Atf2 Cas9-CKO Strategy

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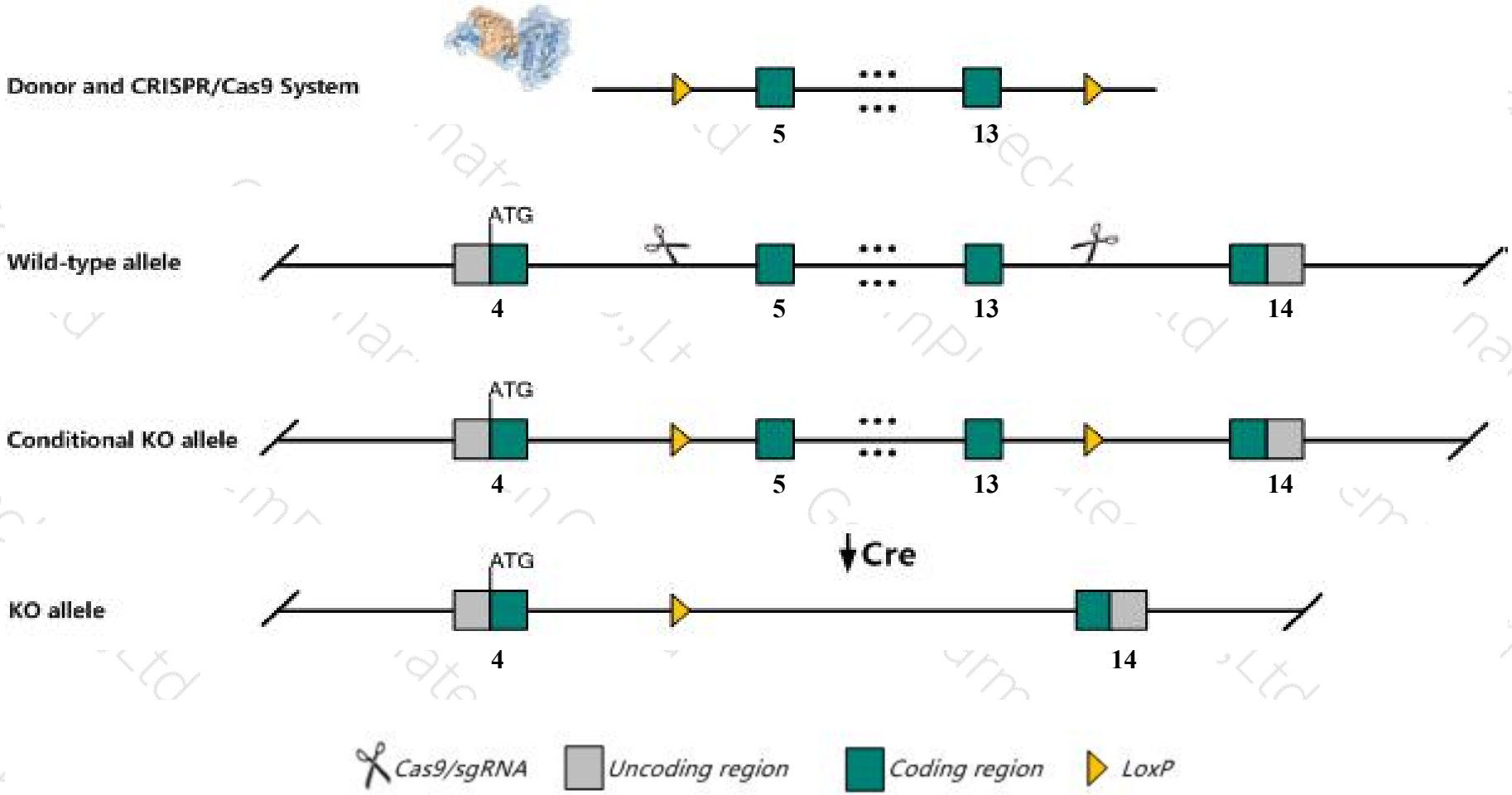
Project Overview

Project Name	***Atf2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Atf2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Atf2* gene has 19 transcripts. According to the structure of *Atf2* gene, exon5-exon13 of *Atf2-201* (ENSMUST00000055833.11) transcript is recommended as the knockout region.The region contains 1189bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Atf2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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,Homozygous mutation of this gene results in increased postnatal lethality, skeletal development defects, runting, decreased hearing, inner ear and brain abnormalities, hyperactivity, and ataxia.

- Transcript *Atf2-209/213/217* may not be affected.

