PCR protocol

Sheet ID PS_05207
Release date : 5/9/2022
Print date : 5/9/2022

RBRC 05802 **Strain name** B6.Cg-Tbc1d25<tm1Mitf>

Primer	primer_name	primer_seq	length		Reference
1	OATL1-genome-fwd1	ACTCTTTACCTTACCCACCCTTTC	24		Depositor's primer
2	OATL1-genome-rvs2	CATGGAAACACACTCTCCTACAAC	24		Depositor's primer
3	NEO-rev#2	CGTTCGAACATAACTTCGTATAGC	24		Depositor's primer

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4	
H2O		2.6	2.6			(μL)
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	1.0			
primer 3	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.4	0.4			
	total	20	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

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	2,482 bp	WT
primer 3	primer 2	2,200 bp	KO

Comment Standard PCR

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