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PCR

1 1, 2 1
, ,

1. , , 266003;
2. , , 266071

: PCR ,
(*Crassostrea nippona*) 4 10 20 30
1 , (*EF1A GAPDH RO21 TUB TUA*) 3
(geNorm NormFinder BestKeeper) , , *GAPDH*
RO21 , ,
q-PCR ,

: ; PCR; ;
: S92 **:** A **:** 1005−8737−(2019)04−0657−07

, (*Crassostrea*
[1], *gigas*) , [12]
,
[2]
,
, , , [3]
, ,
[1] [11-13]
,
(osmoregulator) , PCR (quantitative real-time PCR,
(ECF) qRT-PCR) ,
[4-5], ,
, ,
, [22] , RNA
(osmoconformer)[6] ,
[7] [8] [9],
[10] [16]
, *β*-actin (ACT),
20 m [11] elongation factor-1α (EF1A), *β*-tubulin

: 2018-12-06; **:** 2019-01-17.
: (2016ZDJS06A06); (17-3-3-64-nsh).
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: , , , . E-mail: qili66@ouc.edu.cn

(TUB) [17] , , 3
, , ,
[11], 3
ε6#*bɑɻÉ" Þp“´xV…”= tù= C pÐ0C O¹ Ñ 1 !YA Lª ; W = tgCW

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4 ,
, qRT-PCR

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–80℃

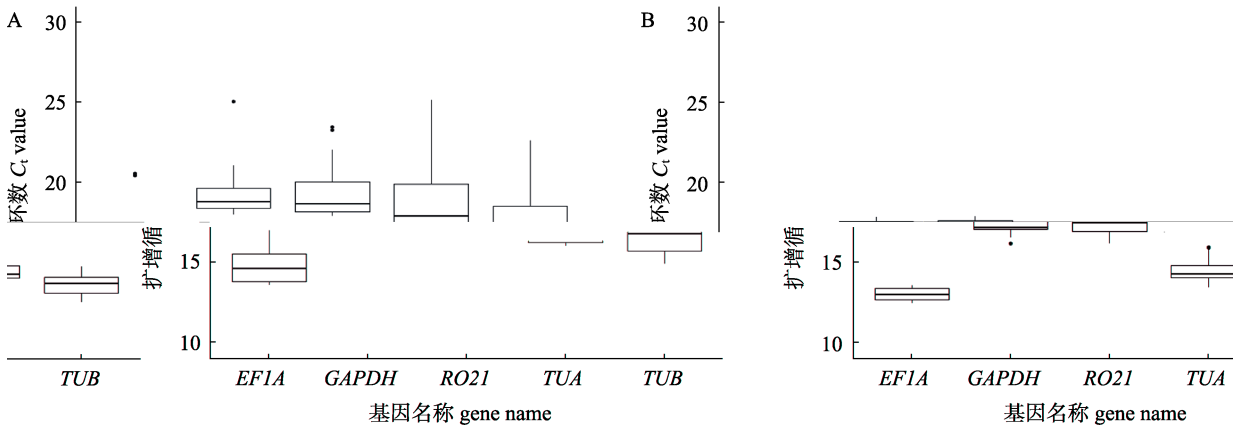
1.2

3 , 10 20
30( ), 3 ,
24 h ( )
70 L
, 30

1.5 RNA cDNA PCR , 1.5% , geNorm NormFinder , BestKeeper 5 , , geNorm NormFinder cDNA qPCR , , (C_t) , BestKeeper C_t 99.99%~104.75% (1) C_t 12.65~28.88, *GAPDH* , *TUA* , *EF1A* (C_t), *RO21* (1) C_t , ,

