

STRAIN: EM00243

cx 32 KO 1 **5'-CCA/TAA/GTC/AGG/TGT/AAA/GGA/GC-3'**

cx 32 KO 2 **5'-ATC/ATG/CGA/AAC/GAT/CCT/CAT/CC-3'**

cx 32 KO 1 15pm / total volume 30ul Tm: 68°C

cx 32 KO 2 15pm / total volume 30ul Tm: 68°C

PCR MIX

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
cx 32 KO 1	(0.5pm/ul)
cx 32 KO 2	(0.5pm/ul)
H2O	
TAQ POLYMERASE (5U/ul)	0.16ul
DNA	1ul
TOTAL	30ul

PCR PROGRAM:

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 66°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: 414 BP