

GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: B6.129-Ctnnd2^{tm1Lxin}/Mmucd MMRRC: 036797-UCD

Protocol: *(PCR protocol provided by Donating Investigator)*

Reagent/Constituent	Volume (μL)
Water	6.3
10x Buffer	2.0
dNTPs (stock concentration is mM)	0.3
Primer 1. (stock concentration is μM)	0.2
Primer 2. (stock concentration is μM)	0.2
Primer 3. (stock concentration is μM)	0.2
Taq Polymerase 5Units/μL	0.4
DNA extracted with <input type="checkbox"/> NaOH <input type="checkbox"/> Proteinase K <input type="checkbox"/> Other:	0.4
TOTAL VOLUME OF REACTION:	
	10.000 μL

Comments on protocol:

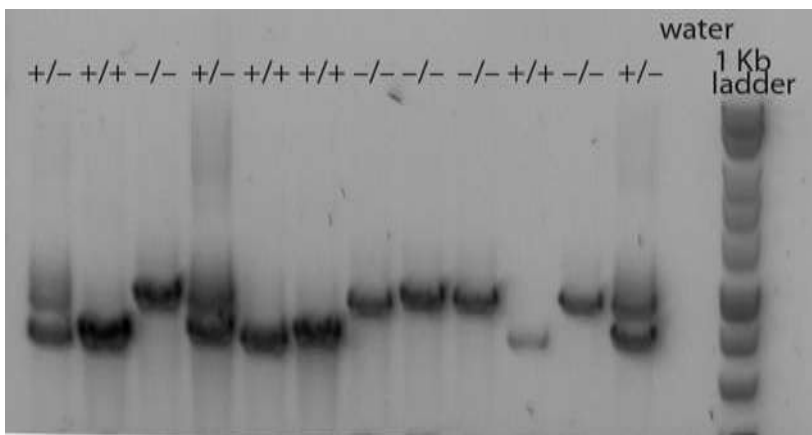
- This PCR amplifies relatively long PCR fragments. LongAmp Taq (NEB, Cat. M0323S) has been successfully used. EtOH precipitation of DNA has been helpful in consistent amplification of PCR products.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	3:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	63	0:30	40x
4. Elongation	68	2:30	
5. Amplification	68	10:00	1
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5%	V: 90
1. INS5	CCCACTTCACAACACTAGCACATGG	Estimated Running: Time: 90 min.	
2. Rev1	GGCAGTAACAGCTCACAGCGTG	Primer Combination	Band (bp)
3. GFPprev	GCTCGTCCATGCCGAGAGTG	1 and 2	2386
		1 and 3	2804
			Genotype
			Wild-type
			KO or MT/-



This PCR is quite robust. So, it is very easy to overload DNA, which hinders separation of both bands.