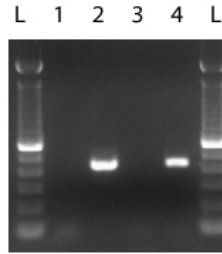


**Ple80 Promoter PCR (pEMS1060)**

**MiniPromoter:** Ple80  
**pEMS#:** 1060  
**Expected product size (bp):** 435

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 oEMS2319	1.25
10 µM oEMS2308	1.25
Taq Pol. (5 U/µl)*	0.1
DNA***	2
Total Volume of Rxn:	25



1- WT mouse DNA  
 2- Control plasmid DNA (pEMS1060)  
 3- No Template  
 4- Knock-in ESC line  
 L- 100 bp ladder  
 expected band size = 435 bp

\* 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	reapeat steps 2-4 34 times
	5	72°C	5 min	
	6	4°C	hold	

**Primers:**

Name	Sequence	Tm (°C)	Notes
oEMS2319	5'- TGGTCCAGAGAGTTTAAGAGCCCT -3'	56.5	Sense primer located in Ple80 MiniP sequence region "2"
oEMS2308	5'- TGTTC CAATAGGCTCAGCGTTTAT -3'	56.1	Anti-sense primer located in Ple80 MiniP sequence region "Prom"