2

2.1 PCR

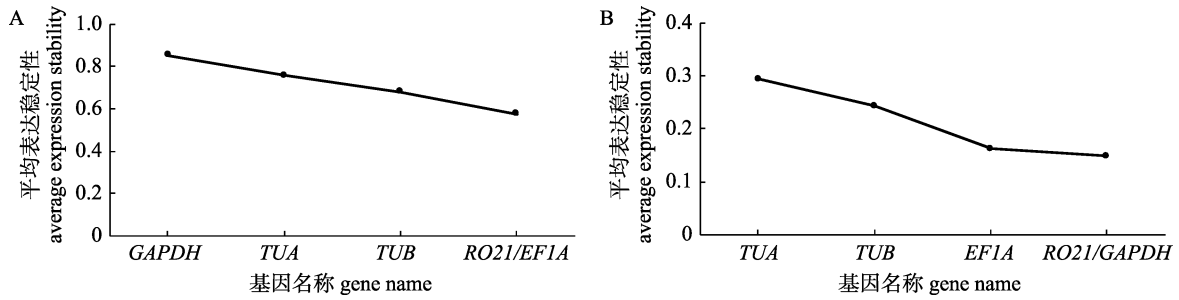


1 (A) (B)

Fig. 1 Expression levels of candidate reference genes in different tissues (A) and salinity stress (B)

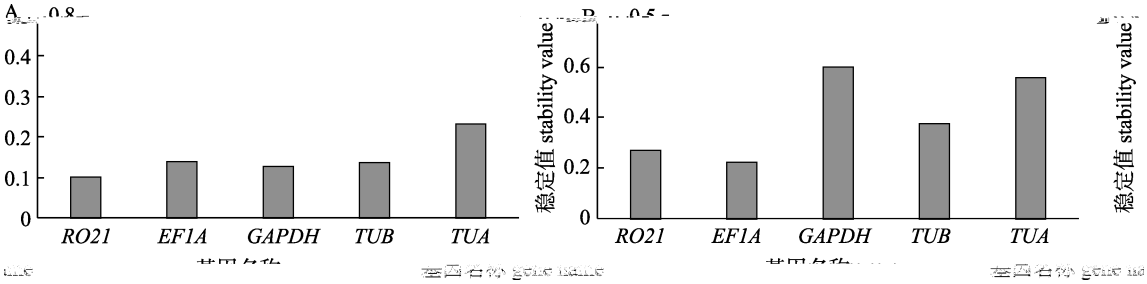
2.2

, geNorm , geNorm *M* geNorm , *M* 2 , , *EF1A* , *M* (0.22), *GAPDH* , geNorm (0.60) , *RO21* , 2 (0.10), *TUA* (0.23) (3) , *M* BestKeeper *GAPDH*>*TUA*>*TUB*>*RO21*/*EF1A*, (CV) (SD) *RO21* *EF1A* SD (0.57) , SD *M* *TUA*>*TUB*>*EF1A*>*RO21*/ , SD , geNorm *GAPDH*, NormFinder , *RO21* *GAPDH* (0.15) (2) , BestKeeper NormFinder *M* *GAPDH* , *TUA* (2)



2 geNorm (A) (B)

Fig. 2 Average expression stability values of the candidate reference genes in different tissues (A) and under salinity stress (B) analyzed by geNorm



3 NormFinder (A)

Fig. 3 Average expression stability values of the candidate reference genes in different tissues (A) and under salinity stress (B) analyzed by NormFinder

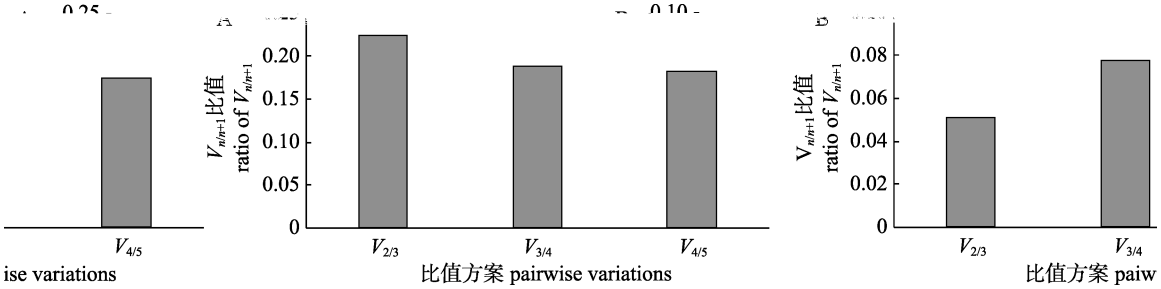
2 BestKeeper

Tab. 2 Expression stability of reference genes analyzed by BestKeeper

	parameter	gene name				
		GAPDH	*EF1A*	*RO21*	*TUB*	*TUA*
different tissues	Stu dev (±CP)	1	1.28	1.28	1.8	1.98
	CV (%CP)	5.19	8.46	6.6	10.32	10.69
different salinities	Stu dev (±CP)	0.31	0.32	0.4	0.55	0.6
	CV (%CP)	1.81	2.48	2.3	4.02	4.13

