

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	383
MMRRC Strain Name	B6.129X1-Ly6a ^{tm1Grau} /Mmnc
Gene Name(s)	Lymphocyte antigen 6 complex, locus A (Ly6a)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	12/2/13

MMRRC# 383 PCR Reaction

	1X
DNA	1.0
5X Buffer	5.0
10mM dNTPs	1.0
20 uM Primer 1 & 2	1.0
Taq	1.0
ddH ₂ O	13.5
25mM MgCl ₂	2.0

NOTE: Two, 2-primer reactions per DNA sample: (Sca1 F & Sca1 R) and (Sca1F & GFP R). Combine PCR products to run out on gel.

Denaturing: 94°C for 45 sec
 Annealing Temp: 58°C for 45 sec
 Extension: 72°C 1 min
 Cycles: 36

Taq: **Apex and Chromataq Buffer**

Bands: WT: 167bp;
 MUTANT: 266bp

Primers are 10uM with respect to each primer (20uM total).

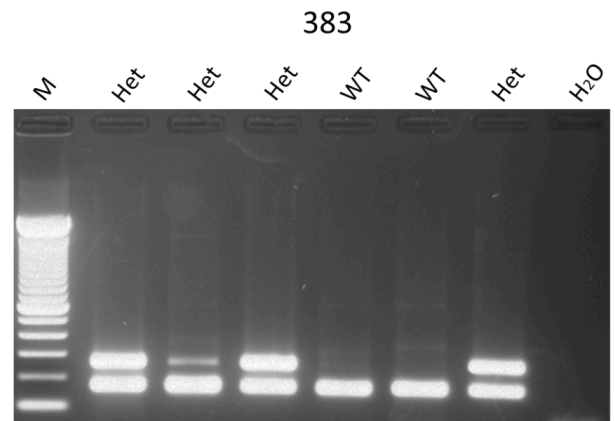
Primer sequences 5' to 3':

Sca1 intron 1 F: GGG AAG ATC GGA TGC CTG AGC

Sca1 exon2 R: GCA CAC AGT AGG GCC ACA AGA AGA

GFP R: ATG TTG CCG GTG GTG CAG AT

Run on 2% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)