



Project Overview

Gene Information

Gene: Ryr1 ENSMUSG00000030592

Description ryanodine receptor 1, skeletal muscle [Source:MGI Symbol;Acc:[MGI:99659](#)]

Synonyms Ryr, calcium release channel isoform 1, skrr

Location [Chromosome 7: 29,003,344-29,125,179](#) reverse strand.

GRCm38:CM001000.2

About this gene This gene has 8 transcripts ([splice variants](#)), [93 orthologues](#), [5 paralogues](#), is a member of [1 Ensembl protein family](#) and is associated with [65 phenotypes](#).

Transcripts

[Hide transcript table](#)

Show/hide columns (1 hidden)								Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags	
Ryr1-201	ENSMUST00000032813.9	15352	5033aa	Protein coding	-	K3W4M2	-	TSL:5	GENCODE basic APPRIS ALT2
Ryr1-202	ENSMUST00000179893.8	15358	5035aa	Protein coding	CCDS39866	E9PZQ0	NM_009109 NP_033135	TSL:5	GENCODE basic APPRIS P2
Ryr1-203	ENSMUST00000207764.1	1028	No protein	Retained intron	-	-	-	TSL:5	
Ryr1-204	ENSMUST00000207783.1	2366	No protein	Retained intron	-	-	-	TSL:2	
Ryr1-205	ENSMUST00000208010.1	698	No protein	Retained intron	-	-	-	TSL:3	
Ryr1-206	ENSMUST00000208318.1	3044	No protein	Retained intron	-	-	-	TSL:2	
Ryr1-207	ENSMUST00000208922.1	2525	No protein	Retained intron	-	-	-	TSL:NA	
Ryr1-208	ENSMUST00000214374.1	15300	5061aa	Protein coding	-	A0A1L1SQG7	-	TSL:5	GENCODE basic

Mouse Models

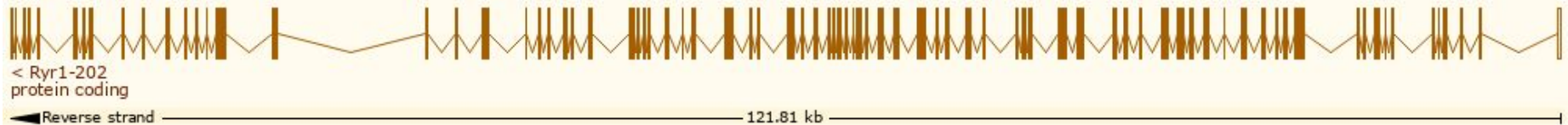
Transcript: Ryr1-202 ENSMUST00000179893.8

Description	ryanodine receptor 1, skeletal muscle [Source:MGI Symbol;Acc: MGI:99659]
Synonyms	skrr, calcium release channel isoform 1, Ryr
Location	Chromosome 7: 29,003,344-29,125,151 reverse strand.
About this transcript	This transcript has 106 exons is annotated with 88 domains and features , is associated with 208 variations and maps to 867 oligo probes .
Gene	This transcript is a product of gene ENSMUSG00000030592 Hide transcript table

RYR1-96 (#1921)
(exon 96)



RYR1-34 (#1922)
(exon 34)





Project RYR1-96 (#1921)

(T4706M)



