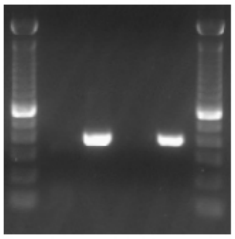
Ple51 Promoter PCR (pEMS1362)

MiniPromoter:	**Ple51**
pEMS#:	**1362**
Expected product size (bp):	**398**

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 oEMS2478	1.25
10 µM oEMS2474	1.25
Taq Pol. (5 U/µl)*	0.1
DNA***	2
Total Volume of Rxn:	25



1- WT mouse DNA
2- Control plasmid DNA (pEMS1362)
3- No Template
4- Knock-in ESC line
L- 100 bp ladder
expected band size = 398 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**
oEMS2478	5′- GGAAACAAGTAGGGAGATGGTTGG -3′	56.8	Sense primer located in Ple51 MiniP sequence region "3"
oEMS2474	5′- CCCTCCTCTAATTGGCACTAATGAC -3′	56.3	Anti-sense primer located in Ple51 MiniP sequence region "Prom"