

# GENOTYPING BY PCR PROTOCOL

## KOMP Repository: UC DAVIS

### Protocol: CR799 Asic4

Reagent/Constituent	Volume (μL)
Water	10.275
10x Buffer	2.5
MgCl <sub>2</sub> (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Primer 3. (stock concentration is 20μM)	0.5
Primer 4. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>25.000 μL</b>

#### Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non-specific amplifications.

#### Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

#### Primers:

#### Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V: 90
1. SEQ-Asic4-F	GCCTGGCAGATACTCAGTCTGC	Estimated Running Time: 90 min
2. CpGf-lacZ-R	CTGTCCTGGCTTCCTCAGAGTTCC	<b>Primer Combination</b>
3. SEQ-Asic4-R	GCCTCCTTCTCACAGCGCAGTC	<b>Band (bp)</b>
		<b>Genotype</b>
		1 & 2 311 mutant
		1 & 3 237 wildtype

**Allele Description:** Exon 4 (ENSMUSE00000265212) has an in-frame LacZ insertion in the Asic4 gene (ENSMUST00000037708.9) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

#### Homologous recombinant

\*Targeting Confirmed