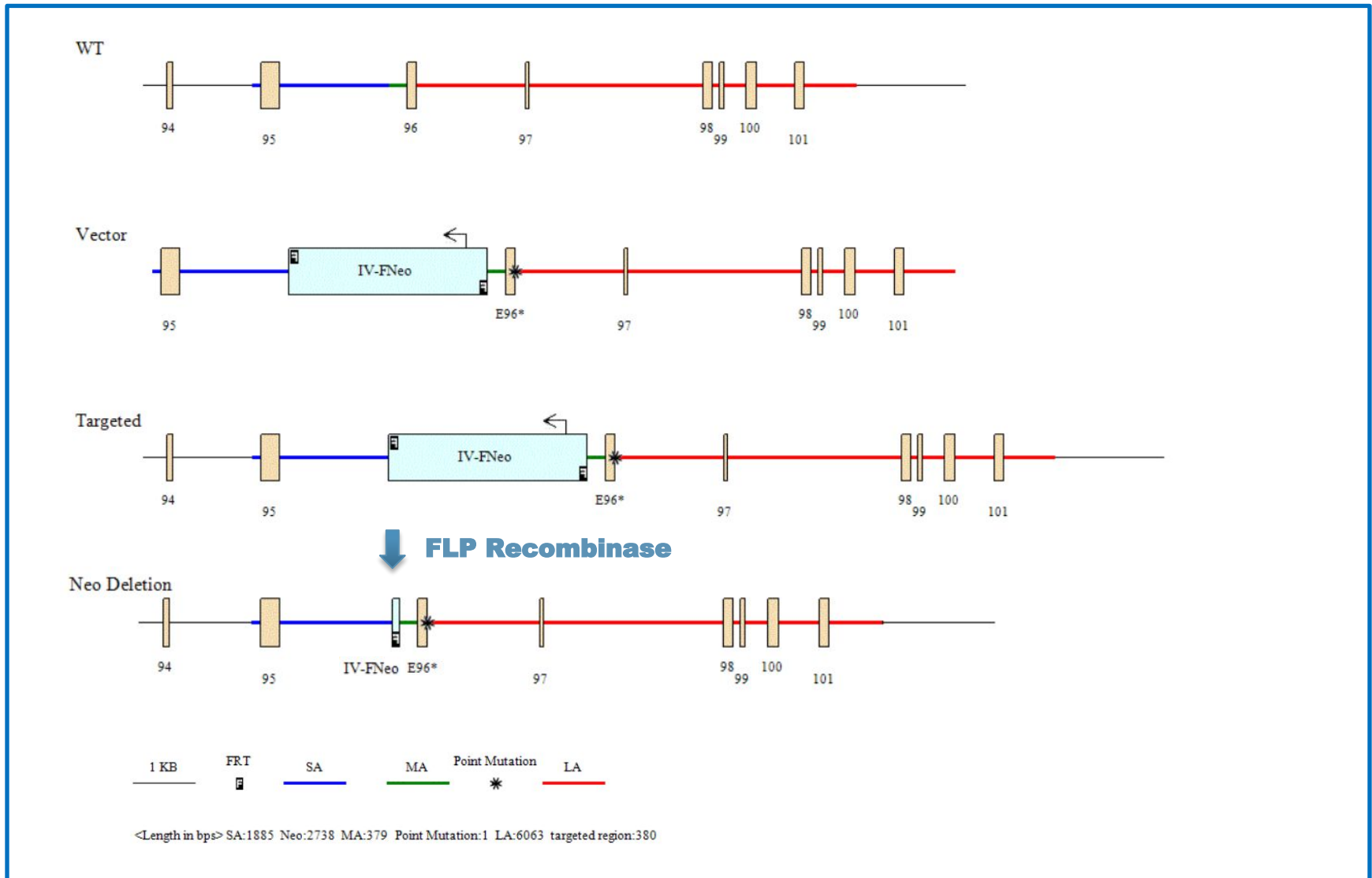
RYR1-96 (#1921): Point Mutation (T4706M) Knock-in

- Target gene: mouse Ryr1 gene; chromosome 7
- Target exon: 96
- Point mutation: Threonine 4706 Methionine (ACG > ATG)

<i>Mouse wild-type DNA sequence</i>	TGGGACCGCCTGGTGCTCAACA <u>C</u> GCCGTCTTTCCCTAGCAACTAC
<i>Mouse wild-type protein sequence</i>	-W--D--R--L--V--L--N-- <u>T</u> --P--S--F--P--S--N--Y-
<i>Mouse DNA sequence after mutation</i>	TGGGACCGCCTGGTGCTCAACA <u>T</u> GCCGTCTTTCCCTAGCAACTAC
<i>Mouse protein sequence after mutation</i>	-W--D--R--L--V--L--N-- <u>M</u> --P--S--F--P--S--N--Y-

