

**Ple17 Promoter PCR (pEMS1487)**

**MiniPromoter:** Ple17  
**pEMS#:** 1487  
**Expected product size (bp):** 440

| 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 Sense primer        | 1.25         |
| 10 µM Antisense primer    | 1.25         |
| Taq Pol. (5 U/µl)*        | 0.1          |
| DNA***                    | 2            |
| Total Volume of Rxn:      | 25           |

\* 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))

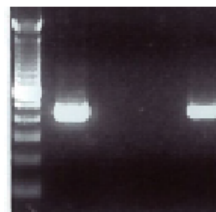
| Cycling conditions: | Step | Temp | Time   | Note                      |
|---------------------|------|------|--------|---------------------------|
|                     | 1    | 94°C | 3 min  |                           |
|                     | 2    | 94°C | 1 min  |                           |
|                     | 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                        | Tm (°C) | Notes                                      |
|-----------------|---------------------------------|---------|--|
| <b>oEMS2364</b> | 5' - GCGTATCAGGAGGCCCTTTC- 3'   | 56.0    | Sense primer in vector backbone            |
| <b>oEMS3624</b> | 5' - GATGAAGCATGCAGCAGGACA - 3' | 56.0    | Anti-Sense primer for Ple17 in region "6". |

**Pleiades Promoter Project**

L 1 2 3 4



1- Control plasmid DNA (pEMS1487)

2- WT mouse DNA

3- No Template

4- Knock-in ESC line

L- 100 bp ladder

expected band size = 440 bp