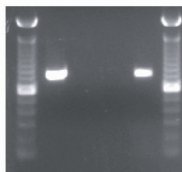


### Ple99 Promoter PCR (pEMS1071)

**MiniPromoter:** Ple99  
**pEMS#:** 1071  
**Expected product size (bp):** 777

| <b>Reaction components</b> | <b>Vol/Rxn (µl)</b> |
|----------------------------|---------------------|
| H <sub>2</sub> O           | 15.15               |
| 10X PCR buffer*            | 2.5                 |
| 50 mM MgCl <sub>2</sub> *  | 0.75                |
| 2.5 mM dNTPs**             | 2                   |
| 10 µM oEMS2335             | 1.25                |
| 10 µM oEMS2349             | 1.25                |
| Taq Pol. (5 U/µl)*         | 0.1                 |
| DNA***                     | 2                   |
| Total Volume of Rxn:       | 25                  |

L 1 2 3 4 L



1 - Control Plasmid DNA (pEMS1071)  
2 - WT mouse DNA  
3 - No Template  
4 - Knock-in ESC line (mEMS733)  
Expected band size = 777 bp

\* Taq Polymerase set from Invitrogen (Cat no.18038-042)

\*\* dNTPs from Invitrogen (Cat no.10297-018)

\*\*\*Approximately 100 ng DNA used for samples, approximately 5 ng used for plasmid control

Samples run on a 2% agarose gel (containing SYBRsafe (Invitrogen Cat no. S33102))

| <b>Cycling conditions:</b> | <b>Step</b> | <b>Temp</b> | <b>Time</b> | <b>Note</b>               |
|----------------------------|-------------|-------------|-------------|---------------------------|
|                            | 1           | 94°C        | 3 min       |                           |
|                            | 2           | 94°C        | 1 min       |                           |
|                            | 3           | 61°C        | 1 min       |                           |
|                            | 4           | 72°C        | 45 sec      | repeat steps 2-4 34 times |
|                            | 5           | 72°C        | 5 min       |                           |
|                            | 6           | 4°C         | hold        |                           |

### Primers:

| <b>Name</b>     | <b>Sequence</b>                | <b>T<sub>m</sub> (°C)</b> | <b>Notes</b>   |
|-----------------|--------------------------------|---------------------------|--|
| <b>oEMS2335</b> | 5'- TGAGGGGTATGTGATGGCAGG-3'   | 56.9                      | Anti-sense primer in 5' region of "HPRT homology region"   |
| <b>oEMS2349</b> | 5'- CTGCTTTATGCCAGTTTCCTAGG-3' | 57.3                      | Sense primer located in Ple99 MiniP sequence region "Prom" |