(Thre) ACG > (Met) ATG

Targeting Strategy for RYR1-96 Model

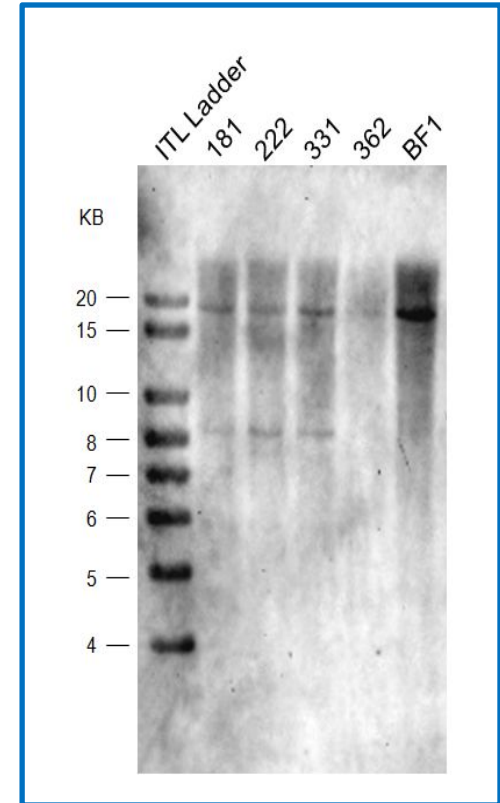
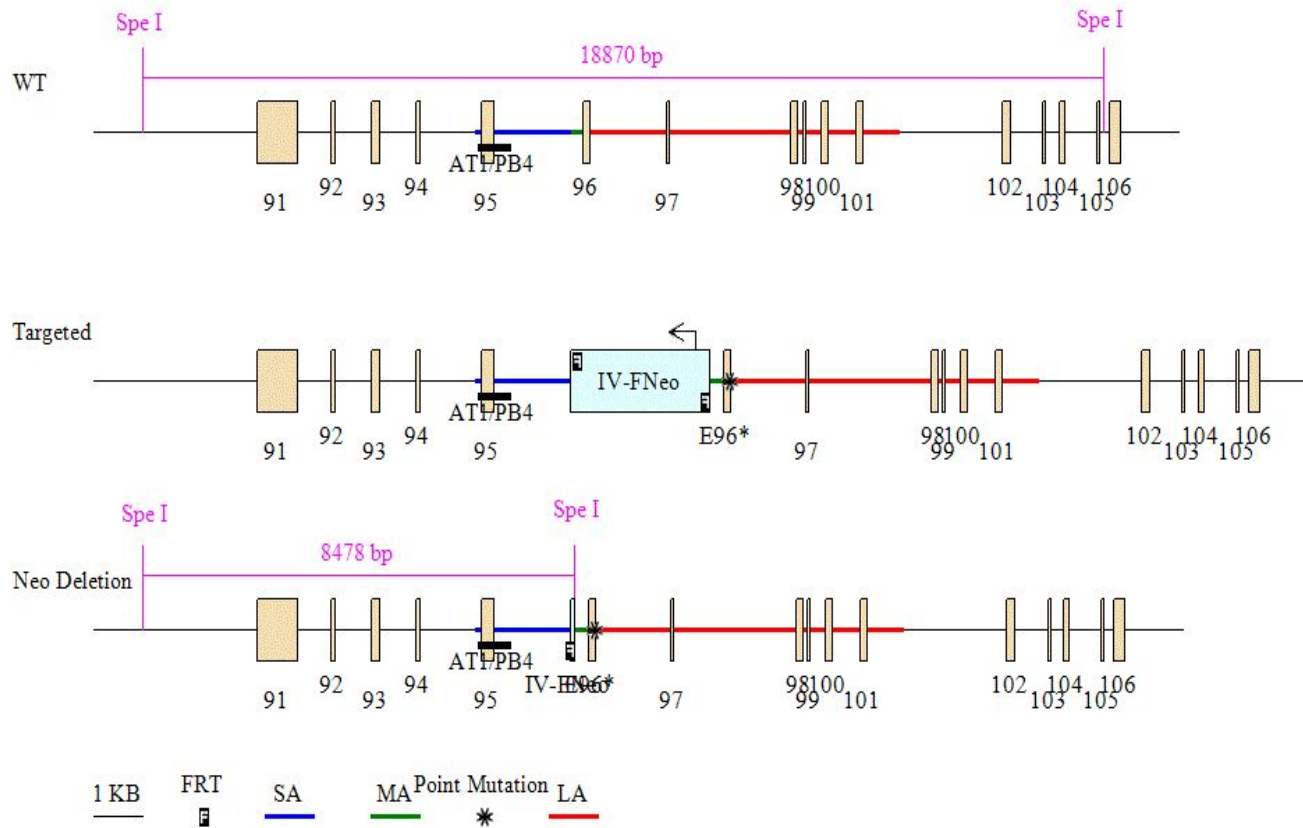




