Ple112 Promoter PCR (pEMS1052) **Pleiades Promoter Project**

MiniPromoter:	**Ple112**
pEMS#:	**1052**
Expected product size (bp):	**491**

Reaction components	Vol/Rxn (µl)
H_20	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
oEMS2263	AGGTCTGAAGAGGAGTTTACGTCCA	56.5	Sense primer in vector 5' to miniP seq
oEMS2297	CTTAGTCCAGGCCTTCTTTCAAGC	56.8	Antisense primer for Ple112, in region "1"