*Sod1*tm2 PCR

Primers: CITE20U21 CCG TCT TTT GGC AAT GTG AGG
CITE355L21 TGG GGT ACC TTC TGG GCA TCC
Sod1EX3/4 113U21 GGC GGA TGA AGA GAG GTG AGC
Sod1EX3/4 360L21 ATT GGC CAC ACC GTC CTT TCC

Primer conc. CITE20U21 0.5 µM final conc. (prepare 5 µM as 10X stock)
CITE355L21 0.5 µM final conc. (prepare 5 µM as 10X stock)
Sod1EX3/4 113U21 0.25 µM final conc. (prepare 2.5 µM as 10X stack)
Sod1EX3/4 360L21 0.25 µM final conc. (prepare 2.5 µM as 10X stack)

PCR fragment size: *CITE* 335 bp indicates the presence of KO allele
Sod1 ~700 bp (amplified from *Sod1* exon 3 to exon 4) indicates the presence of normal *Sod1* allele

PCR buffer: ThermoPol buffer (New England Biolab)

PCR reaction: 2.5 µl 10X ThermoPol buffer
2.5 µl 10X primers
0.25 ul BSA (100x)
14.25 µl H2O
0.5 µl DNA prep
20 µl mineral oil overlay

and 2.5 µl dNTP (2 mM)
0.1 U Tag polymerase (0.5 U)
2.4 µl H2O

PCR profile: 1 cycle at 94oC 5 min. and 80oC 5 min. for hot start
30 cycles at 94oC 30”, 62oC 30” and 72oC 30”
then reduce to 4oC and hold in Perkin Elmer Gene Amp 9700.

Profile name: Use same program as Sod2.

1. Label a set of 0.5 ml tubes with sample ID, plus two tubes – one labeled H2O (as negative control), and one label -/+ (as positive control).
2. Prepare “master mix”.

No. tubes	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55
10x primers	50 µl	62.5 µl	75 µl	87.5 µl	100 µl	112.5 µl	125 µl	137.5 µl	150 µl
10x buffer	50 µl	62.5 µl	75 µl	87.5 µl	100 µl	112.5 µl	125 µl	137.5 µl	150 µl
100x BSA	5 µl	6.3 µl	7.5 µl	8.8 µl	10 µl	11.3 µl	12.5 µl	13.8 µl	15 µl
H2O	285 µl	356 µl	427.5 µl	499 µl	570 µl	641 µl	712 µl	784 µl	855 µl

3. Add 19.5 µl master mix into the bottom of each tube. Use automatic repeat pipetter.
4. Add 0.5 µl H2O to the tube labeled H2O; 0.5 µl -/+ DNA to the tube labeled -/+; 0.5 µl from each DNA sample into their corresponding tubes. Put the 0.5 µl directly into the 19.5 µl master mix.
5. Add 20 µl mineral oil to each tube. Use a repeat pipetter. Let oil run down through the side of the tubes.
6. Load tubes onto PCR machine. Start PCR profile.
7. Prepare dNTP/Taq mix.

No. tubes	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55
10x dNTP	50 µl	62.5 µl	75 µl	87.5 µl	100 µl	112.5 µl	125 µl	137.5 µl	150 µl
Taq (5U/µl)	2 µl	2.5 µl	3 µl	3.5 µl	4 µl	4.5 µl	5 µl	5.5 µl	6 µl
H2O	48 µl	60 µl	72 µl	84 µl	96 µl	108 µl	120 µl	132 µl	144 µl

8. Add 5 µl dNTP/Taq mix to each tube when PCR goes down to 80oC. Put pipette tip all the way down to the bottom of the tube, pipette up and down three times to mix.
9. Close the lid of the tubes as you add dNTP mix. Finish the whole process in 5 minutes. If can’t finish, press pause to stop the timer for a while.
10. Depends on the PCR machine, close down the heated cover or switch to a different profile. The whole process will take 1 to 1.5 hours. When finished, PCR machine will switch to 4oC.
11. Prepare 1% agarose gel. Load 10 µl PCR product on the gel.