

**STRAIN: EM09917 MIX A**

**PRIMER G418F 5' ATTGTCTTCCCAATCCTCCC 3'**

**PRIMER G418R 5' CGACTGTGCCTTCTAGTTGC 3'**

**PRIMER G418F 15pm / total volume 30ul Tm: 60°C**

**PRIMER G418R 15pm / total volume 30ul Tm: 64°C**

**PCR MIX**

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
<b>PRIMER G418F</b>	(0.5pm/ul)
<b>PRIMER G418R</b>	(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 0'30''
- 3) 58°C x 0'30''
- 4) 72°C x 0'30''
- 5) step 2 to step 5 x 50 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

**MUTATION: 200 bp**

**STRAIN: EM09917      MIX B**

**PRIMER INT2 FOR      5' GGAGCCCTCTGTGGTAGTCA 3'**

**PRIMER EX2 REV      5' TTCAAGCAACATGTCCTCCA 3'**

**PRIMER INT2 FOR      15pm / total volume 30ul      Tm: 64°C**

**PRIMER EX2 REV      15pm / total volume 30ul      Tm: 58°C**

**PCR MIX**

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
<b>PRIMER INT2 FOR</b>	(0.5pm/ul)
<b>PRIMER EX2 REV</b>	(0.5pm/ul)
H2O	
TAQ POLYMERASE (5U/ul)	0.3ul
DNA	2ul
<b>TOTAL</b>	30ul

**PCR PROGRAM:**

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 58°C x 0'30''
- 4) 72°C x 0'30''
- 5) step 2 to step 5 x 50 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

**WILD TYPE: 200 bp**