

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: FVB/NJ-Tg(APOL1*)G2Mrpk/Mmucd MMRRC: 069640-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	
15	

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.

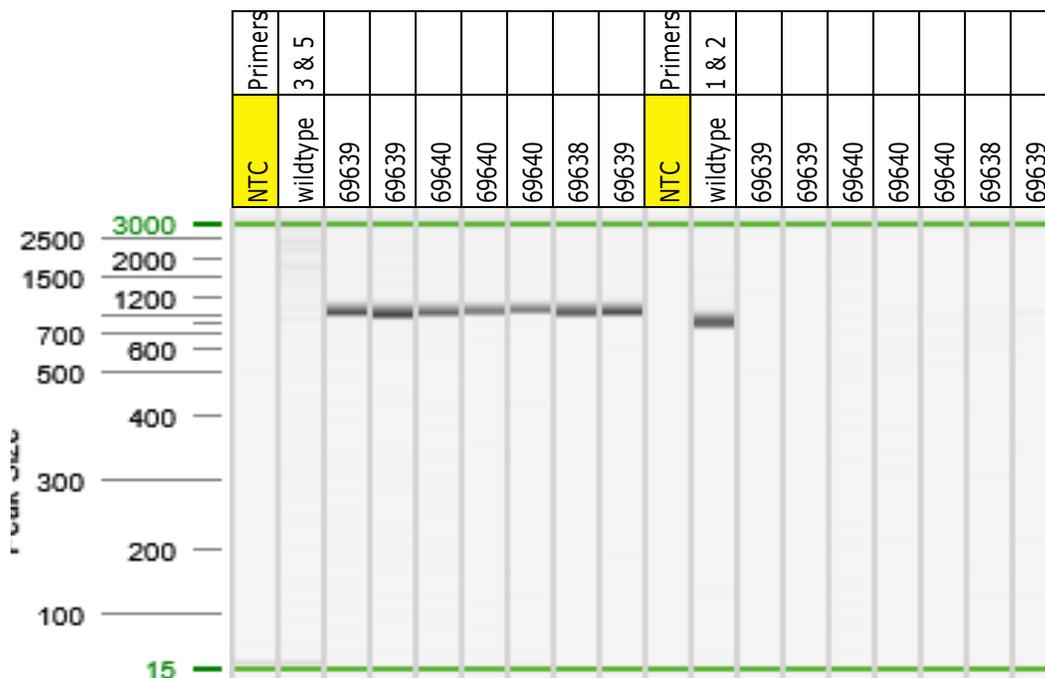
Strategy:

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)	

Primers:

Electrophoresis Protocol:

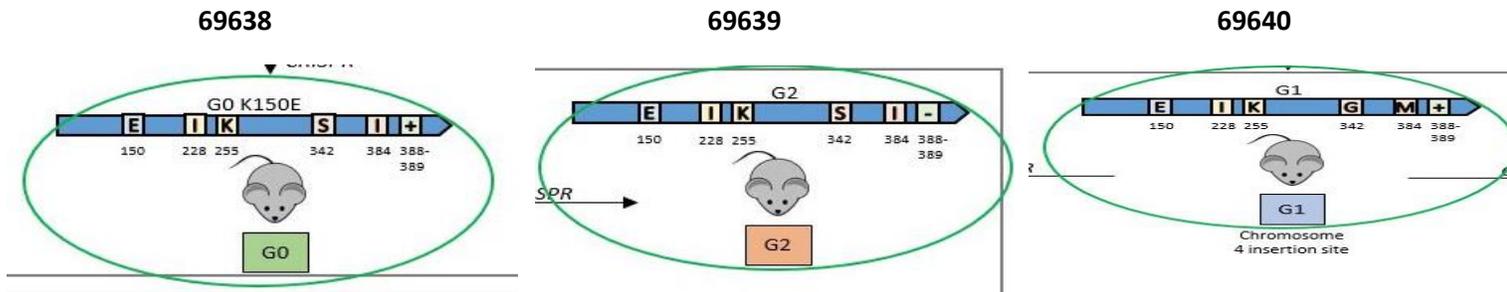
Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5%	V: 90	
1. 69638.39.40-wtF	ATCAAGTTTGGGAAGGTGCTTTATC	Estimated Running:Time: 90 min.		
2. 69638.39.40-wtR	TGCCACTTTGCAATAATCACAT	Primer Combination	Band (bp)	Genotype
3. 69638.39.40-mutF	TGAGGCAGATGAGCTCCGTA	1 & 2	821	wildtype
4. 69638.39.40-mutR	AAGTTTGCATTTTGTCTTGCC	3 & 4	900	mutant
5. 69638.39.40-SeqF2	GCCAATCTCAGCTGAAAGCG			



PCR protocol developed by MMRRC at University of California, Davis

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Sequence with primer 3

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69638-T-0695-mutF | CAGTACAGAAACTGGTTTCTGAAAGAGTTTCCTCGGTTGAAAAGTGAGCTT
69639-T-0722-mutF | CAGTACAGAAACTGGTTTCTGAAAGAGTTTCCTCGGTTGAAAAGTGAGCTT
69640-T-0704-mutF | CAGTACAGAAACTGGTTTCTGAAAGAGTTTCCTCGGTTGAAAAGTGAGCTT
    
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Sequence with primer 5

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) 69638-T-0695-seqF2 | GAGGAGTCAAGCTCACGGATGTGGCCCCTGTAAGCTT
) 69639-T-0721-seqF2 | GAGGAGTCAAGCTCACGGATGTGGCCCCTGTAAGCTT
) 69640-T-0703-seqF2 | GAGGAGTCAAGCTCACGGATGTGGCCCCTGTAAGCTT

69638-T-0695-seqF2 | CACTTACATGAGGGGGCAAAGTCAGAGACAGCTGAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATTC
69639-T-0721-seqF2 | CACTTACATGAGGGGGCAAAGTCAGAGACAGCTGAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATGC
69640-T-0703-seqF2 | CACTTACATGAGGGGGCAAAGTCAGAGACAGCTGAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATTC

69638-T-0695-seqF2 | AGCACTTACATGAGGGGGCAAAGTCAGAGACAGCTGAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATTCTCAAATAATTATAAGAT
69639-T-0721-seqF2 | AGCACTTACATGAGGGGGCAAAGTCAGAGACAGCTGAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATGCTCAAATAATTATAAGAT
69640-T-0703-seqF2 | AGCACTTACATGAGGGGGCAAAGTCAGAGACAGCTGAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATTCTCAAATAA:::GA
    
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