

**Ple234 Promoter PCR (pEMS1206)****Pleiades Promoter Project**

**MiniPromoter:** Ple234  
**pEMS#:** 1206  
**Expected product size (bp):** assay #1: 272  
 assay #2: 468

note: both assays must be the correct size to be sure of correct construct

<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:****assay #1:**

<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>oEMS2885</b>	GCTTTGTCCCCCAGACTGGA	56.2	Antisense primer for Ple234, in region "Prom"

**assay #2:**

<b>Name</b>	<b>Sequence (5' to 3')</b>	<b>Tm (°C)</b>	<b>Notes</b>
<b>oEMS2888</b>	CCGCCTGGCGCAGATATAAG	57.1	Sense primer for Ple234, in region "Prom"
<b>oEMS2639</b>	TGGCGGACTTGAAGAAGTCGT	56.2	Antisense primer in vector 3' to miniP seq in EGFP