PCR protocol

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RBRC 10238 **Strain name** B6;Cg-Tg(Cryd1/Cryaa-Sfrp2)1Jmca

Primer	primer_name	primer_seq	length		Reference
1	delta1-crystallin enhancer	GCTGACGCAAAAATCCCTAATG	22		Dev Biol., 324(1):161-76(2008).
2	Cd1-cry R	ACTGGGAAACTAGCATTGCA	20		Dev Biol., 324(1):161-76(2008).
3	oIMR0015	CAAATGTTGCTTGTCTGGTG	20		JAX Genotyping Protocol
4	oIMR0016	GTCAGTCGAGTGCACAGTTT	20		JAX Genotyping Protocol

Reaction Components

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

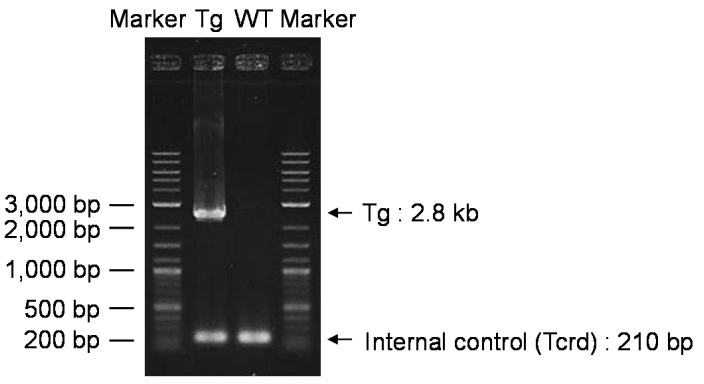
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	240	* *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	2.8 kb	Tg	Non-Tg : 210 bp Tg : 210 bp, 2.8 kb
primer 3	primer 4	210 bp	Internal control (Tcrd)	

Comment Standard PCR

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