Dmd Cas9-KO Strategy

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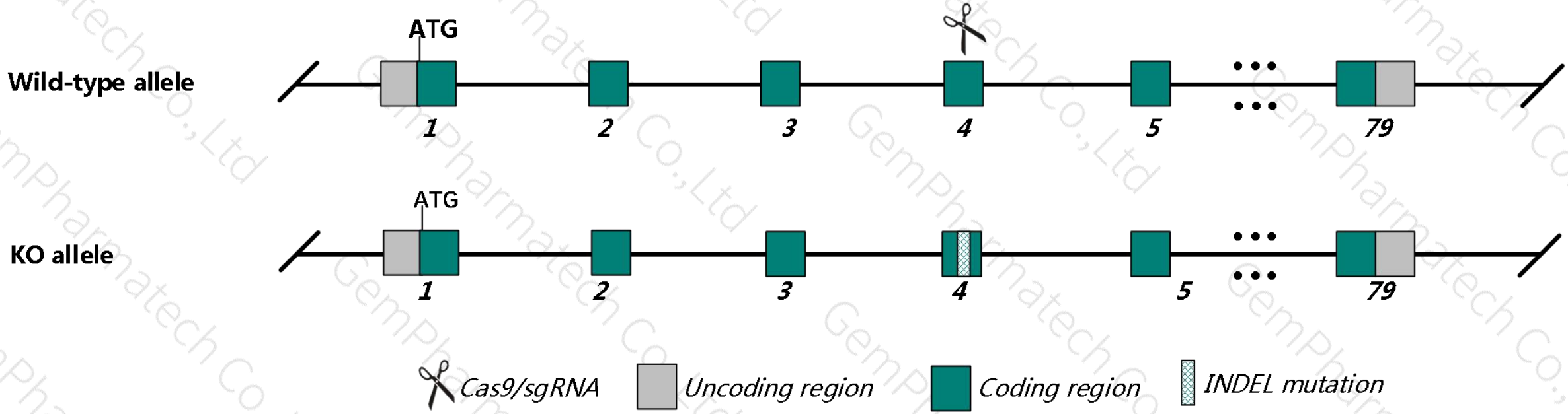
Project Overview

Project Name	***Dmd***
Project type	**Cas9-KO**
Strain background	**C57BL/10ScSnJ**

Knockout strategy

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



Technical routes

➢ The *Dmd* gene has 14 transcripts. According to the structure of *Dmd* gene, part of exon4 of *Dmd-203* (ENSMUST00000114000.7) transcript is recommended as the knockout region. The INDEL mutation on Exon4 will result in frameshift mutation of *Dmd* gene. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Dmd* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/10ScSnJ 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/10ScSnJ mice.

Notice

- According to the existing MGI data,Mutations in this gene cause muscular dystrophy. Phenotypic variation has been observed in different backgrounds.

- The *Dmd* gene is located on the ChrX. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Dmd dystrophin, muscular dystrophy [Mus musculus (house mouse)]

Gene ID: 13405, updated on 9-Apr-2019

Summary

Official Symbol	Dmd provided by MGI
Official Full Name	dystrophin, muscular dystrophy provided by MGI
Primary source	MGI:MGI:94909
See related	Ensembl:ENSMUSG00000045103
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	DXSmh7, DXSmh9, Dp427, Dp71, dys, mdx, pke
Summary	This gene encodes a large, rod-like cytoskeletal protein which is found at the inner surface of muscle fibers in skeletal and cardiac muscles. The encoded protein, dystrophin, is part of the dystrophin-glycoprotein complex, which bridges the inner cytoskeleton (F-actin) and the extra-cellular matrix. This protein is required for proper development and organization of myofibers as contractile units in striated muscles. Mutations in the human gene cause Duchenne and Becker Muscular Dystrophies and a form of heart disease called DMD-associated dilated cardiomyopathy. Several alternatively spliced transcript variants of this gene have been described, but the full-length nature of some of these variants has not been determined. [provided by RefSeq, Sep 2015]
Expression	Broad expression in bladder adult (RPKM 4.4), frontal lobe adult (RPKM 2.2) and 23 other tissuesSee more
Orthologs	human all

