PCR protocol

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RBRC 11097 **Strain name** 129;B6-Pias4<em1Yfj>

Primer	primer_name	primer_seq	length		Reference
1	mPIAS4-F	AGTGCGAGTGTCCCTCATCT	20		Depositor's primer
2	mPIAS4-R	GGTGGGCTTCTTCTCATTCA	20		Depositor's primer

Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

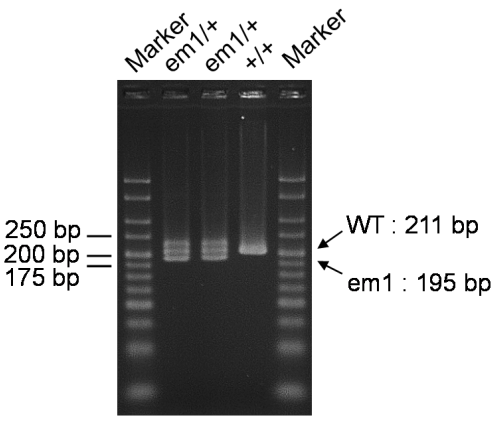
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	60	* *Repeat for 30 cycles

Marker 25bp DNA Step Ladder (Wako, Cat. 292-69001)

Gel 4% Nusieve in 0.5 X TBE (100 V, 60 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	211 bp	WT	+/+ (wild-type): 211 bp em1/+ (heterozygous): 211 bp and 195 bp
primer 1	primer 2	195 bp	em1	

Comment Standard PCR

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.