PCR protocol

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RBRC 11525 **Strain name** STOCK Tg(Clgn-Dcst2/PA)1Nino Dcst1<tm1Osb>

Primer	primer_name	primer_seq	length		Reference
1	196	CCTTCCTGCGGCTTGTTCTCT	21		Depositor's primer
2	PAtag-R	ACCACATCATCTTCGGCACCTGGCAT	26		Depositor's primer
3	Dcst1_WT	GTAAGAGGAGGCTAAAGCTATGGC	24		RIKEN BRC
4	DCST1 R	CCAGGGGATCCAACACCCTT	20		Depositor's primer
5	Neo_F	GGTGGAGAGGCTATTCGGCTATG	23		RIKEN BRC

Reaction Components

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

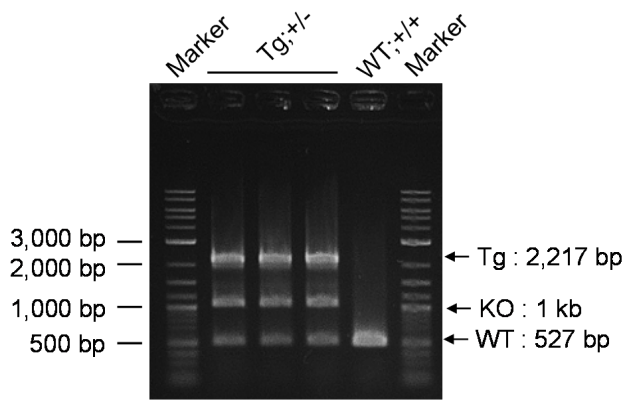
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.3				(μL)
PCR buffer	2 X	10.0				
dNTP	2 mM	4.0				
primer 1	10 pmol/μl	0.5				
primer 2	10 pmol/μl	0.5				
primer 3	10 pmol/μl	0.5				
primer 4	10 pmol/μl	0.5				
primer 5	10 pmol/μl	0.5				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	65	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	2,217 bp	Tg
primer 3	primer 4	527 bp	WT
primer 3	primer 5	1 kb	KO

Non-Tg;+/+ (wild-type): 527 bp
Tg;+/-: 527 bp and 1 kb and 2,217 bp

Comment Standard PCR

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