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Full Length Article

Whole Genomic SNPs and SSRs Development based on High-Throughput Transcript Sequencing in Sea Cucumber (*Apostichopus japonicus*)

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Abstract

As the efficient genomic resources, molecular markers (single nucleotide polymorphism (SNPs) and simple sequence repeats (SSRs)) have become the significant molecular marker of choice for breeding programs and population genetic studies in many species. RNA-Seq has been approved as a cost-effective and efficient way for molecular marker identification and validation. In this study, a large scale of polymorphic functional markers on papillae and skin transcriptome of sea cucumber (*Apostichopus japonicas*) were obtained by high-throughput sequencing. A total of 166,572 and 171,480 putative SNPs were identified in papillae and skin transcriptome, respectively. These putative SNPs included transitions (papillae/skin, 95,713/98,905) and transversions (papillae/skin, 70,859/72,575). Additionally, 12,201 and 12,149 SSRs were discovered from the papillae and skin transcriptome, respectively. Among SSRs, tri-nucleotide repeats were almost equally in UTRs compared to CDSs and non-CDSs, while di-nucleotide repeats were twice more abundant spread over these two regions. And the distribution rate of tri-nucleotide which around CDS were almost five-fold more abundant than that of di-nucleotide. We also analyzed the *A. japonicus* genes that contained molecular markers from both tissues transcriptome by Gene ontology (GO). We also found many growth-related genes contained a lot of SNPs and SSRs. These useful molecular markers can be used for genetics, breeding and genetic diversity analysis in the sea cucumber

Keywords: Sea cucumber (*Apostichopus japonicus*); Papillae; Skin; SNPs; SSRs

Introduction

Sea cucumber (*Apostichopus japonicas*), as a crucial aquaculture species, is intensively cultured in China (Chang *et al*., 2009). Although traditional breeding of *A. japonicus* has been improved for inducing polyploidy, enhancing the rates of metamorphosis and immune activity of *A. japonicus* in the recent years (Ding *et al*., 2007; Ma, 2012), the long breeding cycle and heavy workload make it cannot meet the production needs. Comparison with the traditional breeding technique, molecular marker-assisted selection is a more attractive approach to improve the desirable trait of *A. japonicus*. Marker-assisted selection (MAS) is an indirect approach where a trait of interest is selected based on a molecular marker linked to a desirable trait.

Molecular markers, SNPs and SSRs are essential for organism improvement and better utilization in genetic breeding. Two SNP markers were identified from myostatin genes of *Argopecten irradians* and they were associated with the growth traits of *A. irradians* (Meng *et al.,* 2017). Meanwhile, two polymorphisms of the MSTN gene were detected from *Takifugu rubripes* by polymerase chain reaction-single strand conformation polymorphism and these two SNP markers were also associated with the growth traits (Wang *et al*., 2014). Recently, with the development of high throughout deep sequencing technology, RNA-Seq has been approved as a cost effective and efficient way for molecular markers identification and validation, including SNP and SSR in several aquatic species. Cui *et al*. (2014) found a large number of SNPs from *T. rubripes* by RNA-Seq. Similarly, transcriptome analysis was also used for small and large size of crab (*Portunus trituberculatus*) and nineteen differentially expressed genes were identified and applied to detect growth-related SNP. And a growth associated SNP that was located in hemocyanin by association analysis in *P. trituberculatus* (Lv *et al*., 2015).

SSRs are useful as molecular markers for genetics and biology researches. SSRs are also play a significant role in genetics and biology researches, which owing to its ubiquitous and multiallelic occurrence in genomes, high reproducibility of DNA sequences. Kang *et al*. (2011a) suggested that nine SSRs can be applied to distinguish the color types of *S. japonicus*, including green and red types. In

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addition, Qiu *et al.* (2013) amplified thirteen high polymorphic microsatellites from shrimp using eighteen pairs of primers to analyze the genetic diversity.

