

Genotyping protocol

EMMA ID: EM:09262

Gene symbol: Col15a1

Strain designation: 129-Col15a1^{tm1Pih}/Oulu

Primers:

Primer name	Primer sequence (5'- 3')
XV-wasta	TGGCAGTGTGTTGTCACTGTACAGCTA
XV-ali	CGCCTCATTCTGGACATAAAAGGG
XV-yla	AGGGGAACGGAGTTCAATCCATAGA

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	XV-wasta	XV-ali	200
Mutant	XV-wasta	XV-yla	400

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:

Time	Temperature (+ °C)	Cycles
12 min	95	
1 min	95	35
1 min	62	
45 sec	72	
10 min	72	
∞	10	