GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: C;B6-Tg(Smgb-TAg)8Mir/Mmucd **MMRRC #** 000039-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	17.65
10x Buffer (without 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20µM) Tag 3	0.5
Primer 2 (stock concentration is 20µM) Tag 5	0.5
Primer 3 (stock concentration is 20µM) Tcrd F	0.2
Primer 4 (stock concentration is 20µM) Tcrd R	0.2
Taq Polymerase 5 Units/µL	0.25
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

Tcrd is used as a reporter gene to detect presence of DNA and does not give any indication of zygosity.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	1:30	**1**
2. Denaturation	94	0.20	
3. Annealing } steps 2-3-4 will cycle in sequence	57	0:30	} **30x**
4. Elongation	72	0:35	
5. Amplification	72	2:00	**1**
6. Finish	10	∞	n/a

Primers:

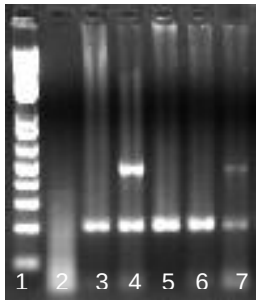
Primer Name	Nucleotide Sequence (5' - 3')
1: Tag 3 oIMR 68	CAG AGC AGA ATT GTG GAG TGG
2: Tag 5 oIMR 69	GGA CAA ACC ACA ACT AGA ATG CAG TG
3: Tcrd F	CAA ATG TTG CTT GTC TGG TG
4: Tcrd R	GTC AGT CGA GTG CAC AGT TT

Electrophoresis Protocol:

% Agarose: 2 **V:** 100

Estimated Running Time (min): 60

Expected Bands	Genotype
474 bp	Transgene
200 bp	Wild-type



Lanes:
1. 1Kb + ladder
2. H_2O
3. B6
4. Tag +
5-6. Wild-type samples
7. Tag + sample