

PCR protocol

Sheet ID PS_07092
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RBRC 09739

Strain name B6;B6CB-Ddhd1 <tm1b(KOMP)Katn>

Primer	primer_name	primer_seq	length	
1	Alpha-genotyping_Fw-1	CTACCGGCAAGTAACTACTT	20	Depositor's primer
2	loxP_RV-3	GAAGTCAGGGCTCAAGAG	18	Depositor's primer
3	loxP_Fw-2	ACCGCGTCGAGAAGTTCCTA	20	Depositor's primer

Reaction Components

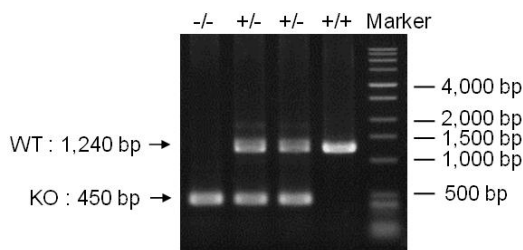
Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reaction Conditions

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

	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			

Standard PCR



Marker

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

Gel

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

Comment

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

PCR products	primer_set	product_size	product
primer 1	primer 2	1,240 bp	WT
primer 1	primer 3	450 bp	KO