



PCR protocol

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RBRC 04865

Strain name B10.B6-Kit<W-sh>/Hir

Primer	primer_name	primer_seq	length	Reference
1	sh for	AGGCTTGCAGCGCATTAT	18	Depositor' s primer
2	Sh	GAGGATTCATAGTTGTTCAATGTCC	25	Am. J. Pathol., 173, 6, 1693-1701(2008).
3	wt for	TTTGACAGTGTTAGTTACAC	19	Depositor' s primer

Reaction Components

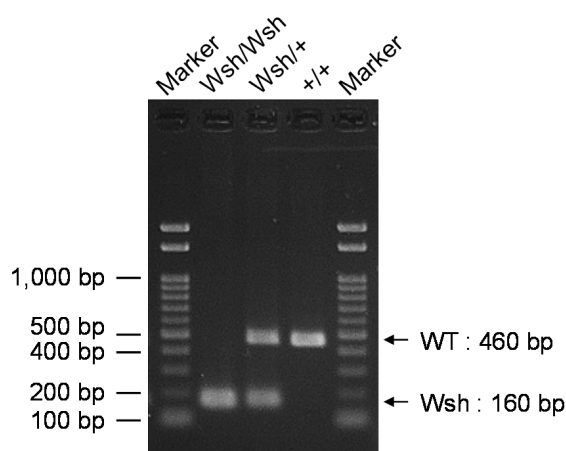
Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	55	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 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	160 bp
primer 2	primer 3	460 bp

+/+ (wild-type): 460 bp
Wsh/Wsh (homozygous): 160 bp
Wsh/+ (heterozygous): 460 bp and 160 bp

Comment [Standard PCR](#)

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