

RBRC 10189

Strain name C57BL/6-Gt(ROSA)26Sor^{tm1.1}(CAG-3XFLAG/dCas9,-EGFP)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)
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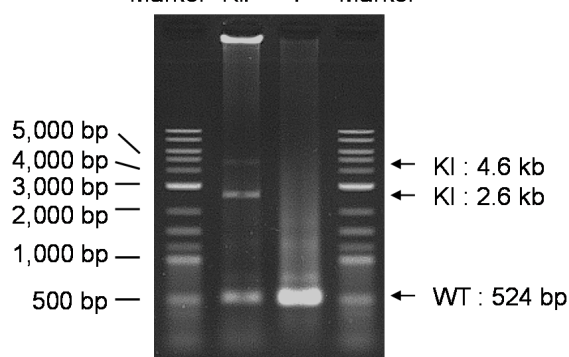
Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	360*	*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)

Marker KI/+ +/- Marker



primer_set	product_size	product (allele type)	
primer 1	primer 2	524 bp	WT
primer 1	primer 3	2.6 kb	KI
primer 2	primer 4	4.6 kb	KI

+/+ (wild-type): 524 bp
KI/+ (heterozygous): 524 bp, 2.6 kb and 4.6 kb

Comment [Standard PCR](#)

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