

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	42289
MMRRC Strain Name	STOCK <i>Btbd18^{tm1Dean}</i> Tg(Btbd18/mCherry)#Dean/Mmnc
Gene Name(s)	BTB (POZ) domain containing 18
Breeding Protocol(s)	Random Intra-strain Mating
Protocol Date	10/19/2020

PCR Reaction

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

3, 2 Primer Reactions Required

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 57C(Tg) or 55C(KO), 30sec

Step 4: 72C, 30sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex and Chromataq 5X Buffer

Bands: mCherry: WT: No Band, Tg: 320bp

KO: MUT: 408bp, WT: 384bp

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

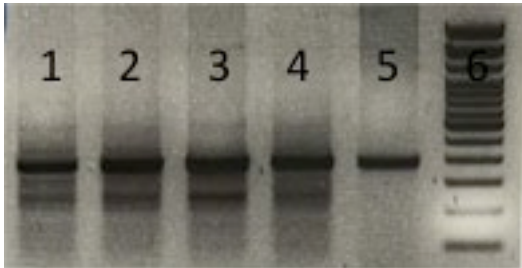
pmcherry267F CCTGTCCCCTCAGTTCATGT

pmcherry591R CTCAGCTTCAGCCTCTGCT

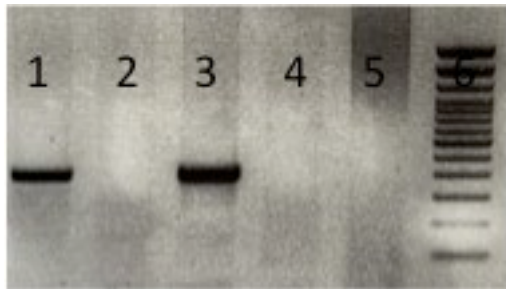
BT(42289)WTF	CTTGAAATTACCAGCTCCC
BT(42289)WTR	GAACTCTAACCTGCCCTCC
BT(42291)KOF	GAAGGTGCCACTCCCCTG
BT(42291)KOR	ACCAGCCAAACAAATGAAA

Run on 2.0% agarose gel in TAE.

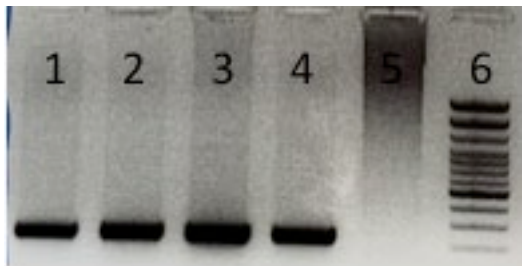
BTBD Wild-type Reaction



BTBD KO Reaction



mCherry Reaction



1. BTB Heterozygous, mCherry+
2. BTB Wild-type, mCherry+
3. BTB Heterozygous, mCherry+
4. BTB Wild-type, mCherry+
5. Wild-type Control
6. 100bp Ladder