

Genotyping protocol

EMMA ID: EM:09872

Gene symbol: Dkk1

Strain designation: B6;129P2-Dkk1^{tm1.1Svo}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
EM:09872 flox1	AGA ACT AAC CCA GCC CCA CAG CAG A
EM:09872 flox2	CTC CTC AGG GAA GAC AAC AAA GCC G
EM:09872 flox3	CCC GGG AAC TAC TGC AAA AAT GGT G
EM:09872 flox4	GTG CTC AAA CAC AAG CCA GTG ACG A

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type 5'	EM:09872 flox1	EM:09872 flox2	288
Mutant 5'	EM:09872 flox1	EM:09872 flox2	358
Wild-type 3'	EM:09872 flox3	EM:09872 flox4	186
Mutant 3'	EM:09872 flox3	EM:09872 flox4	278

Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	2.0
Primer 1 (10 µM)	1.0
Primer 2 (10 µM)	1.0
10x Buffer (15 mM MgCl ₂)	3.0
MgCl ₂ (25 mM)	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.05
H ₂ O	21.7
Total	30.0

PCR program:

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	94	30
1 min	68	
1 min	72	
7 min	72	
∞	4	