PCR protocol

Sheet ID PS_08211
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RBRC 11014 **Strain name** B6D2-Tmprss12<em4Osb>

Primer	primer_name	primer_seq	length		Reference
1	exon 2	GCTGCTCACTGCACAAAAGAGG	22		Depositor's primer
2	exon 5	ACAGTGTTCTGGTGTAGACACAGC	24		RIKEN BRC
3	Tmprss12-ex3.Fw (#7689)	GATCCTCTGAAGTGGAGAGCAGTGATG	27		Biol. Reprod., 103(2):254-263 (2020).
4	Tmprss12-ex3.Rv (#7690)	GGGTTCCTACTTGTGCTTGCGCAA	24		Biol. Reprod., 103(2):254-263 (2020).
5	WT_exon 2	AGTGGAAGGGTCTCGGATTATAGG	24		RIKEN BRC
6	MT_exon 2	AGTGGAAGGGTCTCGGAGGTTC	22		RIKEN BRC
7	exon 2	CCTCTTTTGTGCAGTGAGCAGC	22		Biol. Reprod., 103(2):254-263 (2020).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8	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	1.0	0	0		
primer 4	10 pmol/μl	1.0	0	0		
primer 5	10 pmol/μl	0	1.0	0		
primer 6	10 pmol/μl	0	0	1.0		
primer 7	10 pmol/μl	0	1.0	1.0		
DNA		1.0	1.0	1.0		
Taq	1 U/μl	0.2	0.2	0.2		
	total	20	20	20		

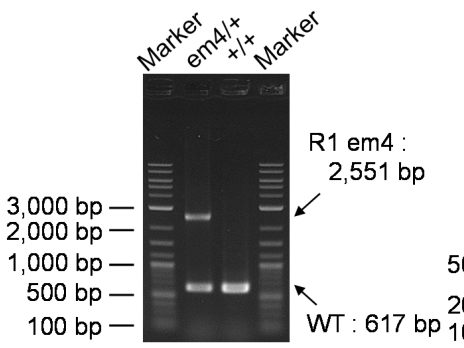
Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	[Reaction 1]
2	98	10	*
3	60	30	*
4	68	240	* *Repeat for 30 cycles
1	94	120	[Reaction 2, 3]
2	98	10	*
3	60	30	*
4	68	60	* *Repeat for 30 cycles

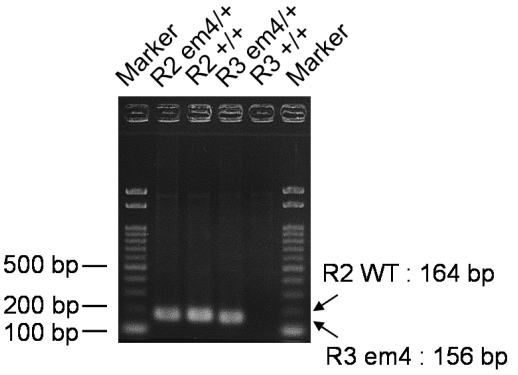
Marker 2-Log DNA Ladder (NEB, Cat. N3200)

Gel Run1:1 % agarose in 1 X TAE (100 V, 30 min)Run2,3:2 % agarose in 1 X TAE (100 V, 30 min)

【Reaction 1 】
7871 bp deletion and 5 bp insertion



【Reaction 2, 3】
8 bp deletion



primer_set		product_size	product (allele type)
primer 1	primer 2	2,551 bp	em4 (7,871 bp del + 5 bp ins)
primer 3	primer 4	617 bp	WT
primer 5	primer 7	164 bp	WT
primer 6	primer 7	156 bp	em4 (8 bp del)

Comment Standard PCR

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