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Identification and characterization of microRNAs from the tube foot in the sea urchin *Strongylocentrotus intermedius*

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Abstract

MicroRNAs (miRNAs) play critical roles in regulating many bio-processes of eukaryotes. The sea urchin *Strongylocentrotus intermedius* (an important fishery resource) is of great economic importance in Japan, North Korea, Russia, and China. In the current study, miRNAs of tube foot in *S. intermedius* were firstly identified and characterized. Data in this study can provide more genomic information for the further understanding of the complex regulation network in sea urchins and present a new way for monitoring the health status of cultured sea urchins.

Keyword: Genetics

1. Introduction

MicroRNAs (miRNAs) are short endogenous non-coding RNAs, with lengths of about 20−25 nucleotides (nt) (Chen et al., 2016). It has been well documented that miRNAs play vital roles in many physiological and biochemical processes of

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eukaryotes (Wei et al., 2014). MiRNAs are also involved in host immune and stress response in eukaryotes, via regulating the expression of their target genes post-transcriptionally (Achkar et al., 2016). As for marine organisms, miRNAs have been identified from many species such as fish (Chen et al., 2017), crustaceans (Zhou et al., 2015), echinoderms (Wang et al., 2014; Mi et al., 2014), shellfish (Picone et al., 2016), and cephalochordates (Liao et al., 2017).

The sea urchin *Strongylocentrotus intermedius* is naturally distributed in northern regions of the Pacific coastal waters, the Sea of Japan, and Korean waters (Lawrence, 2013). In 1989, *S. intermedius* was transplanted from Japan waters by Dalian Ocean University, and it has become one of the most important cultured sea urchin species to date. In China, it is widely cultivated along the coastal areas of Liaoning and Shandong Provinces (Chang et al., 2012). According to China fishery Statistical Yearbook (2015), the annual aquaculture output of the sea urchin *S. intermedius* was 6.79 kilotons in 2014, as a result of the large demand for its gonad which is a highly valuable domestic and export product (Ministry of Agriculture of the People's Republic of China, 2015). However, with the expansion of *S. intermedius* aquaculture and the continuous deterioration of its culture environment, it has been prominent to increase the disease resistance of *S. intermedius* in recent years (Wang et al., 2013).

Tube foot is an important organ for sea urchins. It functions in sensory, movement, attachment, and responding to environmental changes (Kabat-Zinn and Singer, 1981). As tube feet can be sampled non-destructively *in vivo*, the health status of sea urchins can be monitored at any time by tube feet sampling. To date, many genes and proteins of tube feet related to the functions mentioned above have been identified and characterized. However, the information of miRNAs of tube feet in sea urchins is still lacking.

In this study, miRNAs of tube feet in *S. intermedius* were identified and characterized by next-generation high-throughput sequencing techniques. Data observed here can increase our knowledge of tube foot miRNAs in sea urchins. It can also provide information for the further understanding of the complex regulation network in sea urchins when coping with different conditions.

2. Materials and methods

2.1. Sea urchin tube feet sampling

Fifteen healthy *S. intermedius* (average test diameter = 27.89 ± 1.14 mm) provided by the Key Laboratory of Mariculture & Stock Enhancement in North China's Sea were randomly grouped into three groups (five each group) as replicates (SI1, SI2,

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SI3) in this study. Tube feet collected from each individual within each group were pooled.

2.2. RNA extraction, RNA library construction, and sequencing

Total RNA extraction, small RNA library construction, Illumina sequencing, and transcriptome assembly were performed for each replicated pool as described by Zhong et al. (2015). An equal mixture of the tube foot RNA extracted from five individuals within each replicate was used to build the small RNA library. Raw reads were processed for the evaluation of sequencing quality, the removing of low quality reads and adaptor sequences, and the calculation of the length distribution of small RNA reads (Xu et al., 2015).

