

## Genotyping protocol

EMMA ID: EM:10618

Gene symbol: Tg(Tie1-cre)9Ref, Col15a1

Strain designation: B6;129-Col15a1<sup>tm2Pih</sup> Tg(Tie1-cre)9Ref/Oulu

### Primers:

Primer name	Primer sequence (5' - 3')
XV-wasta	TGGCAGTGTGTTGTCACGTACAGCTA
XV-ali	CGCCTCATTCTGGACATAAAAGGG
XV-yla	AGGGGAACGGAGTTCAATCCATAGA
Cref	CCATCTGCCACCAGCCAG
Crer	TCGCCATCTTCCAGCAGG

### Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	XV-wasta	XV-ali	200
LoxP-flanked	XV-wasta	XV-ali	300
Inactivated	XV-wasta	XV-yla	400
Transgene	Cref	Crer	281

### 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 <sub>2</sub> )	3.0
MgCl <sub>2</sub> (25 mM)	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.1
H <sub>2</sub> O	20.65
<b>Total</b>	<b>30.0</b>

### PCR program:

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