Wnt9a Cas9-KO Strategy

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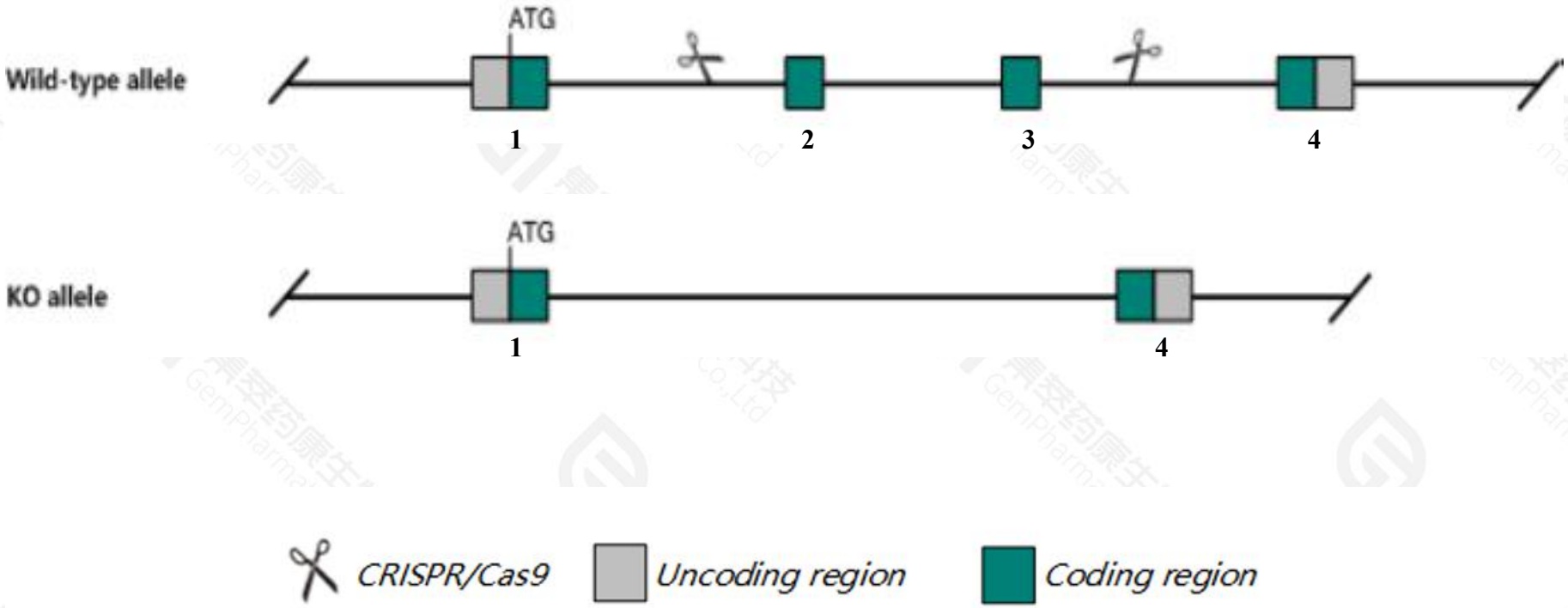
Design Date: 2021-4-13

Project Overview

Project Name	***Wnt9a***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Wnt9a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Wnt9a* gene has 2 transcripts. According to the structure of *Wnt9a* gene, exon2-exon3 of *Wnt9a-202*(ENSMUST00000108783.4) transcript is recommended as the knockout region.The region contains 520bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Wnt9a* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,homozygous inactivation of this gene results in neonatal lethality, altered chondrocyte maturation, cranial defects, and skeletal abnormalities including shortened appendicular long bones, partial joint fusions of carpal and tarsal elements, and chondroid metaplasia in synovial and fibrous joints.

➢ The *Wnt9a* gene is located on the Chr11. 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）

集萃药康 GemPharmatech

Wnt9a wingless-type MMTV integration site family, member 9A [Mus musculus (house mouse)]

Gene ID: 216795, updated on 2-Mar-2021

Summary

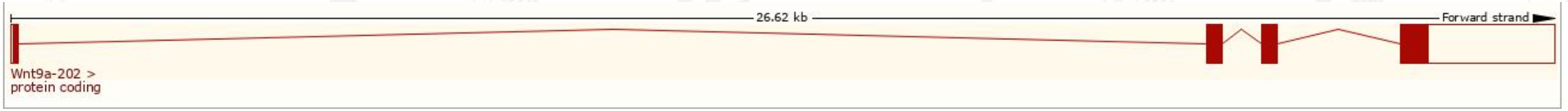
Official Symbol	Wnt9a provided by MGI
Official Full Name	wingless-type MMTV integration site family, member 9A provided byMGI
Primary source	MGI:MGI:2446084
See related	Ensembl:ENSMUSG00000000126
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Wnt1, Wnt14, wnt-14
Expression	Broad expression in ovary adult (RPKM 5.2), subcutaneous fat pad adult (RPKM 5.2) and 21 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

