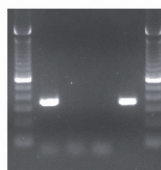


Ple175 Promoter PCR (pEMS1363)

MiniPromoter: Ple175
pEMS#: 1363
Expected product size (bp): 373

L 1 2 3 4 L



1 - Control Plasmid DNA (pEMS1363)
2 - WT mouse DNA
3 - No Template
4 - Knock-in ESC line (mEMS1654)
Expected band size = 373 bp

<u>Reaction components</u>	<u>Vol/Rxn (µl)</u>
H ₂ O	15.15
10X PCR buffer*	2.5
50 mM MgCl ₂ *	0.75
2.5 mM dNTPs**	2
10 µM oEMS2364	1.25
10 µM oEMS2367	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))

<u>Cycling conditions:</u>	<u>Step</u>	<u>Temp</u>	<u>Time</u>	<u>Note</u>
	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:

<u>Name</u>	<u>Sequence</u>	<u>T_m (°C)</u>	<u>Notes</u>
oEMS2364	5'- GCGTATCACGAGGCCCTTTC-3'	56.0	Sense primer located in Vector backbone 5' to MCS
oEMS2367	5'- GTGGAAGGAAAAGCGCAAG-3'	56.2	Anti-sense primer located in Ple175 MiniP sequence region "Prom"