

Genotyping protocol

General information:

Strain name	Mut-flox
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Primers:

Name	Sequence	Primer type
C029.5	5'-CTATGAACGGAGCTGTCATC-3'	gene-specific
C029.6	5'-GGAGGTGGCAAACACATAAG-3'	gene-specific
		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:

dd water	29	μl
10X PCR buffer	5	μl
25mM MgCl ₂	2	μl
10mM dNTP mix	1	μl
10uM Primers C029.5 / C029.6	1	μl
Template Genomic DNA	2	μl
5X Q Solution	10	μl
5U/ul HotStar Taq Qiagen	3/10	μl
Final volume	50	μl

PCR program:

95	°C	15	min	x32
95	°C	60	sec	
58	°C	45	sec	
72	°C	1	min	
72	°C	10	min	

Expected fragment size:

wt	521	bp
mutant	483	bp

Comments/Additonal information:

For further discrimination between wild-type and mutant PCR bands, the PCR product can additionally be digested with SmaI (11.5 ul PCR product, 1.5ul 10X NEB Buffer 1, 1.5ul 10X BSA, 0.5ul SmaI), which does not cut the wild-type PCR band (remains 521 bp), but does cut the mutant PCR band (418bp+65bp). The product should be analyzed on a 2.0% agarose gel.

