PCR protocol

Sheet ID PS_07222
Release date : 5/6/2022
Print date : 5/6/2022

RBRC 09908 **Strain name** C.Cg-Braf<tm1Tumg>

Primer	primer_name	primer_seq	length		Reference
1	mBr1-Fw	GTGTTGTTCTGCCCATACTTACTGC	25		*Hum. Mol. Genet.*, 23, 6553-6566 (2014).
2	mBr1-Rv	GTGACTTAATGTACAGCATGGATCA	25		*Hum. Mol. Genet.*, 23, 6553-6566 (2014).
3	oIMR0214	CCGGAGAACCTGCGTGCAATCC	22		JAX Genotyping Protocol

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		1.8			
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			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

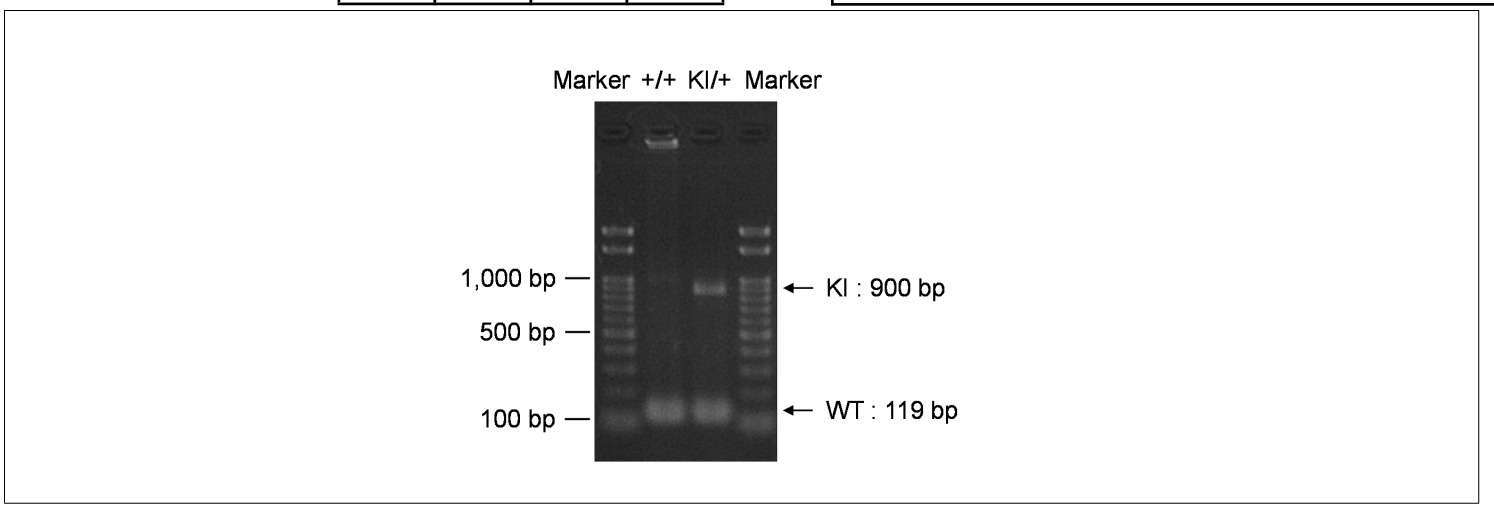
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	120*	*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	119 bp	WT
primer 1	primer 3	900 bp	KI
primer 1	primer 2	1.2 kb	KI (dneo)
primer 1	primer 2	196 bp	KI (After cre mediated excision)

Comment Standard PCR

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

PCR protocol

Sheet ID PS_07224
Release date : 1/1/1990
Print date : 5/6/2022

RBRC 09908 **Strain name** C.Cg-Braf<tm1Tumg>

Primer	primer_name	primer_seq	length		Reference
1		GTCTCGAACTGTCGCACTAGTGG	23		
2	intron7_R	GAGTTGCGTGGACTATTCGTTC	22		
3	WT	GACTTTTGCCGAAAGCTGCTTTTCTA	26		
4	MT	CACATGTTTGACAACGGAAACCTC	24		

Reaction Components

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

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

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	60	90	*
4	72	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	728 bp	Internal control
primer 2	primer 3	264 bp	WT
primer 1	primer 4	513 bp	MT

Comment Separated PCR