PCR protocol

Sheet ID PS_05507
Release date : 5/14/2021
Print date : 5/14/2021

RBRC 06153 **Strain name** B6.Cg-Tg(TOPGAL) Ctnnb1<Rgsc1880>

Primer	primer_name	primer_seq	length		Reference
1	TK5	CAAACCCCGCCCAGCGTCTTGTC	23		RIKEN BRC
2	LacZ-R5	ATGTGAGCGAGTAACAACCCGTCGGATTCT	30		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

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

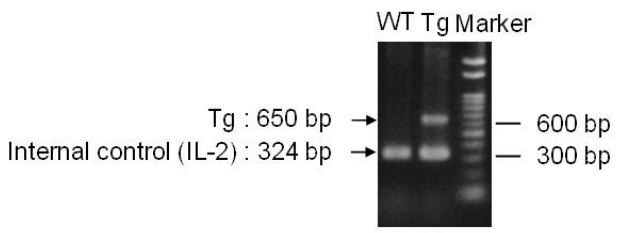
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	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	650 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment Standard PCR

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

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Strain name B6.Cg-Tg(TOPGAL) Ctnnb1<Rgsc1880>

Primer	primer_name	primer_seq	length		Reference
1	wild	CAGCTGGAATTCTCTCTAACCTCACGT	27		RIKEN BRC
2	mut	CAGCTGGAATTCTCTCTAACCTCACGA	27		RIKEN BRC
3	TM024	GTGTTGAGTGAAATGGACGCTTCGG	25		Depositor's primer

Reaction Components

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

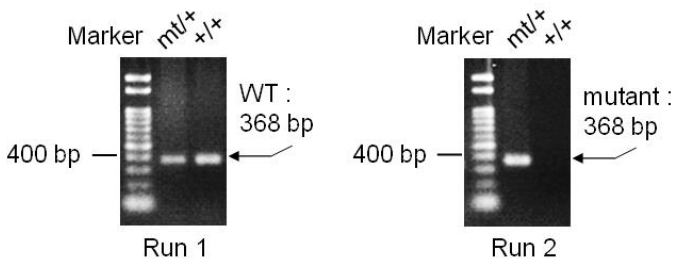
Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		2.0	2.0			
Master Mix	2 X	5.0	5.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	0	1.0			
primer 3	10 pmol/μl	1.0	1.0			
DNA		1.0	1.0			
	total	10	10			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30*	
3	60	90*	
4	72	60*	*Repeat for 30 cycles
5	72	600	

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 3	368 bp	WT
primer 2	primer 3	368 bp	mutant

Comment Allele specific PCR (ASP)