

Genotyping protocol

EMMA ID: EM:07434

Gene symbol: Lox

Strain designation: B6.129-Lox^{tm1Soin}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
Mexlf2	GAGAGGAGGACCGTGCCACGT
Mexlr2	CTCGCACGGGTAGAGGCGGTGC
Mexlf5	GAGTGTGTATTGGGAAATTTGTC
Mintlr1	GGCGTTGTTGAGTCTGCGGGAC

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	Mexlf2	Mexlr2	504
Mutant	Mexlf5	Mintlr1	257

Reaction mixes:

Wild-type Component	Volume (µl)	Mutant Component	Volume (µl)
Genomic DNA (~50-100 ng)	1.0	Genomic DNA (~50-100 ng)	1.0
Primer 1 (10 µM)	1.5	Primer 1 (10 µM)	1.5
Primer 2 (10 µM)	1.5	Primer 2 (10 µM)	1.5
10x Buffer (15 mM MgCl ₂)	3.0	10x Buffer (15 mM MgCl ₂)	3.0
MgCl ₂ (25 mM)	1.0	MgCl ₂ (25 mM)	1.0
dNTPs (25 mM)	0.25	dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.1	DNA polymerase (5 U/µl)	0.1
H ₂ O	21.65	H ₂ O	21.65
Total	30.0	Total	30.0

PCR program:

Wild-type

Time	Temperature (+ °C)	Cycles
4 min	95	
30 sec	95	40
30 sec	65	
1 min	72	
10 min	72	
∞	10	

Mutant

Time	Temperature (+ °C)	Cycles
4 min	95	
30 sec	95	40
30 sec	60	
1 min	72	
10 min	72	
∞	10	