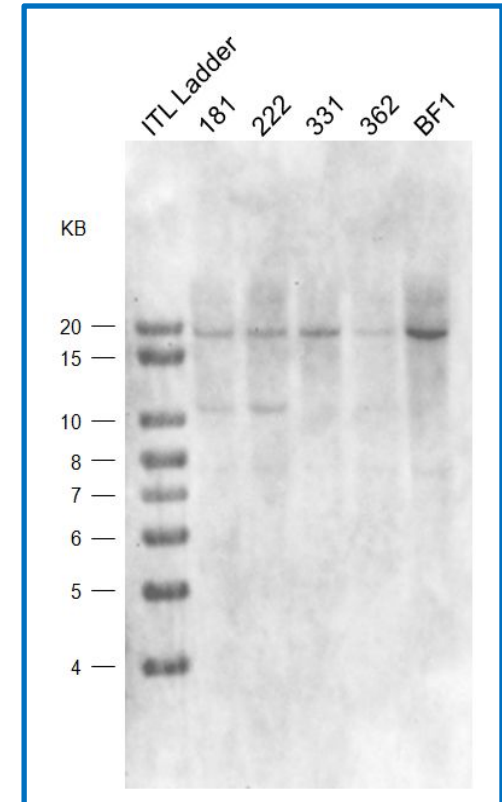
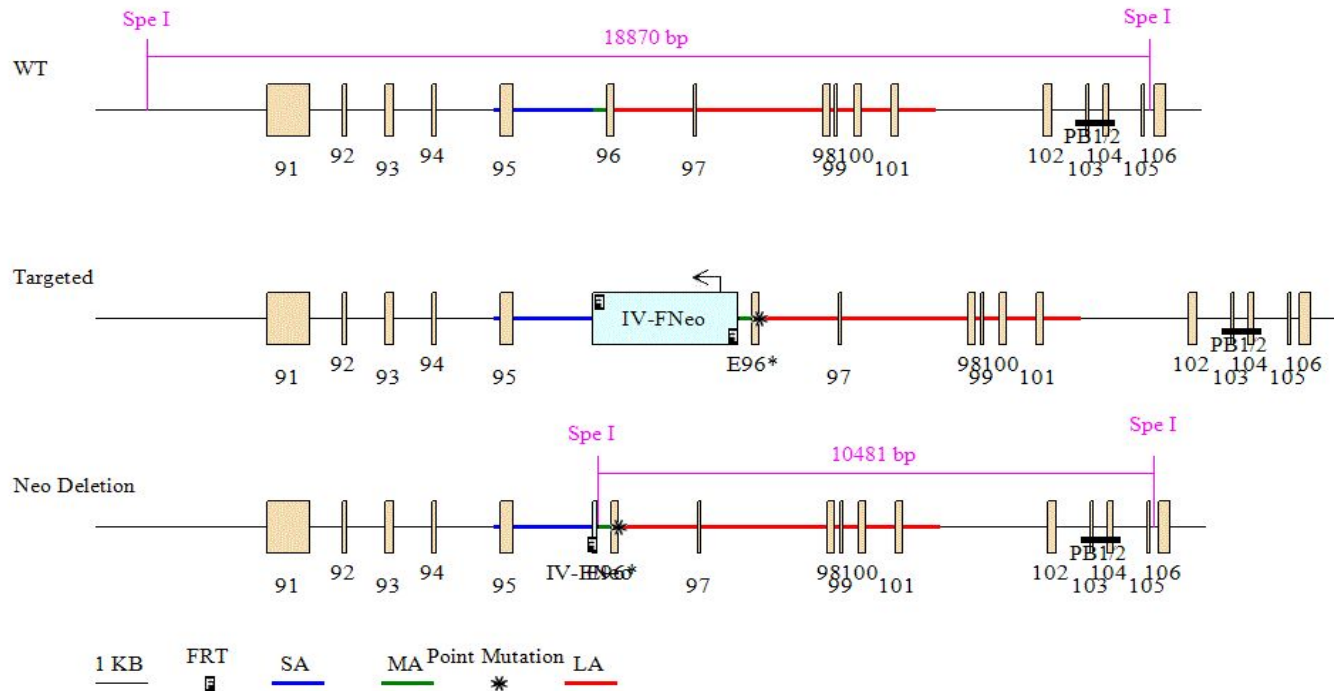
Vector/Screening Details for RYR1-96

