GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **STOCK *Ntf3*^{tm1Lfr}/Mmucd** **MMRRC #** **000191-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer (contains / without 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM) MNT3-IF	0.5
Primer 2 (stock concentration is 20µM) MNT3-IR	0.5
Primer 3 (stock concentration is 20µM) PGK2-mut	0.5
Primer 4 (stock concentration is 20µM) SV40F-mut	0.5
Taq Polymerase 5Units/µL	0.2
DNA extracted with ☐ NaOH ☒ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- Use Touch-Down cycling protocol-first 10 cycles anneal at 65^{o} C decreasing in temperature by 1.0 ^{o} C; next 30 cycles anneal at 55^{o} C.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (^{o}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 - 55 (↓1^{o}C/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: MNT3-IF	ACT ACG GCA ACA GAG ACG CTA C
2: MNT3-IR	ACA GGC TCT CAC TGT CAC ACA C
3: PGK2-mut	GTG CCA GCG GGG CTG CTA AAG CGC
4: SV40F-mut	CTG CAT TCT AGT TGT GGT TTG TCC AAA CTC ATC

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
1 and 2	200 bp	WT +/+
3 and 4	150 bp	KO -/-