

RBRC 00763

Strain name WS4

Primer	primer_name	primer_seq	length	
1		TAAACCAGTGGTCTTCAACCTTCC	24	Depositor's primer
2		GTTAACCTCACAGTCTTGGGATCC	24	Depositor's primer

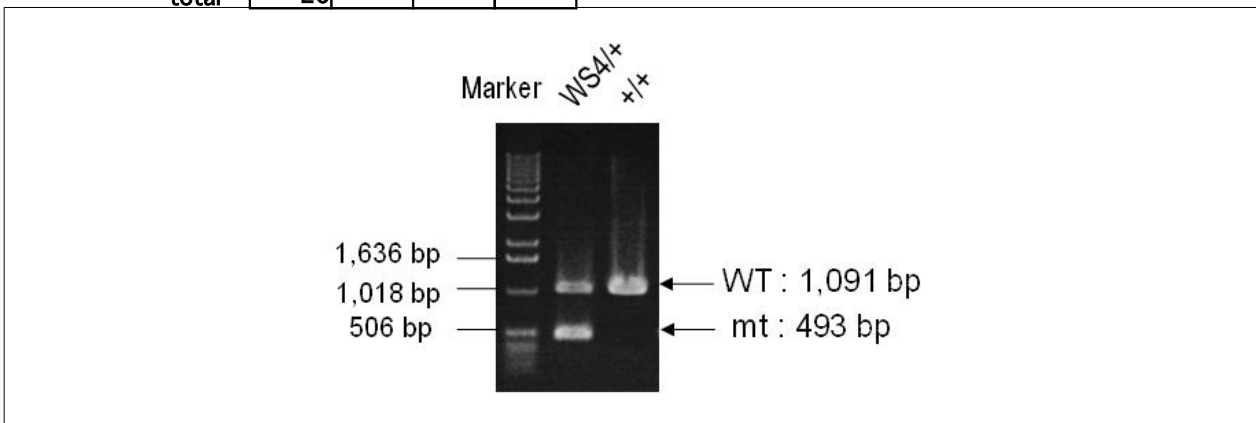
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.8			
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			
DNA		1.0			
Taq	1 U/ μ l	0.2			
total		20			

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	90*	*Repeat for 30 cycles



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

1% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set	product_size	product
primer 1 primer 2	493 bp	mutant
primer 1 primer 2	1,091 bp	WT

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

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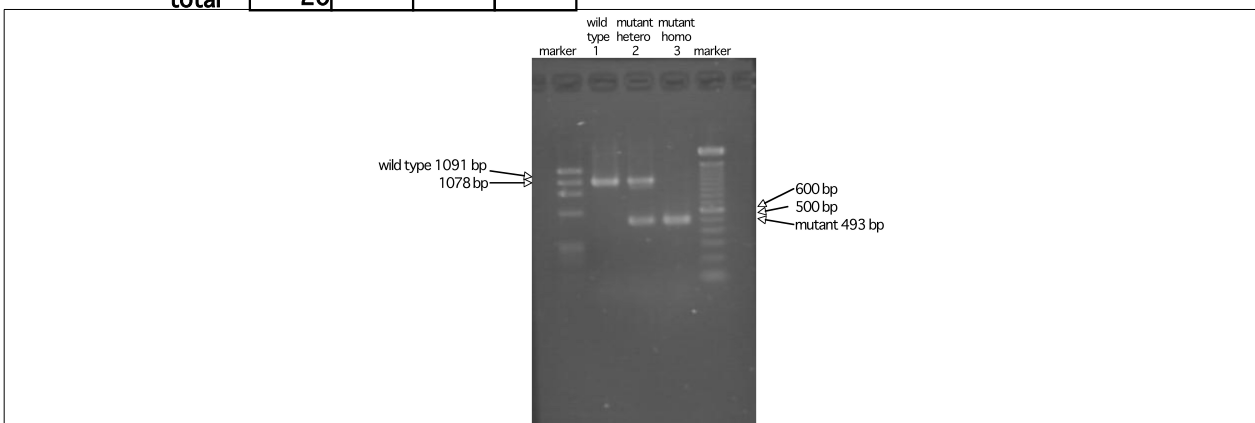
Reaction Components

Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		4.8			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
DNA		1.0			
Taq		0.2			
total		20			

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	90*	*Repeat for 30 cycles
5	72	300	



Marker
marker 1: ϕ X174 Hae III, marker2: 100 bp ladder (Invitrogen, Cat. 15628-050)

Gel
2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set	product_size	product
primer 1	493 bp	mutant
primer 1	1091 bp	WT

Comment