

GENOTYPING BY PCR PROTOCOL

KOMP Repository: UC DAVIS

Protocol: CR930 Pde6a

Reagent/Constituent	Volume (μL)
Water	10.275
10x Buffer	2.5
MgCl ₂ (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
TOTAL VOLUME OF REACTION:	25.000 μL

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 MgCl₂ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps	Temp (°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 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. Pde6a CR930-F	CCTAGAGGACTGCTTGGTGATGC	Estimated Running Time: 90 min
2. Pde6a CR930-R	GAGAGCGTACCTCTTCTGTGTTGG	
		Primer Combination
		1 & 2
		125
		Band (bp)
		112
		Genotype
		Wildtype
		mutant

Allele Description: Exon 1 (ENSMUSE00000351964) received a 13bp deletion (**cgacactccaaa**) from the Pde6a gene (ENSMUST00000025468.13) using CRISPR Cas9 gene editing technology in mouse zygotes. This causes a frameshifted transcript followed by early termination signal. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals

Frameshift Indel

Pde6a Exon 1

13bp Deletion



Contig ggggtgtcgtgggccacgt**cgacactccaaa**aagattgccaatgtcc