- **Strategy confirmed: 3/20/2017**
- **Electroporation of vector: 5/10/2017**
iTL FLP C57BL/6 ESC line; 300 clones selected
- **PCR screening result: 5 clones identified from 1st plate**
(1 clone didn't grow during expansion)
- **Reconfirmation result: 2 out of 4 clones confirmed**

Southern Blot Strategy & Result for 5' Arm Integration



Southern Blot Strategy & Result for 3' Arm Integration



Clones #181 and 222 were confirmed for single targeted integration by Southern blot analysis.



RYR1-96: KI-PM Sequencing Confirmation for Recombinant ES Clones

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Query 17135 CTGAACCTCTACCAGCACCCCCATCTTTCTACCCTGAAGACCGAGGCAGCTCCTTTGGT 17194
|||||
Sbjct 404 CTGAACCTCTACCAGCACCCCCATCTTTCTACCCTGAAGACCGAGGCAGCTCCTTTGGT 345

Query 17195 GCCATTACAGGCTGCAGGCAGGTCTCATGGCTTCTGCAAAGGCCATGGTCTCCCTCCCC 17254
|||||
Sbjct 344 GCCATTACAGGCTGCAGGCAGGTCTCATGGCTTCTGCAAAGGCCATGGTCTCCCTCCCC 285

Query 17255 GCATAACTCATGTGGCCCCTCACCCCTGCCTCCCTCCATCCCTAGGTGCCCTTGTCATC 17314
|||||
Sbjct 284 GCATAACTCATGTGGCCCCTCACCCCTGCCTCCCTCCATCCCTAGGTGCCCTTGTCATC 225

Query 17315 TTCAAGCGGGAGAAGGAGCTGGCCCGGAAGCTGGAGTTTGATGGCCTCTACATTACAGAG 17374
|||||
Sbjct 224 TTCAAGCGGGAGAAGGAGCTGGCCCGGAAGCTGGAGTTTGATGGCCTCTACATTACAGAG 165
                                     (Thre) ACG > (Met) ATG

Query 17375 CAGCCCGAGGATGATGACGTGAAGGGACAGTGGGACCGCCTGGTGCTCAAC ACGCCGTAA 17434
|||||
Sbjct 164 CAGCCCGAGGATGATGACGTGAAGGGACAGTGGGACCGCCTGGTGCTCAAC ATGCCGTAA 105

Query 17435 GTGACCAGCCCCACCTCAGGGGAGCAGAGGGGGCGGGGCTAGGTTCCCCTCCAGCCCAG 17494
|||||
Sbjct 104 GTGACCAGCCCCACCTCAGGGGAGCAGAGGGGGCGGGGCTAGGTTCCCCTCCAGCCCAG 45

