

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	16842
MMRRC Strain Name	B6;129S7- <i>Twist1</i> ^{tm2Bhr} /Mmnc
Gene Name(s)	Twist gene homolog 1 (Twist1)
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	12/2/13

MMRRC# 16842 PCR Reaction

	<u>1X</u>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
10 uM Primer 1 & 2	1.0
Taq	0.5
ddH ₂ O	18.0
25mM MgCl ₂	1.5

Sample Preparation

Lysis Buffer Recipe

50mM Tris pH 8.8

1.0mM EDTA

0.5% Tween-20

Immediately before using, add 200ug/ml proK

1. Spin down sample in tub, add 100ul lysis buffer for ear punch, 200ul for 3-4mm tail snip.
2. Digest at 56°C for twelve hours, tap down condensation, then inactivate at 80°C for 10 minutes (we do this on a thermal cycler in strip tubes, but it's also easy on a heat block)
3. Tap spin down debris - usually 8000 rpm for 5 seconds will do it. Let samples cool to at least room temperature before doing a PCR.

Denaturing: 94°C for 45 sec
 Annealing Temp: 60°C for 45 sec
 Extension: 72°C for 1 min
 Cycles: 36
 Taq: **Apex and Chromataq Buffer**

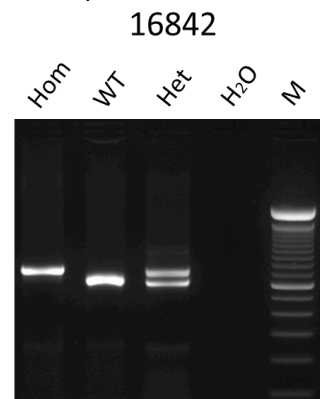
Bands: WT: 630bp
 Flox: 680bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

P112: AGC GGT CAT AGA AAA CAG CC

P114: CCG GAT CTA TTT GCA TTT TAC CAT GGG TCA TC

Run on 2% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)