2.3. MiRNA identification

The remaining clean reads were aligned against known pre-miRNAs in miRbase 21.0 (http://www.mirbase.org/) to identify the conserved miRNAs. Only those small RNAs with their mature and precursor sequences perfectly matched to the known sea urchin miRNAs were considered to be conserved miRNAs. Two software, miREvo (Wen et al., 2012) and miRDeep2 (Friedlander et al., 2011), were used to predict novel miRNAs through exploring the secondary structure, the Dicer cleavage site, and the minimum free energy of the small RNA tags that were not annotated in previous steps. At the same time, custom scripts were applied to obtain the identified miRNA counts. Base bias with certain length on the first position and on each position of all the identified miRNAs were also obtained in this step.

3. Results

3.1. Data description

As shown in the results, an average of 8911741.67 raw reads (raw data) were obtained after sequencing on an Illumina Hiseq 2500 platform (Novogene Bioinformatics Technology Co., Ltd., Beijing, China), and an average of 7083236.67 clean reads (clean data) were then filtered from the raw data (Table 1). An average of 518539.67 unique sequences were observed from clean data, as candidates for miRNA analysis. All unique sequences were aligned against the known miRNAs in miRbase (v21.0, http://www.mirbase.org/) by BLAST (Basic Local Alignment Search Tool).

3.2. Data deposition

All the sequencing clean reads were deposited in the Short Read Archive (SRA) database (http://www.ncbi.nlm.nih.gov/sra/), which are retrievable under the

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Table 1. Summary of the miRNA transcriptome sequencing of the tube foot in *S. intermedius*. SI1, SI2 and SI3 are replicates.

Library	Count of reads			Mean	% of total			Mean
	SI1	SI2	SI3		SI1	SI2	SI3	
Raw reads	9476098.00	8140985.00	9118142.00	**8911741.67**	100	100	100	**100**
N% > 10%	1.00	39.00	33.00	**24.33**	0.00	0.00	0.00	**0.00**
low quality	44565.00	27825.00	35480.00	**35956.67**	0.47	0.34	0.39	**0.40**
5_adapter_contamine	7469.00	5007.00	1526.00	**4667.33**	0.08	0.06	0.02	**0.05**
3_adapter_null or insert_null	2271046.00	1121001.00	1959048.00	**1783698.33**	23.97	13.77	21.49	**19.74**
with ployA/T/G/C	2898.00	5484.00	4093.00	**4158.33**	0.03	0.07	0.04	**0.05**
known_miRNA	1950339.00	1498864.00	2238924.00	**1896042.33**	0.21	0.18	0.25	**0.21**
rRNA	4112.00	5264.00	3322.00	**4232.67**	0	0.00	0.00	**0.00**
tRNA	0.00	1.00	1.00	**0.67**	0.00	0.00	0.00	**0.00**
snRNA	104.00	111.00	98.00	**104.33**	0.00	0.00	0.00	**0.00**
snoRNA	817.00	577.00	504.00	**632.67**	0.00	0.00	0.00	**0.00**
novel_miRNA	116695.00	58033.00	42348.00	**72358.67**	0.01	0.01	0.00	**0.01**
other	1633190.00	2532754.00	2459310.00	**2208418.00**	0.17	0.31	0.27	**0.25**
clean reads	7150119.00	6981629.00	7117962.00	**7083236.67**	75.45	85.06	78.06	**79.52**
18nt	22825.00	21287.00	33469.00	**25860.33**	0.00	0.00	0.00	**0.00**
19nt	52200.00	65623.00	114338.00	**77387.00**	0.01	0.01	0.01	**0.01**
20nt	189334.00	206819.00	333449.00	**243200.67**	0.02	0.03	0.04	**0.03**

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Table 1. (*Continued*)

