

PCR-screening protocol for B6-Tnfrsf1b^{tm1Gkl}/Flmg

PCR REACTION:

PCR component	Volume (μ l)
10x PCR buffer	2
MgCl ₂ 25mM	1.4
dNTP's 2.5mM	1
primer A	5 pmol / reaction
primer B	5 pmol / reaction
primer C	5 pmol / reaction
Taq polymerase	0.1-0.4
DNA	1
milliQ H ₂ O	up to 20

Primer A (flpS): 5' – CACATGTATGTACACCTGTGTG – 3'

Primer B (deflpA): 5' – CTCTCCTGGGCCTAATGTAG – 3'

Primer C (deflxA): 5' – ATTGTCTACTCAGCACTGGG – 3'

PCR CONDITIONS:

1. 95.0, 5 min
 2. 95.0, 30 sec
 3. 50.0, 30 sec
 4. 72.0, 1 min
 5. GO TO 2, for 29 cycles
 6. 72.0, 10 min
- END

PCR PRODUCTS:

- WT band size: 151 bp
- Floxed band size: 200 bp
- DFloxed band size: 321 bp

