

JAK1 Genotyping

Primers:

2nd loxp PCR:

J1	5' ATCCTGGACCTTCACTTCTCACTGAAACG	Wt 469 bp
J2	5' GTCCTTTTACAATGTAGCAAGACCAAGCC	Mutated (loxp) 307 bp
J3	5' GTGAGTTCTGGAAGCCAGGGTCCTGCC	Wt 549 bp
J4	5' CATAATGCATTTTATAAACCAATCTCAC	Mutated (loxp) 389 bp

FLP mediated deletion PCR:

J5	5' TTTGAGGGCCTTGTGAGGTTTTCTGC	Wt 450 bp
J6	5' GAGACCAGACTTATAAGAGATGAAACC	Deleted 539 bp Not deleted: no product
J7	5' CAGGACTGCTACTTTAGTCCTGGCCC	Wt 287 bp
J8	5' CTGGGGTCATGTAACCTATAAGCAACC	Deleted 376 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)