Library	Count of reads			Mean	% of total			Mean
	SI1	SI2	SI3		SI1	SI2	SI3	
21nt	572291.00	521369.00	751698.00	**615119.33**	0.06	0.06	0.08	**0.07**
22nt	3209445.00	2325028.00	2315233.00	**2616568.67**	0.34	0.29	0.25	**0.29**
23nt	1471068.00	947966.00	846541.00	**1088525.00**	0.16	0.12	0.09	**0.12**
24nt	170896.00	119441.00	114960.00	**135099.00**	0.02	0.01	0.01	**0.02**
25nt	82657.00	86359.00	66465.00	**78493.67**	0.01	0.01	0.01	**0.01**
26nt	89247.00	151105.00	112963.00	**117771.67**	0.01	0.02	0.01	**0.01**
27nt	151490.00	242906.00	191590.00	**195328.67**	0.02	0.03	0.02	**0.02**
28nt	528928.00	1301596.00	1283661.00	**1038061.67**	0.06	0.16	0.14	**0.12**
29nt	314882.00	548439.00	530376.00	**464565.67**	0.03	0.07	0.06	**0.05**
30nt	108349.00	171554.00	168642.00	**149515.00**	0.01	0.02	0.02	**0.02**
31nt	45733.00	56481.00	43980.00	**48731.33**	0.00	0.01	0.00	**0.01**
32nt	24132.00	35199.00	24415.00	**27915.33**	0.00	0.00	0.00	**0.00**
33nt	13544.00	24251.00	17755.00	**18516.67**	0.00	0.00	0.00	**0.00**
34nt	10175.00	18322.00	13640.00	**14045.67**	0.00	0.00	0.00	**0.00**
35nt	6635.00	13880.00	10368.00	**10294.33**	0.00	0.00	0.00	**0.00**

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accession number [SRR6251260, SRR6251258, and SRR6251259] in the SRA database of NCBI.

4. Discussion

As shown in the results, miRNAs with a length of 22nt had the highest percentage of all identified miRNAs in the three replicates (Table 1). This is consistent with a previous study showing that miRNAs with a length of 22nt had the highest percentage in *Andrias davidianus* (Huang et al., 2017a,b). In order to search for the miRNAs expressed in all three replicates, miRNA expression levels of each pooled sample were estimated by TPM (transcript per million) through the criteria of Zhou et al. (2010). A total of forty-one known miRNAs and twenty novel miRNAs (TPM $>$ 0) were identified from the three replicates (Table 2 and Table 3). The three most abundantly expressed known miRNAs were spu-miR-184, spu-miR-7, and spu-miR-1. Wang et al. found that miR-184 and miR-1 were two of the most expressed known miRNAs in the tube foot of healthy sea cucumber *Apostichopus japonicas* (Wang et al., 2014). Taken both results together, we hypothesis that the expression trends of miR-184 and miR-1 were consistent in echinoderms. GO (Gene Ontology) analysis (http://www.geneontology.org/) showed that the identified miRNAs might regulate multiple genes involved in cellular components, molecular functions, and several bio-processes such as metabolic process, response to stimulus, and catalytic activity (Figs. 1-b and 2-b). This result could facilitate further studies on the specific roles played by miRNAs in sea urchins. Moreover, it is worthwhile to note that the number of conserved miRNAs in tube feet of the sea urchin *S. intermedius* was less than that in tube feet of the sea cucumber *Apostichopus japonicas*, while the number of novel miRNAs in tube feet of *S. intermedius* was more than that in tube feet of *A. japonicas* (Wang et al., 2014). This observation indicates that miRNA expression profiles might vary among species. Many studies have documented that uracil (U) is the most common base as the first nucleotide located at 5′ end of miRNA (Greagg et al., 1999). A similar result was observed in the current study. An average of 78.56% of known miRNAs and an average of 84.23% of novel miRNAs had a relatively higher percentage of U at the first position in the tube foot of *S. intermedius* (Figs. 1-a and 2-a). The "seed region" (defined as the 2^{nd} to the 8^{th} nucleotides of miRNAs) has been demonstrated to be responsible for targeting mRNAs for gene regulation (Huang et al., 2017a,b). The strong bias of U at the 1^{st} and 9^{th} nucleotides might regulate miRNA-mRNA interaction through flanking the edges of the "seed region" (Zhang et al., 2009). Compared to the results from Mi et al. that 58.3% of known miRNAs from gonads of *Strongylocentrotus nudus* tend to use U as the first base (Mi et al., 2014), conserved miRNAs in the tube foot of *S. intermedius* exhibited a relatively stronger bias of U at the first position. Therefore, we postulate that there are species-specific and tissue-specific variabilities in conserved miRNAs

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Table 2. Known miRNAs identification from the tube foot of *S. intermedius.*

