

**Ple129 Promoter PCR (pEMS1600)**

**MiniPromoter:** Ple129  
**pEMS#:** 1600  
**Expected product size (bp):** 389

Reaction components	Vol/Rxn (µl)
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 Sense primer	1.25
10 µM Antisense 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)

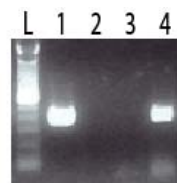
\*\*\*Approximately 100 ng DNA used for samples, approximately 5 ng used for plasmid control

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	T <sub>m</sub> (°C)	Notes
<b>oEMS2364</b>	5' -GCGTATCACGAGGCCCTTTC- 3'	56.0	Sense primer in vector backbone
<b>oEMS3599</b>	5'-CAAATTGAAAAGACTGTATGCCCA- 3'	56.8	Anti-Sense primer for Ple129 in region "4"

**Pleiades Promoter Project**

1- Control plasmid DNA (pEMS1600)  
 2- WT mouse DNA  
 3- No Template  
 4- Knock-in ESC line  
 L- 100 bp ladder  
 expected band size = 389 bp