PCR protocol

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RBRC 11140 **Strain name** B6.Cg-Dnase1l3<tm1Dkit> Dffb<tm1Osa>

Primer	primer_name	primer_seq	length		Reference
1	DNase1L3-sense primer	GCACACAACTCCCGAGACCTCCG	23		Depositor's primer
2	DNase1L3-antisense primer	CACTCCTGGGCTTCTTGATGGTCAG	25		Depositor's primer
3	NeoR primer	GATTCGCAGCGCATCGCCTTCTATCG	26		Mol. Cell. Biol., 24, 6539-6549 (2004).
4	CAD-sense primer	AAAAGAACAGTCGGGACTGC	20		Depositor's primer
5	CAD-antisense primer	TTCACACCAGGAGACATCTG	20		Depositor's primer

Reaction Components

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

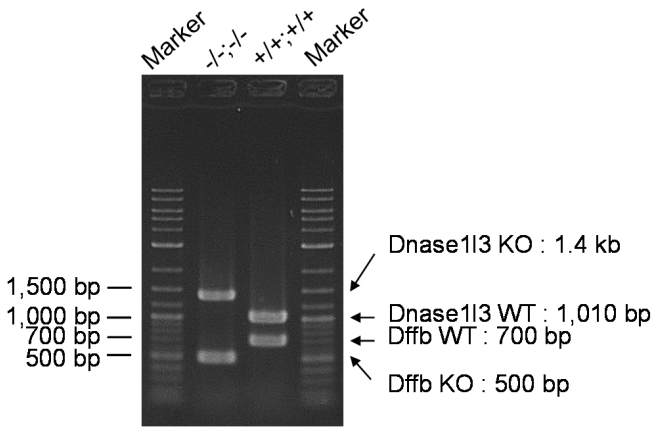
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.3				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	0.5				
primer 2	10 pmol/μl	0.5				
primer 3	10 pmol/μl	0.5				
primer 4	10 pmol/μl	0.5				
primer 5	10 pmol/μl	0.5				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

Gel 1% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	1,010 bp	WT	+/+;+/+ (wild-type): 1,010 bp and 700 bp
primer 2	primer 3	1.4 kb	KO	-/-;-/- (homozygous): 1.4 kb and 500 bp
primer 4	primer 5	700 bp	WT	
primer 3	primer 5	500 bp	KO	

Comment Standard PCR

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