PCR protocol

RBRC 04774 **Strain name** Dnmt1<tm1Okan> (Acc.No.CDB0500K)

Primer

	primer_name	primer_seq	length		
1	AT040F	AAGCGTGTGCCGTCTAGGCA	20		Depositor's primer
2	04774WT	ATGCCACTGAAGCCCTGGTA	20		RIKEN BRC
3	04774MT	ATGCCACTGAAGCCCTGGTT	20		RIKEN BRC

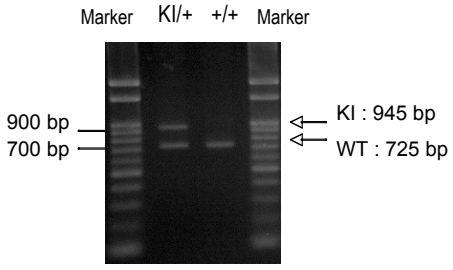
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		13.3	13.3		
PCR buffer	10 X	2.0	2.0		
dNTP	2.5 mM	1.6	1.6		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	5U/μl	0.1	0.1		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	60	30	*
4	72	60	* *Repeat for 35 cycles
5	72	300	



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 2	725 bp	WT
primer 1	primer 3	945 bp	KI

Comment The PCR were performed in two separate reactions. Both PCR products were mixed in an equal volume and electrophoresed.