

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR10726 Tph2 exdel

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.5
Primer 2. (stock concentration is 20µM) wtR	0.5
Primer 3. (stock concentration is 20µM) mutR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	15.00 µL

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Tph2_comF	CGCAGGAGATTCACCTAGAGTCG	Estimated Running Time: 90 min.	
2. CR_Tph2_wtR	TCCTCCTCTGTCGATAGACATTGTCCT	Primer Combination	Band (bp)
3. CR_Tph2_mutR	GCTCACAAACACACAGCCCTCATG	1 & 2, 1 & 3	575, 1137
		1 & 3	574
			wildtype
			mutant

Electrophoresis Protocol:

Allele Description: Exon 5 ENSMUSE00000100959 and flanking splicing regions were constitutively deleted from the Tph2 gene ENSMUST0000006949.8 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)

*wtR and mutR should be run in separate reactions