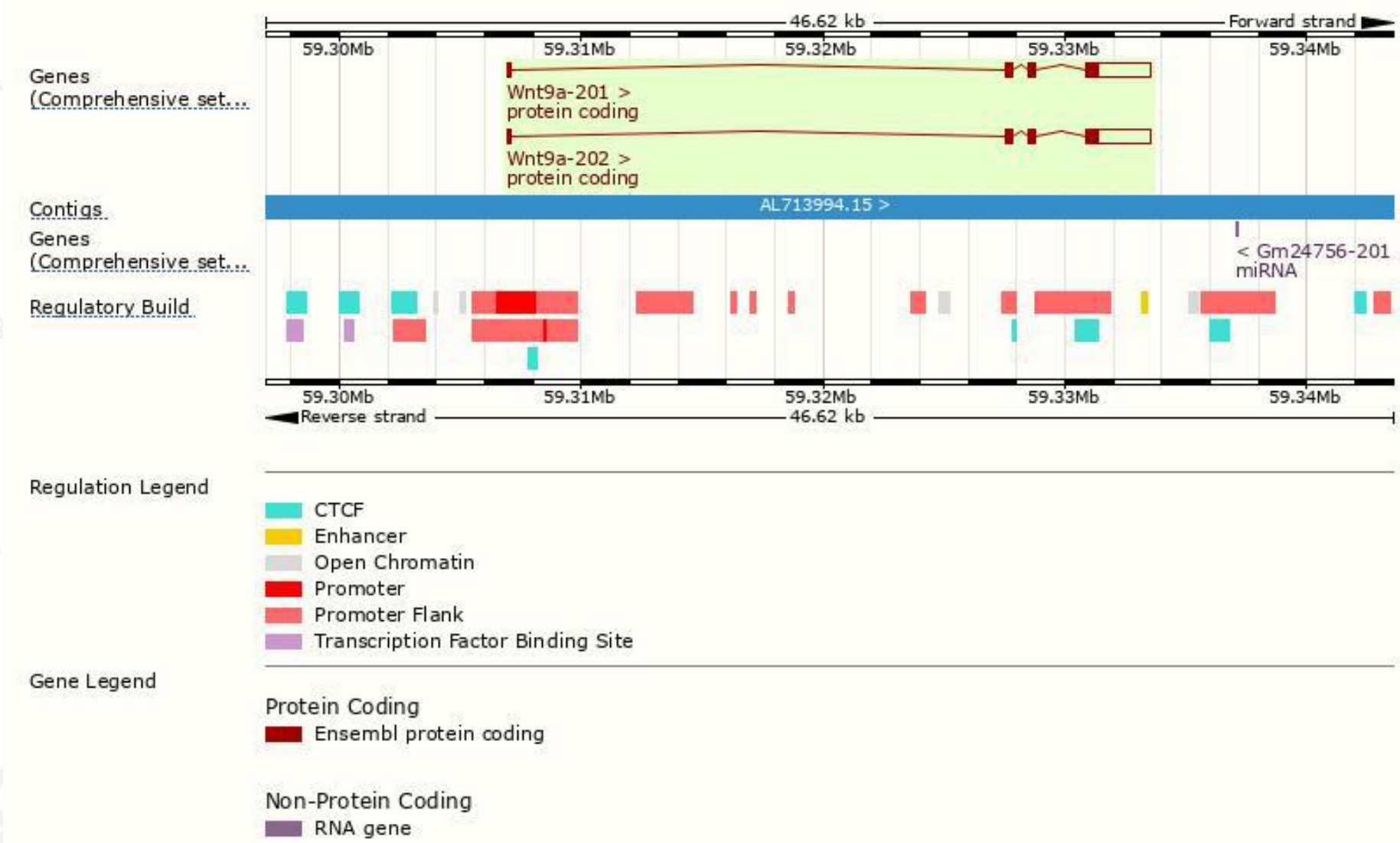
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wnt9a-202	ENSMUST00000108783.4	3318	365aa	Protein coding	CCDS36169		TSL:1 , GENCODE basic , APPRIS P2 ,
Wnt9a-201	ENSMUST00000000128.10	3320	365aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT1 ,

