

**STRAIN: EM00433**

**RB FLOX 1**            **5'-GGC/GTG/TGC/CAT/CAA/TG-3'**

**RB FLOX 2**            **5'-AAC/TCA/AGG/GAG/ACC/TG-3'**

RB FLOX 1            15pm / total volume 30ul            Tm: 54°C

RB FLOX 2            15pm / total volume 30ul            Tm: 52°C

**PCR MIX**

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
RB FLOX 1	(0,5pm/ul)
RB FLOX 2	(0,5pm/ul)
H2O	
TAQ POLYMERASE (5U/ul)	0.3ul
DNA	2ul
<b>TOTAL</b>	<b>30ul</b>

**PCR PROGRAM:**

- 1) 95°C x 2'00''
- 2) 95°C x 0'45''
- 3) 55°C x 0'45''
- 4) 72°C x 1'00''
- 5) step 2 to step 5 x 45 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

**MUTATION: 700 BP            WILD TYPE: 650 BP**