

STRAIN: Tga20

EM:00181

Substrain: B6;129S7-Prnp<tm1Cwe> Tg(Prnp)a20Cwe/CweCnrm

Maintained at the EMMA facility at CNR, Monterotondo (Italy)

(MIX PRP-KO)

PRIMER P3: 5'-ATT-CGC-AGC-GCA-TCG-CCT-TCT-ATC-GCC-3'

PRIMER P10: 5'-GTA-CCA-TAA-TCA-GTG-GAA-CAA-GCC-CAG-C-3'

PRIMER NC: 5'-ATA-ACC-CCT-CCC-CCA-GCC-T-3'

PRIMER P3 3pm / total volume 30ul Tm: 86°C

PRIMER P10 15pm / total volume 30ul Tm: 84°C

PRIMER NC 15pm / total volume 30ul Tm: 62°C

PCR MIX

dNTPS (2mM) 3ul

PCR BUFFER 10x 3ul

PRIMER P3 (0.1pm/ul)

PRIMER P10 (0.5pm/ul)

PRIMER NC (0.5pm/ul)

H2O

TAQ POLYMERASE (5U/ul) 0.16ul

DNA (200ng/ul) 1ul

TOTAL 30ul

PCR PROGRAM:

1) 95°C x 2'00''

2) 95°C x 0'30''

3) 60°C x 0'30''

4) 72°C x 1'30''

5) step 2 to step 5 x 45 repeats

6) 72°C x 10'00''

7) 4°C forever

8) End

MUTATION: 350 BP

WILD TYPE: 550 BP

STRAIN: EM00181

(MIX TG)

PRIMER TG1 5'-GTC GCG CAG CAG GGG GTG TAC -3'

PRIMER TG2 5'-CCC TCG CCC CGC CTA GC -3'

PRIMER TG1 15pm / total volume 30ul Tm: 72°C

PRIMER TG2 15pm / total volume 30ul Tm: 62°C

PCR MIX

dNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
PRIMER TG1 (0.5pm/ul)	
PRIMER TG2 (0.5pm/ul)	
H2O	
TAQ POLYMERASE (5U/ul)	0.2ul
DNA (200ng/ul)	1ul
TOTAL	30ul

PCR PROGRAM:

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 60°C x 0'30''
- 4) 72°C x 1'30''
- 5) step 2 to step 5 x 45 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

MUTATION: 273BP