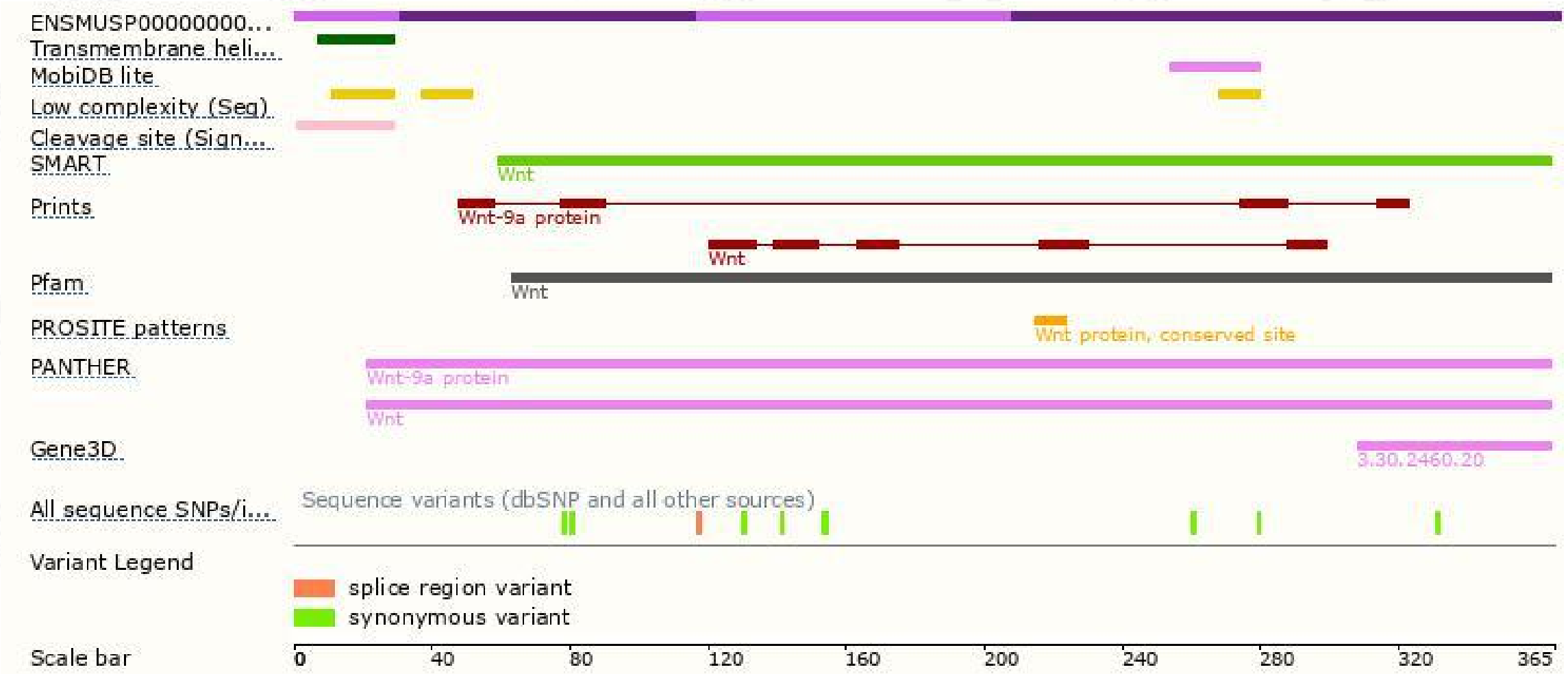
The strategy is based on the design of *Wnt9a-202* transcript,the transcription is shown below:



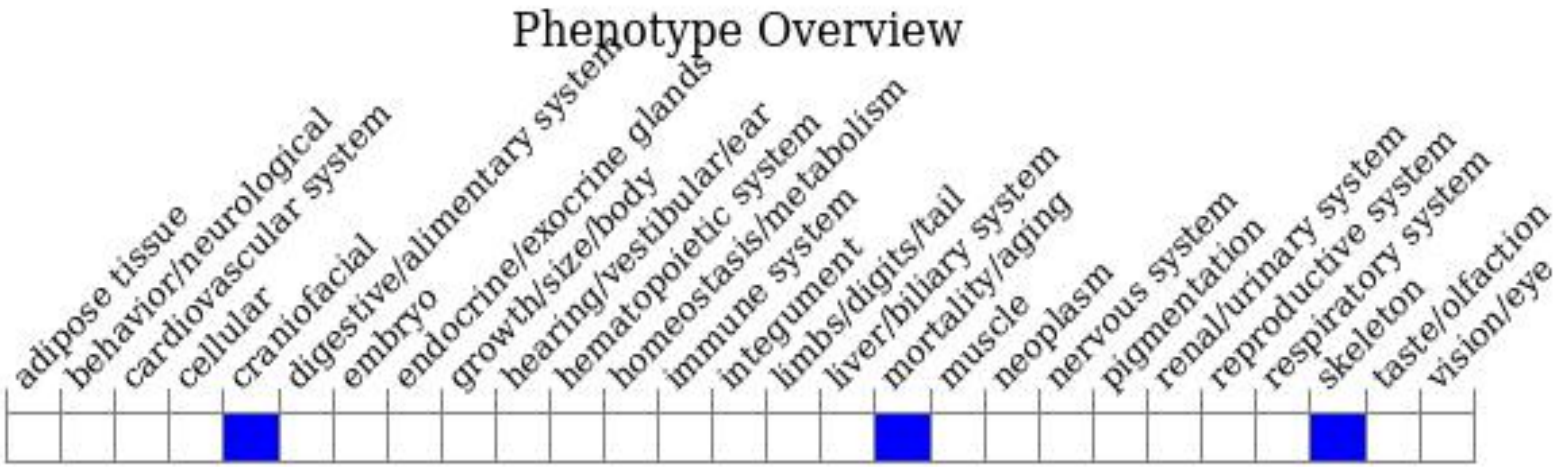
Genomic location distribution



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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