Known-miRNA	Sequences (5′-3′)	Length	Mean readcount	Mean TPM
spu-miR-92d	UAUUGCACUUACCCCGGCUG	20	122.67	65.22
spu-miR-124	UAAGGCACGCGGUGAAUGCCA	21	10.00	5.28
spu-miR-96	UUUGGCACUAGCACAUUUUGC	21	21.67	11.59
spu-miR-2013	UGCAGCAUGAUGUAGUGGUGU	21	106.00	55.23
spu-miR-92e	UAUUGCACUUACCCCGGCUUA	21	161.33	82.26
spu-miR-31	AGGCAAGAUGUUGGCAUAGCU	21	18875.67	9951.81
spu-miR-2010	UUACUGUUGAUGUCAGCCCCUU	22	2.67	1.28
spu-miR-252b	CUAAGUAGUAGUGCCGCAGGUA	22	2.67	1.27
spu-miR-183	UAUGGCACUAUAGAAUUCACUG	22	3.00	1.42
spu-miR-210	UUGUGCGUGCGACAGCGACUGA	22	5.33	2.78
spu-miR-137	UAUUGCUUGAGAAUACACGUAG	22	11.33	6.22
spu-miR-92b-3p	UAUUGCACUUGUCCCGGCCUGC	22	17.33	9.21
spu-miR-2001	AUGUGACCGAUAUAAUGGGCAU	22	38.33	20.92
spu-miR-278-5p	UGGAAUGAAAGCCUCGCCAAUC	22	55.33	28.64
spu-miR-9-3p	AUAAAGCUAGGUUACCAAAGAU	22	60.00	31.03
spu-miR-278-3p	UCGGUGGGACUUUCGUUCGAUU	22	61.00	30.88
spu-miR-4852	AAUUCUAUCAUUUUGGCUGCAU	22	78.67	41.27
spu-miR-2011	ACCAAGGUGUGCUAGUGAUGAC	22	500.00	248.08
spu-miR-125-3p	ACAGGUUGGUAUCUCAGGAAUU	22	631.00	325.71
spu-miR-9-5p	UCUUUGGUUAUCUAGCUGUAUG	22	802.00	410.52
spu-miR-153-3p	UUGCAUAGUCACAAAAGUGAUU	22	1706.33	906.22
spu-miR-200-5p	CAUCAUACUGGACAGCAUUGGA	22	2654.67	1362.21
spu-miR-4847	UAAUGAUGGCGCGGUGCGGUGC	22	3506.00	1902.39
spu-let-7	UGAGGUAGUAGGUUAUAUAGUU	22	6961.00	3571.52
spu-miR-375	UUGUUCGUUCGGCUCGCGUCAA	22	10442.00	5430.47
spu-miR-2004	UCACACACAACCACAGGAAGUU	22	12090.67	6153.14
spu-miR-29a	AAGCACCAGUUGAAAUCAGAGC	22	13320.33	7146.20
spu-miR-2012	UAGUACUGGCAUAUGGACAUUG	22	20244.33	10439.92
spu-miR-125-5p	UCCCUGAGACCCUAACUUGUGA	22	23805.00	12701.23
spu-miR-34	CGGCAGUGUAGUUAGCUGGUUG	22	29303.33	15381.47
spu-miR-1	UGGAAUGUAAAGAAGUAUGUAU	22	266969.00	135440.97
spu-miR-184	UGGACGGAGAACUGAUAAGGGC	22	947961.00	479823.98
spu-miR-2003-3p	CAGGUUAUGCCCUUUGGGUAGUA	23	1.00	0.52
spu-miR-92a	UAUUGCACUUGUCCCGGCCUACU	23	17.33	9.21
spu-miR-10	AACCCUGUAGAUCCGAAUUUGUG	23	70.00	39.08
spu-miR-2007	UAUUUCAGGCAGUAUACUGGUAA	23	249.33	131.06
spu-miR-71	UGAAAGACAUGGGUAGUGAGAUU	23	2640.00	1423.26
spu-miR-2002-3p	UGAAUACAUCUGCUGGUUUUUAU	23	2793.33	1439.96

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Table 2. (*Continued*)

Known-miRNA	Sequences (5′-3′)	Length	Mean readcount	Mean TPM
spu-miR-200-3p	UAAUACUGUCUGGUGAUGAUGUU	23	43303.00	22430.70
spu-miR-7	UGGAAGACUAGUGAUUUUGUUGU	23	483537.67	245439.18
spu-miR-182	UUUGGCAAUUGAUAGAAUUCACACU	25	26.67	13.18

miRNA expression levels were estimated by TPM (transcript per million) through the Normalization formula: Normalized expression = mapped read count/Total reads*1000000.

