PCR protocol

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RBRC 06054 **Strain name** C57BL/6-Polk<tm3(mpolk)Csk> Tg(gptdelta)1Nmi

Primer	primer_name	primer_seq	length		
1	GT-PCR-F	CTTTCTGTTCCTACCAGAATAATGC	25		Depositor's primer
2	WT	CTGTGTTATATTCAAGTAGGCTTCGT	26		RIKEN BRC
3	MT	CTGTGTTATATTCAAGTAGGCTGCAG	26		RIKEN BRC
4	F1	GTTGTACTTCCAACCATGCCAAAG	24		Genes and Environment (2015) 37:24
5	R3	CCCAGGTAATGAATAATTGCCTCTTTG	27		Genes and Environment (2015) 37:24
6	R1	CAGAAATCATTCCAGGTCCTTGC	23		Genes and Environment (2015) 37:24

Reaction Components

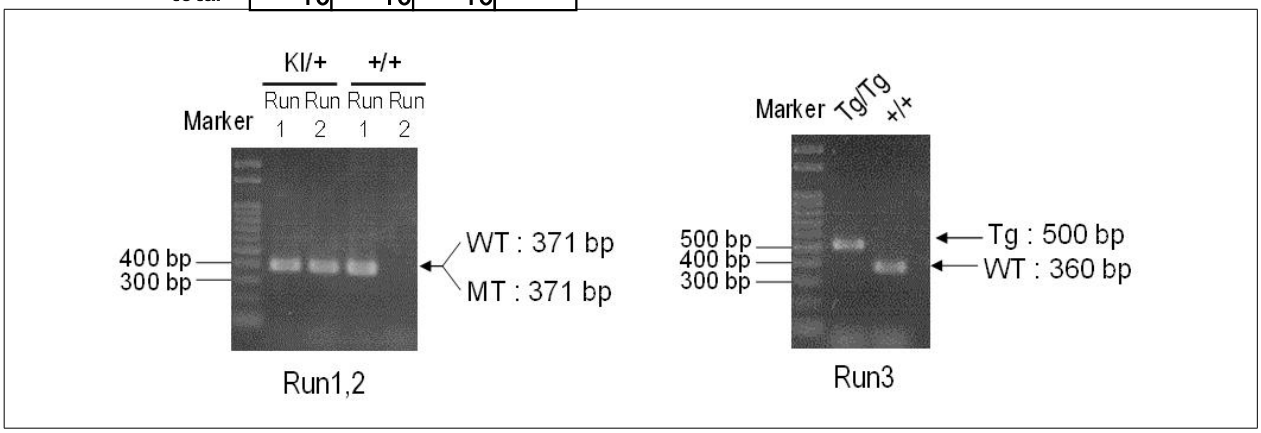
Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.0	2.0	1.0	
Master Mix	2 X	5.0	5.0	5.0	
primer 1	10 pmol/μl	1.0	1.0	0	
primer 2	10 pmol/μl	1.0	0	0	
primer 3	10 pmol/μl	0	1.0	0	
primer 4	10 pmol/μl	0	0	1.0	
primer 5	10 pmol/μl	0	0	1.0	
primer 6	10 pmol/μl	0	0	1.0	
DNA		1.0	1.0	1.0	
	total	10	10	10	

Reaction Conditions

step	temp (℃)	time (sec)	note	
1	95	900		
2	94	30	*	
3	60	90	*	
4	72	60	*	*Repeat for 30 cycles

Separated PCR



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	371 bp	WT(Polk)
primer 1	primer 3	371 bp	KI(Polk)
primer 4	primer 5	500 bp	Tg
primer 4	primer 6	360 bp	WT

Comment