

RBRC 01206

Strain name BTBRTF/ArtRbrc

Primer	primer_name	primer_seq	length	Reference
1	common	CCAGAGACTCAAGCAGAGGATGC	23	RIKEN BRC
2	wild	GTTTCATCCTGAACGTACGCTTAGAC	25	RIKEN BRC
3	tf_mut	GTTTCATCCTGAACGTACGCATCTC	24	RIKEN BRC

Reaction Components

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

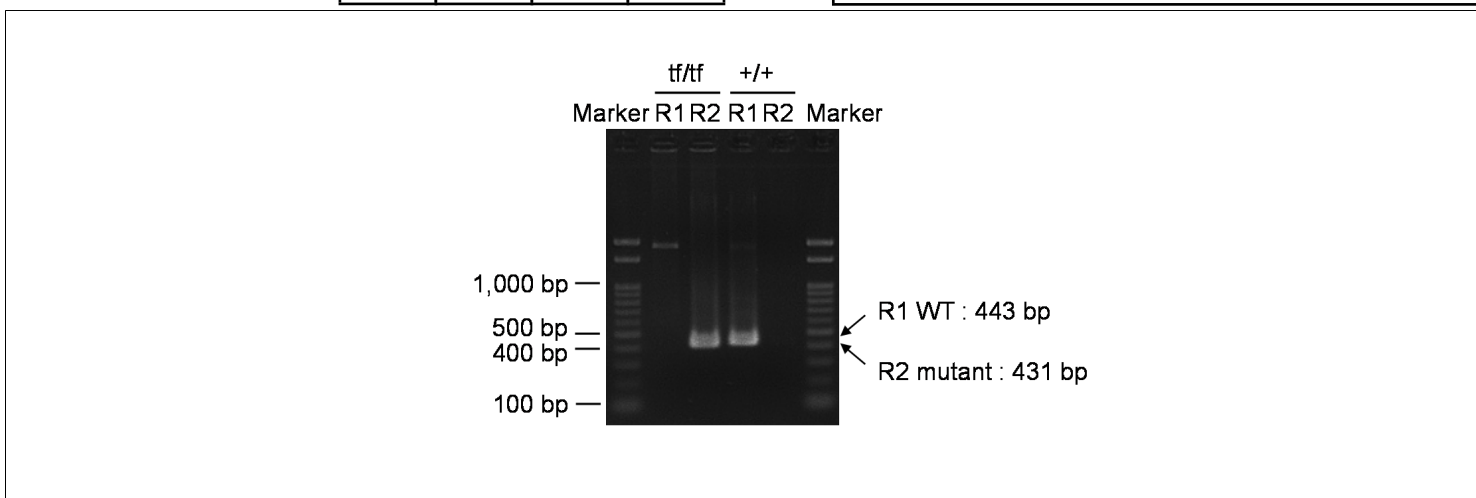
Reagent	Conc	Reaction 1	2	3	4
H2O		2.8	2.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
total		20	20		

(μL)

Reaction Conditions

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

Marker	Gene Ladder 100 (Wako, Cat. 316-06951)
Gel	2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1	primer 2	443 bp	WT
primer 1	primer 3	431 bp	mutant
			Reaction 1
			+/+ : 443 bp
			tf/tf : none
			Reaction 2
			+/+ : none
			tf/tf : 431 bp

Comment Allele specific PCR (ASP)

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