Sea cucumber, as a crucial aquaculture species, is intensively cultured in China. As far as we know, some SNPs and SSRs makers that involvement with various traits has been recognized from body color-related gene, *HSP70* (Kang *et al*., 2011b), heat-tolerance-related gene, *HSP90* (Xu *et al*., 2014) and growth-related gene *Aj-MSTN* (Li *et al*., 2016). Other transcriptome researches were also administrated with the focus on investigate large scale of molecular markers (Guo *et al*., 2013; Chen and Storey, 2014). Echinodermata belong to deuterostome, which is the most advanced group of invertebrates. Due to the important position of *A. japonicus* in aquaculture, the development of molecular markers can promote the implementation of genetic analysis and breeding programs for *A. japonicus*.

Although some molecular markers have been exploitation for *A. japonicus*, more SNP mutant and SSRs loci are still lacked to promote the implementation of genetic analysis and breeding programs. Previously, we has released a large amount of transcriptome data by Illumina 2500 sequencing of the *A. japonicus* (Zhou *et al*., 2016a). By mining our transcriptome data, a total of 166,572 and 171,480 explored SNPs was identified from papillae and skin transcriptome, respectively. The whole set of generated unigenes contained SNPs from both two tissues were used to classify gene product function by GO analysis. Moreover, of more than 12,000 SSRs, almost 3,700 were omitted perfect primers from the two tissues, respectively. As conclude, the developed SSRs in the present research showed highly availability. And the high mutagenicity of SSRs makes it play an important role in genome evolution. Compare with marine animals, comparative analysis of available genomic SSRs in various taxonomic groups usually focuses on land species. The present research has provided biological processes, molecular markers and key genes that related to growth, which will enhance the efficiency of selective breeding programs for *A. japonicus*.

Materials and Methods

Data Resource

To identify a large scale of molecular genetic markers, we used our data resources (Zhou *et al*., 2016a). Briefly, the sample of papillae and skin of 45 sea cucumbers were collected and placed into RNA later® Solution (Ambion) until RNA isolation. Then total RNA was extracted from papillae and skin by using the TRIzol Reagent (Invitrogen, C.A., U.S.A.). High quality RNA was used for the construction of cDNA library. Paired-end sequencing was conducted on an Illumina HiSeq 2500 generating 125bp reads. These data resources were submitted to the NCBI, the accession numbers were SRX1097860 and SRX1081978.

SNP Identification

The unigenes were used as references to detect SNPs with samtools and GATK (https://software.broadinstitute.org/gatk/). BWA was used to align all clean reads to the assembly of *A. japonicus*. Then, the mapped reads were sorted, and PCR duplicates were marked in GATK. Variants were then identified in GATK with emitting and calling standard confidence thresholds at 10.0 and 30.0, respectively. The SNPs location in non-synonymous SNPs (nsSNPs) and synonymous SNPs (sSNPs) were analyzed by Perl script, then transitions and transversions were identified.

SSR Identification

A perl script MISA (http://pgrc.ipk-gatersleben.de/misa/misa.html) was used to scan the tandem repeats to identify SSRs based on RNA-Seq data from *A. japonicus* papillae and skin, respectively. SSR analysis was performed by screening the unigenes larger than 1kbp in length. The parameters are as described as before (Zhou *et al*., 2016b): the minimum repeat number for penta-, tetre- and tri-nucleotides, six for di-nucleotide and ten for mono-nucleotide was five repeats, respectively. Only SSRs with flanking sequences longer than 50 bp on both sides were considered for subsequent validation.

GO Analysis

To understand the molecular function of the unigenes containing SNPs, GO classification was conducted. First, the unigenes containing SNPs were annotated to assembly data and obtained the GO number of every unigenes. Then, the GO annotation was categorized with respect to molecular function (MF), biological process (BP) and cellular component (CC) at level 2 by WEGO (http://wego.genomics.org.cn/cgi-bin/wego/index.pl) as described in the previous study (Zhou *et al*., 2016a).

Results

SNP Marker Detection

A total of 166,572 putative SNPs, including 95,713 transitions (Ts) and 70,859 transversions (Tv) were identified from RNA-Seq data of *A. japonicus* papillae, and these SNPs were obtained from 29,469 unigenes. As shown in Fig. 1A, the Ts of A/G (47,892) and C/T (47,821) accounted for 28.75% and 28.71%, respectively, and each Tv in A/C (17,399), A/T (25,445), C/G (10,437) and G/T (17,578) accounted for 6.27%~15.28% of all SNP types.