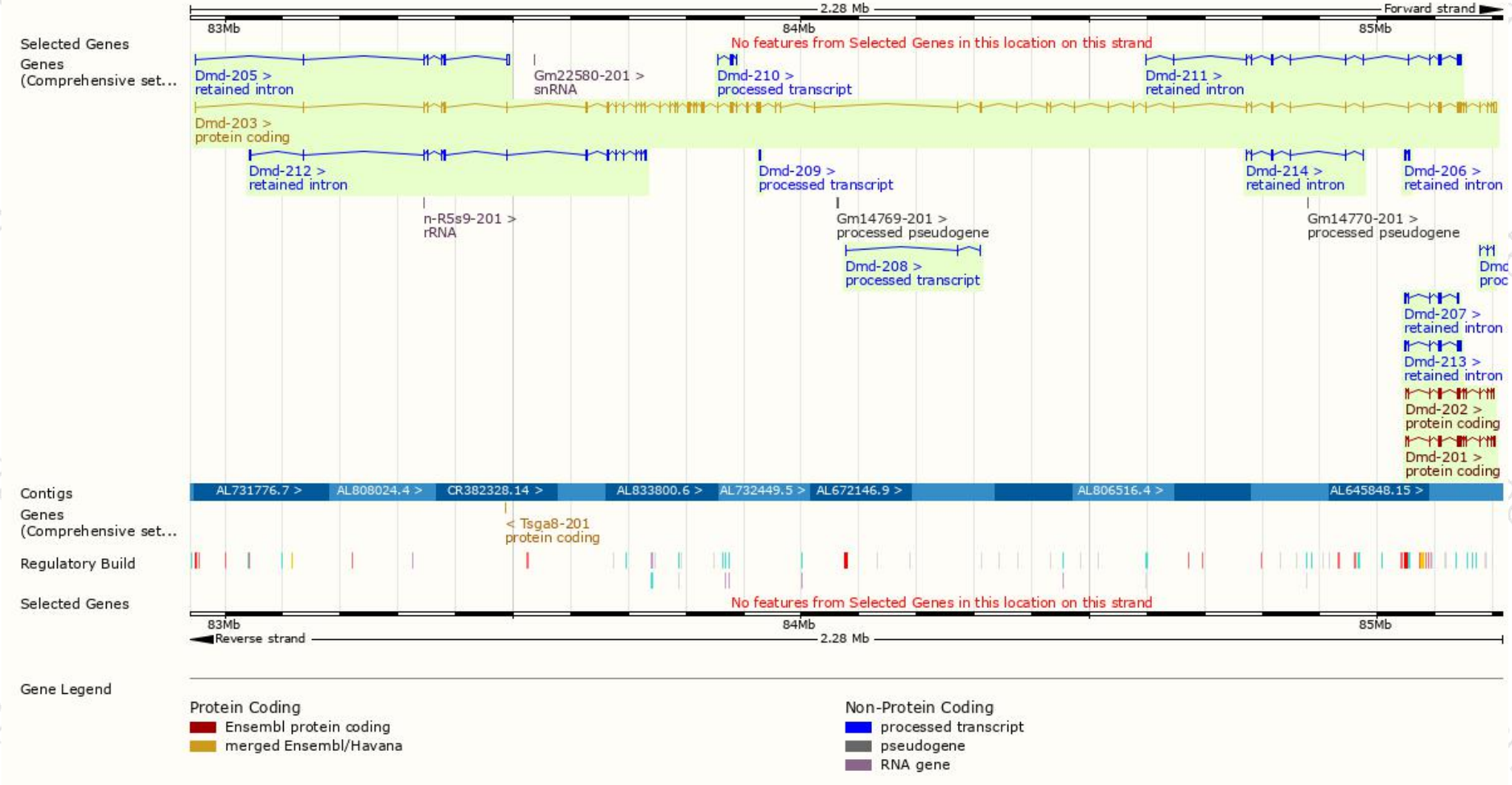
Transcript information（Ensembl）

The gene has 14 transcripts,all transcripts are shown below（C57BL/10ScSnJ）:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dmd-203	ENSMUST00000114000.7	14910	3678aa	Protein coding	CCDS41047	P11531	TSL:1 GENCODE basic APPRIS P1
Dmd-201	ENSMUST00000113991.7	4438	617aa	Protein coding	CCDS85787	A2A9Z2	TSL:1 GENCODE basic
Dmd-202	ENSMUST00000113992.2	1858	604aa	Protein coding	CCDS85788	A2A9Z1	TSL:1 GENCODE basic
Dmd-210	ENSMUST00000141778.1	868	No protein	Processed transcript	-	-	TSL:3
Dmd-209	ENSMUST00000141261.1	704	No protein	Processed transcript	-	-	TSL:3
Dmd-204	ENSMUST00000123308.1	596	No protein	Processed transcript	-	-	TSL:5
Dmd-208	ENSMUST00000139998.1	369	No protein	Processed transcript	-	-	TSL:2
