

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36738
MMRRC Strain Name	B6.129S6- <i>Steap3</i> ^{tm1.1Mdf} /Mmnc
Gene Name(s)	STEAP family member 3; targeted mutation 1, Mark D Fleming (<i>Steap3</i> ^{tm1Mdf})
Breeding Protocol(s)	Sib-mating
Protocol Date	8/12/13

MMRRC #36738 PCR Reaction

Steap3 (36738) wild type PCR assays may yield false positives. PCR for the KO allele, followed by a peripheral blood smear or CBC to phenotype is needed to distinguish heterozygotes from homozygotes.

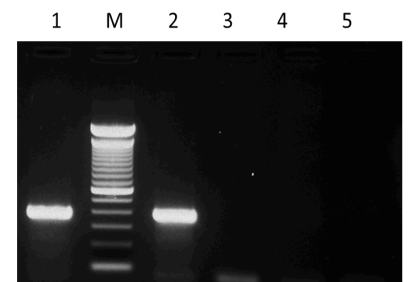
	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 60°C for 45 sec
 Step 4: 72°C for 45 sec
 Step 5: Step 2 to 4; Cycles: 32
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

36738 (Mut Rxn)



Lane 1: Hom; Lane 2: Het; Lane 3, 4: WT; Lane 4: H₂O;
 M: 100 bp DNA ladder Invitrogen

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

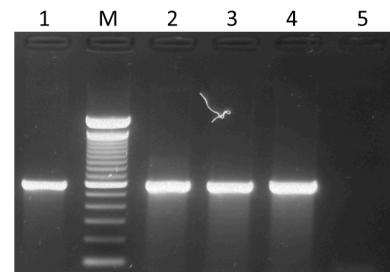
Com-F: TTGACTCACCCGCCAATGTGT
 WT-R: AGCCAGACTGCCATCACTGTCCACTA
 Mut-R: GTCCTACTCAGCTTTAGCTCTCAACATGG

Bands expected:

Primers Com-F + WT-R: WT, Het and Homo all show 596 bp
 Primers Com-F + Mut-R: Mut 390 bp
 WT 1927 bp (Maybe inhibited)

Run on 2% agarose gel in TAE.

36738 (WT Rxn)



Primers : Com-F + WT-R

Lane 1: Hom; Lane 2: Het; Lane 3, 4: WT; Lane 4: H₂O;
 M: 100 bp DNA ladder Invitrogen