



PCR protocol

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RBRC 10190

Strain name C57BL/6-Gt(ROSA)26Sor<tm1.2(CAG-3XFLAG/dCas9)Hfuj>

Primer	primer_name	primer_seq	length	Reference
1	intron 1	AGTAAGGGAGCTGCAGTGGAG	21	RIKEN BRC
2	intron 1	TTACATAGTCTAACTCGCGACACTG	25	RIKEN BRC
3	Cas9_R 27643 (28110)	ATGGTTGGGTACTTTTCATGGT	22	Genes Cells. 23(4):318-325 (2018).
4		CAAGGACTTCCTGGACAATGAGG	23	RIKEN BRC

Reaction Components

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

Reaction Conditions

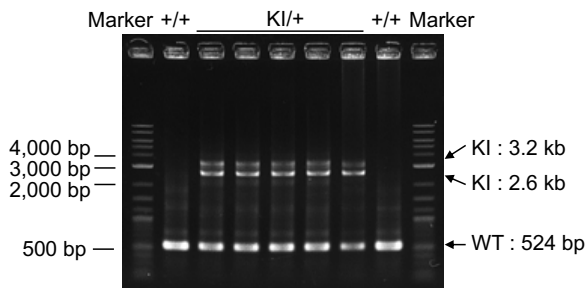
step temp (°C) time (sec) note

1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4
H2O		0.8			
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			
primer 4	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
total		20			

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

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



primer_set	product_size	product (allele type)
primer 1	primer 2	524 bp
primer 1	primer 3	2.6 kb
primer 2	primer 4	3.2 kb

Comment Standard PCR

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