

RBRC 00757

Strain name B6;129P2-Ptpn7^{tm1Bwz}/Rbrc

[illegible]

Reaction Components

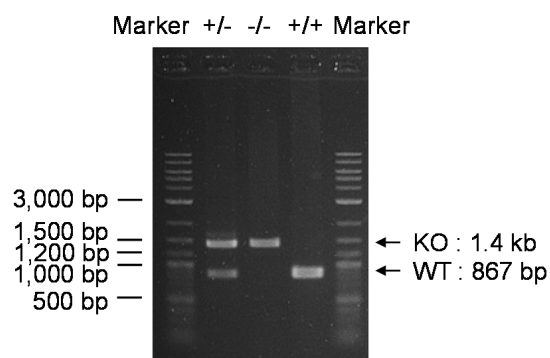
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

[illegible][illegible]

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

Gel	1% agarose in 1 X TAE (100 V, 30 min)
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primer_set		product_size	product (allele type)
primer 1	primer 2	867 bp	WT
primer 1	primer 3	1.4 kb	KO

Comment

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

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Primer	primer_name	primer_seq	length	Reference
1	HePTP common	ATTCCTCCTCTAGCTGCTTG	20	Depositor' s primer
2	HePTP WT	AGTCCTCATGGCTGAATGAG	20	Depositor' s primer
3	PMC1neo antisense1	AACCACACTGCTCGACATTG	20	Depositor' s primer

Reaction Components

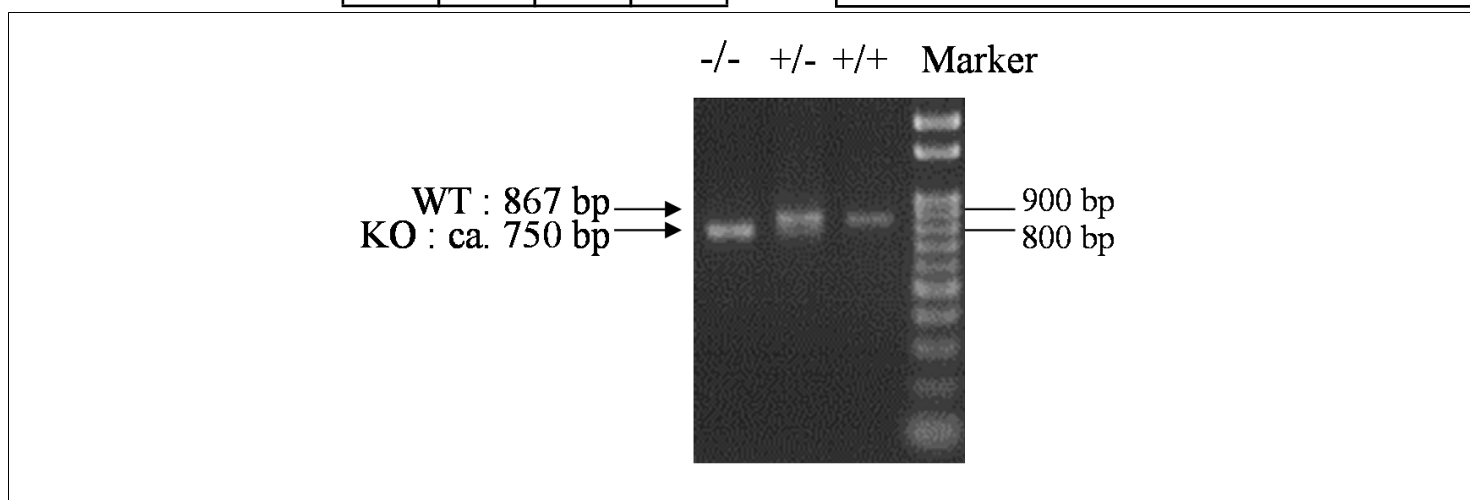
Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reaction Conditions

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
H2O		1.8			
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			
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	867 bp WT
primer 1	primer 3	ca. 750 bp KO

Comment [Standard PCR](#)

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[illegible]

Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

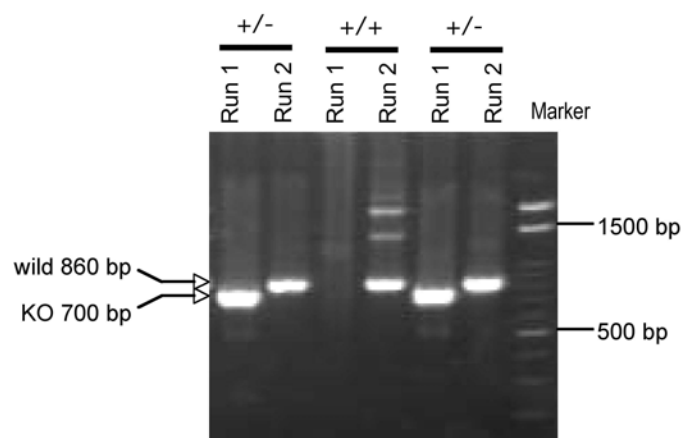
Reaction Conditions

[illegible]

Reagent	Conc	Reaction 1	2	3	4	
H ₂ O		17.3	17.3			(μL)
PCR buffer	10 X	2.5	2.5			
dNTP	2.5 mM	2.0	2.0			
primer 1	10 pmol/μl	1.0	1.0			
primer 2	10 pmol/μl	0	1.0			
primer 3	10 pmol/μl	1.0	0			
DNA		1.0	1.0			
Taq	5 U/μl	0.2	0.2			
						Mark
						Gel
	total	25	25			

Marker 100 bp DNA Ladder (TOYOBO)

Gel	2% agarose in 1 X TAE (100 V, 30 min)
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primer_set		product_size	product (allele type)
primer 1	primer 3	700 bp	KO
primer 1	primer 2	860 bp	WT

Comment Separated PCR