PCR protocol

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RBRC 04749 **Strain name** C57BL/6J-Tg(DBH-hMOR)A

Primer	primer_name	primer_seq	length		Reference
1	hDBH	GAAGGGACAGCATCCGCCTGTC	22		RIKEN BRC
2	MOR3L	GGCAGCTGTTTGTGTAAC	18		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

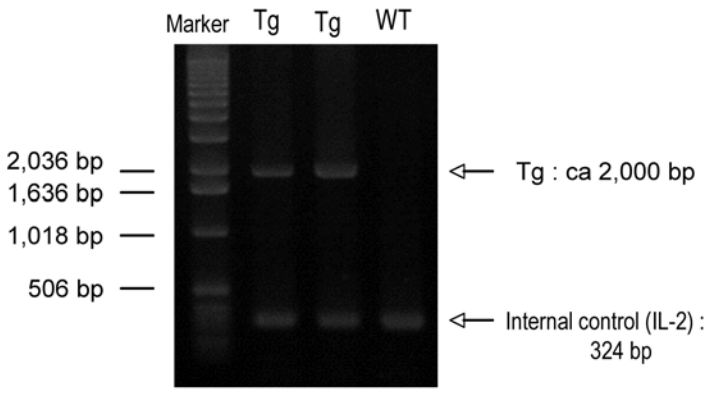
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.3				(μL)
PCR buffer	2 X GC I	10.0				
dNTP	2.5 mM	1.6				
primer 1	10 pmol/μl	2.0				
primer 2	10 pmol/μl	2.0				
primer 3	10 pmol/μl	0.5				
primer 4	10 pmol/μl	0.5				
DNA		1.0				
Taq	5 U/μl	0.1				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	60	30	*
4	72	120	* *Repeat for 30 cycles
5	72	300	

Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	ca 2,000 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment