PCR protocol

Sheet ID PS_06654
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RBRC 09484 **Strain name** B6-Obif<tm2Tfur>

Primer	primer_name	primer_seq	length		Reference
1	Obif-51	GATTCTGGGAGCCTGGCCGGATAAC	25		Depositor's primer
2	OBIF-WT31	GCTTCCCCTTGGCCCTCACTGGTGGCA	27		Depositor's primer
3	MC-3	CAATATCACGGGTAGCCAACGC	22		Depositor's primer

Reaction Components

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

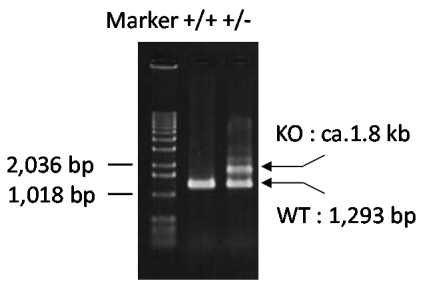
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	0.5				
primer 3	10 pmol/μl	1.5				
DNA		1.0				
Taq		0.2				
total		20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	1,293 bp	WT
primer 1	primer 3	ca 1.8 kb	KO

Comment

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