

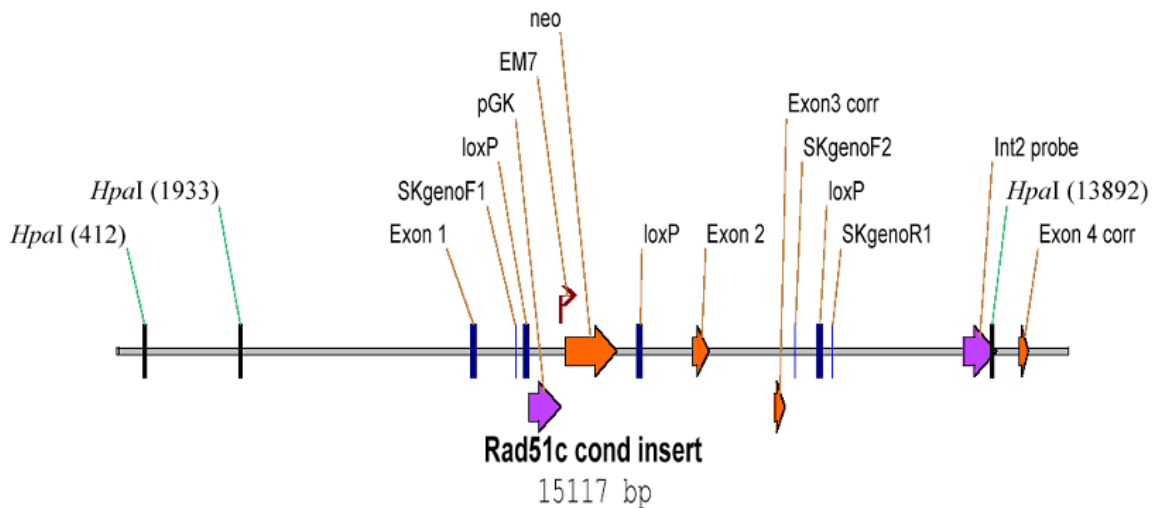
11.13.09 MS
 08.02.10 HB updated
 04.02.12 MS

Genotyping Protocol: **MMRRC 30741**

Assay Type: PCR - can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain Description: This strain has a *neo* resistance gene flanked by two *loxP* sites inserted into intron 1 and a single *loxP* inserted into intron 3 of the Rad51 homolog c gene (*Rad51c*). Details can be found in Kuznetsov et al (2007) Journal of Cell Biology 176:581-592.



Primer Information:

- 1) Name: SKgenoF1 Sequence: 5'-ACC GGG CAG TGG TGG CGC ACG CCT TTA ATC CCA GCA CTT G-3'
- 2) Name: SKgenoF2 Sequence: 5'-CAA AAT GCT GGA ATA ATA GAC CTG TGT CAT ACC CAA AGT G-3'
- 3) Name: SKgenoR1 Sequence: 5'-GGG TAT CCA TAT CAC AGC CAC TGT ACT CTA GCT CCA GGA G-3'

Primer location: SKgenoF1 is located in intron 1. SKgenoF2 is located 162bp from the end of exon 3. SKgenoR1 is located between exons 3 and 4.

Assay Name: Rad51c Conditional PCR

PCR Master Mix Components:

Component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
SKgenoF1	Sigma	25µM	0.3
SKgenoF2	Sigma	25µM	0.3
SKgenoR1	Sigma	25µM	0.3
FastStart Taq	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

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All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 5 minutes
- 2) 94°C 30 seconds
- 3) 70°C 30 seconds
- 4) 72°C 30 seconds
- 5) Repeat steps 2-3 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

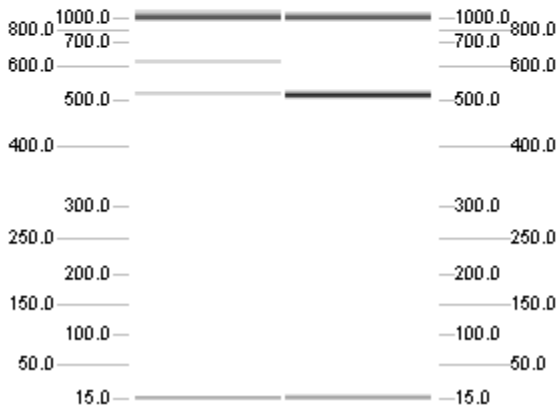
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/1000bp (Cat# 929521)
Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)
Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected Products:

Mutant: 627bp
WT: 524bp

Example Gel:



Lane A07 displays a heterozygous sample (627bp and 524bp bands).
Lane A10 displays a WT sample (524bp band).
Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.