

## **STRAIN: EM01183 (MIX A)**

**Nestin-rtA2 For**                    **5'-GTC/CAG/ATC/GAA/ATC/GTC/TAG-3'**

**Nestin-rtA1 Rev**                    **5'-AGA/GCT/GCT/TAA/TGA/GGT/CG-3'**

Nestin-rtA2 For            15pm / total volume 30ul            Tm: 62°C

Nestin-rtA1 Rev            15pm / total volume 30ul            Tm: 60°C

### **PCR MIX**

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
Nestin-rtA2 For        (0.5pm/ul)	
Nestin-rtA1 Rev        (0.5pm/ul)	
H2O	
TAQ POLYMERASE (5U/ul)	0.3ul
DNA	2ul
<b>TOTAL</b>	<b>30ul</b>

### **PCR PROGRAM:**

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

**MUTATION: 800 BP**

## **STRAIN: EM01183 (MIX B)**

**ftTA2**        **5'-AAG/TAA/AGT/GAT/TAA/CAG/CGC-3'**

**rtTA2**        **5'-CTA/CCC/ACC/GTA/CTC/GTC-3'**

ftTA2 15pm / total volume 30ul        Tm: 58°C

rtTA2 15pm / total volume 30ul        Tm: 58°C

### **PCR MIX**

DNTPS (2mM)                                3ul

PCR BUFFER 10x                             3ul

ftTA2 (0.5pm/ul)

rtTA2 (0.5pm/ul)

H2O

TAQ POLYMERASE (5U/ul)                0.3ul

DNA     2ul

**TOTAL**                                        30ul

### **PCR PROGRAM:**

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 53°C x 0'30''
- 4) 72°C x 2'00''
- 5) step 2 to step 5 x 50 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

**MUTATION: 900 BP**