

RBRC 05944

Strain name B6.Cg-Tg(Mt1-RET)304Ina Tg(Dct-LacZ)A12Jkn

Primer	primer_name	primer_seq	length	
1	mt1	GTCCTCTAAGCGTCACCACGACTTC	25	RIKEN BRC
2	RFP/RET(~1500)	ACTCGGGGAGGCGTTC	16	Depositor's primer
3	LFp	CAGGACACGGCTTGTCATCATGGTGT	26	Depositor's primer
4	DRp	CATTGATCGTCTCTCAGGAATTCA	24	Depositor's primer
5	LRp	CCACACAGACACCTACCACATGCGT	25	Depositor's primer

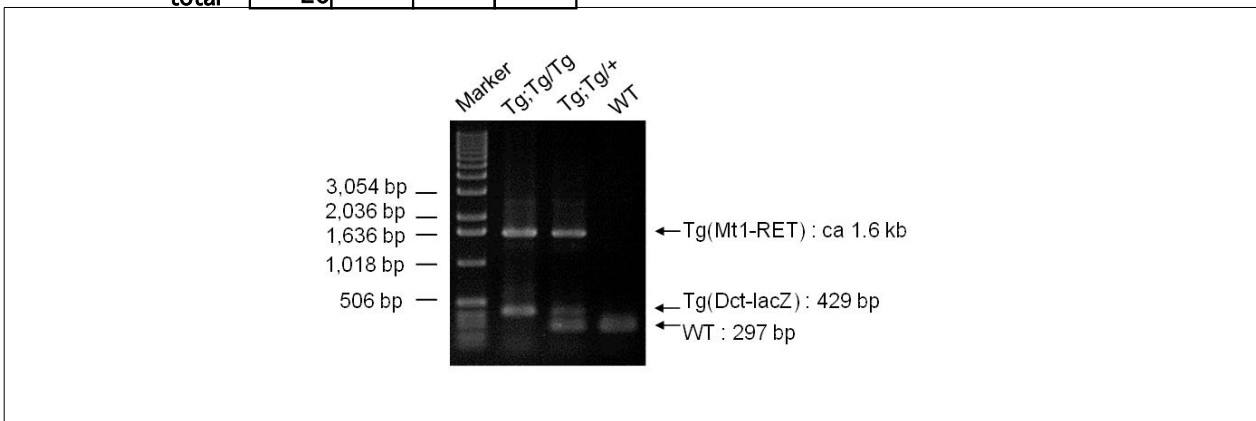
Reaction Components

Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.9			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
primer 4	10 pmol/ μ l	1.0			
primer 5	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	120*	*Repeat for 30 cycles
5	72	300	



Marker
1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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

PCR products			
primer_set	product_size	product	
primer 1	primer 2	ca 1.6 kb	Tg(Mt1-RET)
primer 3	primer 4	429 bp	Tg(Dct-LacZ)
primer 3	primer 5	297 bp	WT

Comment

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Primer	primer_name	primer_seq	length	
1	mt1	GTCCTCTAAGCGTCACCACGACTTC	25	RIKEN BRC
2	RFP/RET(~1500)	ACTCGGGGAGGCGTTC	16	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol
5	LFp	CAGGACACGGCTTGTCATCATGGTGT	26	Depositor's primer
6	DRp	CATTCATCGTCTCTCAGGAATTCA	24	Depositor's primer
7	LRp	CCACACAGACACCTACCACATGCGT	25	Depositor's primer

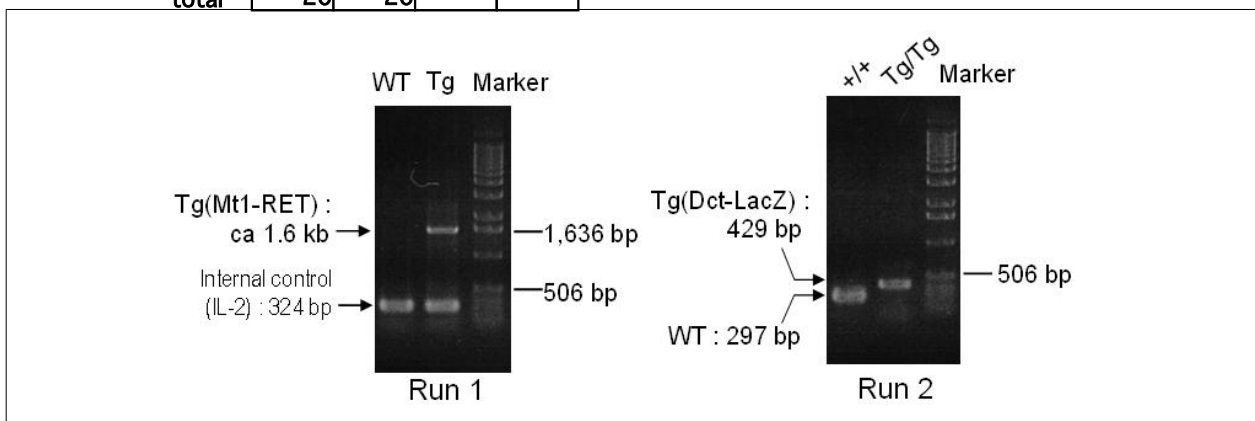
Reaction Components

Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.9	3.9		
PCR buffer	10 X	10.0	10.0		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/ μ l	1.0	0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	1.0	0		
primer 4	10 pmol/ μ l	1.0	0		
primer 5	10 pmol/ μ l	0	1.0		
primer 6	10 pmol/ μ l	0	1.0		
primer 7	10 pmol/ μ l	0	1.0		
DNA		1.0	1.0		
Taq	5 U/ μ l	0.1	0.1		
total		20	20		

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	120*	*Repeat for 30 cycles
5	72	300	



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set	product_size	product
primer 1	ca 1.6 kb	Tg(Mt1-RET)
primer 3	324 bp	Internal control (IL-2)
primer 5	429 bp	Tg(Dct-LacZ)
primer 5	297 bp	WT

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