

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	10626
MMRRC Strain Name	129S4/SvJae- <i>Tnfsf4</i> ^{tm15hr} /Mmnc
Gene Name(s)	Tumor necrosis factor superfamily, member 4 (Tnfsf4)
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	2/10/14

MMRRC #10626 PCR Reaction

	<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: 55°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 35x from step 2 to step 4
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

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

A25 (10626): AAA CTA TGG AGG TGC AGA

A26 (10626): CAG AAG CAA TGT GTC TTG

OXNeo3 (10626): ATT GAA CAA GAT GGA TTG CAC

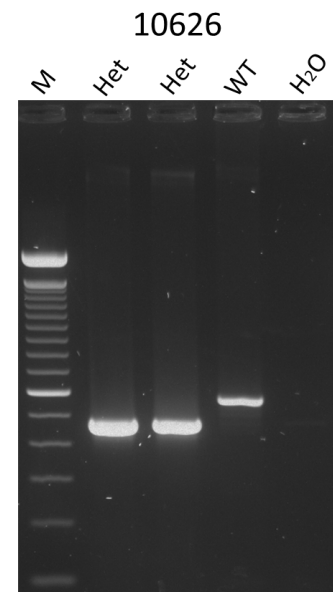
OXNeo4 (10626): CGT CCA GAT CAT CCT GAT C

Bands Expected:

WT [A25 (10626) + A26 (10626)]: 576 bp

MUTANT [OXNeo3 (10626) + OXNeo4 (10626)] ~475 bp

Run on 2% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)