PCR protocol

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RBRC 09365 **Strain name** STOCK Gt(ROSA)26Sor<tm5(ACTB-tTA)Luo>Igs7<tm92.1(tetO-YCX2.60)Hze>/J

Primer	primer_name	primer_seq	length		
1	Rosa10	CTCTGCTGCCTCCTGGCTTCT	21		
2	Rosa11	CGAGGCGGATCACAAGCAATA	21		
3	NeoP	CTTCTATCGCCTTCTTGACGAGTT	24		
4	22159	GTGTAGCCCTGGCTTTTCTG	20		JAX Genotyping Protocol
5	22330	GAACTCACAGTGGCCAGTCA	20		JAX Genotyping Protocol
6	HS4_Fw	CGTAAGGCCATTATCTCTCATCC	23		RIKEN BRC

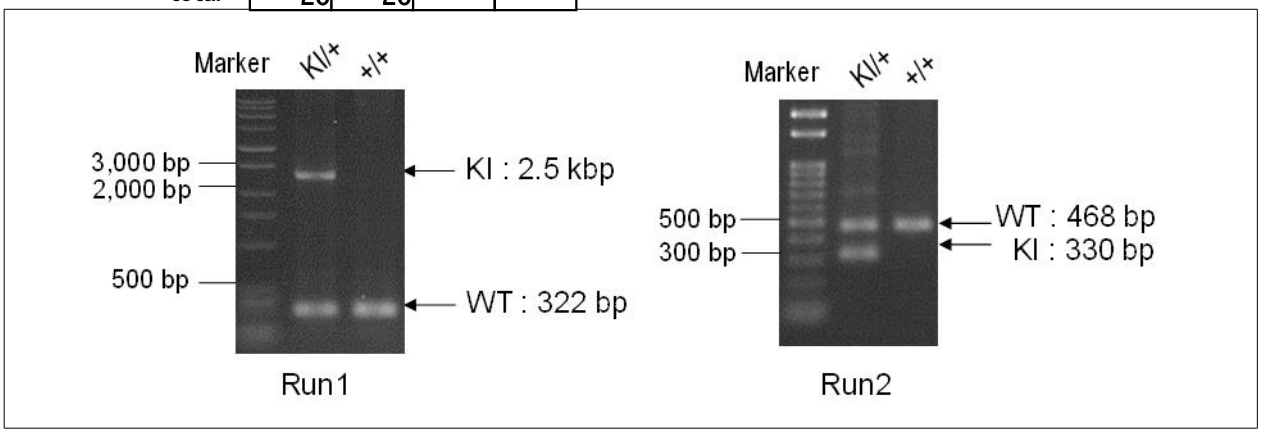
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	1.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	120		
2	98	10	*	
3	60	30	*	
4	68	240	*	*Repeat for 30 cycles



Marker
Run1: DNA Ladder,1 kb Plus (Affymetrix, Cat. 76714 500 UL) ,

Gel
Run1:1 % agarose in 1 X TAE (100 V, 30 min),Run2:2 % agarose in 1 X TAE

PCR products	primer_set	product_size	product
primer 1	primer 2	322 bp	WT (Gt(ROSA)26Sor)
primer 3	primer 2	2.5 kbp	KI (Gt(ROSA)26Sor)
primer 4	primer 5	468 bp	WT (Igs7)
primer 4	primer 6	330 bp	KI (Igs7)

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