We also identified 171,480 putative SNPs over 12,899 unigenes from the transcriptome data of *A. japonicus* skin. Among these SNPs, 90,905 were Ts and 72,575 were Tv. The A/G (49,479) and C/T (49,426) were the most common

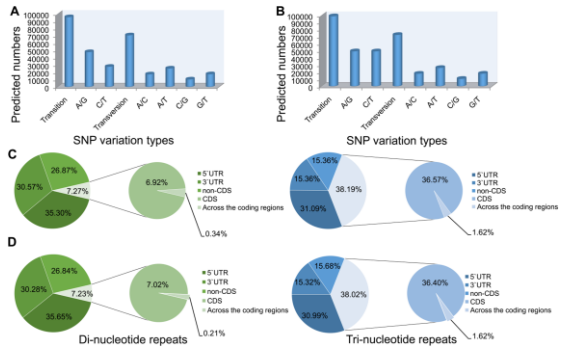

Fig. 1: The preliminary statistics of scanned molecular markers from papilla (**A**, **C**) and skin (**B**, **D**) transcriptome of *A. japonicus*

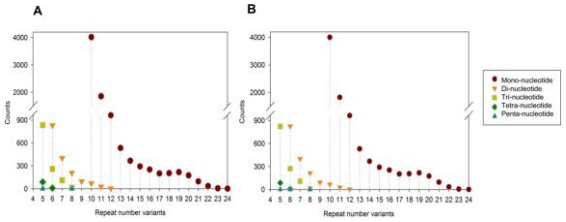

Fig. 2: Repeat number variants distribution for detected polymorphic SSRs. Papilla transcriptome (**A**), Skin transcriptome (**B**)

SNPs types. By contrast, A/C (17,878), A/T (26,046), C/G (10,670) and G/T (17,981) were the least common types (Fig. 1B).

Mining for SSR Markers

SSR markers have been widely used in genetic breeding and biological research caused by its stability and extensive distribution along genomes and transcriptomes. A total of 12,201 SSRs were detected in 7,427 unigenes that derived from the papillae transcriptome data. Similarly, 12,149 SSRs were obtained from 7,411 unigenes by analyzing the skin transcriptome data. Among all unigenes from the RNA-Seq data, more than 51% unigenes contained SSR makers. As shown in Fig. 2, the low motif has main proportion in the present study. According to statistics, the most abundant motif type in our study is the mono-nucleotide (AT/AT), followed by AC/GT, AG/CT, AAT/ATT (Table 1).

To further analysis the distribution of SSRs in various gene structure, we selected di-nucleotide and tri-nucleotide to statistic the amount of these two motifs in 5'untranslated regions (5'UTR), 3'untranslated regions (3'UTR), coding sequence (CDS), non-CDS and region which across the coding and UTR regions (ACR), respectively. We found that di-nucleotide repeats are more likely to appear in in the CDS region than in the UTR regions. The proportion of di-nucleotide motif repeats in CDS (7%) is significantly lower than that in UTR regions (66%) (Fig. 1C and 1D). However, the similar frequencies of tri-nucleotide between over UTR (30%) and CDS (36%) regions.

