Rspo2 Cas9-CKO Strategy

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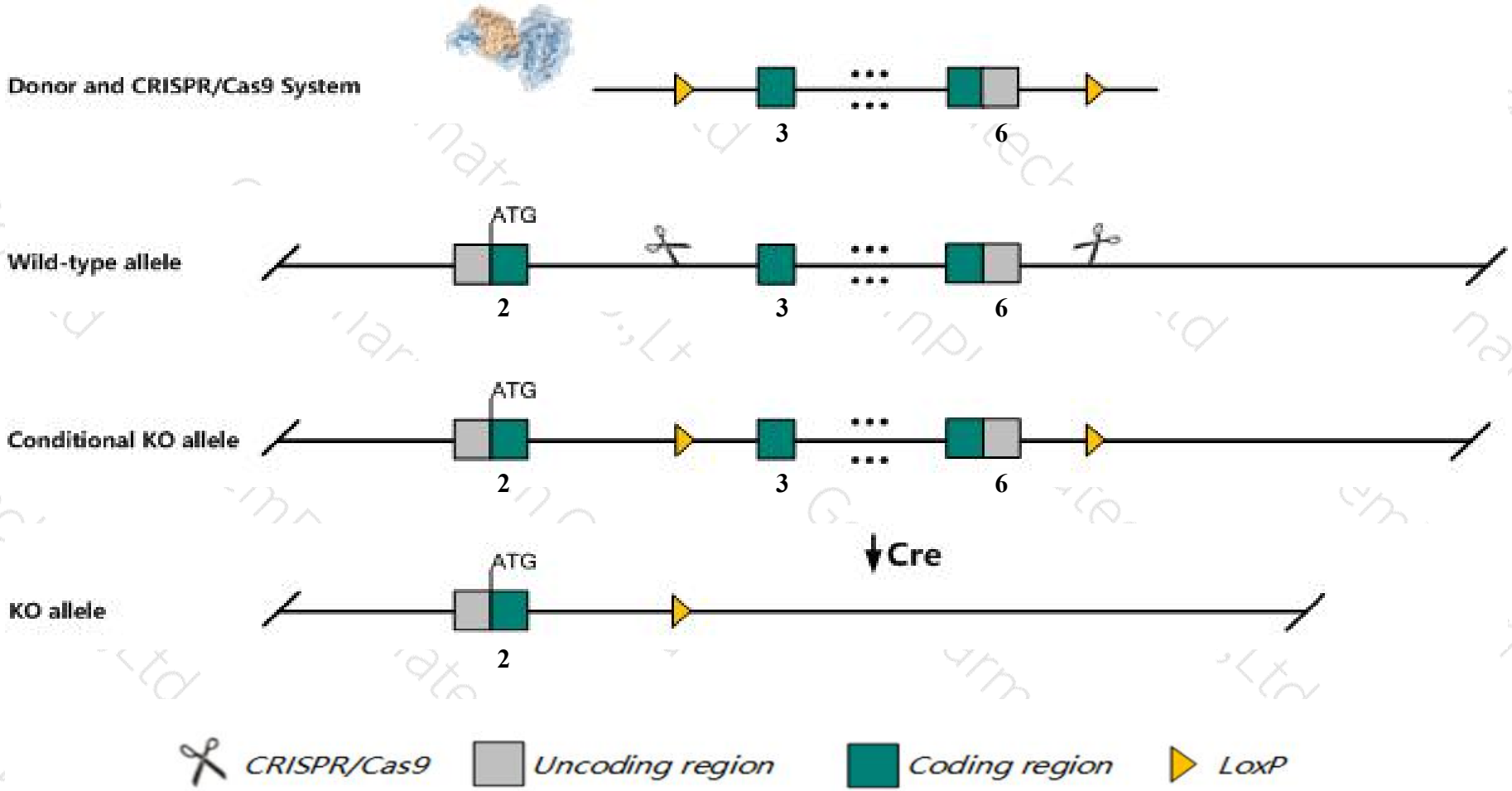
Project Overview

Project Name	***Rspo2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rspo2* gene. The schematic diagram is as follows:



Technical routes

- The *Rspo2* gene has 3 transcripts. According to the structure of *Rspo2* gene, exon3-exon6 of *Rspo2-201* (ENSMUST00000063492.7) transcript is recommended as the knockout region.The region contains most of coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rspo2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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,Homozygous mice display perinatal lethality, cleft palate, lung hypoplasia, asymmetric limb malformations and abnormal renal development. Heterozygous females display reduced fertility with age.

