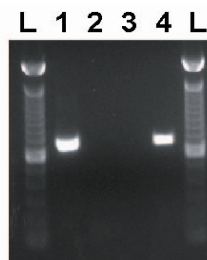


### Ple30 Promoter PCR (pEMS1158)

**MiniPromoter:** Ple30  
**pEMS#:** 1158  
**Expected product size (bp):** 727

| Reaction components       | Vol/Rxn (µl) |
|---------------------------|--------------|
| 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 oEMS2364            | 1.25         |
| 10 µM oEMS2611            | 1.25         |
| Taq Pol. (5 U/µl)*        | 0.1          |
| DNA***                    | 2            |
| Total Volume of Rxn:      | 25           |



1 - Control Plasmid DNA (pEMS1158)  
2 - WT mouse DNA  
3 - No Template  
4 - Knock-in ESC line (mEMS1431)  
expected band size = 727 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))

| Cycling conditions: | Step | Temp | Time   | Note                      |
|---------------------|------|------|--------|---------------------------|
|                     | 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:

| Name     | Sequence                        | T <sub>m</sub> (°C) | Notes  |
|----------|---------------------------------|---------------------|--|
| oEMS2364 | 5'- GCGTATCACGAGGCCCTTTC -3'    | 56.0                | Sense primer located in Vector backbone 5' to MCS        |
| oEMS2611 | 5'- CAGGGCAAAGTCAACAGTGTCAC -3' | 56                  | Anti-Sense primer for Ple30 MiniP sequence region "Prom" |