

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36722
MMRRC Strain Name	<i>C.129S-Tpsb2^{tm1.1Mfg}/Mmnc</i>
Gene Name(s)	tryptase beta 2 (Tpsb2)
Breeding Protocol(s)	Sib-mating
Protocol Date	4/12/13

MMRRC #36722 PCR Reaction

Note: 2,2 Primer Reaction

Wild-type Reaction: mMCP6 + mMCP9

Mutant Reaction: mMCP9 + EGFP

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 65°C for 30 sec

Step 4: 72°C for 45 sec

Step 2 to 4 Cycles: 32

Step 5: 72°C for 7 min

	1X
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM WT Primer	1
10uM Mut Primer	1
Taq	0.5
DNA	2

Taq: **Apex Taq and Chromataq 5X Buffer**

Bands: MUTANT: ~800bp

WT: 264bp

Note: There is a ~300bp nonspecific band in homozygous samples

Primer sequences 5' to 3':

mMCP6 (36722): CAC TCG CTG ATT GGC TGG GCC TGG AGA GAG A

mMCP9 (36722): TTT AGC TGG ACT CAG GCT GTG CTC CTC ACT

EGFP(36722): GACCATGTGATCGCGCTTCT;

Run on 2% agarose gel in TAE.