- The *Rspo2* gene is located on the Chr15. 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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Rspo2 R-spondin 2 [Mus musculus (house mouse)]

Gene ID: 239405, updated on 19-Mar-2019

Summary

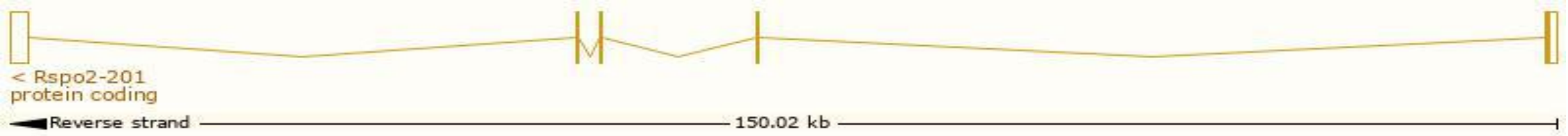
Official Symbol	Rspo2 provided by MGI
Official Full Name	R-spondin 2 provided by MGI
Primary source	MGI:MGI:1922667
See related	Ensembl:ENSMUSG00000051920
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2610028F08Rik, AA673245, D430027K22, ftls
Expression	Biased expression in CNS E11.5 (RPKM 3.5), CNS E18 (RPKM 1.5) and 8 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

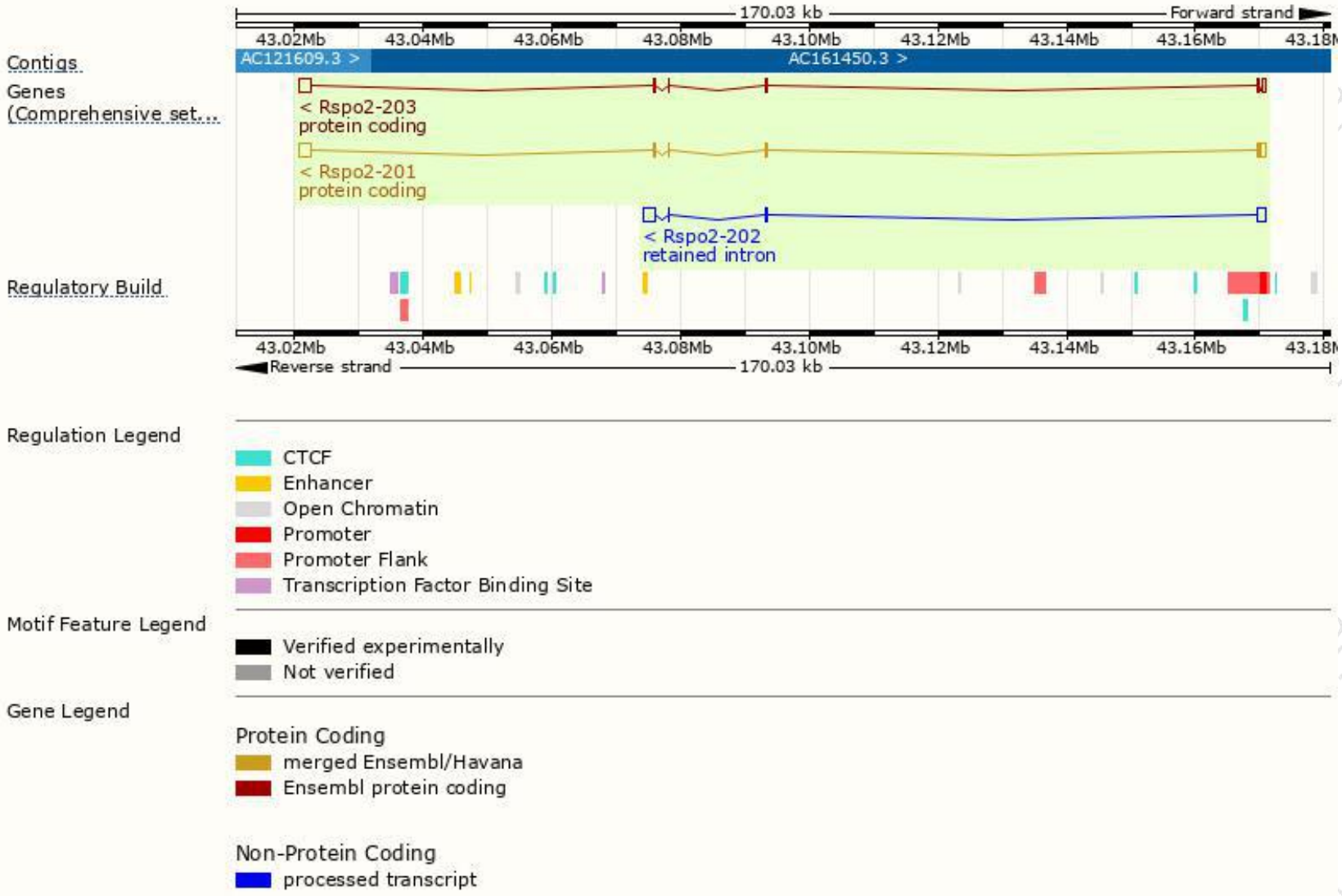
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rspo2-201	ENSMUST00000063492.7	3338	243aa	Protein coding	CCDS27451	Q8BFU0	TSL:1 GENCODE basic APPRIS P1
Rspo2-203	ENSMUST00000226810.1	3091	243aa	Protein coding	CCDS27451	Q8BFU0	GENCODE basic APPRIS P1
Rspo2-202	ENSMUST00000226402.1	3259	No protein	Retained intron	-	-	