Dmd-205	ENSMUST00000127295.7	3690	No protein	Retained intron	-	-	TSL:1
Dmd-212	ENSMUST00000147740.1	3055	No protein	Retained intron	-	-	TSL:1
Dmd-211	ENSMUST00000146331.7	2494	No protein	Retained intron	-	-	TSL:2
Dmd-214	ENSMUST00000156107.1	2145	No protein	Retained intron	-	-	TSL:2
Dmd-207	ENSMUST00000132333.7	2133	No protein	Retained intron	-	-	TSL:2
Dmd-206	ENSMUST00000128983.1	1792	No protein	Retained intron	-	-	TSL:1
Dmd-213	ENSMUST00000149433.1	1383	No protein	Retained intron	-	-	TSL:1

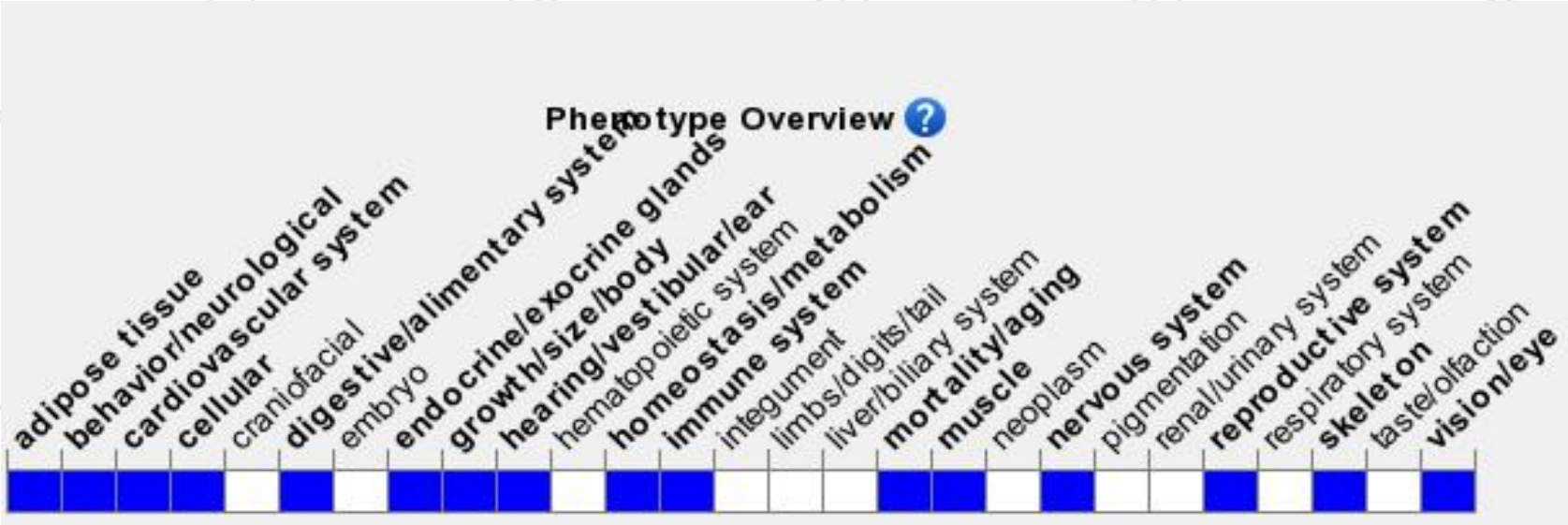
The strategy is based on the design of *Dmd-203* transcript,The transcription is shown below

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/).

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

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