

GENOTYPING BY PCR PROTOCOL

KOMP Repository: UC DAVIS

Protocol: CR930 Pde6a

Reagent/Constituent	Volume (μ L)
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 20 μ M)	0.5
Primer 2. (stock concentration is 20 μ M)	0.5
Primer 3. (stock concentration is 20 μ M)	0.5
Primer 4. (stock concentration is 20 μ M)	0.5
Taq Polymerase 5Units/ μ L	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? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 ($\downarrow 1^{\circ}\text{C}/\text{cycle}$)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

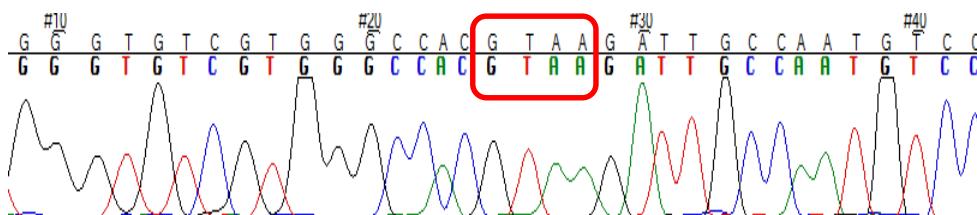
Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V: 90
1. Pde6a CR930-F	CCTAGAGGACTGCTGGTGATGC	Estimated Running Time: 90 min
2. Pde6a CR930-R	GAGAGCGTACCTCTCTGTGTTGG	Primer Combination
		Band (bp)
		1 & 2
		125
		Wildtype
		1 & 2
		112
		mutant

Allele Description: Exon 1 (ENSMUSE00000351964) received a 13bp deletion (**cgcacactccaaa**) 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 gggtgtcggtggccacgt**cgcacactccaaaagattgccaatgtcc**

