

STRAIN: EM01831 (C57BL/6-wr)

PRIMER EX23

5'-TTTTTACTACTGGAAATCTTCAAGCCTTAAAAGGCCTTAAAAATCTGGATC -3'

PRIMER EX23

**5'-GATGAACGACCTGGGTCTCCAGTCTGTCATCACCTCTTCTGTTCCCAGATTTCCG
GCCATA -3'**

PRIMER EX23 15pm / total volume 30ul Tm: 132°C

PRIMER EX23 15pm / total volume 30ul Tm: 182°C

PCR MIX

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
PRIMER EX23 (0.5pm/ul)	
PRIMER EX23 (0.5pm/ul)	
H2O	
TAQ POLYMERASE (5U/ul)	0.2ul
DNA	1ul
TOTAL	30ul

PCR PROGRAM:

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 62°C x 0'20''
- 4) 72°C x 0'40''
- 5) step 2 to step 5 x 45 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

PCR product : 114 BP

Digest 10 ul of amplified DNA with restriction enzyme BstI 1 overnight at 60°C:

MUTATION:114+74+40 BP **WT:** 74+40 BP