

Smyd5 without FRT sites (homo & het) protocol

PCR reaction to check if the region between the FRT sites was excised following Flp-recombination.

DNA (stock 100ng/ul or less)	2ul
10X buffer	5ul
MgCl2 (stock 25mM)	4ul (2mM final)
dNTPs (stock 10mM each)	1ul (0.2mM final)
Primers (stock 10um each)	2ul
Taq polymerase	1ul
Betaine (stock 5M)	10ul
Total	50ul

Band sizes:

- Smyd5 mice without FRT sites: 1267bp
- Smyd5 mice with FRT sites: no product is expected as 6667 bp can't be amplified in these conditions

SMYD5_F (forward): 5' – GGTCTCATGGGGAAGTGGAGG – 3'

SMYDR1 (reverse): 5' – GGCGAGCTCAGACCATAACT – 3'

Smyd5 Mice without FRT sites:

gttcccaggaggcgttgggagccggttctaggatgagttcagtgctttgtgctggctcact
tctccccaaactaggctgctttctggtgggataggtatgctctgagaccctggagag
cagcacaaggtctcatggggaactgaggttagtgccatcctagtagccactgctggtgtaa
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cccagagctatgcagtggtgtaaggacctccaccagaactgccccattggcaggtgag
caccctggggaaatgcataggaaggaggcagcccaaggaggctttagttccgtcttcac
tgggtgagacaactgagcagaactggttcggttccctcaaggctcatggtgtagtagacagt

Smyd5-F

FRT

loxP

Exon 2

loxP

SMYDR1

Exon 3

acaagggcctagtcattggttcagccttttctttgctgccagtgtccattgtttagaaaat

Program:

- 95°C: 5 min
 - 95°C: 30 sec
 - 55°C: 30 sec
 - 72°C: 1.40 min
 - 72°C: 5 min
 - 16°C/4°C/depending on the machine: forever
- 36 cycles** (step 2, 3 and 4)