- The *Atf2* gene is located on the Chr2. 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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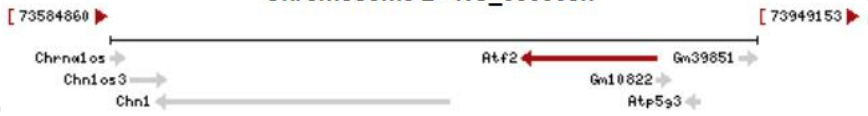
Atf2 activating transcription factor 2 [*Mus musculus* (house mouse)]

Gene ID: 11909, updated on 27-Aug-2019

Summary

Official Symbol	Atf2 provided by MGI
Official Full Name	activating transcription factor 2 provided by MGI
Primary source	MGI:MGI:109349
See related	Ensembl:ENSMUSG00000027104
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	mXBP; Atf-2; Creb2; CRE-BP; D18875; D130078H02Rik; Tg(Gzma-Klra1)7Wum
Expression	Ubiquitous expression in CNS E14 (RPKM 7.9), frontal lobe adult (RPKM 7.8) and 28 other tissues See more
Orthologs	human all

Chromosome 2 - NC_000068.7

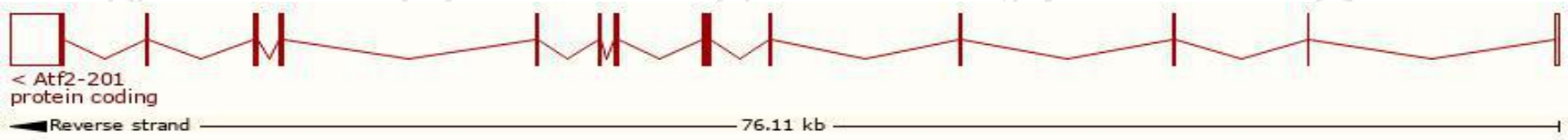


Transcript information（Ensembl）

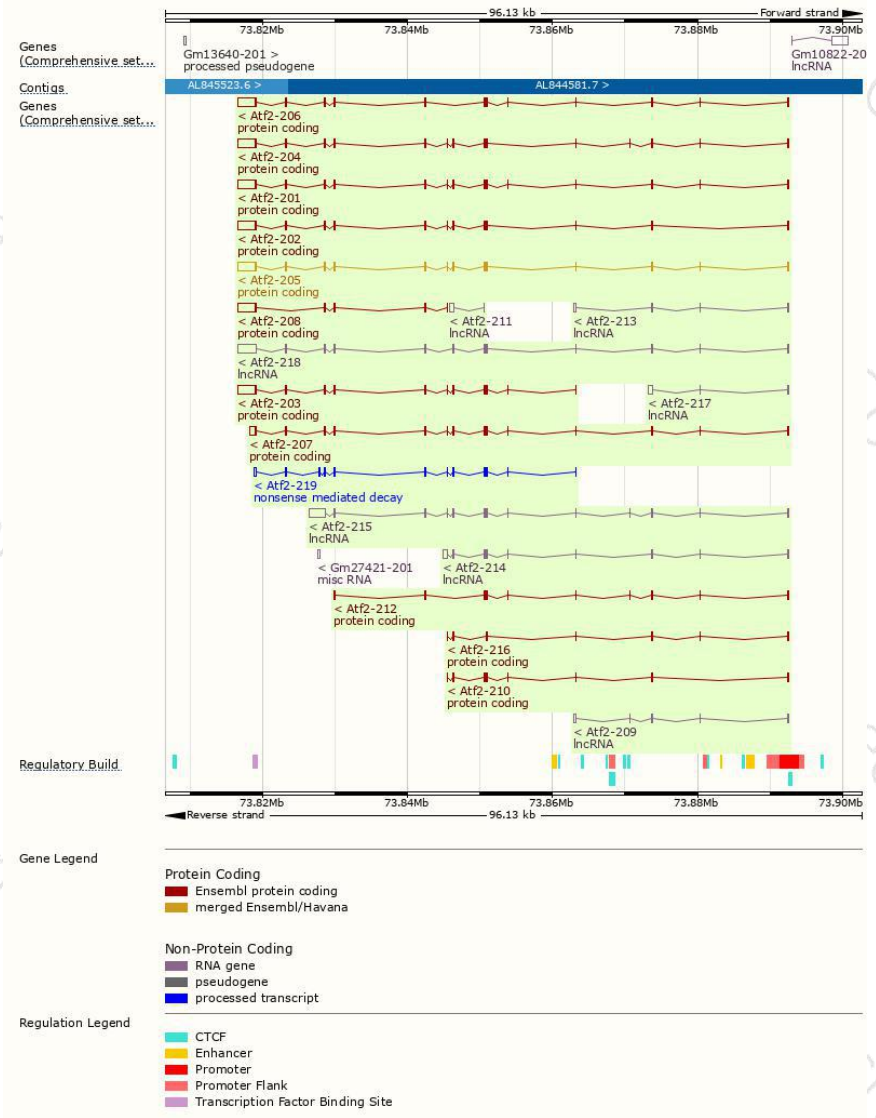
The gene has 19 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Atf2-201	ENSMUST00000055833.11	4210	487aa	ENSMUSP00000058521.5	Protein coding	CCDS16134	P16951	TSL:5 GENCODE basic APPRIS P3
Atf2-204	ENSMUST00000112007.7	4195	447aa	ENSMUSP00000107638.1	Protein coding	CCDS16135	P16951 Q543G2	TSL:1 GENCODE basic
Atf2-205	ENSMUST00000112010.8	4037	447aa	ENSMUSP00000107641.2	Protein coding	CCDS16135	P16951 Q543G2	TSL:1 GENCODE basic
Atf2-202	ENSMUST00000090802.10	4016	447aa	ENSMUSP00000088311.4	Protein coding	CCDS16135	P16951 Q543G2	TSL:5 GENCODE basic
Atf2-206	ENSMUST00000112016.8	3943	389aa	ENSMUSP00000107647.2	Protein coding	CCDS71076	P16951 Q640L6	TSL:1 GENCODE basic APPRIS ALT2
Atf2-203	ENSMUST00000100009.10	3936	487aa	ENSMUSP00000097588.4	Protein coding	CCDS16134	P16951	TSL:1 GENCODE basic APPRIS P3
Atf2-207	ENSMUST00000112017.7	2396	487aa	ENSMUSP00000107648.1	Protein coding	CCDS16134	P16951	TSL:5 GENCODE basic APPRIS P3
Atf2-208	ENSMUST00000124737.7	3151	212aa	ENSMUSP00000114828.1	Protein coding	-	F6Z2B2	CDS 5' incomplete TSL:3
Atf2-212	ENSMUST00000136958.7	935	207aa	ENSMUSP00000118357.1	Protein coding	-	A2AQE9	CDS 3' incomplete TSL:3
Atf2-210	ENSMUST00000128531.7	863	228aa	ENSMUSP00000118560.1	Protein coding	-	A2AQF0	CDS 3' incomplete TSL:3
Atf2-216	ENSMUST00000154456.7	775	146aa	ENSMUSP00000118719.1	Protein coding	-	A2AQF1	CDS 3' incomplete TSL:5
Atf2-219	ENSMUST00000173010.7	1658	377aa	ENSMUSP00000133632.1	Nonsense mediated decay	-	G3UXC3	TSL:5
Atf2-218	ENSMUST00000156455.7	4003	No protein	-	lncRNA	-	-	TSL:1
Atf2-215	ENSMUST00000143714.7	3641	No protein	-	lncRNA	-	-	TSL:1
Atf2-214	ENSMUST00000141050.7	1604	No protein	-	lncRNA	-	-	TSL:1
Atf2-209	ENSMUST00000125159.7	660	No protein	-	lncRNA	-	-	TSL:2
Atf2-211	ENSMUST00000129555.1	658	No protein	-	lncRNA	-	-	TSL:5
Atf2-213	ENSMUST00000138098.7	656	No protein	-	lncRNA	-	-	TSL:2
Atf2-217	ENSMUST00000154965.1	645	No protein	-	lncRNA	-	-	TSL:2