Table 3. Novel miRNAs identification from the tube foot of *S. intermedius.*

Novel_miRNA	Sequences (5′-3′)	Length	Mean readcount	Mean TPM
novel_137	uuaauacacuuguggcucca	20	5.00	2.43
novel_98	uguaaaaauguguagaacagg	21	18.67	10.03
novel_181	aauccgguccuagaagcaaga	21	41.33	20.34
novel_50	aaauacuggcccuucuauuacc	22	2.00	1.04
novel_79	uccguuucguugacgaucagcc	22	2.33	1.12
novel_121	uuuuccgucucuuucguucguu	22	2.67	1.35
novel_147	augggggccuguaucacgacuau	22	3.00	1.42
novel_87	uuuucacaaagugacgguagug	22	3.33	1.66
novel_45	aaauuuguagcggcguuguagc	22	4.67	2.41
novel_39	auggccgucgcgcuguuggagu	22	6.67	3.80
novel_194	ucgacaucucuucaaacgcgug	22	11.67	6.67
novel_70	uugacuaucccauugaaacgug	22	13.33	6.91
novel_134	uggugucugucugcaugcuacu	22	28.67	15.78
novel_20	uuucacacuggucuagacaagg	22	84.67	44.86
novel_202	cugauugucaacgaaacggagu	22	127.00	63.95
novel_7	ugagguaguagguuguauaguu	22	36356.00	18847.28
novel_118	uuuguucguucggcucgcgucau	23	327.33	169.86
novel_8	uaaugcugucuggugaugauguu	23	35236.00	18243.18
novel_163	acaauggucguuggcaguguaccu	24	6.33	3.16
novel_113	aaggacaucagggugcaacugcca	24	9.67	5.54

miRNA expression levels were estimated by TPM (transcript per million) through the Normalization formula: Normalized expression = mapped read count/Total reads*1000000.

when regulating target mRNAs in echinoderms. We also found that novel miRNAs with lengths of 24nt and 26nt exhibited a bias about 40.15%−50.00% of adenine (A) at the first position, which may need further research to study and clarify.

In conclusion, an overview of miRNAs in the tube foot of sea urchin *S. intermedius is provided* in this preliminary study. Observations in this study increase the knowledge of non-coding RNAs in sea urchins and provide a new way for monitoring the health status of cultured sea urchins.

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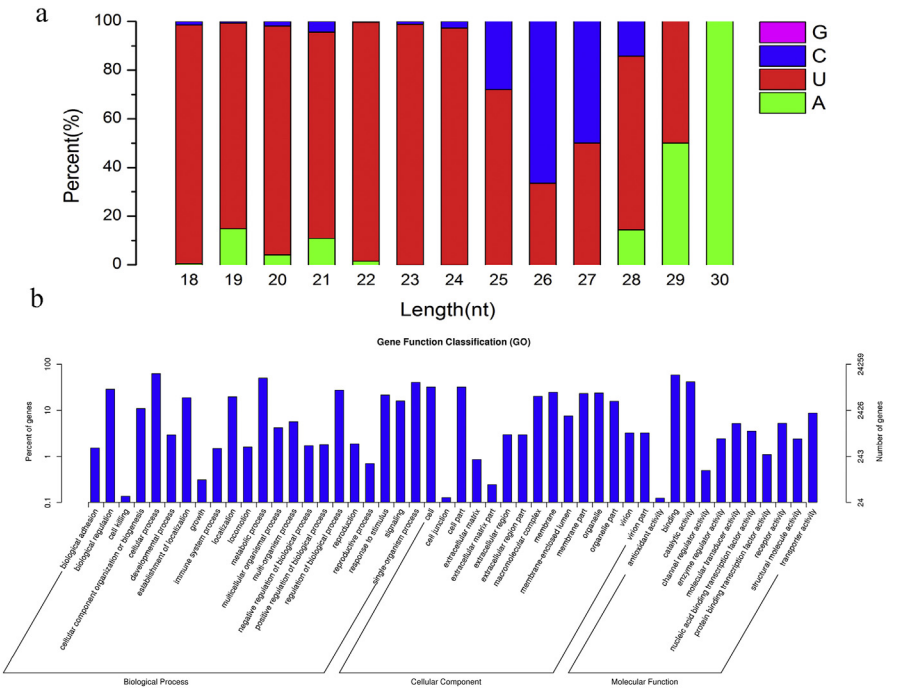

