PCR protocol

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RBRC 11332 **Strain name** JF1/Ms-Ngly1<em1Ta>

Primer	primer_name	primer_seq	length		Reference
1	mNgly1_SF1	AACCCAGGTAGAGATGTGAGTTTCA	25		Proc. Jpn. Acad. Ser. B Phys. Biol. Sci., 97(2):89-102 (2021).
2	mNgly1_SR1	CCTGAGTCTATGGCCTTACACTTCA	25		Proc. Jpn. Acad. Ser. B Phys. Biol. Sci., 97(2):89-102 (2021).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

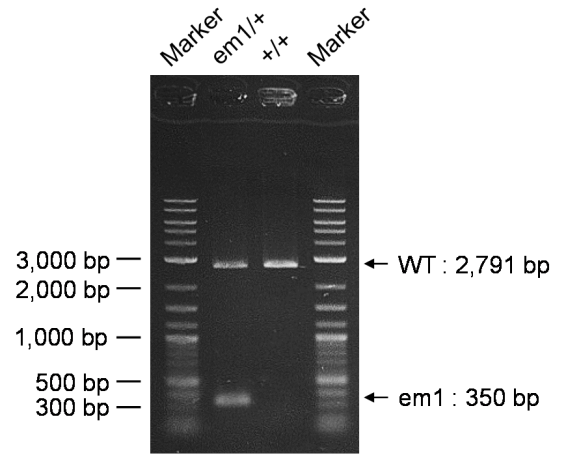
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	240	* *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	2,791 bp	WT	+/+ (wild-type): 2,791 bp em1/+ (heterozygous): 2,791 bp and 350 bp
primer 1	primer 2	350 bp	em1	

Comment Standard PCR

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