PCR protocol

Sheet ID PS_08027
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RBRC 10941

Strain name C57BL/6N-Diaph1<em1Rbrc>

Primer	primer_name	primer_seq	length		Reference
1	ck_MGE203-F	TTTCAGGTAGTAGGCAAGGAGTAAT	25		RIKEN DNA Bank
2	ck_MGE203-R	AGAAGGTAGATTTCTACTCAAATAG	25		RIKEN DNA Bank

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8			
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			

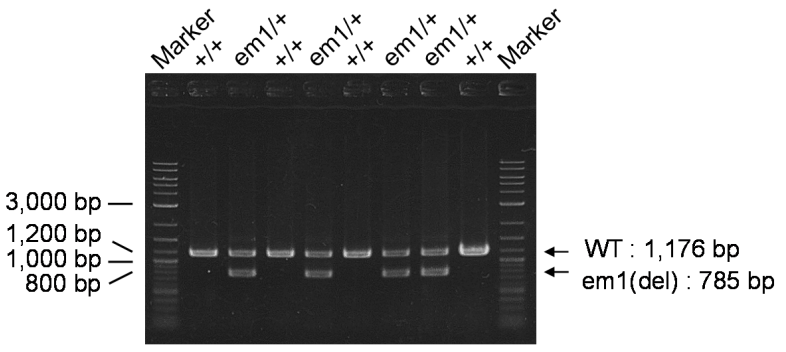
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	90*	*Repeat for 30 cycles

Marker: 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	1,176 bp	WT
primer 1	primer 2	785 bp	em1(del)

+/+ (wild-type): 1,176 bp
em1/+ (heterozygous): 1,176 bp and 785 bp

Comment Standard PCR

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