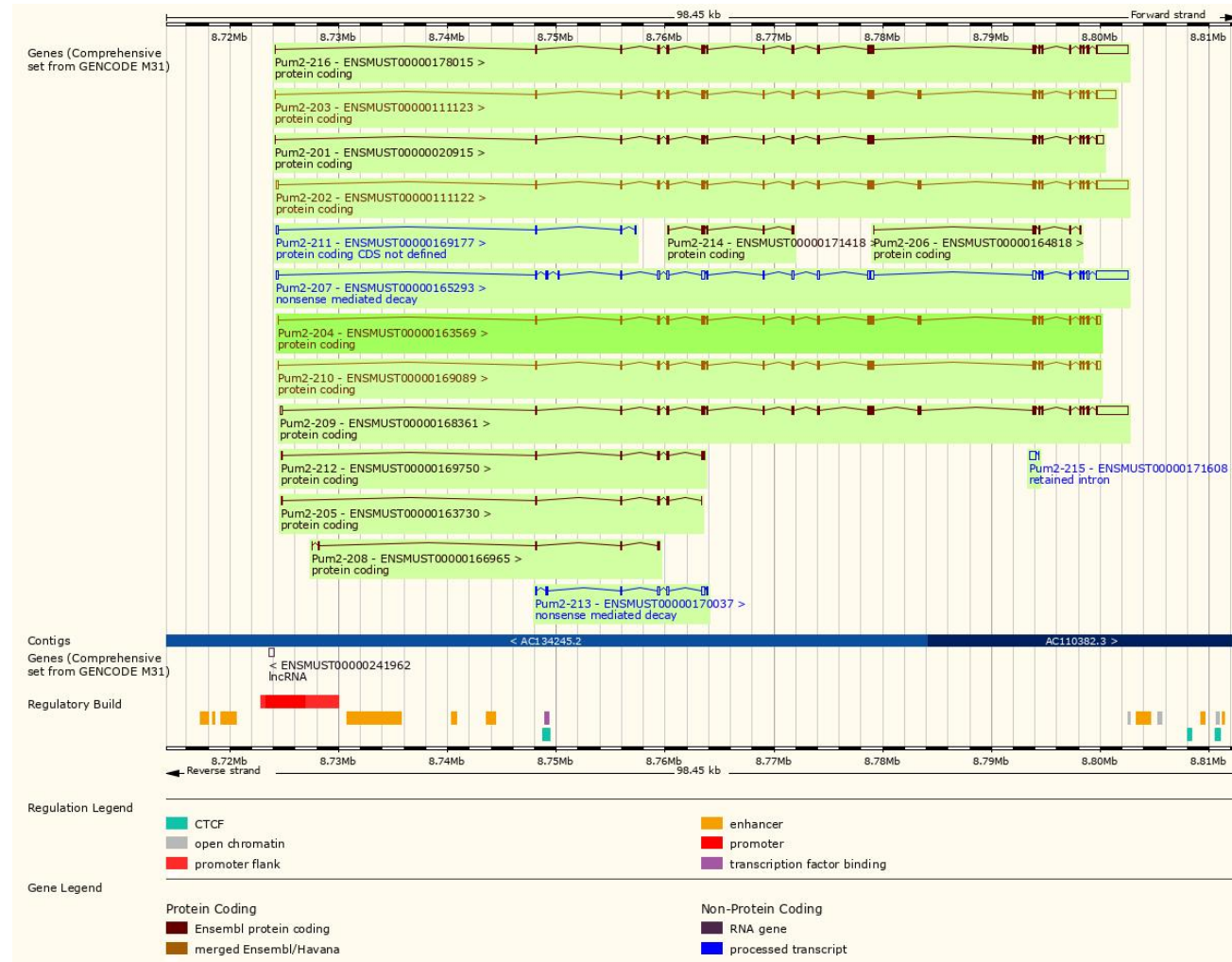
The gene has 16 transcripts, all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST0000020915.10	Pum2-201	3587	980aa	Protein coding	CCDS79105	Q3TQ29	GENCODE basic APPRIS ALT1 TSL:1
ENSMUST00000111122.9	Pum2-202	6311	1064aa	Protein coding	CCDS49025	Q3UR91	GENCODE basic APPRIS P4 TSL:1
ENSMUST00000111123.9	Pum2-203	4984	1064aa	Protein coding	CCDS49025	Q3UR91	GENCODE basic APPRIS P4 TSL:1
ENSMUST00000163569.8	Pum2-204	3560	1066aa	Protein coding	CCDS25802	Q80U58-1	GENCODE basic APPRIS ALT1 TSL:1
ENSMUST00000163730.8	Pum2-205	648	185aa	Protein coding		E9Q4Q6	TSL:5 CDS 3' incomplete
ENSMUST00000164818.3	Pum2-206	665	222aa	Protein coding		F6YD01	TSL:5 CDS 5' and 3' incomplete
ENSMUST00000165293.8	Pum2-207	6218	35aa	Nonsense mediated decay		E9Q0P1	TSL:1
ENSMUST00000166965.2	Pum2-208	472	109aa	Protein coding		E9Q765	TSL:3 CDS 3' incomplete
ENSMUST00000168361.8	Pum2-209	6226	1066aa	Protein coding	CCDS25802	Q80U58-1	Ensembl Canonical GENCODE basic APPRIS ALT1 TSL:5
ENSMUST00000169089.8	Pum2-210	3295	985aa	Protein coding	CCDS49026	Q80U58-2	GENCODE basic APPRIS ALT1 TSL:5
ENSMUST00000169177.8	Pum2-211	518	No protein	Protein coding CDS not defined		-	TSL:2
ENSMUST00000169750.8	Pum2-212	902	262aa	Protein coding		E9PW51	TSL:5 CDS 3' incomplete
ENSMUST00000170037.2	Pum2-213	994	35aa	Nonsense mediated decay		E9Q0P1	TSL:5
ENSMUST00000171418.3	Pum2-214	706	235aa	Protein coding		F7A034	TSL:3 CDS 5' and 3' incomplete
ENSMUST00000171608.2	Pum2-215	612	No protein	Retained intron		-	TSL:2
ENSMUST00000178015.8	Pum2-216	5874	985aa	Protein coding	CCDS49026	Q80U58-2	GENCODE basic APPRIS ALT1 TSL:5

