11.11.09 MS
08.02.10 HB updated
03.04.14 MLS

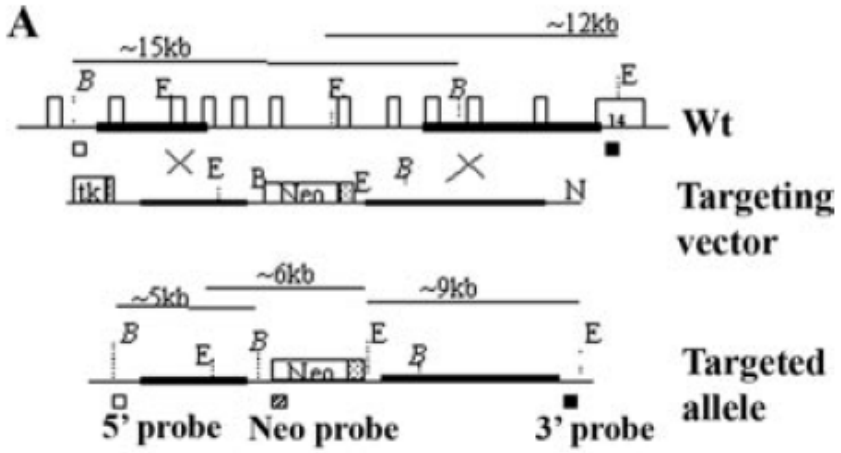
Genotyping Protocol: **MMRRC 30963**

Current Background Strain of M30963: C57BL/6
Details on the importance of background strain can be found in Xiao et al (2009) Biol Blood Marrow Transplant 15:1-11.
This strain has an identical genetic alteration to M30964 – the two strains differ in current background strain.*

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 200ul of AE buffer once.

Strain Description: This strain has a targeting construct inserted into the DNA cross-link repair 1C, PSO2 homolog gene (*Dclre1c*) on Chromosome 2, resulting in the deletion of exons 6-11 of *Dclre1c*. The targeting construct is made up of a 1.8kb Neomycin gene flanked by exons 4-6 and 11-14 of *Dclre1c*. Details can be found in Li et al (2005) J. Immunol. 174;2420-2428.



Primer Information:
1) Name: M30964 E6 F Sequence: 5'-TCA GGG CAG TAA TGG AAC TGT C-3'
2) Name: M30964 E6 R Sequence: 5'-CTG TGT GCT CAC ACA TGC AC-3'
3) Name: M30964 Neo R Sequence: 5'-TGC CTG CTC TTT ACT GAA GG-3'

Primer location: M30964 E6 F is located in exon 6, and M30964 E6 R is located in intron 6 of the *Dclre1c* gene on Chromosome 2. M30964 Neo R is located in the targeting construct.

Assay name: Dclre1c 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
M30964 E6 F	Sigma	25µM	0.3
M30964 E6 R	Sigma	25µM	0.3
M30964 Neo R	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

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

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:

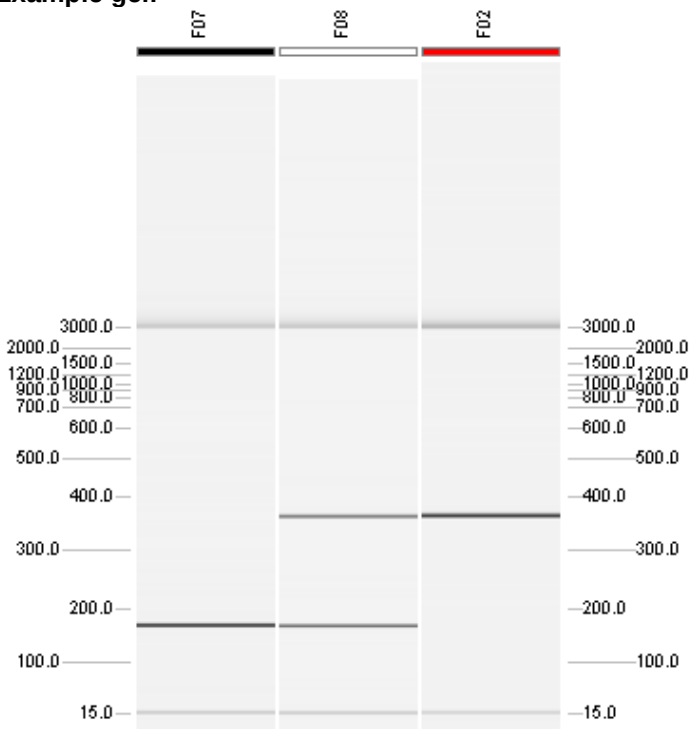
1)	95°C	5 minutes
2)	94°C	30 seconds
3)	63°C	30 seconds
4)	72°C	30 seconds
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10 minutes
7)	4°C	hold until refrigerate product

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/3Kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Heterozygous: 169bp, 400bp
Homozygous for mutant allele: 400bp
Wild Type: 169bp

Example gel:



Lane F07 displays a WT sample (169bp band).
Lane F08 displays a heterozygous sample (169bp and 400bp bands).
Lane F02 displays a homozygous mutant sample (400bp band).

Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.