GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

- *Open the original graphic in the program that created it*
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These instructions are very generic. The menu options for your graphics program may be different.

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Strain Name ***Pik3ap1*<m1Btlr>; alternatively, *sothe***		MMRRC Stock Number **36402**

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NAME OF PCR: **PCR & Sequencing Protocol for *sothe*** **MMRRC:** **36402-UCD**

Protocol: ***(PCR protocol provided by Donating Investigator)***

Reagent/Constituent	Volume (µL)
Water	36
10x Buffer (JumpStart Mix with $MgCl_2$)	5
dNTPs (stock concentration is 10mM)	4
Primer 1. (stock concentration is 20µM)	1
Primer 2. (stock concentration is 20µM)	1
Taq Polymerase 0.5Units/µL (JumpStart)	1
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	2
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume. **TOTAL VOLUME OF REACTION:**	**50.000 µL**

Comments on protocol:

- *Sothe* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the nucleotide change.

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	94	2:00	**1**
2. Denaturation	94	0:30	
3. Annealing steps 2-3-4 cycle in sequence	57	0:30	**30x**
4. Elongation	72	1:00	
5. Amplification	72	7:00	**1**
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Sothe(F)_PCR	TGAGCAAATGGCACAGAAGATACCC
2. Sothe(R)_PCR	TTCCTGCTGAGGCTCTCAGAAGAC
3. Sothe(F)_seq	GGCACAGAAGATACCCCAAGG
4. Sothe(R)_seq	GGCCACACTTACCTTTAGAAATG

on reaction, and the Sothe_seq primers are used for the .

nomic region: NC_000085.5 of the linear genomic sequence of

```
      tgagcaaat ggcacagaag
tttg gcaacagaga acattcgctc
acgg ttctgtactc agtccccctt
tggg agagagaaca acaccatccc
gaga ccttctctgt ggacctagcc
agcg ctcctggccc tcccccaccg
ggcc agggatggtg ctcagcaggg
gcag gaa
```

d; Sequencing primer binding sites are highlighted; the mutated + strand) at 41,356,683 bp on Chr. 19).

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Protocol / Gel Comments:

PCR protocol provided by Donating Investigator