Pik3cd Cas9-CKO Strategy

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Reviewer: Xueting Zhang

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Project Overview

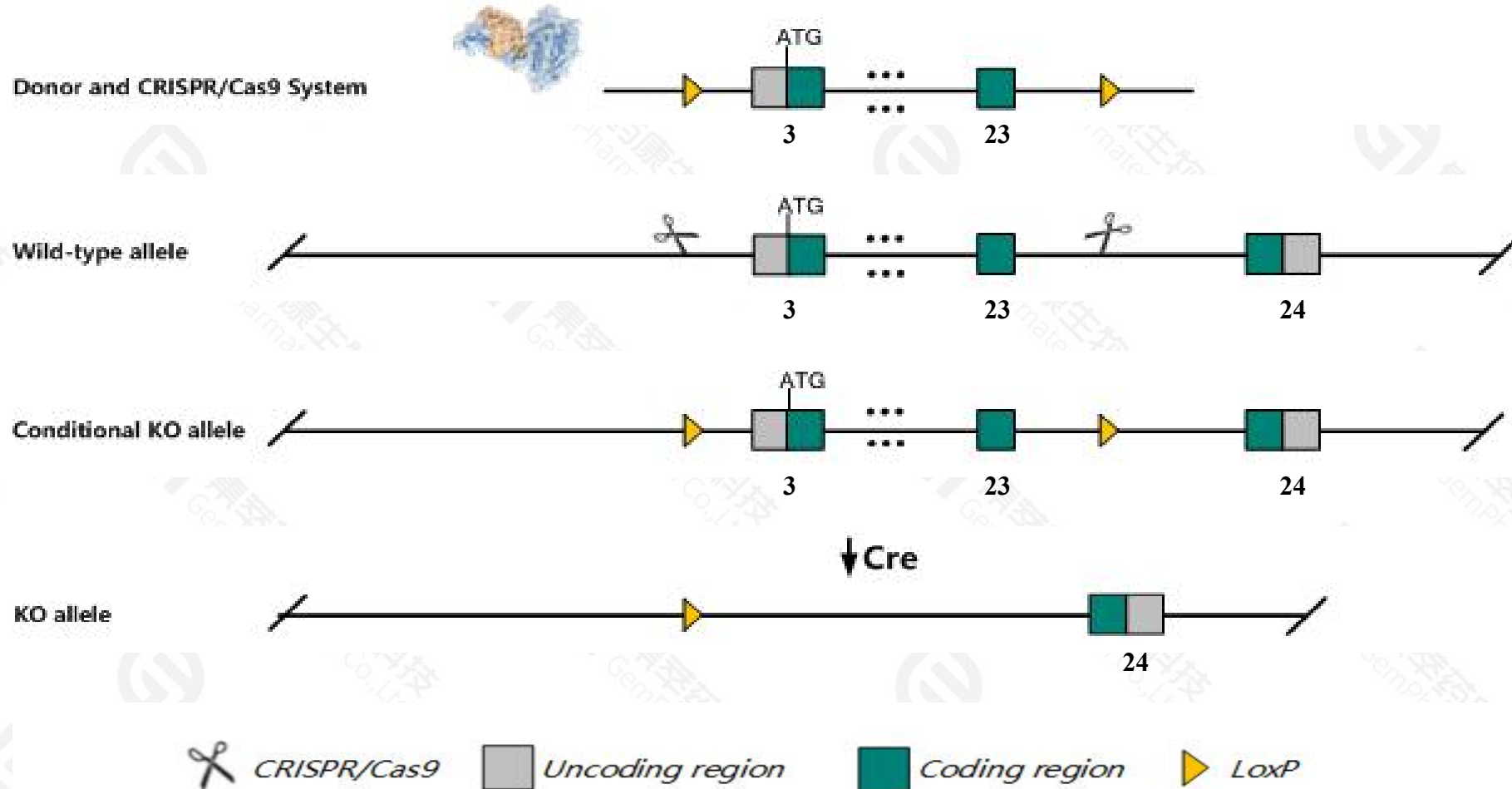|	
Project Name	*Pik3cd*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pik3cd* gene. The schematic diagram is as follows:

Technical routes

➤ The *Pik3cd* gene has 14 transcripts. According to the structure of *Pik3cd* gene, exon3-exon23 of *Pik3cd-204*(ENSMUST00000105690.9) transcript is recommended as the knockout region.The region contains start codon ATG.Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Pik3cd* 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 exhibit impaired B and T cell antigen receptor signaling, reduced or ablated immune responses and decreased immunoglobulin levels. Mutants also develop inflammatory bowel disease.