Query: Genomic sequence from Ensembl
Sbjct: Sequencing result from clone

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Injection Result for RYR1-96

Injection of clone #222:

3 male chimeras (medium); 1 female chimera (medium)

% of Chimerism	
Perfect	100%
High	80-99%
Medium	50-79%
Low	< 50%



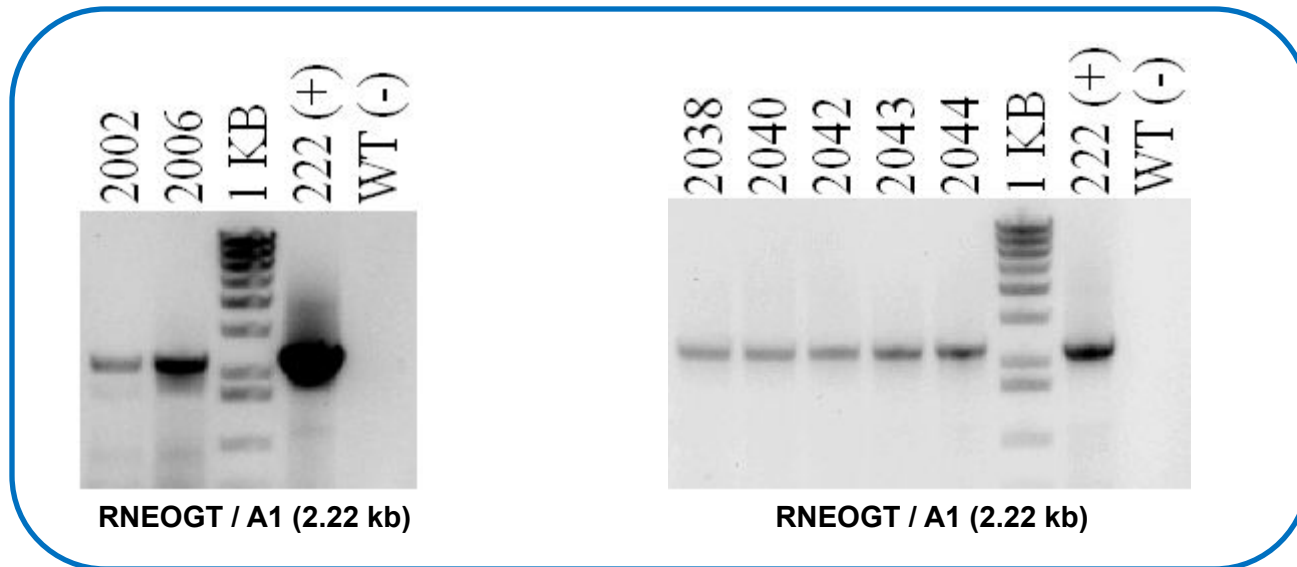
Medium male chimera for RYR1-96

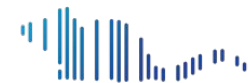
Breeding/Genotyping Status for RYR1-96

Chimeras mated to C57BL/6J WT mice on 12/6/2017



Germline-transmitted mice identified and weaned on 1/28/2018
(5 additional mice were identified subsequently)





RYR1-96: KI-PM Sequencing Confirmation for F1 Het Mice

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Query  169      CCTGAAGACCGAGGCAGCTCCTTTGGTGCCATTACAGGCTGCAGGCAGGTCTCATGGCT  228
      |||||
Sbjct  17168     CCTGAAGACCGAGGCAGCTCCTTTGGTGCCATTACAGGCTGCAGGCAGGTCTCATGGCT  17227

Query  229      TCTGCAAAGGCCATGGTCTCCCTCCCCGCATAACTCATGTGGCCCCCTCACCCCTGCCTCC  288
      |||||
Sbjct  17228     TCTGCAAAGGCCATGGTCTCCCTCCCCGCATAACTCATGTGGCCCCCTCACCCCTGCCTCC  17287