Table 1: The SSR repeat types from papillae/skin transcriptome of *A. japonicus*

SSR Repeat Types	Repeats	Total Counts
Mono-uncleotide	A/T	6,644/6,616
	C/G	2,589/2,568
Di-uncleotide	AC/GT	431/428
	AG/CT	357/357
	AT/AT	858/851
Tri-uncleotide	AAC/GTT	137/137
	AAG/CTT	236/237
	AAT/ATT	282/284
	ACC/GGT	60/59
	ACG/CGT	13/15
	ACT/AGT	47/46
	AGC/CTG	42/44
	AGG/CCT	204/195
	ATC/ATG	198/198
	CCG/CGG	2/2
Tetra-uncleotide	AAAC/GTTT	5/7
	AAAG/CTTT	12/10
	AAAT/ATTT	9/7
	AACG/CGTT	4/4
	AAGC/CTTG	1/1
	AAGG/CCTT	0/1
	AATC/ATTG	5/5
	AATG/ATTC	7/7
	AATT/AATT	5/6
	ACAG/CTGT	11/11
	ACAT/ATGT	22/22
	ACCC/GGGT	1/1
	ACGG/CCGT	5/5
	ACTC/AGTG	1/1
	ACTG/AGTC	2/2
	AGAT/ATCT	3/3
	ATCC/ATGG	4/4
	ATCG/ATCG	1/1
Penta-uncleotide	AAGAG/CTCTT	0/1
	AAGGG/CCCTT	1/1
	AGGGG/CCCCT	1/1
	ATATC/ATATG	1/1

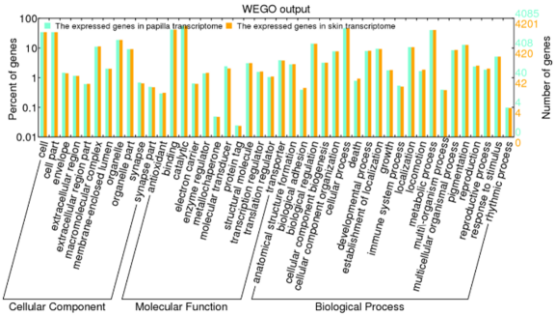

Fig. 3: Gene Ontology of genes containing putative SNPs

GO Analysis

GO classification was performed for the unigenes containing molecular markers. The distribution of the unigenes in various GO terms was shown in Fig. 3. Some over-represented terms linked with the catalytic (GO:0003824), pigmentation (GO:0048066), response to

Table 2: Growth-related genes that containing molecular markers

Gene ID	Annotation	SNP number (papillae/skin)	SSR number (papillae/skin)
c46471.graph_c0	serine/threonine-protein kinase PLK2-like	4/0	-
c56639.graph_c0	heat shock protein 70	1/0	-
c35104.graph_c0	eukaryotic translation elongation factor 1A	3/0	-
c73895.graph_c0	eukaryotic translation elongation factor 1 epsilon-1-like	18/19	1/1
c73320.graph_c0	bone morphogenetic protein 3-like	4/4	2/2
c73997.graph_c0	transforming growth factor beta regulator 1-like	8/8	-
c60335.graph_c0	transforming growth factor-beta-induced protein ig-h3-like	6/6	-
c41857.graph_c0	transforming growth factor beta-2-like	0/1	1/1
c60567.graph_c0	epidermal growth factor receptor kinase substrate 8-like	1/2	1/1
c82653.graph_c0	multiple epidermal growth factor-like domains protein 6-like isoform 2	0/2	-
c70682.graph_c0	multiple epidermal growth factor-like domains protein 10-like isoform 2	13/10	-
c70322.graph_c1	epidermal growth factor receptor	25/11	-
c50670.graph_c0	multiple epidermal growth factor-like domains protein 11-like	2/1	-
c69604.graph_c0	epidermal growth factor receptor substrate 15-like 1-like	18/14	-
c61913.graph_c1	insulin-like growth factor 2 mRNA-binding protein 3-like	11/9	2/2

stimulus were annotated. For biological process, except for the immune system process, the number of unigenes that containing molecular markers in biological adhesion (GO:0007155), death (GO:0035071), locomotion (GO:0040011), reproduction (GO:0000003) and reproductive process (GO:0022414) for papillae is less than that of in skin transcriptome data. The quantity difference between these two tissues was to be of interest, and further studies on functional markers and practical applications in aquaculture breeding should warrant the evidence to explain this difference.

Growth-related Genes Containing Molecular Markers

We also identified several growth-related genes that containing molecular markers. In papillae, epidermal growth factor receptor gene contained the greatest number of SNPs, and eukaryotic translation elongation factor 1 epsilon-1-like gene contained the greater number of SNPs (Table 2). In addition, the part genes contained SSR sequence, such as eukaryotic translation elongation factor 1 epsilon-1-like, bone morphogenetic protein 3-like, transforming growth factor beta-2-like, epidermal growth factor receptor kinase substrate 8-like and insulin-like growth factor 2 mRNA-binding protein 3-like genes.

