

Ple214 Promoter PCR (pEMS1260)
 requires 2 assays to confirm

Pleiades Promoter Project

MiniPromoter: Ple214
pEMS#: 1260
Expected product size (bp): Assay A 451 Assay B 650

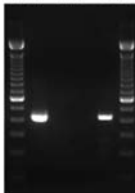
Reaction components A	Reaction components B	Vol/Rxn (ul)
H ₂ O	H ₂ O	15.15
10X PCR buffer*	10X PCR buffer*	2.5
50 mM MgCl ₂ *	50 mM MgCl ₂ *	0.75
2.5 mM dNTPs**	2.5 mM dNTPs**	2
10 μM oEMS2354	10 μM oEMS3577	1.25
10 μM oEMS3565	10 μM oEMS2639	1.25
Taq Pol. (5 U/ul)*	Taq Pol. (5 U/ul)*	0.1
DNA***	DNA***	2
Total Volume of Rxn:	Total Volume of Rxn:	25

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

** dNTPs from Invitrogen (Cat no.10297-018)

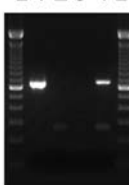
***Approximately 100 ng DNA used for samples, approximately 5 ng used for plasmid control

L 1 2 3 4 L



L - 100bp Ladder
 1 - Control Plasmid DNA (pEMS1260)
 2 - WT mouse DNA
 3 - No Template
 4 - Transgenic mouse DNA
 Expected band size = 451bp

L 1 2 3 4 L



L - 100bp Ladder
 1 - Control Plasmid DNA (pEMS1260)
 2 - WT mouse DNA
 3 - No Template
 4 - Transgenic mouse DNA
 Expected band size = 650bp

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	repeat steps 2-4 34 times
	5	72°C	5 min	
	6	4°C	hold	

Primers:

For Assay A:

Name	Sequence	Tm (°C)	Notes
2364	5'-GCGTATCACGAGGCCCTTTC-3'	56.0	Sense primer for 5' end of the vector backbone.
3565	5'-GTAACATATTTCCCAGGGAGCTCA-3'	56.7	Anti-Sense primer for TAC1-A in region "1A"

For Assay B:

3577	5'-GAGACTGGCTTCCCAAACGC-3'	56.1	Sense Primer in LongProm region of TAC1-A/B
2639	5'-TGCGGACTTGAAGAAGTCGT-3'	56.2	Anti-Sense primer in the 5' seq of EGFP