

Ple250 Promoter PCR (pEMS1281)**Pleiades Promoter Project**

MiniPromoter: Ple250
pEMS#: 1281
Expected product size (bp): 386

<u>Reaction components</u>	<u>Vol/Rxn (µl)</u>
H ₂ O	15.15
10X PCR buffer*	2.5
50 mM MgCl ₂ *	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))

<u>Cycling conditions:</u>	<u>Step</u>	<u>Temp</u>	<u>Time</u>	<u>Note</u>
	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:

<u>Name</u>	<u>Sequence (5' to 3')</u>	<u>Tm (°C)</u>	<u>Notes</u>
oEMS2364	GCGTATCACGAGGCCCTTTC	56.0	Sense primer in vector 5' to miniP seq
oEMS3213	GGAATCATAGCACCATCTGCAGG	57.3	Antisense primer for Ple250, in region "Prom2"