

Aire Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Aire

Project type

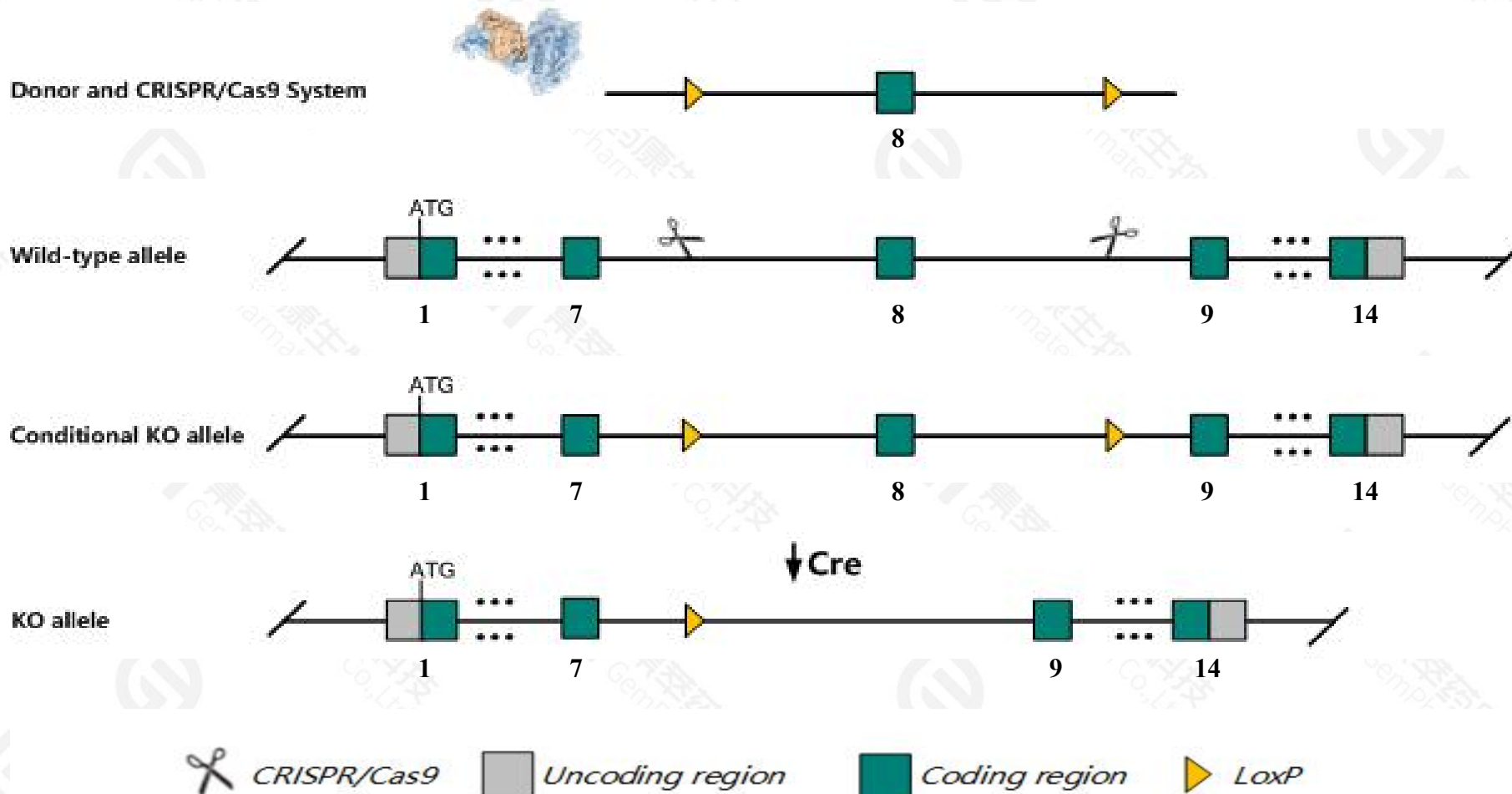
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Aire* gene has 16 transcripts. According to the structure of *Aire* gene, exon8 of *Aire-204*(ENSMUST00000128241.8) transcript is recommended as the knockout region. The region contains 116bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Aire* 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.

