PCR protocol

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RBRC 10967 **Strain name** B6(Cg)-Sox1<em1(cre)Okn> T<em1(dre)Okn> Sox17<em1(phiC31<*>)Okn>/2-3

Primer	primer_name	primer_seq	length		Reference
1	SOX1-Fw1	CCCTCGGATCTCTGGTCAAGTCGGA	25		Depositor's primer
2	SOX1-Rv1	GCTCTCCAGAGCGGCCTTTTATCGAGA	27		Depositor's primer
3	T-Fw1	CTTTCGAGGCTCCCCTGCACATTACAC	27		Depositor's primer
4	T-Rv1	CTAAGGCAACAAGGGAGGACATTAGAGGTG	30		Depositor's primer
5	SOX17-Fw1	ACGGAATCCAACCAGCCCACTGAGC	25		Depositor's primer
6	SOX17-Rv1	AACACTGCTTCTGGCCCTCAGGTC	24		Depositor's primer

Reaction Components

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

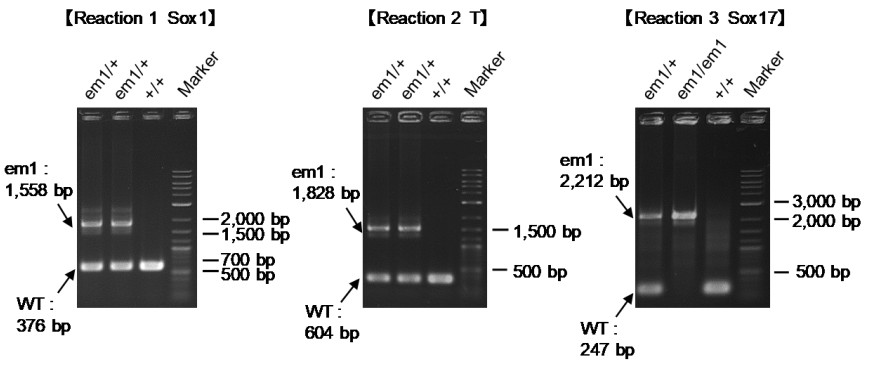
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8	2.8	2.8		(μL)
PCR buffer	2 X	10.0	10.0	10.0		
dNTP	2 mM	4.0	4.0	4.0		
primer 1	10 pmol/μl	1.0	0	0		
primer 2	10 pmol/μl	1.0	0	0		
primer 3	10 pmol/μl	0	1.0	0		
primer 4	10 pmol/μl	0	1.0	0		
primer 5	10 pmol/μl	0	0	1.0		
primer 6	10 pmol/μl	0	0	1.0		
DNA		1.0	1.0	1.0		
Taq	1 U/μl	0.2	0.2	0.2		
	total	20	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	65	30*	
4	68	180*	*Repeat for 30 cycles

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)	
primer 1	primer 2	376 bp	WT	[Reaction 1] Sox1 +/+ : 376 bp, em1/+ : 376 bp and 1,558 bp, em1/em1 : 1,558 bp
primer 1	primer 2	1,558 bp	em1	
primer 3	primer 4	604 bp	WT	[Reaction 2] T +/+ : 604 bp, em1/+ : 604 bp and 1,828 bp, em1/em1 : 1,828 bp
primer 3	primer 4	1,828 bp	em1	
primer 5	primer 6	247 bp	WT	[Reaction 3] Sox17 +/+ : 247 bp, em1/+ : 247 bp and 2,212 bp, em1/em1 : 2,212 bp
primer 5	primer 6	2,212 bp	em1	

Comment Standard PCR

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