

# Genotyping protocol

EM:09496

**B6;129S6-Sp140<sup>tm1Ptsn</sup>/Kctt**

## Primers

Sp140F	5' AAA GCC ACT TTG AGT GGA CGA C 3'
Sp140R1	5' CCT CAG AGG CTG AAC ACA TTC 3'
Sp140R2	5' TCC CTT CAA GTT ACC ACA CGT C 3'

## PCR profile

95 °C, 5 min	
94 °C, 30 s	35 cycles
58 °C, 45s	
72 °C, 60 s	
72 °C, 10 min	
4 °C, ∞	

## PCR mix

10 x PCR Buffer II (Life technologies)	3.0 µl
MgCl <sub>2</sub> (25 mM)	1.8 µl
dNTP mix (25 mM of each nucleotide)	0.24 µl
Sp140F (100 µM)	0.15 µl
Sp140R1 or Sp140R2 (100 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.15 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H <sub>2</sub> O	<u>22.51 µl</u>
	30 µl

## Post-PCR analysis

1.5% agarose gel.  
 Expected pattern is **wt: 617 bp**  
**Ko: 500 bp**