PCR protocol

Sheet ID PS_07617
Release date : 7/11/2018
Print date : 7/11/2018

RBRC 05428 **Strain name** B6;129-Engase<tm1Tasuz>

Primer	primer_name	primer_seq	length		
1	ENG-WT-F1	TGGTTAGCGTACGCATTGGG	20		PLoS Genet., 13(4):e1006696(2017).
2	ENG-WT-R3	GCAATCTGGACCAGCCGATC	20		PLoS Genet., 13(4):e1006696(2017).
3	oIMR0214	CCGGAGAACCTGCGTGCAATCC	22		JAX Genotyping Protocol

Reaction Components

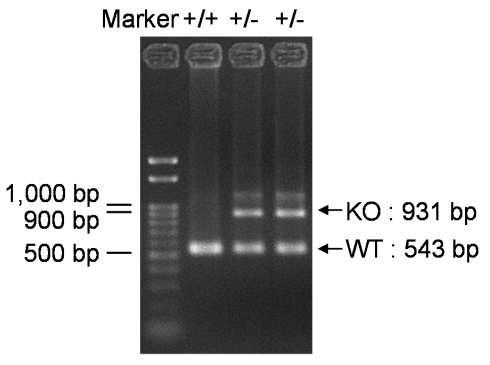
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	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	60	30	*	
4	68	60	*	*Repeat for 30 cycles

Standard PCR



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	543 bp	WT
primer 1	primer 3	931 bp	KO

Comment

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