Sqstm1 Cas9-KO Strategy

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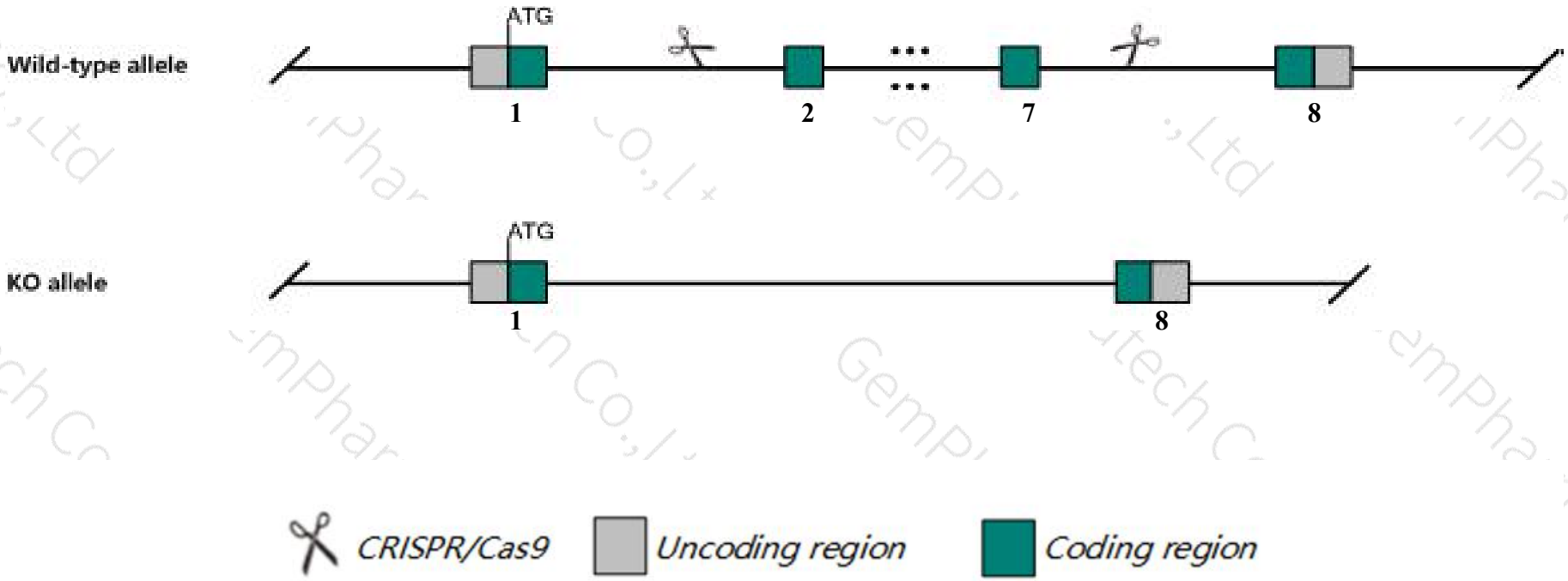
Design Date: 2019-11-13

Project Overview

Project Name	***Sqstm1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sqstm1* gene. The schematic diagram is as follows:



Technical routes

The *Sqstm1* gene has 7 transcripts. According to the structure of *Sqstm1* gene, exon2-exon7 of *Sqstm1-201* (ENSMUST00000015981.11) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Sqstm1* gene. The brief process is as follows: CRISPR/Cas9 systen

Notice

According to the existing MGI data,Mice homozygous for one knock-out allele exhibit impaired osteoclastogenesis in response to osteoclastogenic factors. Mice homozygous and heterozygous for a knock-in allele exhibit osteolytic lesion with increased bone formation, mineral apposition rate,and osteoclast numbers.

The KO region deletes most of the coding sequence, but does not result in frameshift.

The floxed region is 2.5kb away from *Mgat4b* *Mgat4b* may be affected.

