

**Backbone Vector + CAG PCR - pEMS1164****Pleiades Promoter Project**

**MiniPromoter:** CAG  
**pEMS#:** 1164  
**Expected product size (bp):** 370

| <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 forward primer       | 1.25                |
| 10 µM reverse 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))

| <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 (5' to 3')</b> | <b>Tm (°C)</b> | <b>Notes</b>                           |
|-----------------|----------------------------|----------------|----------------------------------------|
| <b>oEMS2364</b> | GCGTATCACGAGGCCCTTTC       | 56.0           | Sense primer in vector 5' to miniP seq |
| <b>oEMS2668</b> | GCCAAGTAGGAAAGTCCCATAAGG   | 56.3           | Antisense primer in CAG                |