- According to the existing MGI data, targeted mutations that inactivate the gene result in immune system dysfunction characterized by multiorgan lymphocytic infiltration and circulating autoantibodies. Whereas one line is fertile, another exhibits male and female sterility.
- This strategy will retain the N-terminal amino acid sequence, and the function is unknown.
- The *Aire* gene is located on the Chr10. 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)

Aire autoimmune regulator (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy) [Mus musculus (house mouse)]

Gene ID: 11634, updated on 13-Mar-2020

Summary

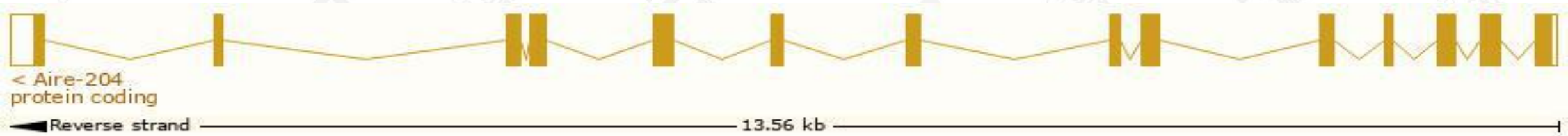
Official Symbol	Aire provided by MGI
Official Full Name	autoimmune regulator (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy) provided by MGI
Primary source	MGI:MGI:1338803
See related	Ensembl:ENSMUSG00000000731
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in thymus adult (RPKM 20.4), spleen adult (RPKM 1.4) and 1 other tissue See more
Orthologs	human all

Transcript information (Ensembl)

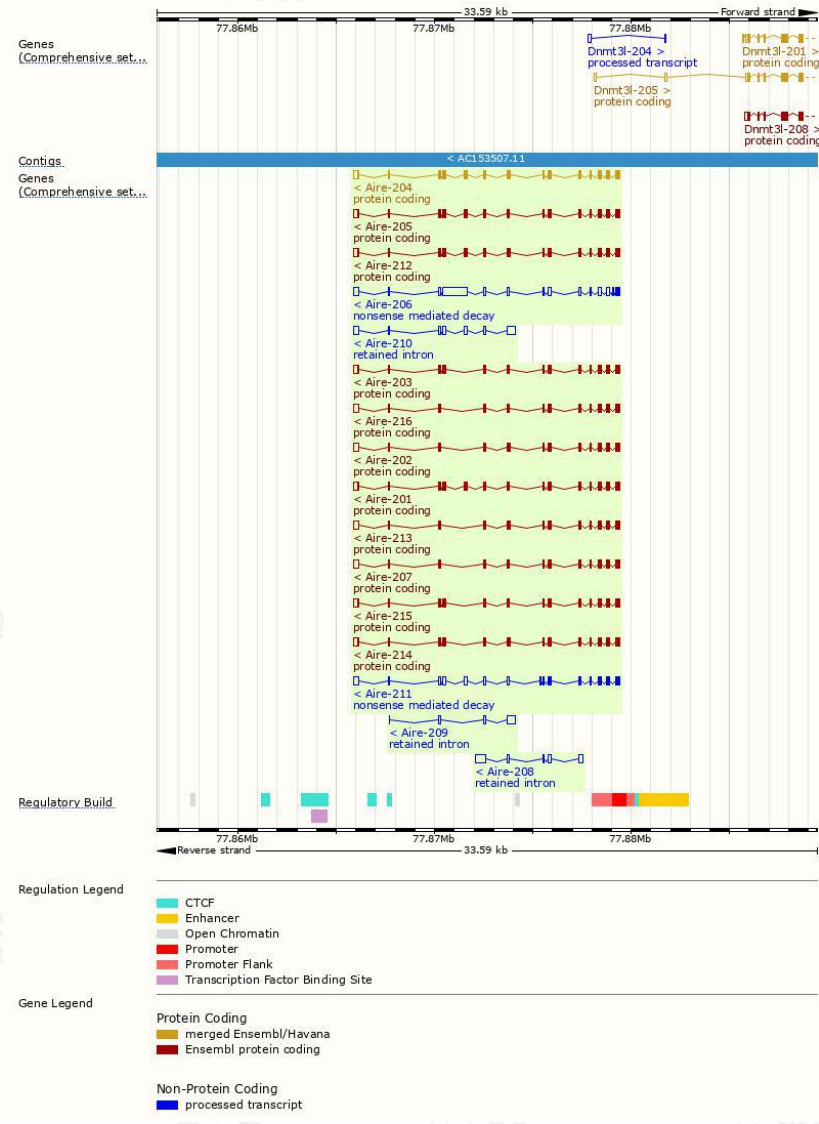
The gene has 16 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Aire-204	ENSMUST00000128241.7	1938	552aa	Protein coding	CCDS23961	B2MVU6 Q9Z0E3	TSL:1 GENCODE basic APPRIS P3
Aire-201	ENSMUST0000019257.14	1926	551aa	Protein coding	CCDS70053	Q3ZB65 Q9Z0E3	TSL:1 GENCODE basic APPRIS ALT2
Aire-212	ENSMUST00000145975.7	1926	548aa	Protein coding	CCDS70060	Q9Z0E3	TSL:1 GENCODE basic APPRIS ALT2
Aire-205	ENSMUST00000130972.7	1923	547aa	Protein coding	CCDS70058	Q9Z0E3	TSL:1 GENCODE basic APPRIS ALT2
Aire-203	ENSMUST00000105396.8	1749	492aa	Protein coding	CCDS70052	Q9Z0E3	TSL:1 GENCODE basic APPRIS ALT2
Aire-215	ENSMUST00000155021.7	1737	488aa	Protein coding	CCDS70057	Q9Z0E3	TSL:1 GENCODE basic APPRIS ALT2
Aire-214	ENSMUST00000154374.1	1712	493aa	Protein coding	CCDS70055	Q3ZB71 Q9Z0E3	TSL:1 GENCODE basic APPRIS ALT2
Aire-202	ENSMUST00000105395.8	1630	409aa	Protein coding	CCDS70054	Q9Z0E3	TSL:1 GENCODE basic
Aire-216	ENSMUST00000156417.7	1627	408aa	Protein coding	CCDS70051	Q9Z0E3	TSL:1 GENCODE basic
Aire-213	ENSMUST00000148469.7	1618	405aa	Protein coding	CCDS70059	Q9Z0E3	TSL:1 GENCODE basic
Aire-207	ENSMUST00000140636.7	1615	404aa	Protein coding	CCDS70056	Q9Z0E3	TSL:1 GENCODE basic
Aire-206	ENSMUST00000131028.7	2966	67aa	Nonsense mediated decay	-	D6RDQ5	TSL:1
Aire-211	ENSMUST00000143735.7	1936	317aa	Nonsense mediated decay	-	E9Q441	TSL:1
Aire-210	ENSMUST00000143548.7	1265	No protein	Retained intron	-	-	TSL:5
Aire-208	ENSMUST00000141255.1	1077	No protein	Retained intron	-	-	TSL:2
Aire-209	ENSMUST00000143452.1	632	No protein	Retained intron	-	-	TSL:3