The strategy is based on the design of *Pum2-204* transcript, the transcription is shown below:

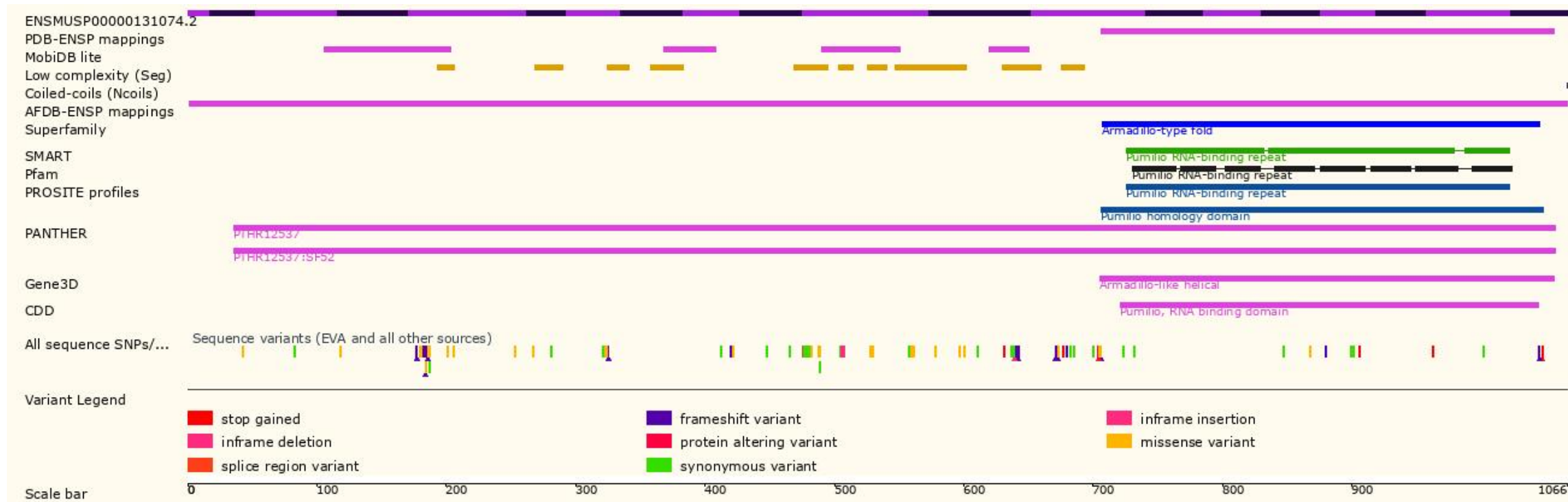


Genomic Information



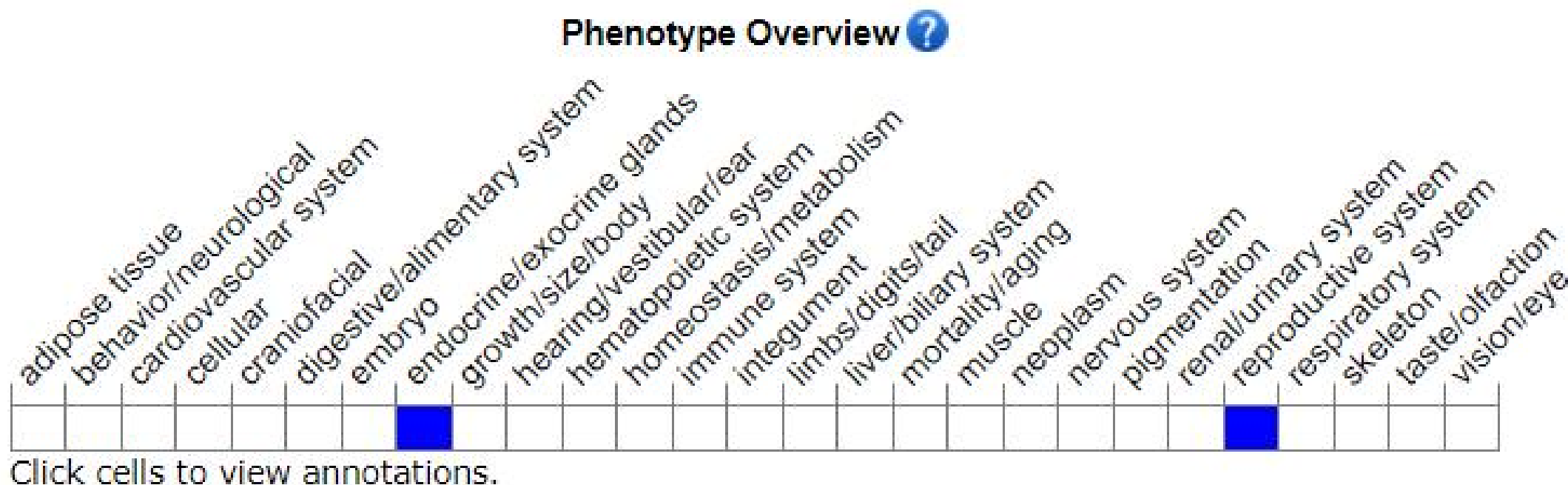
Source: <http://asia.ensembl.org/>

Protein Information



Source: <https://www.ensembl.org>

Mouse Phenotype Information (MGI)

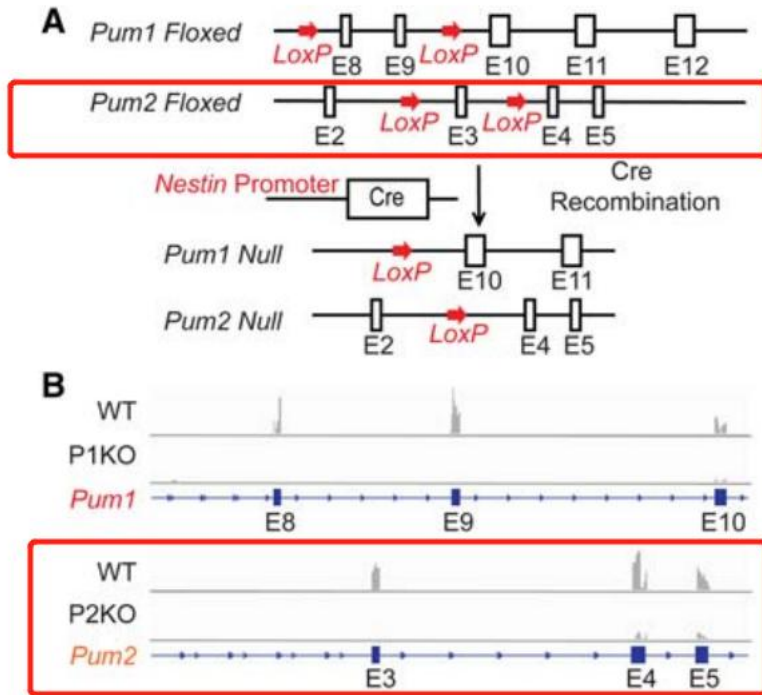


Mice homozygous for a gene trapped allele exhibit significantly smaller testes and seminiferous tubule degeneration but are otherwise viable and fertile.

Important Information

- According to the existing MGI data, mice homozygous for a gene trapped allele exhibit significantly smaller testes and seminiferous tubule degeneration but are otherwise viable and fertile.
- This strategy may not affect *Pum2*-206, *Pum2*-214 and *Pum2*-215 transcript.
- *Pum2* is located on Chr 12. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

Reference



Since *Pum1* and *Pum2* global double knockout mice are lethal by E8.5 (Uyhazi 2012), we generated neural-specific *Pum1* and *Pum2* double conditional knockout mice to study the role of *Pum1* and *Pum2* in neurogenesis. We crossed *Pum1* and *Pum2* double *Flox* mice (*LoxP* sites flanking exons 8 and 9 of *Pum1* and exon 3 of *Pum2*) with *Nestin* promoter-driven *Cre* mice (Fig. 2A; Tronche et al. 1999). *Nestin*-*Cre*-mediated conditional knockout starts from the beginning of neurogenesis and occurs in the entire central nervous system by E15.5 (Dubois et al. 2006). We bred *Pum1*^{+/-};*Pum2*^{+/-};*Nestin-Cre*⁺ with

[1] Zhang M, Chen D, Xia J, Han W, Cui X, Neuenkirchen N, Hermes G, Sestan N, Lin H. Post-transcriptional regulation of mouse neurogenesis by Pumilio proteins. *Genes Dev.* 2017 Jul 1;31(13):1354-1369. doi: 10.1101/gad.298752.117. Epub 2017 Aug 9. PMID: 28794184; PMCID: PMC5580656.