Query  289      CTCCATCCCTAGGTGCCCCTTGTCATCTTCAAGCGGGAGAAGGAGCTGGCCCCGGAAGCTG  348
      |||||
Sbjct  17288     CTCCATCCCTAGGTGCCCCTTGTCATCTTCAAGCGGGAGAAGGAGCTGGCCCCGGAAGCTG  17347

Query  349      GAGTTTGATGGCCTCTACATTACAGAGCAGCCCCGAGGATGATGACGTGAAGGGACAGTGG  408
      |||||
Sbjct  17348     GAGTTTGATGGCCTCTACATTACAGAGCAGCCCCGAGGATGATGACGTGAAGGGACAGTGG  17407
                        (Thre)ACG > (Met)ATG

Query  409      GACCGCCTGGTGCTCAAC  ATGCCGTAAGTGACCAGCCCCACCTCAGGGGAGCAGAGGGGG  468
      |||||
Sbjct  17408     GACCGCCTGGTGCTCAAC  ACGCCGTAAGTGACCAGCCCCACCTCAGGGGAGCAGAGGGGG  17467

Query  469      CGGGGCTAGGTTCCCACTCCAGCCCAGGCCTGGGCTTCCAGAGAGCAAGACCCCTGAGGC  528
      |||||
Sbjct  17468     CGGGGCTAGGTTCCCACTCCAGCCCAGGCCTGGGCTTCCAGAGAGCAAGACCCCTGAGGC  17527

Query: Sequencing data from PCR products
Sbjct: Genomic wild-type sequence

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RYR1-96: Mouse Information of F1 Heterozygotes

Mouse #	Sex	DOB	Clone #	Parent Info
2002*	M	1/07/2018	222	CH X C57BL/6 WT
2006*	M	1/07/2018	222	CH X C57BL/6 WT
2038*	M	1/13/2018	222	CH X C57BL/6 WT
2040	M	1/13/2018	222	CH X C57BL/6 WT
2042*	F	1/13/2018	222	CH X C57BL/6 WT
2043*	F	1/13/2018	222	CH X C57BL/6 WT
2044	F	1/13/2018	222	CH X C57BL/6 WT

* Mice retain the FLP transgene.



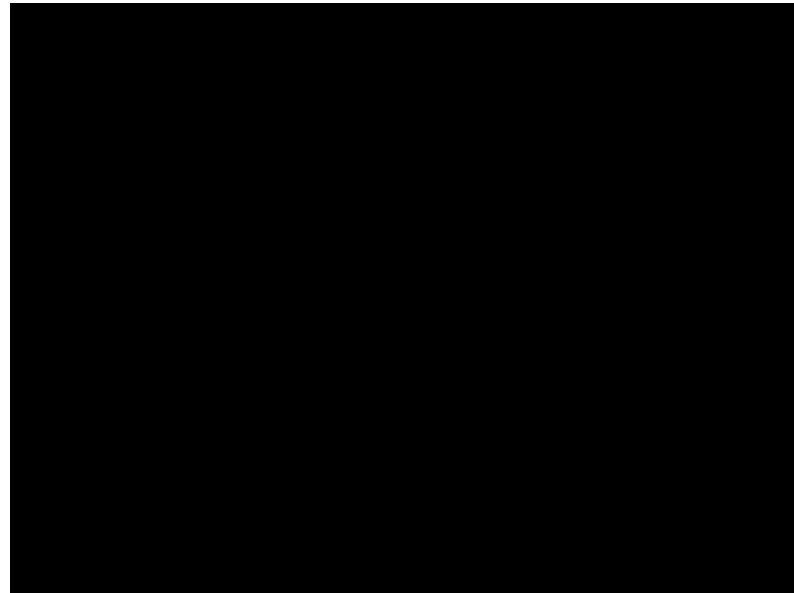
RYR1-96: Generating Homozygotes

- **Hets x WT mice to segregate the FLP transgene.**
- **Het x Het breeders to generate homozygotes.**

