

MMRRC UNC – Genotyping Protocol

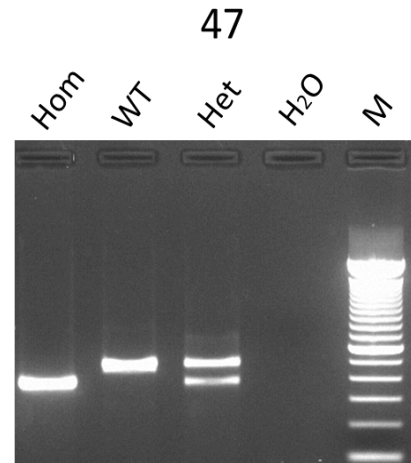
MMRRC Strain ID	47
MMRRC Strain Name	B6;129- <i>Dspp</i> ^{tm1Kul} /Mmnc
Gene Name(s)	Dentin sialophosphoprotein (<i>Dspp</i>)
Breeding Protocol(s)	Intra-strain mating (Sib-mating)
Protocol Date	10/14/13

MMRRC #47 PCR Reaction

	<u>1X</u>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
40 uM Primer F/R?lacZ	1.5
Taq	1.0
ddH ₂ O	14.0
25mM MgCl ₂	2.0

Thermal Cycler:

94°C for 45 sec
 56°C for 45 sec
 72°C for 60 sec
 36x



Taq: **Apex and Chromataq 10X Buffer**

Bands: MUTANT: 389bp
 WT: 489bp

M: 100 bp DNA ladder (Invitrogen)

Primers are 10uM with respect to each primer (40uM total). BUT, the WT R primer is 2x the concentration of the lacZ R.

Primer sequences 5' to 3':

DsppFOR1: GTATCTTCATGGCTGTTGCTTC

DsppREV1: TGTGTTTGCCTTCATCGAGA

LacZREV1: CCTCTTCGCTATTACGCCAG

Run on 2.0% agarose gel in TAE.