PCR protocol

Sheet ID PS_08214
Release date : 8/17/2022
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RBRC 11403

Strain name C57BL/6N-Cdkn2a<em1Matsu>

Primer	primer_name	primer_seq	length		Reference
1	P16/19-fw	GCCGTGATCCCTCTACTTTTT	21		Depositor's primer
2	P16/19-rv	TATCGCACGATGTCTTGATGT	21		Depositor's primer

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			

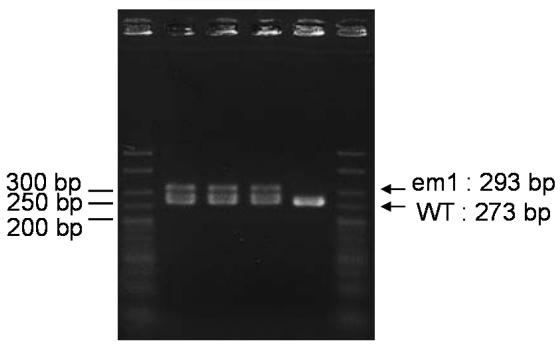
(μL)

Reaction Conditions

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

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

Gel: 3% Nusieve in 0.5 X TBE (100 V, 60 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	273 bp	WT
primer 1	primer 2	293 bp	em1

+/+ (wild-type): 272 bp
em1/+ (heterozygous): 272 bp and 293 bp

Comment Standard PCR

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