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



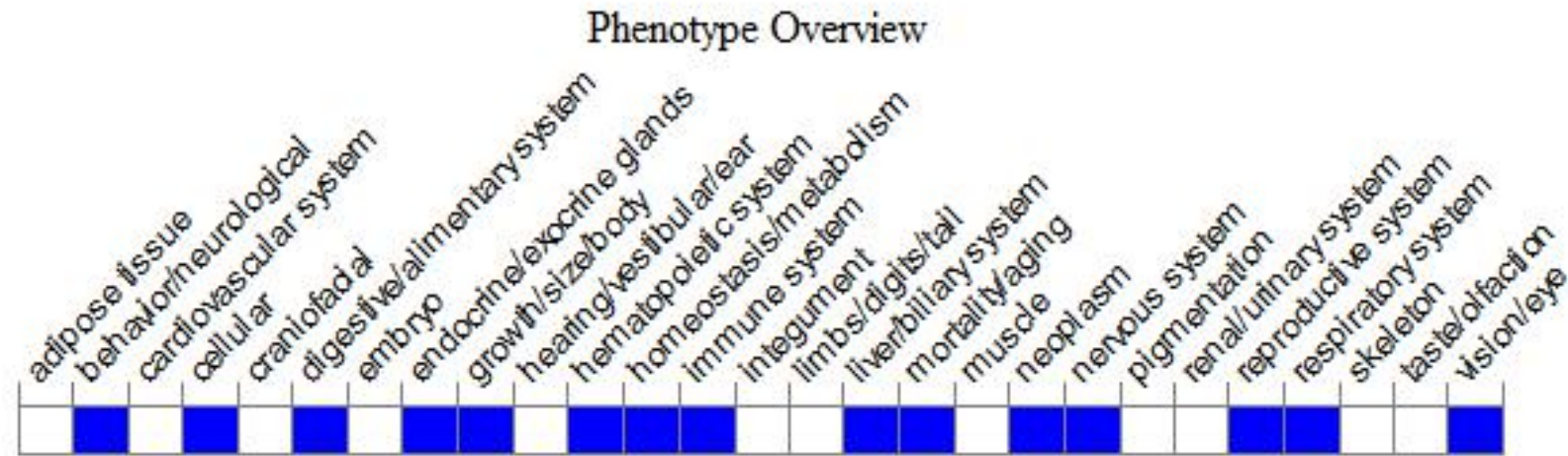
Genomic location distribution



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, targeted mutations that inactivate the gene result in immune system dysfunction characterized by multiorgan lymphocytic infiltration and circulating autoantibodies. Whereas one line is fertile, another exhibits male and female sterility.

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