

Genotyping protocol

Carbonic anhydrase 9; targeted mutation 1

Car9^{tm1Spas}

Primers:

Primer name	Primer sequence (5' - 3')
wt1	CCA GTC AGC TGC ATG GCC
wt2	AGG AGC CTC GGG AGT CGA
mut1	AGG AGC AAA GCT GCT ATT GG

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Mutant	mut1	wt2	~400
Wild type	wt1	wt2	318

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)	0.1
H ₂ O	22.65
Total	30.0

PCR program:

Time	Temperature (+ °C)	Cycles
5 min	96	
30 s	96	
1 min	56	35
1 min	72	
7 min	72	
∞	4	