

# Genotyping protocol

## General information:

Strain name	B6.129S2-Trp53<tm1Thst>
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## Primers:

Name	Sequence	Primer type
4791	TCTTTGTGAAGGAACCTTACT	please select one
4792	CATTCATCAGTTCCATAGGTT	please select one
6035	CCCTGAGAAGAGCAAGGC	please select one
6036	AACCAGATCAGGAGGGTCAC	please select one

In case more than two primers are introduced, please indicate how they should be combined:

	Forward primer	Reverse primer
LSL	4791	4792
wt	6035	6036

## Reaction mix:

4791 10 $\mu$ M	1	$\mu$ l
4792 10 $\mu$ M	1	$\mu$ l
6035 10 $\mu$ M	1	$\mu$ l
6036 10 $\mu$ M	1	$\mu$ l
PCR buffer 5X	4	$\mu$ l
dNTP 10mM	0.4	$\mu$ l
Taq polymerase 5U/ $\mu$ l	0.2	$\mu$ l
water	10.4	$\mu$ l
Final volume	20	$\mu$ l

## PCR program:

94	$^{\circ}$ C	3	min	X10 - 0.5C/cycle
94	$^{\circ}$ C	30	sec	
60	$^{\circ}$ C	60	sec	
72	$^{\circ}$ C	30	sec	
94	$^{\circ}$ C	30	sec	X25
55	$^{\circ}$ C	60	sec	
72	$^{\circ}$ C	30	sec	
72	$^{\circ}$ C	3	min	

## Expected fragment size:

wt	139	bp
LSL	166	bp
recombinant	175	

## Comments/Additonal information:

Unspecific band ~200bp can be present in heterozygous +/-S180A recombinant samples

