PCR protocol

Sheet ID PS_07607
Release date : 03/15/2022
Print date : 3/15/2022

RBRC 09920 **Strain name** C57BL/6N-Pdx1<em1(cre/ERT2)Rbrc>

Primer	primer_name	primer_seq	length		Reference
1	Pdx1 5 short F2	ACCCGAGGAAAACAAGAGGACCCGT	25		Depositor's primer
2	Pdx1 3 arm R8	CTAGCCTCCTCCCCTCTCATTTGG	24		Depositor's primer
3	Pdx1 3 long F3 rGpA F	TGGCTGCCATGAACAAAGGTGGCTA	25		Depositor's primer

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		1.8			
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			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

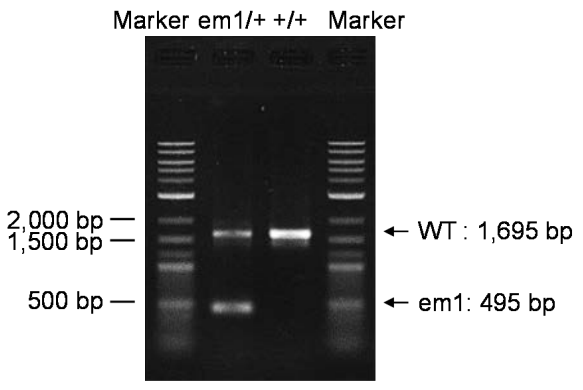
(μL)

Reaction Conditions

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

Marker DNA Ladder,1 kb Plus (Affymetrix, Cat. 76714 500 UL)

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



primer_set		product_size	product (allele type)	
primer 1	primer 2	1,695 bp	WT	+/+ (wild-type): 1,695 bp em1/+ (heterozygous): 1,695 bp and 495 bp
primer 2	primer 3	495 bp	em1	

Comment Separated PCR

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