

**STRAIN: EM01647 (Cx45flox)**

**PRIMER I5FC FOR 5'- GGA-TTA-AAG-GCA-TAT-GTC-ACC-ACT-CTT-GGC -3'**

**PRIMER I3F REV 5'- CTC-TAG-GAA-CAC-TGT-AAC-CTG-AGA-TGT-CCC -3'**

**PRIMER CX45 FLOX INT.2 5'- TAT-ACG-AAG-TTA-TTC-GAG-GTC -3'**

PRIMER I5FC FOR 15pm / total volume 30ul Tm: 88°C

PRIMER I3F REV 15pm / total volume 30ul Tm: 90°C

PRIMER CX45 FLOX INT.2 15pm / total volume 30ul Tm: 54°C

**PCR MIX**

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
PRIMER I5FC FOR	(0.5pm/ul)
PRIMER I3F REV	(0.5pm/ul)
PRIMER CX45 FLOX INT.2	(0.5pm/ul)
H2O	
TAQ POLYMERASE (5U/ul)	0.2ul
DNA	2ul
<b>TOTAL</b>	<b>30ul</b>

**PCR PROGRAM:**

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 56°C x 0'30''
- 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

**MUTATION: 473/170 BP WILD TYPE: 380 BP**