

Ple189 Promoter PCR (pEMS1392)**Pleiades Promoter Project**

MiniPromoter: Ple189
pEMS#: 1392
Expected product size (bp): 201

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 oEMS2364	1.25
10 µM oEMS3114	1.25
Taq Pol. (5 U/µl)*	0.1
DNA***	2
Total Volume of Rxn:	25

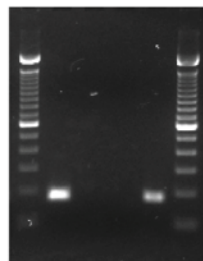
* Taq Polymerase set from Invitrogen (Cat no.18038-042)

** 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))

L 1 2 3 4 L



L - 100bp Ladder

1 - Control Plasmid DNA (pEMS1392)

2 - WT mouse DNA

3 - No Template

4 - Transgenic mouse DNA

Expected band size = 201bp

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.
3114	5'-CTTGATCAGGGGACAATAGGCATAT-3'	56.5	Anti-Sense primer for SLC6A2-C very close to 5' end of region "3".