

RBRC 00993

Strain name B6.129S4-Sh2b3<tm1Rmp>/Rbrc

Primer	primer_name	primer_seq	length	Reference
1	Lnk-Fw	TCAAAGCCCAAGCTCCAAGAG	21	Depositor's primer
2	Lnk-Rv	ATCCATCGCCTGTGGCATGGAACC	24	Depositor's primer
3	Neo	CAGGATCTCCTGTCATCTCACCTT	24	Immunology, 102(3):289-300 (2001).

Reaction Components

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

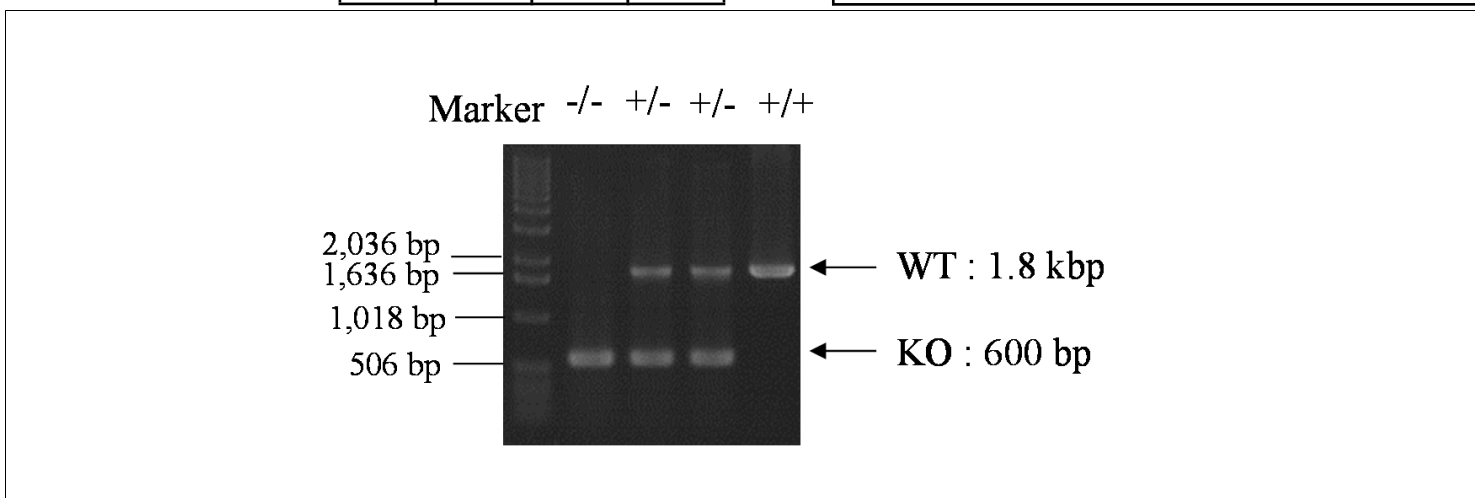
Reagent	Conc	Reaction 1	2	3	4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
total		20			

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

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

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



primer_set	product_size	product (allele type)
primer 1	primer 2	1.8 kbp WT
primer 2	primer 3	600 bp KO

Comment [Standard PCR](#)

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

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Reaction Components

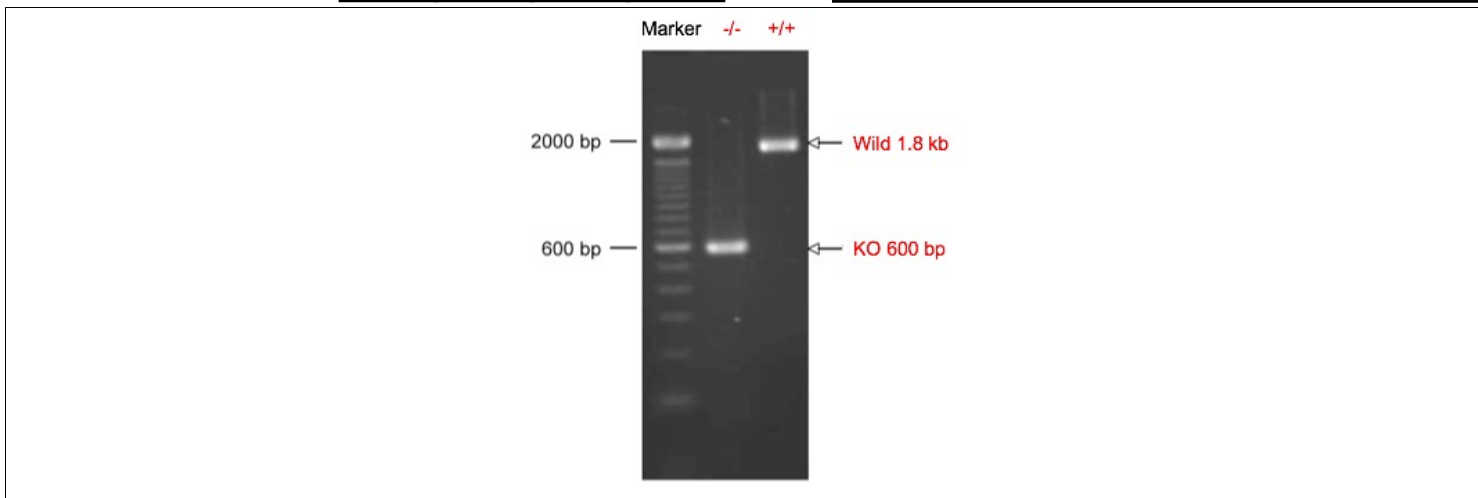
Enzyme Roche Taq (Cat. No. BM1418432)

Reagent	Conc	Reaction 1	2	3	4	(μ L)
H2O		16.3				
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				
DNA		0.2				
Taq		1.0				
total		25				

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	64	30*	
4	72	120*	*Repeat for 30 cycles
5	72	300	

Marker 100 bp ladder (Invitrogen, Cat. 15628-050)
Gel 1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	primer 2	1.8 kb WT
primer 2	primer 3	600 bp KO

Comment Standard PCR