PCR protocol

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IMPC

RBRC 06369 **Strain name** C57BL/6N-Fam135b<em1Rbrc>

Primer	primer_name	primer_seq	length		
1	wild 44	GAAGTTTCCATAAATGACGCCATGC	25	EAD_8849	RIKEN BRC
2	delta 44	CGTGTCTTCCAGATTCCGGG	20	EAD_8850	RIKEN BRC
3	common	GAAGCTCCTCTGTGAATGATTTCC	24	EAD_8851	RIKEN BRC

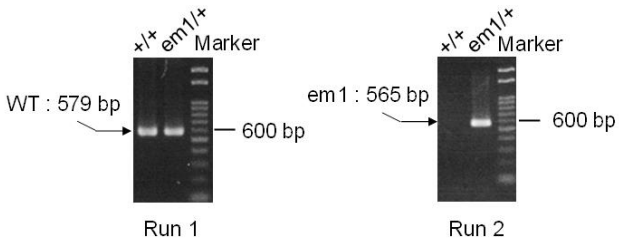
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.8	2.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	0	1.0		
primer 3	10 pmol/μl	1.0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	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

Gene Ladder 100 (Wako, Cat. 316-06951)

Gel

2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set		product_size	product
primer 1	primer 3	579 bp	WT
primer 2	primer 3	565 bp	em1

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