➢ Knockout the region may affect the function of *Mir7023* gene.

➢ The *Pik3cd* gene is located on the Chr4. 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）

Pik3cd phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta [Mus musculus (house mouse)]

Gene ID: 18707, updated on 13-Mar-2020

▲ Summary

|	
Official Symbol	Pik3cd provided by MGI
Official Full Name	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta provided by MGI
Primary source	MGI:MGI:1098211
See related	Ensembl:ENSMUSG00000039936
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	2410099E07Rik, 2610208K16Rik, AW545373, p110delta
Expression	Broad expression in spleen adult (RPKM 42.3), thymus adult (RPKM 28.3) and 18 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pik3cd-204	ENSMUST00000105690.8	4921	1047aa	Protein coding	CCDS51384	Q3T9Y0	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 P4
Pik3cd-201	ENSMUST00000038859.13	4905	1045aa	Protein coding	CCDS51383	Q8CI98	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 ALT1
Pik3cd-205	ENSMUST00000118704.7	4894	1046aa	Protein coding	CCDS51381	Q3TBW3	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 ALT1
Pik3cd-214	ENSMUST00000177654.7	4765	1047aa	Protein coding	CCDS51384	Q3T9Y0	TSL:5 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 P4
Pik3cd-202	ENSMUST00000105688.9	4335	1044aa	Protein coding	CCDS51382	Q3UDT3	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 ALT1
Pik3cd-203	ENSMUST00000105689.7	3132	1043aa	Protein coding	CCDS51380	O35904	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 ALT1
Pik3cd-206	ENSMUST00000122059.7	4832	1040aa	Protein coding	-	B0QZL5	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 ALT1
Pik3cd-212	ENSMUST00000146612.7	729	117aa	Protein coding	-	B1AS26	CDS 3' incomplete TSL:2
Pik3cd-208	ENSMUST00000127273.7	716	171aa	Protein coding	-	B1AS25	CDS 3' incomplete TSL:3
Pik3cd-209	ENSMUST00000131224.7	699	145aa	Protein coding	-	B1AS24	CDS 3' incomplete TSL:3
Pik3cd-210	ENSMUST00000134534.7	436	62aa	Protein coding	-	B1AS27	CDS 3' incomplete TSL:3
Pik3cd-213	ENSMUST00000156309.1	327	44aa	Protein coding	-	B1AS28	CDS 3' incomplete TSL:3
Pik3cd-211	ENSMUST00000137430.7	824	No protein	Processed transcript	-	-	TSL:2
Pik3cd-207	ENSMUST00000124510.1	404	No protein	Processed transcript	-	-	TSL:2

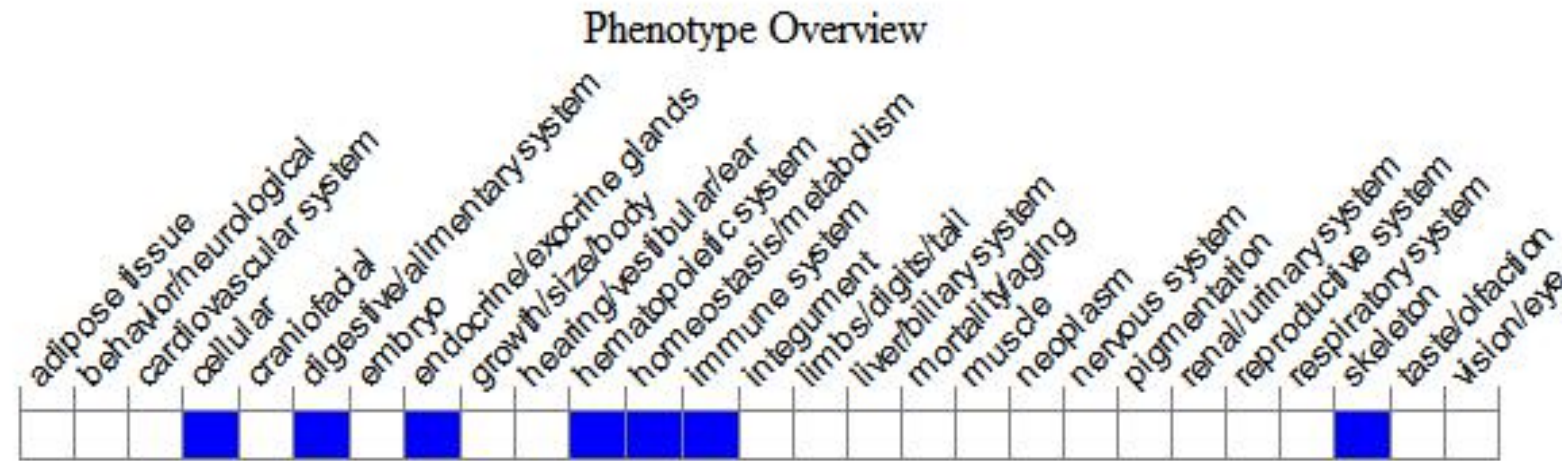The strategy is based on the design of *Pik3cd-204* transcript,the transcription is shown below:

< Pik3cd-204
protein coding

◄ Reverse strand

49.54 kb
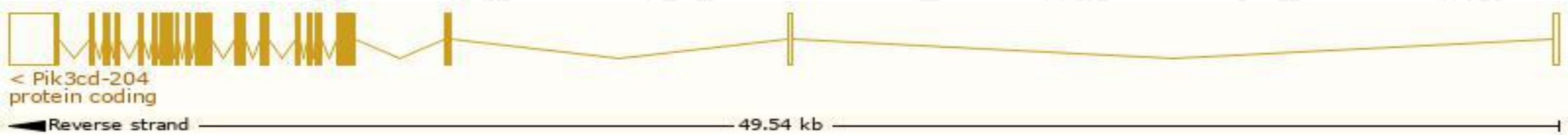
Genomic location distribution
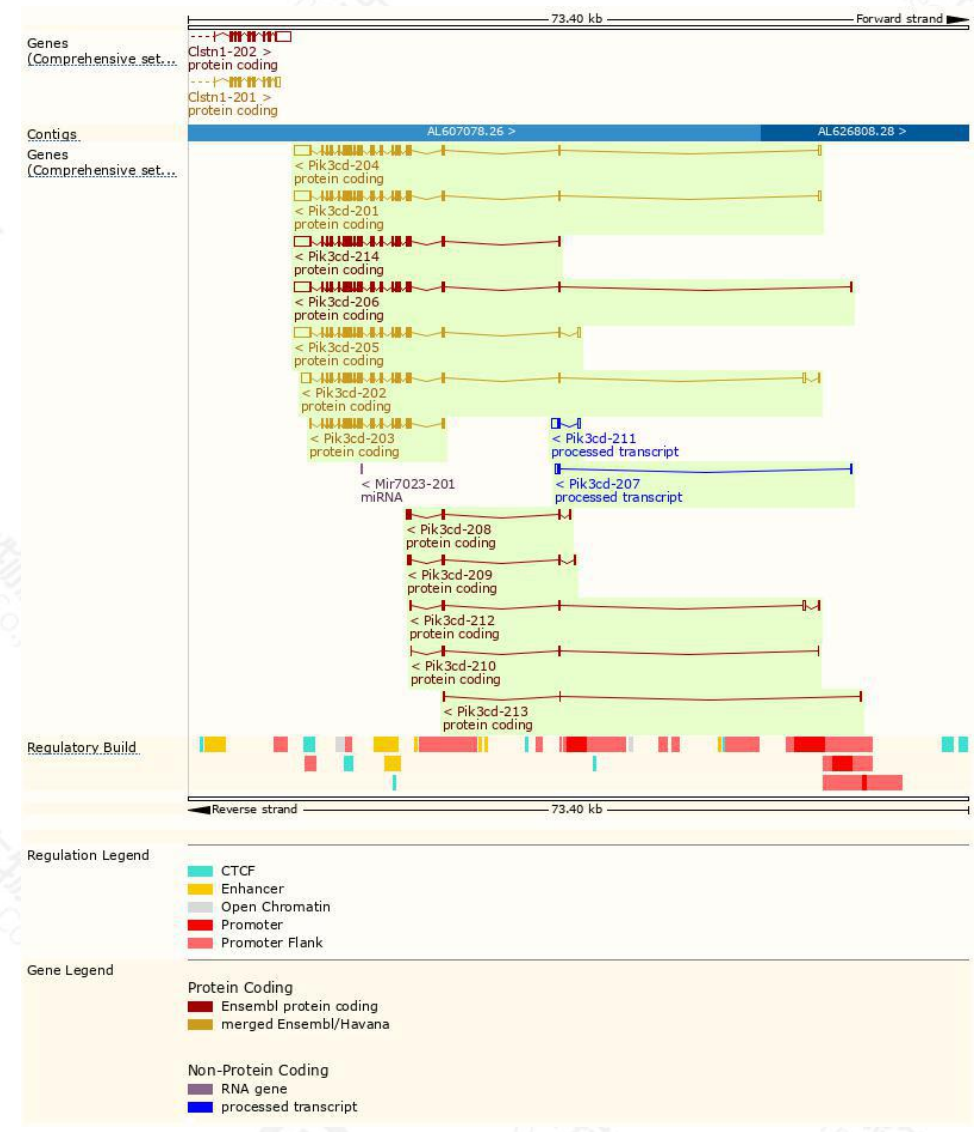
Protein domain