The strategy is based on the design of *Atf2-201* transcript,The transcription is shown below

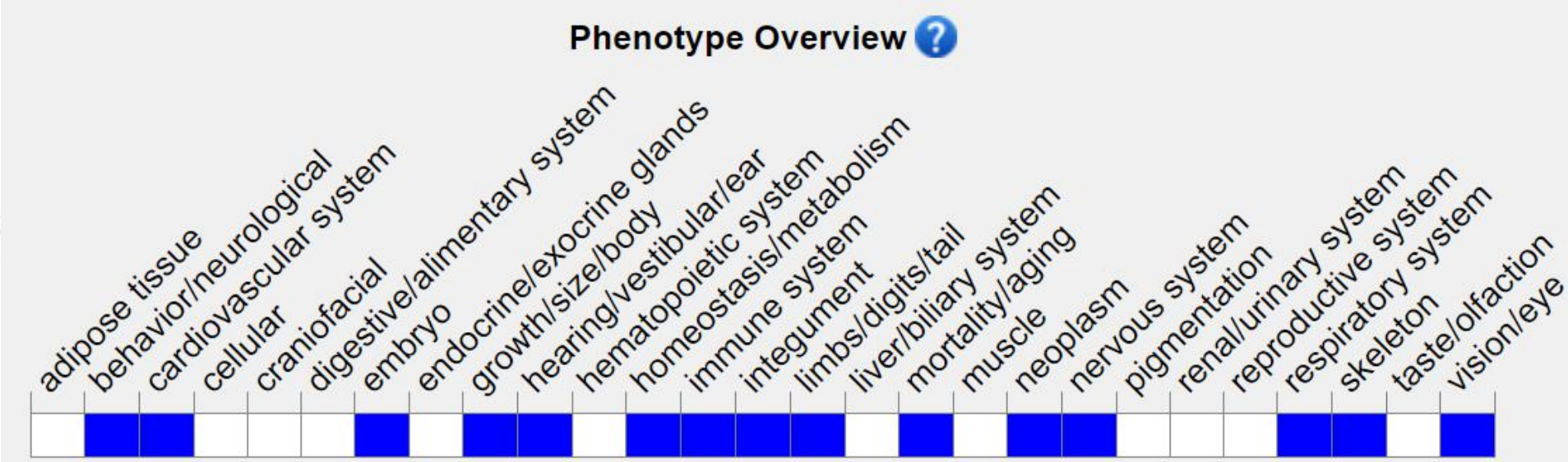


Genomic location distribution



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,Homozygous mutation of this gene results in increased postnatal lethality, skeletal development defects, runting, decreased hearing, inner ear and brain abnormalities, hyperactivity, and ataxia.

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

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