PCR protocol

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RBRC 10042

Strain name B6.Cg-Mapt<tm1.1(MAPT)Tcs> App<tm2(NL-F)Tcs>

Primer	primer_name	primer_seq	length		Reference
1	Tau-KI-F	GTCAGATCACTAGACTCAGC	20		Depositor's primer
2	Tau-WT-R	CTGTGCTCCACTGTGACTGG	20		Depositor's primer
3	Tau-KI-R	CTGCTTGAGTTATCTTGGCC	20		Depositor's primer
4		CTCCTTGTGGCTGGCGGTCACAC	23		RIKEN BRC
5	common_1	CTATCGTGGACCGAGAATGGTCATG	25		RIKEN BRC

Reaction Components

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

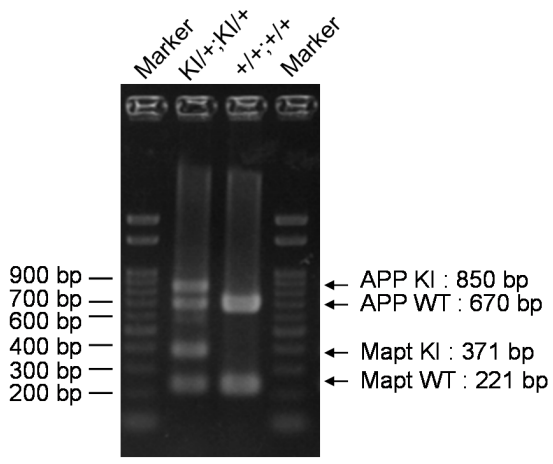
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.3				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	0.5				
primer 2	10 pmol/μl	0.5				
primer 3	10 pmol/μl	0.5				
primer 4	10 pmol/μl	0.5				
primer 5	10 pmol/μl	0.5				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	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	221 bp	WT (Mapt)
primer 1	primer 3	371 bp	KI (Mapt)
primer 4	primer 5	670 bp	WT (App)
primer 4	primer 5	850 bp	KI (App)

+/+;+/+ (wild-type): 221 bp and 670 bp
KI/+;KI/+ (heterozygous): 221 bp and 371 bp and 670 bp and 850 bp

Comment Standard PCR

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