GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER

sacoord@mmrrc.org

800-910-2291 North America, +1-530-757-5710 International

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

- *Open the original graphic in the program that created it*
- *Choose File, Save As*
- *Select No Compression in the save options.*
- *Save as JPG or PNG or similar format that's compatible with both PC and Mac Word versions.*
- *Switch to Word, choose Insert, Picture, From File and choose the newly saved picture.*

These instructions are very generic. The menu options for your graphics program may be different.

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Strain Name **CD45-minigene transgenics B,F,H,LP4,LP5H,LP5L,LP8,LP26,CS**		MMRRC Stock Number **44051, 46054-46270**

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NAME OF PCR: CD45-minigene transgenics **MMRRC:** 0-CTR

Protocol: ***(PCR protocol provided by Donating Investigator)***

Reagent/Constituent	Volume (µL)
Water	22.75
5x Buffer	10.0
$MgCl_2$ (stock concentration is mM)	0
Betaine (stock concentration is 5M) *Optional*	0
dNTPs (stock concentration is 10mM)	1.0
DMSO *Optional*	0
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
Primer 3. (stock concentration is 20µM)	0
Primer 4. (stock concentration is 20µM)	0
Taq Polymerase 5Units/µL	0.25
DNA (50-200ng/ µL) extracted as described under "Comments on protocol" below	15.0
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume. **TOTAL VOLUME OF REACTION:**	**50.000 µL**

Comments on protocol:

- DNA prepared according to Truett et al *BioTechniques 29:52-54, 2000* (PMID: 10907076) using
  2mm tail snip
  50ul fresh lysis buffer
  100ul neutralization buffer

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	1:00	**1**
2. Denaturation	94	0:22	
3. Annealing steps 2-3-4 cycle in sequence	63	0:30	35
4. Elongation	68	1:00	
5. Amplification	68	5:00	**1**
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. VGI CD363	gccagctacattgatggcttc
2. VGI CD365	cctgtatgaaggaagtctctgg
3. VGI 321	ggtcactggaatgaaaacctcccg
4. VGI 332	gcataggaaatggccatagtc
5.	

Electrophoresis Protocol:

Agarose: 1.5% V: 68

Estimated Running:Time: 30 min.

Primer Combination	Band	Genotype
1 + 2	734 bp	CD45 Tg B
3 + 4	0 bp	CD45 Tg B
	bp	

Please size gel images to fit in this space

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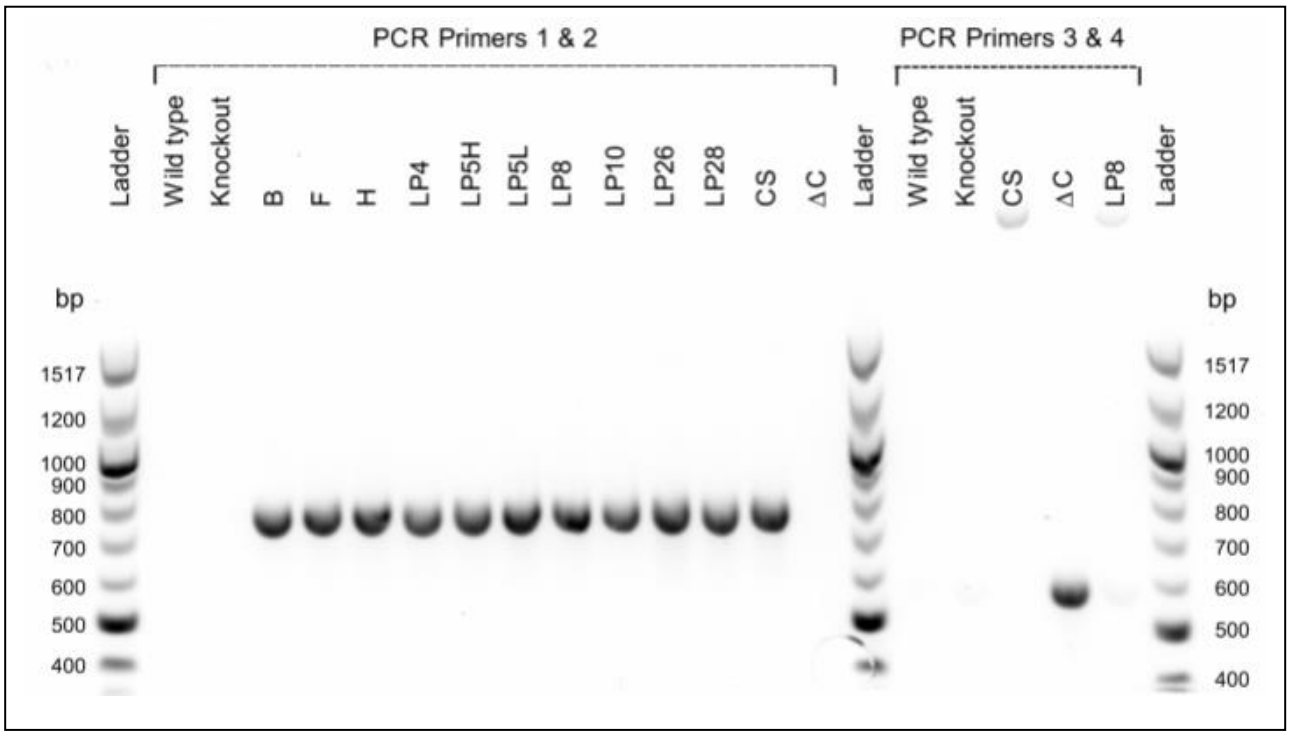
Protocol / Gel Comments:

The PCR primers are designed to detect the CD45 minigene sequence and not the endogenous CD45 gene sequence in wild type mice and CD45 knockout mice. The primers are located in the cDNA portion of the minigene, such that the primer sequences are separated by 5 large introns in the endogenous sequence but amplify a 734 bp sequence from the cDNA section of the minigene. Furthermore, part of each primer sequence is positioned to span an intron in the endogenous sequence to provide additional specificity for the minigene sequence.

Thus, using these PCR primers DNA from wild type and knockout mice will not produce a PCR fragment whereas DNA from each of the CD45-minigene transgenic strains (B, F, H, LP4, LP4H, LP4L, LP8, LP26 and CS strains) will produce a 734 bp PCR fragment. (The ΔC mutant transgenic strain, which lacks the cytoplasmic domain where these primers are located, is the exception and will not produce an amplified fragment with this primer set. Separate primers, primers 3 & 4, are used to detect the ΔC transgenic strain.)

Furthermore, the CD45 minigene transgenic strains with non-mutated coding sequence (strains B, F, H, LP4, LP5H, LP5L, LP8, LP10, LP26 and LP28) can be distinguished from the CS transgenic with the C817S mutation in the minigene by digestion of the PCR fragment with PstI. The point mutation that converts the catalytic cysteine at position 817 to the non-catalytic serine disrupts a PstI site present in the non-mutated sequence. Thus, digestion of PCR fragments with PstI will produce 431 bp and 303 bp fragments from minigene transgenic strains with non-mutated CD45 coding sequence (e.g., LP8 in the second figure below) but will not cut the 731 bp PCR fragment from the CS strain.

Gel pictures:



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