

Genotyping protocol

General information:

Strain name	Sall3
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Primers:

Name	Sequence	Primer type
Fw	GAGCTGCCTTCTCAGGTGTT	please select one
Rv	TGGATGCCATCAGAAGAGGC	please select one
		please select one
		please select one

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

	Forward primer	Reverse primer
e.g. wt		
e.g. mut		

Reaction mix:

dNTPs 2mM	2.5	µl
Buffer Taq polymerase with MgCl 10x	2.5	µl
Sall3 Fw 10 µM	0.25	µl
Sall3 Rv 10 µM	0.25	µl
Taq Polymerase	0.25	µl
H ₂ O dd	18.25	µl
DNA	1	µl
		µl
Final volume	25	µl

PCR program:

94	°C	5	min	X35
94	°C	30	sec	
58	°C	30	sec	
72	°C	90	sec	
72	°C	10	min	

Expected fragment size:

wt	692	bp
mutant	692	bp

Comments/Additonal information:

Analysis of founder mice by cloning and Sanger sequencing, and digest PCR Product with SphI: 692 pb= 312 pb +380 bp.