PCR protocol

Sheet ID PS_07754
Release date : 3/11/2019
Print date : 3/11/2019

RBRC 09758 **Strain name** B6;129P2-Chd8<tm1Kei>

Primer	primer_name	primer_seq	length		Reference
1	promoter	GCTCCTATGTGTGCTGTCCTG	21		RIKEN BRC
2		GTTCACTCAGTAAATTTGTGTGCCTAC	27		RIKEN BRC
3		GCAGCGCATCGCCTTCTATCGC	22		RIKEN BRC

Reaction Components

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

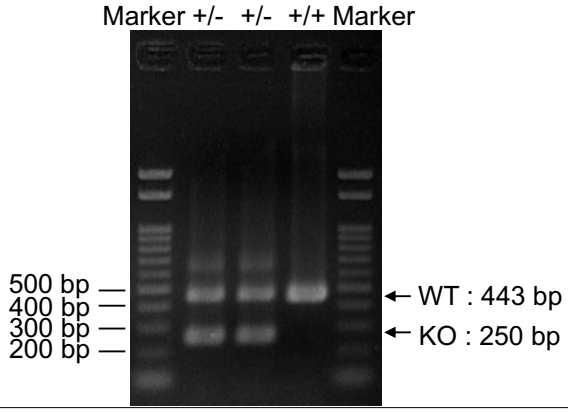
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

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	443 bp	WT	+/+ : 443 bp
primer 1	primer 3	250 bp	KO	+/- : 443 bp, 250 bp

Comment Standard PCR

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

PCR protocol

Sheet ID PS_07131
Release date : 2/1/2017
Print date : 3/11/2019

RBRC 09758 **Strain name** B6;129P2-Chd8<tm1Kei>

Primer	primer_name	primer_seq	length		Reference
1	Endo	TATAGATTTCCTGTTTGATTTTCC	24		Mol. Cell Biol., 29, 8386-8394 (2004).
2	RV1	AACTCCGTAACCATTTGTCTATTC	24		Mol. Cell Biol., 29, 8386-8394 (2004).
3	olMR0214	CCGGAGAACCTGCGTGCAATCC	22		JAX Genotyping Protocol

Reaction Components

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

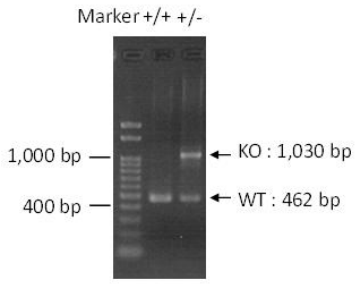
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

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	462 bp	WT
primer 2	primer 3	1,030 bp	KO

There are reports from a user that it is difficult to detect with this protocol. (Dec. 25, 2018)

Comment

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