Discussion

In the present study, we conducted whole genomic SNPs and SSRs based on RNA-Seq in *A. japonicus*. A total of 166,572 and 171,480 putative SNPs was identified in papillae and skin transcriptome, respectively. This result lay the foundation to identify molecular markers that were potentially involved in the growth of the papillae and skin. The generated genomic resources should be valuable for other genetic and genomic studies in the *A. japonicus*.

Molecular markers that distribute in CDS region possess more influence on gene performance and economic traits than those in other regions. Many mutations that located in CDSs could result in the deficiency of gene function, which gives rise to species extinction. The retained traits can suppose beneficial mutations during evolution (Zhu *et al*., 2012). Thus, the influences of natural selection at gene or protein levels can be explained by identifying the mutations that sit in CDSs (Ellegren, 2008). The distribution of molecular markers in different gene regions were analyzed. For the tri-nucleotide repeats over the CDS, the dominance of tri-nucleotide repeats may be expected on account of the length variance of tri-nucleotide motifs does not result in frame shifts in coding genes (Huang *et al*., 2011). Moreover, Yu *et al*. (2004) found that di-nucleotide repeats were high dominance in the region of 5' UTR and 3'UTR. We found that di-nucleotide repeats of SSRs are more likely to appear in the UTR regions than in the CDS region. It's proved that motif type in our study is the mono-nucleotide (AT/AT), the proportion of di-nucleotide repeats in the 3' UTR and 5' UTR regions was about 30 and 35% both in two tissues, which higher than that of tri-nucleotide repeats. The low motif of SSRs hold main proportion in the present study. And in the previous study (Dreisigacker *et al.*, 2004), the higher potentially polymorphisms will appear in low motif than advanced motif was concluded. According to statistics, the most abundant followed by tri-nucleotide and tetra-nucleotide. This finding in the present study is in agreement with previous findings (Zhou *et al*., 2014). It's revealed that the majority of the obtained SSRs in *A. japonicus* papillae and skin may possess hypervariability.

Many SNPs and SSRs makers are identified associated with various traits, including body color, heat-tolerance, and growth and development (Li *et al*., 2016). Other transcriptome researches were also administrated with the focus on investigate large scale of molecular markers (Guo *et al*., 2013; Chen and Storey, 2014). In the present study, many growth-related genes containing molecular markers were developed. As a conserved family of RNA-binding, insulin-like growth factor 2 mRNA-binding protein 1-like (IGF2BP1), IGF2BP2 and IGF2BP3 act in various important aspects of cell function, such as cell migration, polarization, proliferation, morphology, metabolism, and differentiation (Bell *et al*., 2013). In the previous study, transforming

growth factor-beta-induced protein (TGFBIp) is associated with collagen, fibronectin, laminin, and glycosaminoglycan that all belongs to ECM components (Kim *et al*., 2009). As one of the ECM components, TGFBIp/βig-h3 protein and its functions play an extensive role in extracellular matrix interactions, morphogenesis, adhesion/migration, corneal dystrophy, tumorigenesis, angiogenesis, nephropathies, osteogenesis, wound healing and inflammation (Thapa *et al*., 2007). Epidermal growth factor receptor (EGFR) is a member of ErbB family and the signal plays a basic role on human tumor cells development and growth (Zhou *et al*., 2016a). These newly developed molecular markers will provide a vital information platform for accelerating *A. japonicus* breeding and stimulate the application of MAS systems in *A. japonicus* breeding.

Conclusion

A large number of molecular markers were screened by mining the transcriptome sequencing of the *A. japonicus* papillae and skin. It was found that many growth-related genes also contained SNPs and SSRs. These useful molecular markers can be used for genetics, breeding and genetic diversity analysis in the sea cucumber.

