Genotyping Protocol: **MMRRC 30289**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain Characteristics: This strain has exon 1 and part of intron 1 of the *Dmbtl* gene replaced by Neo and GFP cassettes. The GFP is non-functional.

Primer Information:
1) Name: M30289 WT1 Sequence: 5'- TTC TAC TCA AAT CGA GAG GAA -3'
2) Name: M30289 WT2 Sequence: 5'- CTG GGG ACT TCA GTC AAT CA -3'
3) Name: M30289 GFP Sequence: 5'- CGA TGC CCT TCA GCT CGA T -3'

Primer location: M30289 WT1 and WT2 are located on Chromosome 7 in the *Dmbt1* gene. M30289 GFP is located within the inserted GFP.

Assay name: Dmbt1 KO PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M30289 WT1	Sigma or IDT	25µM	0.3
M30289 WT2	Sigma or IDT	25µM	0.3
M30289 GFP	Sigma or IDT	25µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			12.7

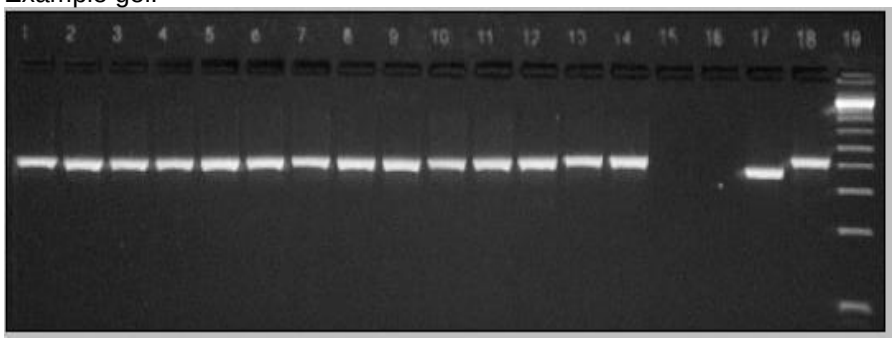
PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 95°C 5 minutes
2) 94°C 1 minute
3) 60°C 1 minute
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining. Run for 1 hr for good separation.
WT product: 369bp
KO product: 421bp

Example gel:



Wells 1-14 are homozygous mutant (KO) (421bp band).
Wells 15 and 16 are blanks.
Well 17 is a wild-type control (369bp band).
Well 18 is a homozygous mutant (KO) control (421bp band).
Well 19 is 1Kb+ Ladder (Invitrogen Cat#10787-018).