GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **B6.129S-*Slc12a*^{1tm1Tkh}/Mmucd** **MMRRC #** **000017-UCD**

DNA Extraction Method: ☒ NaOH ☐ Proteinase K ☐ Other: _____

Protocol:

Reagent/ Constituent	Volume (µL)
Water	17.25
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	3.73
dNTPs (stock concentration is 10mM)	0.5
DMSO	2.0
Primer 1 (stock concentration is 20µM)	0.5
Primer 2 (stock concentration is 20µM)	0.5
Primer 3 (stock concentration is 20µM)	0.5
Taq Polymerase 5 Units/µL	0.25
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

Use commercial 10X buffer without $MgCl_2$, or make 10X buffer containing: 67 uM EDTA, 166 mM Ammonium Sulfate, 670 mM Tris (pH 8.5), 67 mM $MgCl_2$, 50 mM 2-mercapto ethanol.

Strategy:

Steps	Temp (^oC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐	--	--	**--**
2. Denaturation } steps 2-3 will cycle in sequence	94	0:30	} **40x**
3. Annealing } steps 2-3 will cycle in sequence	60	3:00	} **40x**
4. Elongation } steps 2-3 will cycle in sequence	--	--	} **40x**
5. Amplification	--	--	**--**
6. Finish	4	∞	n/a

Primers:

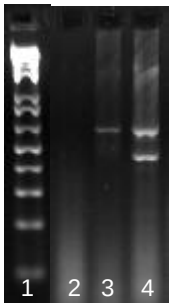
Primer Name	Nucleotide Sequence (5' - 3')
1: Neo R2	CTT CTA TCG CCT TCT TGA CG
2: Fib-2	CAA TAG GCT GCT GAG ATG AG
3: F74-B	GCA TCT TTA CTC TTG GGA GC

Electrophoresis Protocol:

% Agarose: 2 **mV:** 80

Estimated Running Time (min): 90

Expected Bands	Genotype
620 bp	WT +/+
620 / 490 bp	Het +/-
490 bp	KO -/-



Lanes:
1. 1KB+ ladder
2. H_2O
3. B6
4. Het