

MK2 Genotyping

Primers:

2nd loxp PCR:

A1	5´ GACATGTGGTCCTTGGGTGTCATCATG	Wt 433 bp
A2	5´ GAGATGGCAAGGCCGTGATTGGAATAG	Mutated (loxp) 492 bp
A3	5´ CTGGGCCCCGGAGAAGTATGACAAGTCC	Wt 534 bp
A4	5´ CTGATACTTCTGACCACTCCGGGTTAGG	Mutated (loxp) 593 bp

FLP mediated deletion PCR:

A5	5´ CCCTTTCTTGTCCTTGAAGTGGTTCC	Wt 423 bp
A6	5´ CTCTGCGCGCCTTCGGACAGTCCTGG	Deleted 643 bp Not deleted: no product
A7	5´ CTGTCCCTGAGGATTCTGGAGTCGC	Wt 476 bp
A8	5´ GGGAGGCCCTCCAGTGCAGCTCCACC	Deleted 696 bp Not deleted: no product

PCR Thermocycler program:

Step	Temperature (°C)	Time (min)
1	94	10:00
2	94	00:30
3	60	01:30
4	72	01:00
5	cycle to step 2	34x
6	72	02:00
7	8	forever

1% Agarosegel mit 0.5X TBE
„running“ buffer (in der Kammer): 1X TBE
20min für halbes Gel (2 Reihen)
30 min für ganzes Gel (1 Reihe)