

Ple139 Promoter PCR (pEMS1611)**Pleiades Promoter Project**

MiniPromoter: Ple139
pEMS#: 1611
Expected product size (bp): 439

| Reaction components | Vol/Rxn (µl) |
|----------------------------|---------------------|
| H ₂ O | 13.9 |
| 10X PCR buffer* | 2.5 |
| 50 mM MgCl ₂ * | 0.75 |
| 2.5 mM dNTPs** | 2 |
| 10 µM oEMS2364 | 1.25 |
| 10 µM oEMS3656 | 1.25 |
| DMSO | 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 | T_m (°C) | Notes |
|-----------------|--------------------------------|---------------------------|--|
| oEMS2364 | 5'- GCGTATCACGAGGCCCTTTC -3' | 56.0 | Sense primer in vector 5' to MiniP |
| oEMS3656 | 5'- TCTCTATTGTGGTGTGGGCGAA -3' | 56.8 | Antisense primer for Ple139, in region "6" |