GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR10063 Platr14**

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 (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1x**
2. Denaturation		94	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/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')
1. Platr14-5F	GTGTTTGTGGGCACCCATGCTAC
2. Platr14-gtiresR	CTAGGAATGCTCGTCAAGAAGACAGG
3. Platr14_gfp3F1	GGGATCACTCTCGGCATGGAC
4. Platr14_3R1	ACCAAAGAACCATGTCTGAACAGG
5. Platr14-3R	GCTAACTCTGCAACGCTTCTCG

Electrophoresis Protocol:

Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2	418	5' Positive
3 & 4	433	3' Positive
1 & 5	619	Wildtype

Allele Description: An IRES-EGFP cassette was inserted using CRISPR technology into a common exon conserved in all transcripts. This allele is designed to interrupt the function of the long non-coding RNA while producing a polyA to assist in exportation from the nucleus.

Note: Primer combination 1 and 2 amplifies across the 5' junction and primer combination 3 and 4 amplifies across the 3' junction. Either assay can be used to genotype for KI animals.

Either primer combinations (1&2 or 3&4) can be multiplexed with 1&6.