

RBRC 05147 Strain name B6;129S6-Gt(ROSA)26Sor<tm1.1(CAG-mTFP1)Imayo>

Primer	primer_name	primer_seq	length	
1	R26F2	AAAGTCGCTCTGAGTTGTTAT	21	Depositor's primer
2	oIMR0316	GGAGCGGGAGAAATGGATATG	21	JAX Genotyping Protocol
3	oIMR0315	GCGAAGAGTTTGTCTCAACC	21	JAX Genotyping Protocol

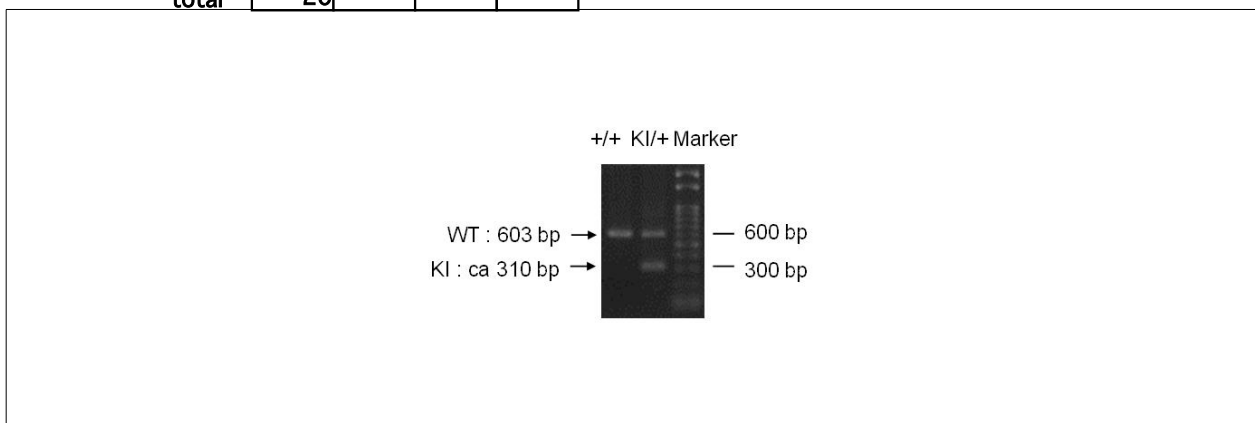
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		12.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
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	5 U/ μ l	0.1			
total		20			

step	temp (°C)	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	603 bp	WT
primer 1	primer 3	ca 310 bp	KI

Comment

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3	oIMR0315	GCGAAGAGTTTGTCTCAACC	21	JAX Genotyping Protocol
4	LFcheck-common-fw	AATTCCTCGACGGGGAATTCGGG	23	Depositor's primer
5	LFcheck-mTFP1-rv	CAGCGTAATCTGGTACGTCGTATG	24	Depositor's primer
6	mTFP1-400bp-fw	GAATGGCCACGCCTTCGTGATCGAG	25	Depositor's primer
7	mTFP1-400bp-rv	TTCAGCACGCCGTCGCGCACGTACATC	27	Depositor's primer

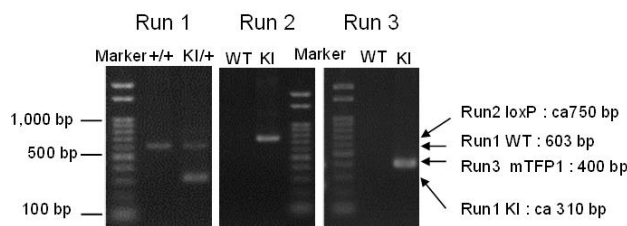
Reaction Components

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

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	63	30*	
4	68	120*	*Repeat for 30 cycles

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	2.8	2.8	
PCR buffer	2 X	10.0	10.0	10.0	
dNTP	2.0 mM	4.0	4.0	4.0	
primer 1	10 pmol/μl	1.0	0	0	
primer 2	10 pmol/μl	1.0	0	0	
primer 3	10 pmol/μl	1.0	0	0	
primer 4	10 pmol/μl	0	1.0	0	
primer 5	10 pmol/μl	0	1.0	0	
primer 6	10 pmol/μl	0	0	1.0	
primer 7	10 pmol/μl	0	0	1.0	
DNA		1.0	1.0	1.0	
Taq	1 U/μl	0.2	0.2	0.2	
total		20	20	20	



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	603 bp	WT
primer 1	ca 310 bp	KI
primer 4	ca 750 bp	loxP
primer 6	400 bp	mTFP1

Comment