

Genotyping protocol

EMMA ID: 07458

Gene symbol: Marco

Strain designation: B6.129S6-Marco^{tm1Ktry}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
Marco/WT-1	CAG CTG GGT CCA TAC CAG C
Marco/WT-2	CTG GAG AGC CTC GTT CAC C
Lam1	TGT CAT CTC ACC TTG CTC CTG
Lam2	TCA AGA AGG CGA TAG AAG GCG

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	Marco/WT-1	Marco/WT-2	500
Mutant	Lam1	Lam2	490

Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	2.0
Primer 1 (100 ng/µl)	1.0
Primer 2 (100 ng/ µl)	1.0
10x Buffer (15 mM MgCl ₂)	3.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.05
H ₂ O	22.7
Total	30.0

PCR program:

Wild-type

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	94	30
1 min	56	
1 min	72	
7 min	72	
∞	4	

Mutant

Time	Temperature (+ °C)	Cycles
5 min	95	
30 sec	94	30
30 sec	55	
30 sec	72	
7 min	72	
∞	4	