

GENOTYPING PROTOCOL

UC Davis Mouse Biology Program

Protocol Name: C57BL/6N-Nadkem1(IMPC)Bay/Mmucd MMRRC: 048726

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	5.15
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
Primer 3. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
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? ☐	94	5:00	1x
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓ 1°C/cycle)	0:30	10x
4. Elongation	72	0:40	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	30X
7. Elongation	72	0:40	
8. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	:	90
1. 48726-5loxp-F	CCAGGACAGAGTCTTGAATCC	Estimated Running 90 min.		
2. 48726-5loxp-R	CCTGCTTGCCAACTGTACCT	Primers	Band (bp)	Genotype
3. 48726-3loxp-F	ACCTAAAGCAGACGGGCTCT	1 & 2	150/190	Wildtype/floxed
4. 48726-3loxp-R	CGAAGACACTTATGCCATGC	3 & 4	136/170	Wildtype/floxed
5. 48726-KO-F	AGACCCAGGACAGAGTCTTGA	5 & 6	244	KO
6. 48726-KO-R	TGAAGTTGATGGTGGGCACT	7 & 8	516	wildtype
7. 48726-endo-F	AGACCCAGGACAGAGTCTTGA			
8. 48726-endo-R	AGCACACTCTTGGGAGACTTG			