



Sheet ID PS_08225
Release date : 9/5/2022
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Strain name B6.129S4-Tob2<tm1Tya>/TyaRbrc

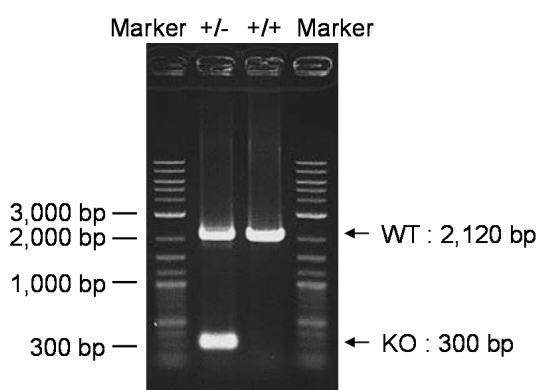
[illegible]

Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μ L)
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				
						Mark
						Gel
	total	20				

[illegible]

Marker	2-Log DNA Ladder (NEB, Cat. N3200)
Gel	1% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	2,120 bp	WT
primer 1	primer 3	300 bp	KO

Comment Standard PCR

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

RBRC 01766

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Primer	primer_name	primer_seq	length	Reference
1	Tob2mut-F	CCTTGCTTCTCTCCTCTCCTCCAGG	25	Depositor' s primer
2	LacZ	TGCGCAACTGTTGGGAAGGGCGATC	25	Depositor' s primer
3	Tob2WT-F	GCATTCATGCCTGTGAGTGTGTGGCTTGC	29	Depositor' s primer
4	Tob2WT-R	GGGTTTCTCGGGCTTAGCCTCGTGTCA	27	Depositor' s primer

Reaction Components

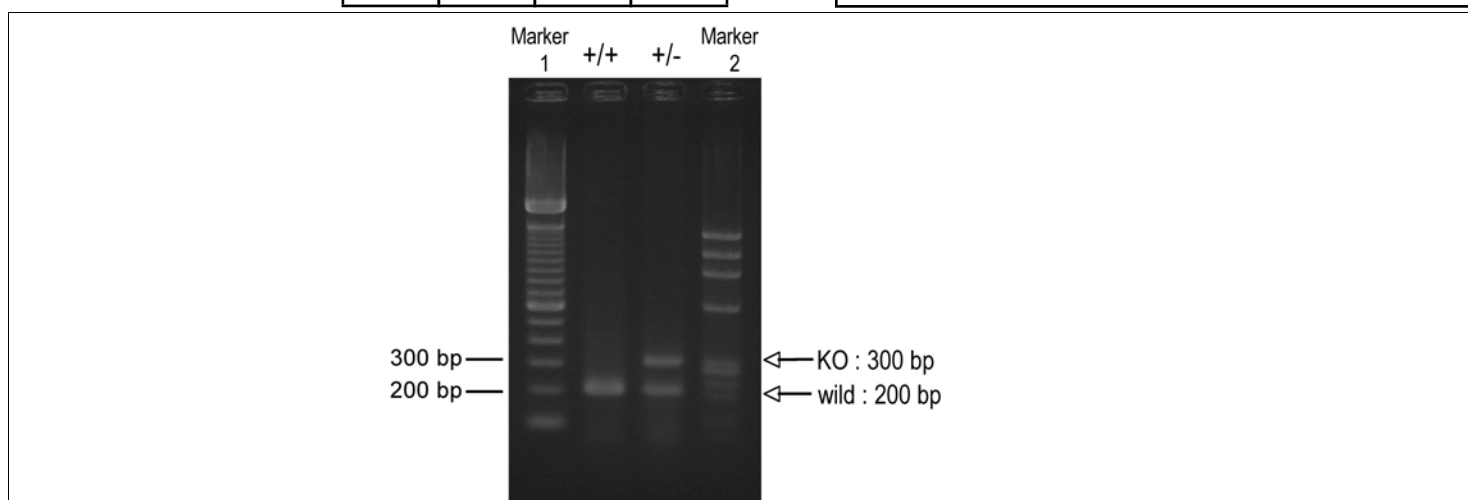
Enzyme	TaKaRa Ex Taq (Cat. No. RR001A)
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Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	58	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

Reagent	Conc	Reaction 1	2	3	4
H2O		15.3			
PCR buffer	10 X	2.5			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
primer 4	10 pmol/ μ l	1.0			
DNA		1.0			
Taq		0.2			
total		25			

Marker	marker 1:100 bp ladder (Invitrogen, Cat. 15628-050), marker2: ϕ X174 Hae III
Gel	2% agarose in 1 X TAE (100 V, 35 min)



primer_set	product_size	product (allele type)
primer 1	primer 2	300 bp
primer 3	primer 4	200 bp

Comment Standard PCR