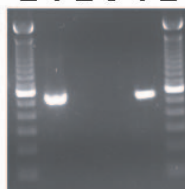


### Ple54 Promoter PCR (pEMS1200)

**MiniPromoter:** Ple54  
**pEMS#:** 1200  
**Expected product size (bp):** 546

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 oEMS2754	1.25
10 µM oEMS2749	1.25
Taq Pol. (5 U/µl)*	0.1
DNA***	2
Total Volume of Rxn:	25

L 1 2 3 4 L



1 - Control Plasmid DNA (pEMS1200)  
2- WT mouse DNA  
3 - No Template  
4- Knock-in ESC line (mEMS1927)  
Expected band size = 546 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
oEMS2754	5'- CTTCAAATCACAGCCTTTTTACGC -3'	56.2	Sense primer located in Ple54 MiniP sequence region "2"
oEMS2749	5'- CTCTAATGAGCAATGTGCTAGCCC -3'	56.2	Anti-sense primer located in Ple54 MiniP sequence region "Prom"