PCR protocol

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RBRC 05539 **Strain name** B6.129-Gt(ROSA)26Sor<tm1(Stop-Bmi1,EGFP)>

Primer	primer_name	primer_seq	length		
1	STOP-ROSA. SA. F1	TGTTGCAATACCTTTCTGGGAGTTC	25		
2	STOP-ROSA. neo. Re1	GCTATCAGGACATAGCGTTGGCTAC	25		
3	STOP-ROSA. FLAG. R1	TGTCATCGTCATCCTTGTAATCG	23		
4	HL54	TAAGCCTGCCCAGAAGACTCC	21		

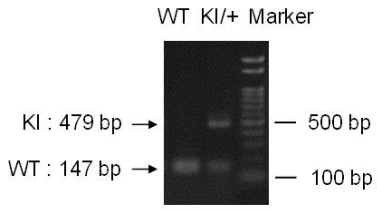
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		11.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
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	5 U/μl	0.1			
	total	20			

Reaction Conditions

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



Marker

Gene Ladder 100 (Wako, Cat. 316-06951)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	479 bp	KI
primer 1	primer 3	351 bp	KI (delta Neo)
primer 1	primer 4	147 bp	WT

Comment