

Primer	primer_name	primer_seq	length	Reference
1	EGFP-F	GCCACCATGGTGAGC	15	Blood, 110, 107-115(2007).
2		TTACTTGTACAGCTCGTCC	19	Blood, 110, 107-115(2007).
3	acro	GTGGAGCTTTGTGAGGTCACAG	22	Depositor's primer
4	gfp	CAGCTTGCCGGTGGTGCAGATG	22	Depositor's primer
5	olMR0015	CAAATGTTGCTTGTCTGGTG	20	JAX Genotyping Protocol
6	olMR0016	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	0.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/ μ l	1.0	0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	0	1.0		
primer 4	10 pmol/ μ l	0	1.0		
primer 5	10 pmol/ μ l	1.0	1.0		
primer 6	10 pmol/ μ l	1.0	1.0		
DNA		1.0	1.0		
Taq	1 U/ μ l	0.2	0.2		
total		20	20		

Reaction Conditions

step	temp (°C)	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	725 bp CAG-EGFP
primer 3	primer 4	300 bp acro-EGFP
primer 5	primer 6	210 bp Internal control (Tcrd)

Comment Standard PCR

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