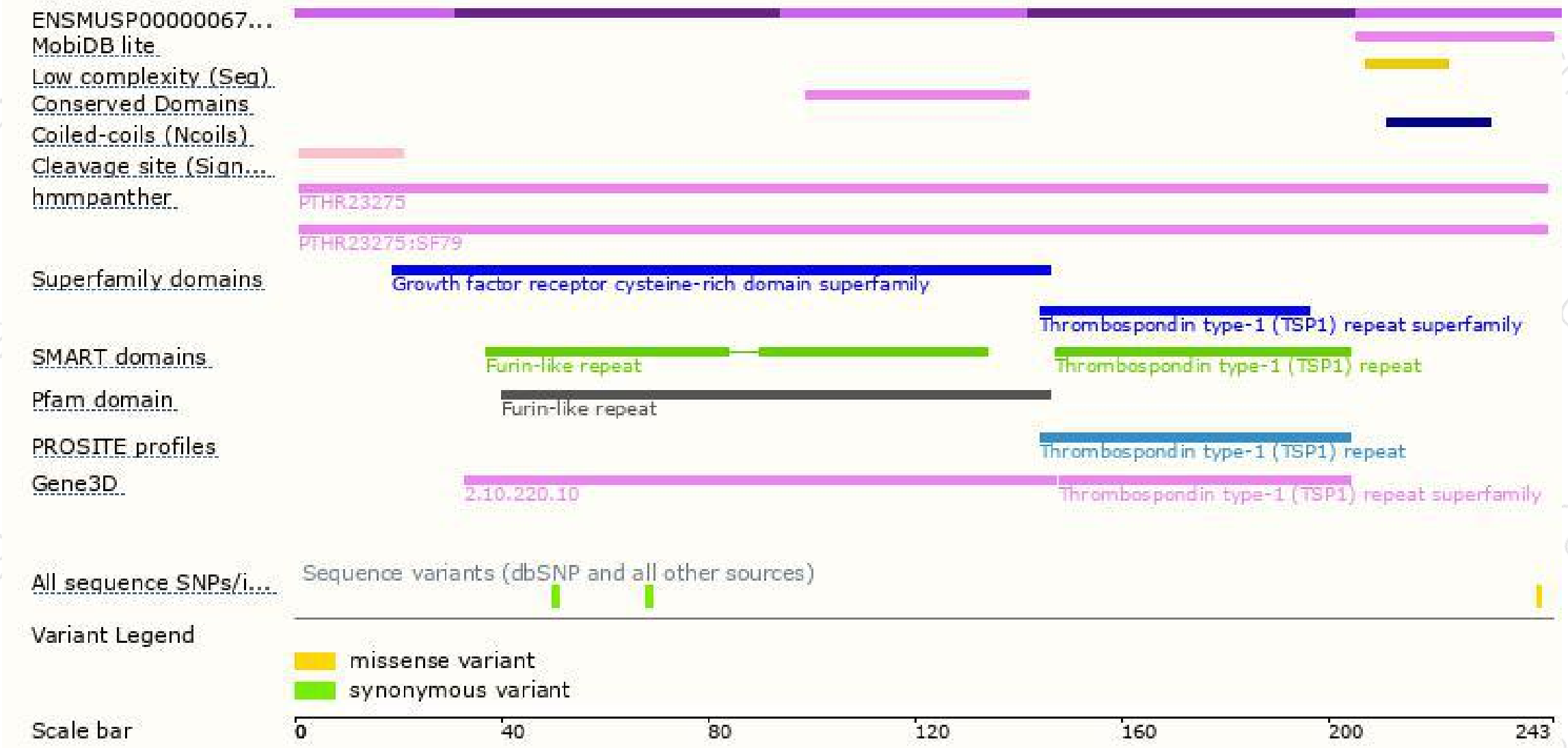
The strategy is based on the design of *Rspo2-201* transcript,The transcription is shown below



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 mice display perinatal lethality, cleft palate, lung hypoplasia, asymmetric limb malformations and abnormal renal development. Heterozygous females display reduced fertility with age.

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

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