PCR protocol

Sheet ID PS_05230
Release date : 1/7/2014
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RBRC 01736 Strain name CFO-hea/+

Primer	primer_name	primer_seq	length		
1	01736_R2	CCCTAGTCTGAGGTCTGCAGG	21		RIKEN BRC
2	01736_F5	TGCCCTCCCGTGCCCACACTC	21		RIKEN BRC
3	01736_R4	CGTCACCGCCACAGCCTGAGG	21		RIKEN BRC

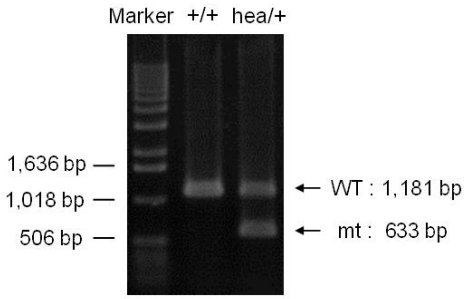
Reaction Components

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

	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/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			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	65	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	primer 2	633 bp	mutant
primer 2	primer 3	1,181 bp	WT

Comment