The *Sqstm1* 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

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Sqstm1 sequestosome 1 [Mus musculus (house mouse)]

Gene ID: 18412, updated on 9-Apr-2019

Summary

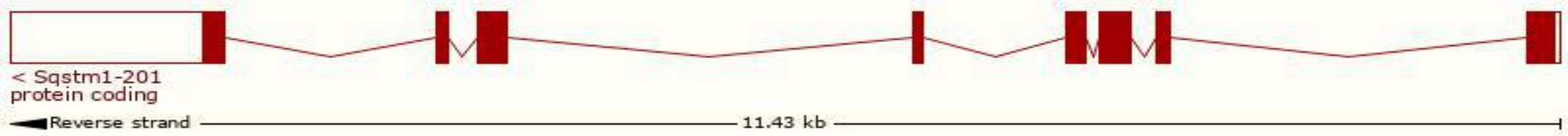
Official Symbol	Sqstm1 provided by MGI
Official Full Name	sequestosome 1 provided by MGI
Primary source	MGI:MGI:107931
See related	Ensembl:ENSMUSG00000015837
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A170, OSF-6, Osi, STAP, STONE14, p62
Expression	Ubiquitous expression in adrenal adult (RPKM 275.7), placenta adult (RPKM 170.1) and 28 other tissues See more
Orthologs	human all

Transcript information Ensembl

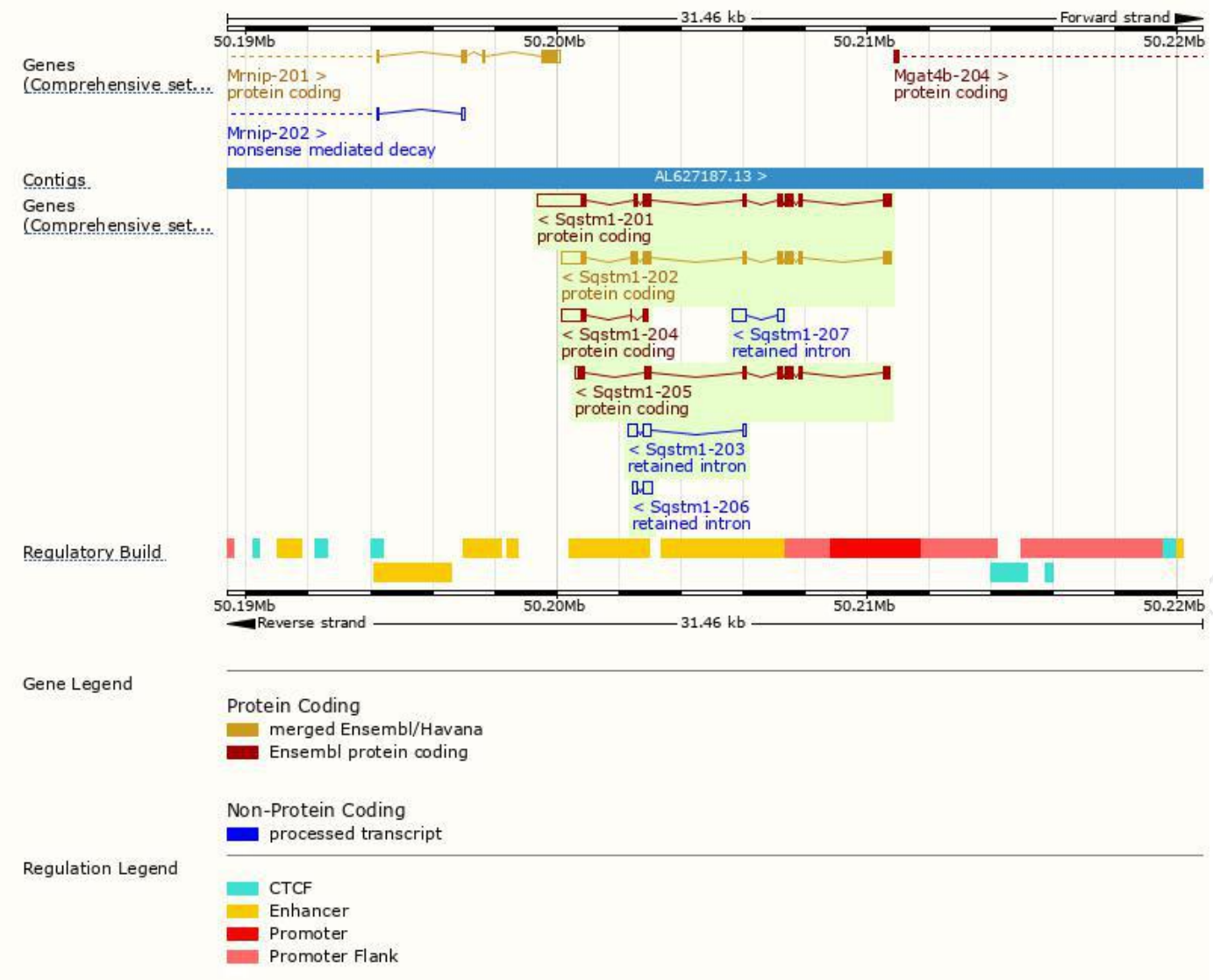
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sqstm1-201	ENSMUST00000015981.11	2673	404aa	Protein coding	CCDS70176	Q64337	TSL:1 GENCODE basic APPRIS ALT2
Sqstm1-202	ENSMUST00000102774.10	2037	442aa	Protein coding	CCDS24629	Q64337	TSL:1 GENCODE basic APPRIS P3
Sqstm1-205	ENSMUST00000143379.1	1266	382aa	Protein coding	-	D3YZJ1	TSL:5 GENCODE basic
Sqstm1-204	ENSMUST00000136936.1	953	109aa	Protein coding	-	F6VD69	CDS 5' incomplete TSL:3
Sqstm1-207	ENSMUST00000154805.1	585	No protein	Retained intron	-	-	TSL:2
Sqstm1-203	ENSMUST00000131214.1	582	No protein	Retained intron	-	-	TSL:3
Sqstm1-206	ENSMUST00000147846.1	457	No protein	Retained intron	-	-	TSL:2

