GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **C57BL/6J-*Gimap5*^{m1Btlr}/Mmcd, (*sphinx*)** **MMRRC #** **030019-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	37.0
10x Buffer Sigma Red Taq Buffer	5.0
dNTPs (stock concentration is 25mM) (D-7295- Sigma)	2.5
Primer 1 (stock concentration is 50µM) Sphinx F	1.0
Primer 2 (stock concentration is 50µM) Sphinx R	1.0
RED Taq	2.5
gDNA template (50-100ng/µl) extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	2.0
TOTAL VOLUME OF REACTION:	**50µL**

Comments on protocol:

- The sphinx mutation destroys an *Aci* I restriction enzyme site in the Gimap5 genomic DNA sequence.
- Sphinx genotyping is performed by amplifying the region containing the mutation using PCR, followed by *Aci* I restriction enzyme digestion.
- Use SIGMA Red Taq, associated buffers and dNTPs; no product is amplified by Accu Taq. PCR reaction mix can be scaled down to a volume of 25 µL if the genomic DNA is of high quality.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	10:00	**1**
2. Denaturation	94	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	55	0:30	} **32x**
4. Elongation	68	1:00	
5. Amplification	68	10:00	**1**
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Sphinx F	CCC TTG GCT GAC TTC CAA TA
2: Sphinx R	GAC CTC CTT CAC CAT CCT CA

Electrophoresis Protocol:

Agarose: 3% **mV:** 80 **Estimated Running Time:** 90 **min**

Primer Combination	Band	Genotype
1 and 2 (before digest)	556 bp	*sphinx*
Restriction Digest w/ *Aci* I	258 bp, 298 bp	WT

Mutation site (red) and flanking sequence:

WT ctggctgcGgtaaaag
sphinx ctggctgcTgtaaaag