PCR protocol

Sheet ID PS_08344
Release date : 6/2/2022
Print date : 6/2/2022

RBRC 03731 **Strain name** B6;129S4-Dnmt3a<tm3.1Enl>

Primer	primer_name	primer_seq	length		Reference
1	NT004F	CTGGTGATTGGAGGCAGTCCATGCA	25		Depositor's primer
2	NT007R	TAGCTGAGGCTGTCTGCATCGGACA	25		Depositor's primer
3	Ex18_Fw	ACAGGGCTCCTGGTGCTGAAGGAC	24		RIKEN BRC

Reaction Components

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

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

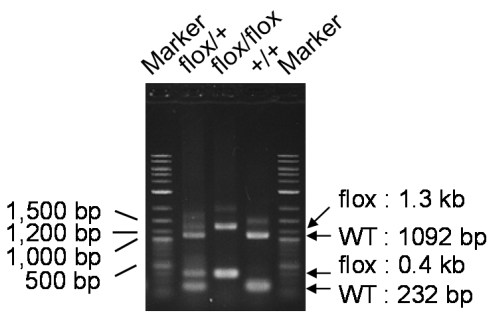
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30 *	
3	60	90 *	
4	72	90 *	*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	232 bp	WT
primer 1	primer 2	0.4 kb	flox
primer 3	primer 2	1092 bp	WT
primer 3	primer 2	1.3 kb	flox
primer 3	primer 2	0.6 kb	null (After cre mediated excision)

flox/+ (heterozygous): 232 bp, 0.4 kb, 1092 bp and 1.3 kb
flox/flox (homozygous): 0.4 kb and 1.3 kb
+/+ (wild-type): 232 bp and 1092 bp

Comment Standard PCR

PCR protocol

Sheet ID PS_03251
Release date : 03/04/2011
Print date : 6/2/2022

RBRC 03731

Strain name B6;129S4-Dnmt3a<tm3.1Enl>

Primer	primer_name	primer_seq	length		Reference
1	NT004F	CTGGTGATTGGAGGCAGTCCATGCA	25		Depositor's primer
2	NT007R	TAGCTGAGGCTGTCTGCATCGGACA	25		Depositor's primer

Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

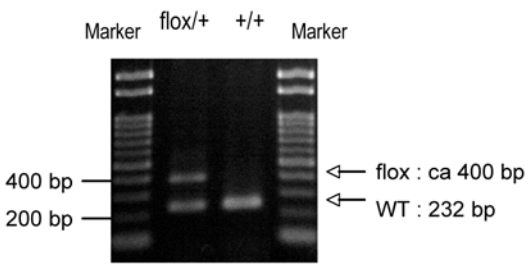
Reagent	Conc	Reaction 1	2	3	4	
H2O		13.3				(μL)
PCR buffer	10 X	2.0				
dNTP	2.5 mM	1.6				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
DNA		1.0				
Taq	5 U/μl	0.1				
	total	20				

Reaction Conditions

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

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	232 bp	WT
primer 1	primer 2	ca 400 bp	flox

Comment Standard PCR