2.3

geNorm $V_{n/n+1}$ $V_{n/n+1}$<0.15 , *n*



4 geNorm (A) (B)

Fig. 4 The number of reference genes calculated by geNorm in different tissues (A) and under salinity stress (B) analyzed by geNorm

, , , 1~2 (4)

2.4

3 , [20] , : *EF1A*>*RO21*> *GAPDH*>*TUB*>*TUA*; , *GAPDH*>*RO21*>*EF1A*>*TUB*>*TUA* (3)

3 geNorm NormFinder BestKeeper 3

Tab. 3 Ranking of reference genes by geNorm, NormFinder, BestKeeper and overall rank

	rank	geNorm	NormFinder	BestKeeper	overall
different tissues	1	*RO21*/*EF1A*	*EF1A*	*GAPDH*	*EF1A*
	2		*RO21*	*EF1A*	*RO21*
	3	*TUB*	*TUB*	*RO21*	*GAPDH*
	4	*TUA*	*TUA*	*TUB*	*TUB*
	5	*GAPDH*	*GAPDH*	*TUA*	*TUA*
different salinities	1	*RO21*/*GAPDH*	*RO21*	*GAPDH*	*GAPDH*
	2		*GAPDH*	*EF1A*	*RO21*
	3	*EF1A*	*TUB*	*RO21*	*EF1A*
	4	*TUB*	*EF1A*	*TUB*	*TUB*
	5	*TUA*	*TUA*	*TUA*	*TUA*

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Evaluation of potential reference genes for quantitative RT-PCR analysis in Iwagaki oyster (*Crassostrea nippona*) under normal and low salinity stress conditions

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Abstract: *Crassostrea nippona* is a commercially important oyster species in East Asia as it is edible during the summer when the other oyster species are unavailable. Salinity is one of the important limiting factors to the survival and distribution of this stenohaline species. The molecular mechanism behind the response of this species to hypo-salinity stress remains unclear. Quantitative Real-Time PCR (qRT-PCR) has been widely used for the analysis of gene expression. The optimal reference gene is constantly transcribed in different types of cells, tissues, and species and under various experimental conditions. However, reference genes that meet all of these conditions are almost non-existent. The selection of a proper reference gene is a precondition for accurate analysis of the expression level of a target gene in quantitative real-time PCR. A total of five candidate reference genes, elongation factor 1α (EF1A), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), heterogeneous nuclear ribonucleoprotein A2/B1 (RO21), *β*-tubulin (TUB), and α-tubulin (TUA), were analyzed by qRT-PCR in four tissues (gill, mantle, visceral mass, and adductor muscle) under three salinity conditions of 10, 20, and 30 psu for one week. Three algorithms, geNorm, NormFinder, and BestKeeper, were used to evaluate the expression stability of the candidate reference genes. The results showed that EF1A was most stable in the different tissues under normal conditions. Under salinity stress, GAPDH was the most stable gene according to overall ranking. In contrast, TUB and TUA were the least stable genes and were not suitable as reference genes. This study showed that different algorithms may generate inconsistent results. Therefore, a combination of several reference genes should be selected to accurately calibrate system errors, especially for studies of different tissues in which candidate reference genes have more unstable expression. The present study was the first to select *C. nippona* reference genes by qRT-PCR and to provide a useful basis for selecting appropriate *C. nippona* reference genes. The present study also has important implications for gene expression and functional genomics research related to salinity stress in this species or other bivalve species.

Key words: *Crassostrea nippona*; quantitative real-time PCR; reference gene; low salinity stress

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