

Ple230 Promoter PCR (pEMS11422)

Pleiades Promoter Project

MiniPromoter: Ple230
pEMS#: 1422
Expected product size (bp): 563

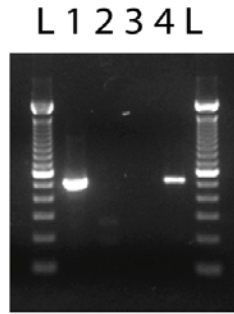
Reaction components	Vol/Rxn (µl)
H ₂ O	15.15
10X PCR buffer*	2.5
50 mM MgCl ₂ *	0.75
2.5 mM dNTPs**	2
10 µM oEMS22364	1.25
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Taq Pol. (5 U/µl)*	0.1
DNA***	2
Total Volume of Rxn:	25

* 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))



L - 100bp Ladder
 1 - Control Plasmid DNA (pEMS1422)
 2 - WT mouse DNA
 3 - No Template
 4 - Transgenic mouse DNA
 Expected band size = 563bp

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	Tm (°C)	Notes
2364	5'-GCGTATCACGAGGCCCTTTC-3'	56.0	Sense primer for 5' end of the vector backbone.
2264	5'-GGGTGTTTTTCAGTGTGGAGCA-3'	58.6	Antisense primer for Ple230 in promoter region "P".