Fig. 1. First position nucleotide percentage and GO terms for predicted target genes of known miRNAs analyses of the tube foot in *S. intermedius*. a. Analysis of the nucleotide percentage at the first position of known miRNAs. b. GO terms for predicted target genes of known miRNAs of the tube foot in *S. intermedius*.

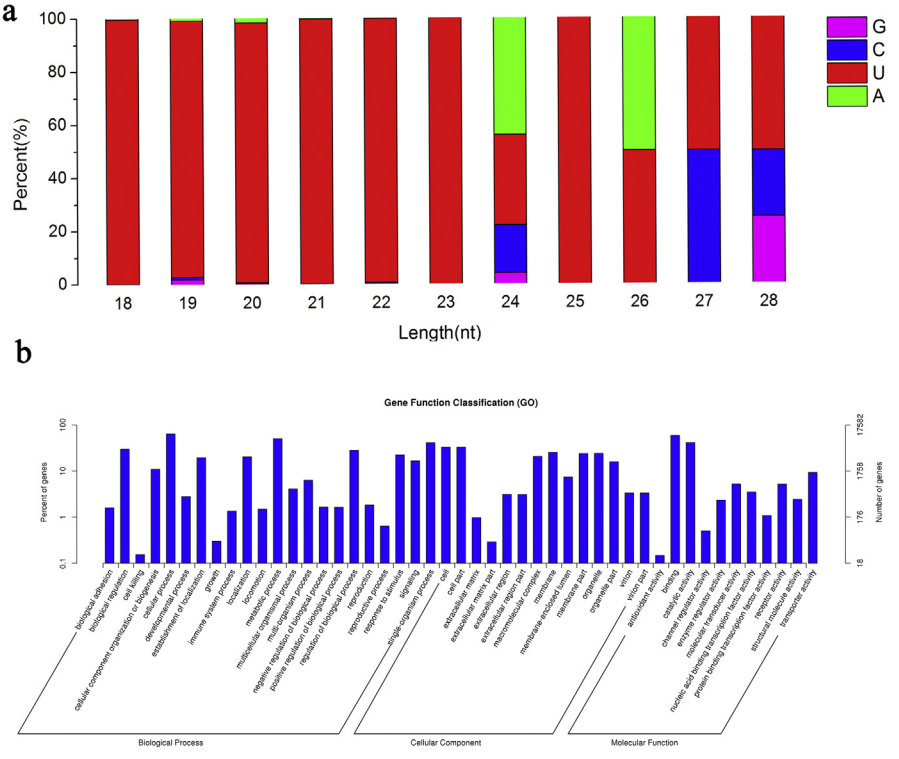

Fig. 2. First position nucleotide percentage and GO terms for predicted target genes of novel miRNAs analyses of the tube foot in *S. intermedius*. a. Analysis of the nucleotide percentage at the first position of novel miRNAs. b. GO terms for predicted target genes of novel miRNAs of the tube foot in *S. intermedius*.

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Declarations

Author contribution statement

Yaoyao Zhan: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yingying Li: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dongyao Cui, Jingxian Sun: Performed the experiments.

Qiantong Pei: Analyzed and interpreted the data; Wrote the paper.

Weijie Zhang: Analyzed and interpreted the data.

Yaqing Chang: Conceived and designed the experiments.

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Competing interest statement

All authors have no conflict of interest.

Additional information

Data associated with this study (all the sequencing clean reads) has been deposited at the Short Read Archive (SRA) database (http://www.ncbi.nlm.nih.gov/sra/) under the accession numbers SRR6251260 (SI1), SRR6251258 (SI2), SRR6251259 (SI3).

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