

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	31695
MMRRC Strain Name	STOCK Tg(Acp5-CFP,Ibsp-YFP,Dmp1-RFP)28-4-3Pmay/Mmnc
Gene Name(s)	Acid phosphatase 5, tartrate resistant (Acp5) Dentin Matrix protein 1 (Dmp1) Integrin binding sialoprotein (Ibsp)
Breeding Protocol(s)	Outcross to CD-1 (Charles River)
Protocol Date	10/7/13

MMRRC# 31695 PCR Reaction

NOTE: MMRRC# 31695 is genotyped with respect to 3 different fluorescent transgenes (ACP5, DMP1, and IBSP). There are 3 primer sets that are used for each DNA sample, and each reaction should be run separately.

	<u>1X</u>
DNA	1.0
10X Buffer	2.5
10mM dNTPs	1.0
20 uM Primer 1 & 2	1.0
Taq	1.0
ddH ₂ O	16.5
25mM MgCl ₂	2.0

Thermal Cycler:

Denaturing: 95°C for 30 sec
Annealing Temp.: 60°C for 30 sec
Extension: 72°C for 1 min
Cycles: 30

Taq: Apex and Chromataq Buffer

Bands: ACP5⁺: 382bp
DMP1⁺: 300bp
IBSP⁺: 358bp

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

ACP5 Genotype: GTC TGT GGA ACT GAC GGC TGT AGA TGG CTA

CT Genotype: GTT TAC GTC GCC GTC CAG CTC GAC CAG GAT

DMP1 Genotype: CTG ACA AGG CTC CTC TCC TTT GTG GCA TCT

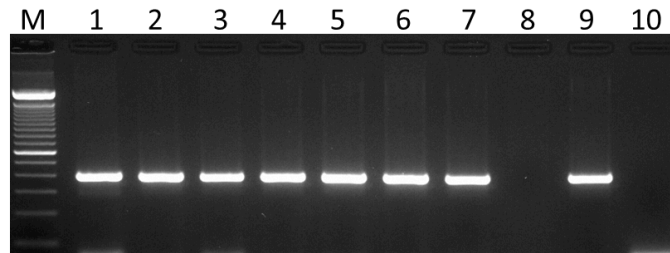
mCherry Genotype: GCA CCT TGA AGC GCA TGA ACT CCT TGA TGA

IBSP Genotype: GCA GGG ATT CAA TGT CCT TGA CCG ACA AAC

CT Genotype: GTT TAC GTC GCC GTC CAG CTC GAC CAG GAT

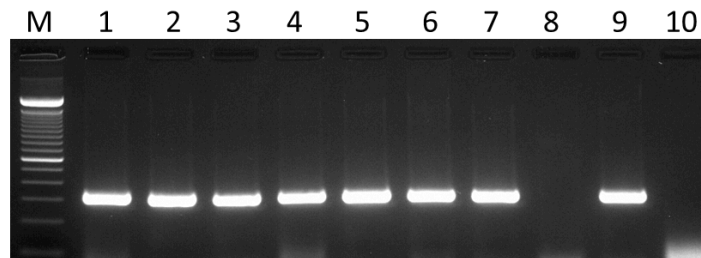
Run on 2% agarose gel in TAE

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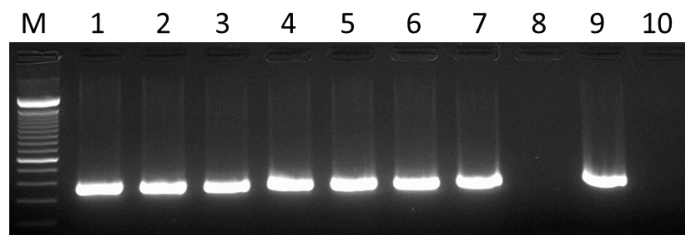
Primers: ACP5 Genotype + CT Genotype
Lane 1 to 7, 9: Tg+; Lane 8: WT; Lane 10: H₂O
M: 100 bp DNA ladder (Invitrogen)

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Primers: DMP1 Genotype + mCherry Genotype
Lane 1 to 7, 9: Tg+; Lane 8: WT; Lane 10: H₂O
M: 100 bp DNA ladder (Invitrogen)

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Primers: IBSP Genotype + CT Genotype
Lane 1 to 7, 9: Tg+; Lane 8: WT; Lane 10: H₂O
M: 100 bp DNA ladder (Invitrogen)