

RBRC 11127

Strain name B6.129S2-Eqtn<tm1Ktos>

Primer	primer_name	primer_seq	length	Reference
1	EqtnKoTyping5' fwd	AAACAAAACCCCCAAATTCC	20	Reproduction, 156(6):579-590 (2018).
2	EqtnKoTyping5' rev1	GGCAGGAGTGTCCATTTCAT	20	Reproduction, 156(6):579-590 (2018).
3	EqtnKoTyping5' rev3	GCTTGGCTGGACGTAAACTC	20	Reproduction, 156(6):579-590 (2018).

Reaction Components

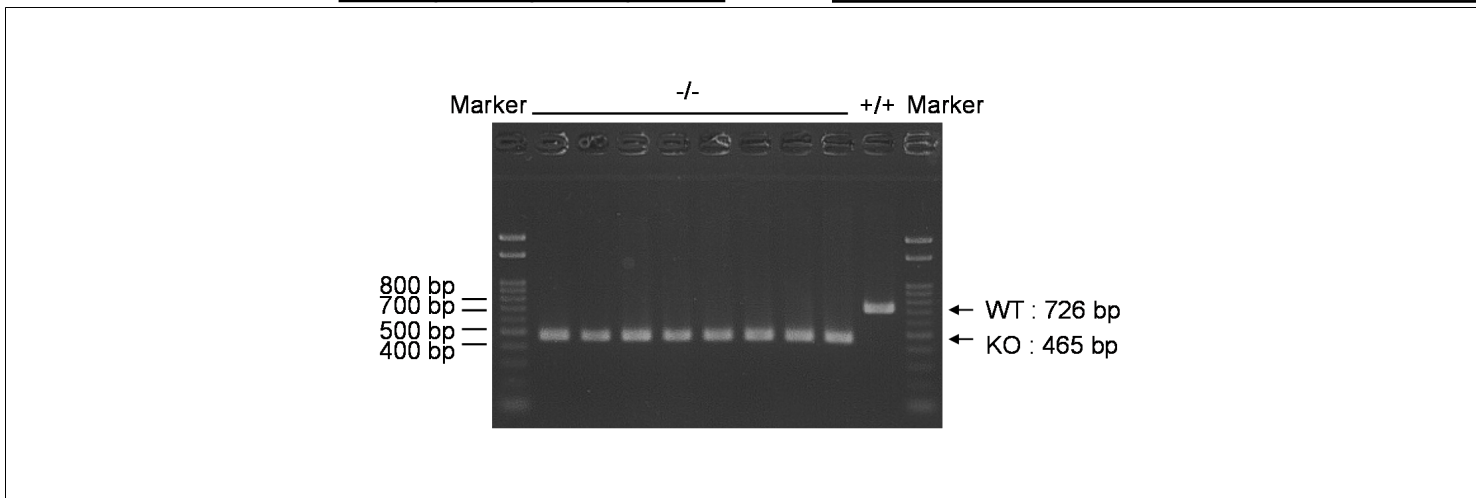
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(μL)
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				

Marker Gene Ladder 100 (Wako, Cat. 316-06951)
Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1	primer 2	726 bp	WT
primer 1	primer 3	465 bp	KO

+/+ (wild-type): 726 bp
-/- (homozygous): 465 bp

Comment Standard PCR

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