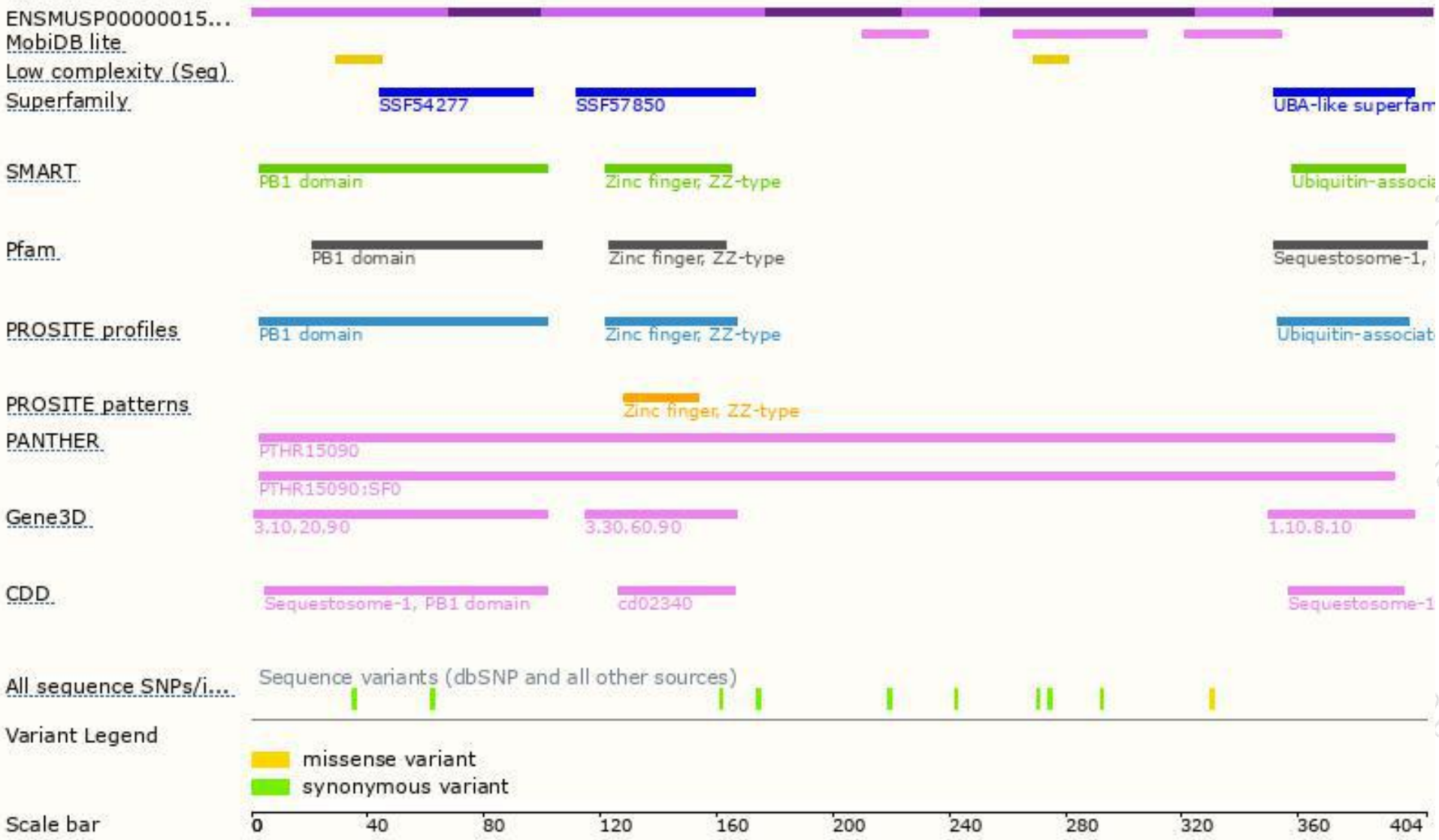
The strategy is based on the design of *Sqstm1-201* transcript,The transcription is shown below



