

Ple183 Promoter PCR (pEMS1382)**Pleiades Promoter Project**

MiniPromoter: Ple183
pEMS#: 1382
Expected product size (bp): 469

Reaction components	Vol/Rxn (μl)
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))

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 (5' to 3')	Tm (°C)	Notes
oEMS2364	GCGTATCACGAGGCCCTTTC	56.0	Sense primer in vector 5' to miniP seq
oEMS2487	CCGTGAAAGGGTTTCCCACA	56.9	Antisense primer for Ple183, in region "Prom"