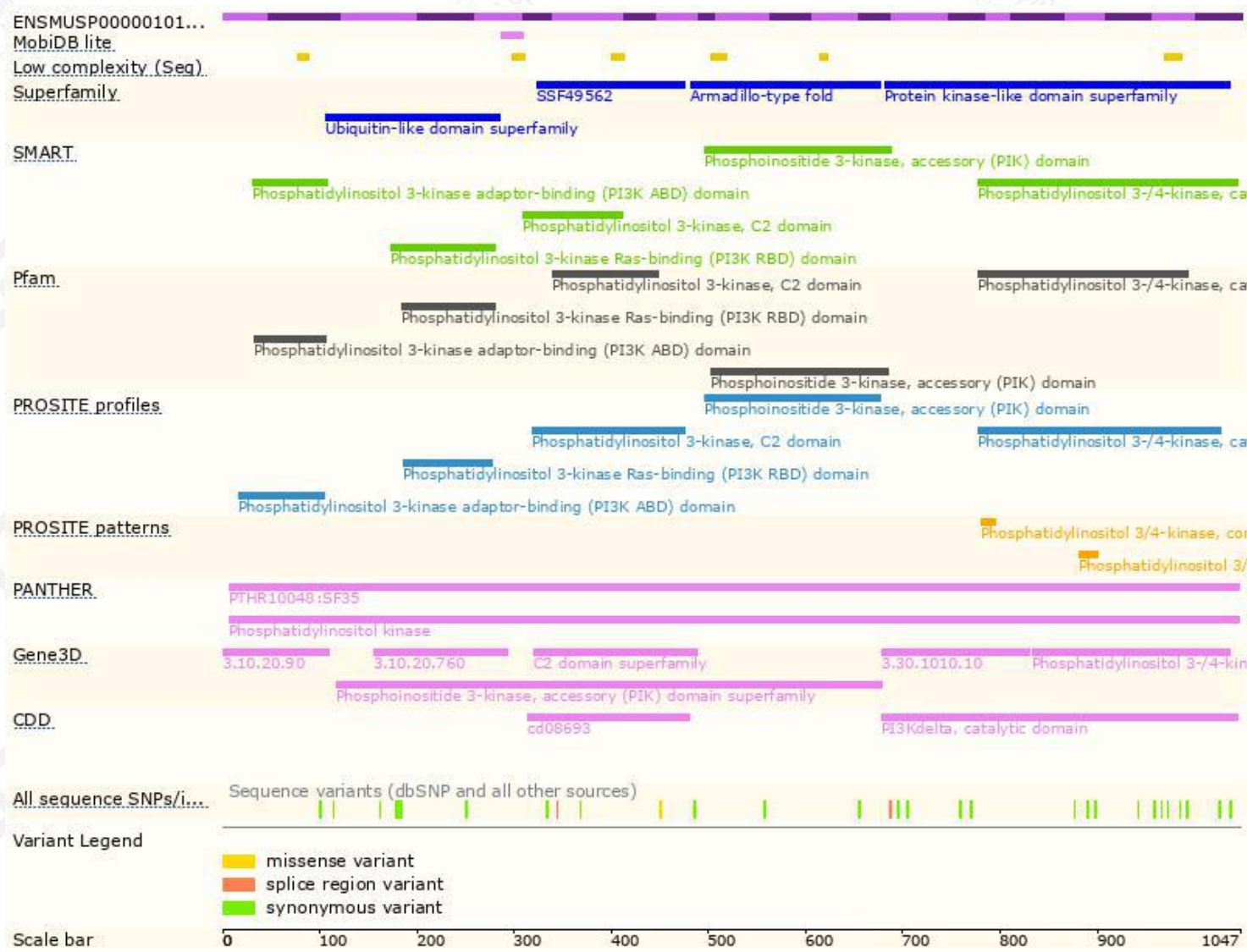
Mouse phenotype description(MGI)

Phenotype Overview

Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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