

Genotyping protocol

EMMA ID: EM:08305

Gene symbol: Amacr

Strain designation: B6.129-Amacr^{tm1Jkh}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
Wtknock	AAC CTT CCT GAC TGT CCA AAG TAT
Knock	AAG CAA AGG ACC TCT TGC CAG A
Neoknock2	TCG CAT TGT CTG AGT AGG TGT CA

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	Wtknock	Knock	750
Mutant	Neoknock2	Knock	500

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	58	
1 min	72	
7 min	72	
∞	4	