PCR protocol

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RBRC 09462 **Strain name** STOCK Tc (CYP3A-MAC)-Cyp3a

Primer	primer_name	primer_seq	length		
1	m11_6L	GTTCAACCTGAGCTCCACATCATGCTC	27		ACS Synth. Biol., (2012).
2	m11_5R	CTAAAGACCCTCATGCTCCTGTGTGGAA	28		ACS Synth. Biol., (2012).
3	HS4_Rv	GGATGAGAGATAATGGCCTTACG	23		RIKEN BRC
4	CYP3A7-3L	TCCCCCTGAAATTACGCTTT	20		Hum Mol Genet., 22(3), 578-592 (2013).
5	exon13_R	GTGGAGATAGTCCTATGAGAAGCAG	25		RIKEN BRC

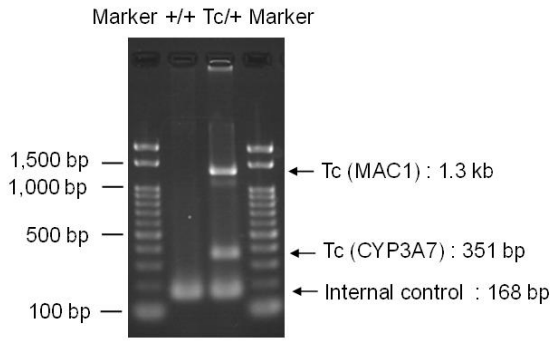
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.6			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	1.5			
primer 2	10 pmol/μl	0.5			
primer 3	10 pmol/μl	1.0			
primer 4	10 pmol/μl	0.5			
primer 5	10 pmol/μl	0.5			
DNA		1.0			
Taq		0.4			
	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
Gene Ladder 100 (Wako, Cat. 316 -06951)

Gel
2% agarose in 1 X TAE (100 V, 30 min)

PCR products	primer_set	product_size	product
primer 1	primer 2	168 bp	Internal control
primer 1	primer 3	1.3 kb	Tc(MAC1)
primer 4	primer 5	351 bp	Tc(CYP3A7)

Comment

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