GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **C57BL/6-*Tirap*^{m1Btlr}/Mmcd, (*torpid*)** **MMRRC #** **016982-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	12.675
10x Buffer (contains 15mM $MgCl_2$)	2.5
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 25mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM) Torpid PCR F1	0.5
Primer 2 (stock concentration is 20µM) Torpid PCR R1	0.5
Taq Polymerase	0.5
DNA sample extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- The *torpid* mutation destroys a *Bgl* II restriction enzyme site in the *Tirap* genomic DNA sequence. *Torpid* genotyping is performed by amplifying the region containing the mutation using PCR, followed by *Bgl* II restriction enzyme digestion.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐		94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 will cycle in sequence	55	0:30	**30x**
7. Elongation		72	0:40	
8. Amplification		72	5:00	**1**
9. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Torpid PCR F1	GTG AAA GGT AAC AGA AAC CAG TCA CCT CC
2: Torpid PCR R1	CGT GCC TGA TGC CAG AGG AAG AAG AC

Electrophoresis Protocol:

Agarose: 2% **mV:** 80 **Estimated Running Time:** 90 **min**

Primer Combination	Band	Genotype
1 and 2	553 bp	*torpid*
the *Bgl* II site destroyed by the *torpid* mutation is highlighted in gray		
Restriction Digest w/ *Bgl* II	159, 393 bp	WT +/+
	159, 393, 553 bp	HET +/-
	553 bp	HOM -/-

Mutation site (red) and flanking sequence:

SNP found at position ~ 243 of sequencing

WT ctctttcAgatctgg
torpid ctctttcTgatctgg