

## B6;129S-Sod1<sup>tm1Leb</sup>/J

Stock No: 002972

Protocol 26277: Standard PCR Assay - Sod1<sup>tm1Leb</sup>

Version 2.2

### Notes

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

### Expected Results

#### Melt Peaks

Wt = 83°C +/- 1

Mut = 92°C +/- 1

Mutant (HPRT) = ~240 bp

Wild Type (Sod1) = ~123 bp

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR0781		TGT TCT CCT CTT CCT CAT CTC C		Mutant	A	
oIMR0782		ACC CTT TCC AAA TCC TCA GC		Mutant	A	
oIMR0878		TGA ACC AGT TGT GTT GTC AGG		Wild type	A	
oIMR0888		TCC ATC ACT GGT CAC TAG CC		Wild type	A	

#### Reaction A

COMPONENT	FINAL CONCENTRATION
ddH <sub>2</sub> O	
Kapa 2G HS buffer	1.30 X
MgCl <sub>2</sub>	2.60 mM
dNTP KAPA	0.26 mM
oIMR0781	0.50 uM
oIMR0782	0.50 uM
oIMR0878	0.50 uM
oIMR0888	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

#### Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.

