

Genotyping protocol

EMMA ID: EM:10948

Gene symbol: Wnt4

Strain designation: B6N.129-Wnt4^{tm3(Wnt11)^{Svo}}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
JDPL 044	CAG ACG CAC TGC CAG CCC
JDPU 032	ACT CCT CGT CTT CGC CGT GTT CT
JDPL 040	TGC TCG GAG GCT GAG CCC CGC
Wnt11r	AGC CAC TTG ATG CCA TAG CAC AC

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	JDPL 044	JDPU 032	~ 300
Mutant	JDPL 040	Wnt11r	~ 300

Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	2.0
Primer 1 (10 µM)	1.5
Primer 2 (10 µM)	1.5
10x Buffer (15 mM MgCl ₂)	3.0
MgCl ₂ (25 mM)	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.1
H ₂ O	20.65
Total	30.0

PCR program:

Wild-type

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	94	30
1 min	60	
1 min	72	
10 min	72	
∞	10	

Mutant

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	94	30
1 min	68	
1 min	72	
10 min	72	
∞	10	