GENOTYPING BY PCR PROTOCOL
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Protocol Name: **C57BL/6-*Poli*^{tm1Pjg}/Mmucd** **MMRRC:** **042060-UCD**

Protocol:

Reagent/Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
Taq Polymerase 5Units/µL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	**1**
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1ºC/cycle)	0:30	**40x**
4. Elongation	72	0:40	
5. Amplification	72	5:00	**1**
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1.42060-F	ACACACACAATACTACACA
2.42060-R	AAGCTGCTGGAGTCTTTT

Electrophoresis Protocol:

Agarose: **2%** V: **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	320	Wt
1 & 2	320+270+50	Wt/KI
1 & 2	270+50	KI/KI

Protocol / Gel Comments: After the PCR, set up the digestion using NEB.com: buffer3 3 µl, ApeK1 1 µl, PCR product 16µl, dH2O 10 µl, for a total of 30 µl. Put the tube on the 75C heating block for 1 to 2 hrs. Spin down and add loading dye. Run 2% agarose gel for 60 min or longer. ApeK1 will only digest KI DNA, yielding 270 and 50 bp bands. Wild type DNA will not be digested. **Note:** ApeK1 has the same cutting pattern as Tse1 (65C).