

PCR protocol

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RBRC 03515

Strain name Aire(GFP) (Acc.No. CDB0479K)

Primer	primer_name	primer_seq	length	
1		CTCTCCGGATAGCACCACGAC	21	RIKEN BRC
2		CATCAATCAGCACACGCCCAC	21	RIKEN BRC
3		AAGAACTCGTCAAGAAGGCGATAGAAGGCG	30	

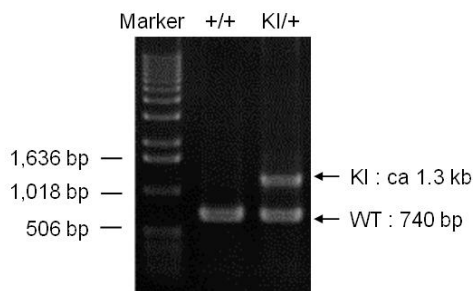
Reaction Components

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

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	1 U/ μ l	0.2			
total		20			

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



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set	product_size	product
primer 1	740 bp	WT
primer 1	ca 1.3 kb	KI

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