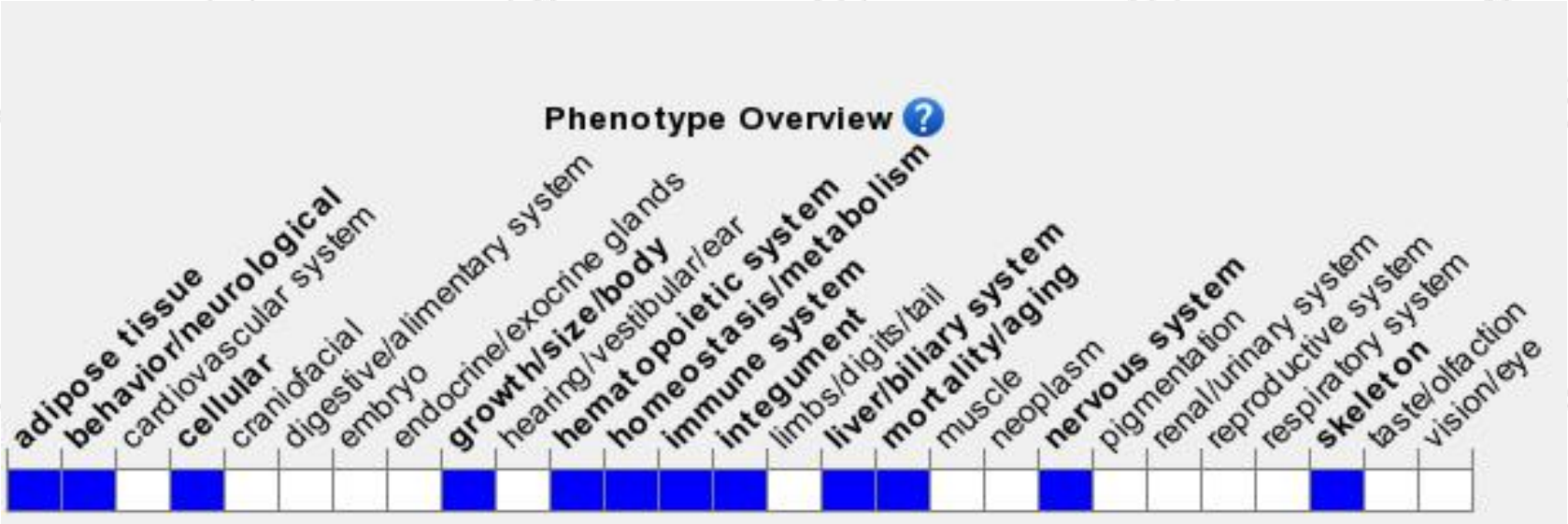
Genomic location distribution



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,Mice homozygous for one knock-out allele exhibit impaired osteoclastogenesis in response to osteoclastogenic factors. Mice homozygous and heterozygous for a knock-in allele exhibit osteolytic lesion with increased bone formation, mineral apposition rate,and osteoclast numbers.

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

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