GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: **C57BL/6J-*Card11*^{m1Btlr}/Mmcd, (*king*)** **MMRRC #** **030114-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	37.0
10x Buffer Sigma Red Taq Buffer	5.0
dNTPs (stock concentration is 25mM)	2.5
Primer 1 (stock concentration is 50mM) King PCR F	0.5
Primer 2 (stock concentration is 50mM) King PCR R	0.5
RED Taq	2.5
gDNA template (50-100ng/µl) extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	2.0
TOTAL VOLUME OF REACTION:	**50µL**

Comments on protocol:

- The *king* mutation introduces a *Dde* I restriction enzyme site in the *Card11* genomic DNA sequence. *King* genotyping is performed by amplifying the region containing the mutation using PCR, followed by *Dde* I restriction enzyme digestion.
- Use SIGMA RedTaq, associated buffers and dNTPs.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	10:00	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	94	0:30	} **34x**
3. Annealing }	55	0:30	
4. Elongation }	68	1:00	
5. Amplification	68	7:00	**1**
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: King PCR F1	ATG CTT CTT CAT TGG GTG GA
2: King PCR R1	AAT TAC GGC AGC TCA CCA TC

Electrophoresis Protocol:

Agarose: 3% **mV:** 80 **Estimated Running Time:** 90 **min**

Primer Combination	Band	Genotype
1 and 2	444 bp	WT
SNP found at position ~ 335 of sequencing		
the novel *Dde I* site is highlighted in gray		
Restriction Digest w/ *Dde I*	332 bp, 113 bp	*king*

Mutation site (red) and flanking sequence:

WT cccgcggctcTgatgagg
king cccgcggctcAgatgagg