GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: B6;SJL-Tg(Amelx-Enam)3Jcch/Mmucd **MMRRC #** 036988-UCD

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

Reagent/Constituent	Volume (µL)
Water	
10x Buffer	
dNTPs (stock concentration is 10mM)	
DMSO *Optional*	
Primer 1 (stock concentration is 20µM) Enam TgF	1.00
Primer 2 (stock concentration is 20µM) Enam TgR	1.00
Primer 3 (stock concentration is 20µM) Enam 4&5F	1.00
Primer 4 (stock concentration is 20µM) Enam 4&5R	1.00
Taq Polymerase 5Units/µL	
Additives / Other (if applicable): Supermix from Invitrogen	17.00
DNA sample extracted with ☐ NaOH ☒ Proteinase K ☐ Other:	1.00
TOTAL VOLUME OF REACTION:	**.µL**

Comments on protocol:

- Please note that primers 1&2 are for amplification of the transgenic construct sequence, total reaction volumn is 20 µl. Primers 3&4 are used (separately from primers 1&2) to amplify enamelin wild-type sequence at Exon 4 and 5, total reaction volumn is 20 µl

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☒	94	5:00	**1**
2. Denaturation	} steps 2-3-4 cycle in sequence	94	0:30	} **25x**
3. Annealing		58	1:00	
4. Elongation		72	0:30	
5. Amplification		72	1:00	**1**
6. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Enam TgF	GCCGCACCTTCTTTTTGAT
2. Enam TgR	CAGACCCAGGAAAACAAGGA
3. Enam 4&5F	GCCCAAAGCACAGTCATTTT
4. Enam 4&5R	TAGGACCTGGCACGTGTCTC

Electrophoresis Protocol:

Agarose: % **V:**

Estimated Running Time: **min.**

Primer Combination	Band	Genotype
1&2	236 bp	Enam Tg
3&4	324 bp	WT+/+