RYR1-96: Generating Homozygotes



**Male Hom with littermates
(4-5 weeks old)**



**Male Hom with Male Het
(please refer to the video on
www.ryr1.org/mice)**

RYR1-96: Generating Homozygotes

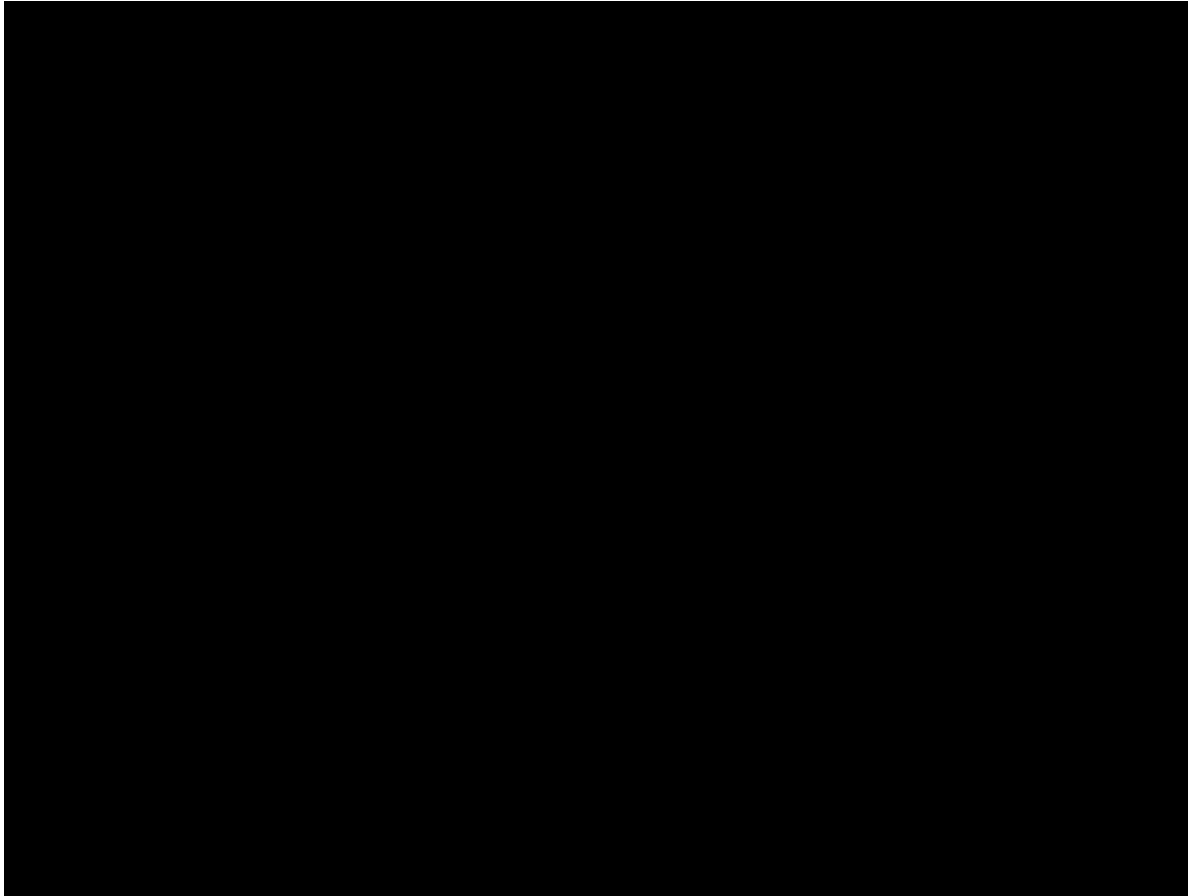


Malocclusion



Hunched back

RYR1-96: Generating Homozygotes



**Female Hom at ~8 months old
(please refer to the video on
www.ryr1.org/mice)**



RYR1-96: Generating Homozygotes

- **Identified 20 Homs (9 males, 11 females)**
- **Relative size to littermates when weaned**
- **At 4-5 weeks old, some Homs were runty, hunched; had malocclusion (provided gel packs/soft food; trimmed teeth)**
- **Died at ~2 months old**
- **Homs that survived are inactive and have hunched back, abnormal gait, and malocclusion**