

RBRC 10290

Strain name B6.129-Itr3<tm1Kmik>

Primer	primer_name	primer_seq	length	Reference
1	PIII#5	TCAGTCCTCTGCACCGAGCC	20	Depositor's primer
2	PIII#2	AAGCAAGCCTCTATCAAGG	19	Depositor's primer
3	Pgk-4	GTGGTTCTAAGTACTGTGGT	20	Depositor's primer

**Reaction Components**

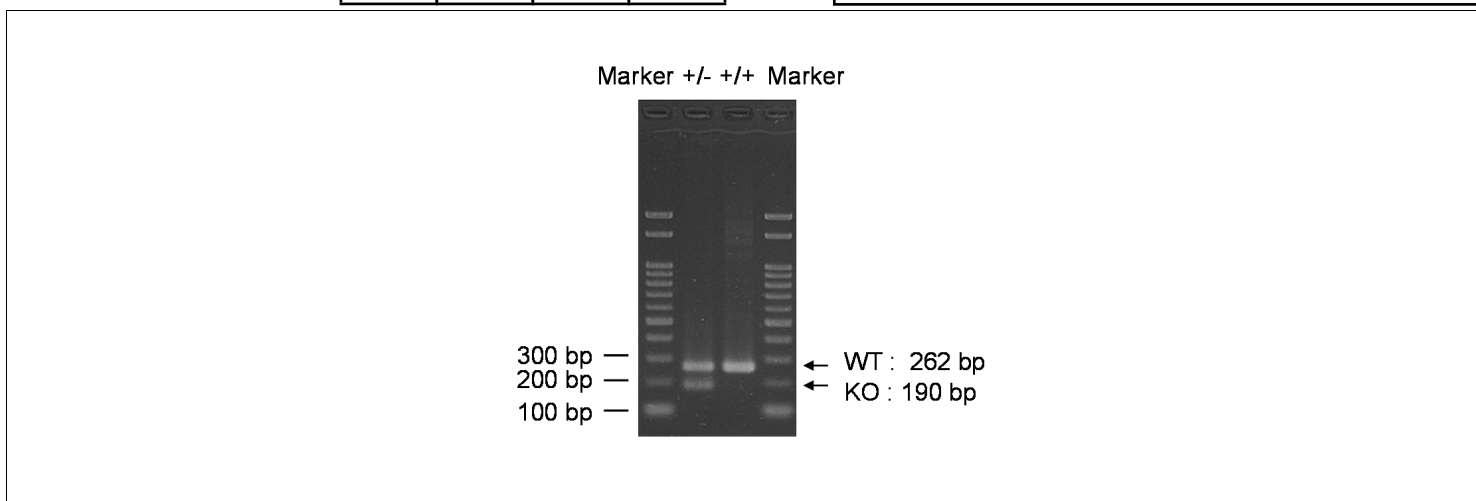
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

**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				
<b>total</b>		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	262 bp	WT
primer 1 primer 3	190 bp	KO

**Comment**

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