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References

Bell, J.L., K. Wachter, B. Muhleck, N. Pazaitis, M. Kohn, M. Lederer and S. Huttelmaier, 2013. Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? *Cell Mol. Life Sci*., 70: 2657–2675

Chang, Y.Q., F. Zhu, J. Yu and J. Ding, 2009. Genetic variability analysis in five populations of the sea cucumber *Stichopus* (*Apostichopus japonicus*) from China, Russia, South Korea and Japan as revealed by microsatellite markers. *Mar. Ecol.*, 30: 455–461

Chen, M.Y. and K.B. Storey, 2014. Large-scale identification and comparative analysis of miRNA expression profile in the respiratory tree of the sea cucumber *Apostichopus japonicus* during aestivation. *Mar. Genom.,* 13: 39–44

Cui, J., H. Wang, S. Liu, X. Qiu, Z. Jiang and X. Wang, 2014. Transcriptome analysis of the gill of *Takifugu rubripes* using Illumina sequencing for discovery of SNPs. *Compar. Biochem. Physiol. D Genom. Proteom.,* 10: 44–51

Ding, J., Y. Chang, Z. Wang and J. Song, 2007. Polyploidy induction by hydrostatic pressure shock and embryo development of sea cucumber *Apostichopus japonicus*. *Chin. J. Oceanol. Limnol.*, 25: 184–190

Dreisigacker, S., P. Zhang, M.L. Warburton, M.V. Ginkel, D. Hoisington, M. Bohn and A.E. Melchinger, 2004. SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different mega environments. *Crop Sci*., 44: 381–388

Ellegren, H., 2008. Comparative genomics and the study of evolution by natural selection. *Mol. Ecol*., 17: 4586–4596

Guo, H., A.X. Jian, A.L. Wang, X.Y. Chao and T.M. Yu, 2013. Trascriptome analysis of the Pacific white shrimp *Litopenaeus vannamei* exposed to nitrite by RNA-seq. *Fish Shellf. Immunol*., 35: 2008–2016

Huang, H., J. Lu, Z.B. Ren, W. Hunter, S.E. Dowd and P. Dang, 2011. Mining and validating grape (*Vitis L*.) ESTs to develop EST-SSR markers for genotyping and mapping. *Mol. Breed.*, 28: 241–254

Kang, J.H., Y.K. Kim, M.J. Kim, J.Y. Park, C.M. An, B.S. Kim, J.C. Jun and S.K. Kim, 2011a. Genetic differentiation among populations and color variants of sea cucumbers (*Stichopus japonicus*) from Korea and China. *Intl. J. Biol. Sci.,* 7: 323–332

Kang, J.H., K.H. Yu, J.Y. Park, C.M. An, J.C. Jun and S.J. Lee, 2011b. Allele-specific PCR genotyping of the HSP70 gene polymorphism discriminating the green and red color variants sea cucumber (*Apostichopus japonicus*). *J. Genet. Genom.*, 38: 351–355

Kim, H.J., P.K. Kim, S. Bae, D.S. Thoudam, J.E. Kim, B.H. Lee, R.W. Park and I.S. Kim, 2009. Transforming growth factor-beta-induced protein (TGFBIp/beta ig-h3) activates platelets and promotes thrombogenesis. *Blood*, 114: 5206–5215

Li, S., Z. Zhou, Y. Dong, H. Sun, S. Gao, Z. Chen, A. Yang, W. Liu and Q. Wang, 2016. Molecular characterization, expression analysis of the myostatin gene and its association with growth traits in sea cucumber (*Apostichopus japonicus*). *Compar. Biochem. Physiol. B Biochem. Mol. Biol.,* 201: 12–20

Lv, J., D. Zhang, B. Gao, P. Liu and J. Li, 2015. Transcriptome and MassARRAY analysis for identification of transcripts and SNPs for growth traits of the swimming crab *Portunus trituberculatus*. *Gene,* 566: 229–235

Ma, C., 2012. *Improvement of The Stratum for Larval Settlement and Metamorphosis of Sea Cucumber Apostichopus japonicuus by Superficial Material and Biofilms*. Ocean University of China. Qingdao, China (In Chinese)

Meng, X., H. Wang, X. Qiu, H. Liu and X. Wang, 2017. SNPs of myostatin (MSTN) gene and their association with growth traits in three bay scallop (*Argopecten irradians*) populations. *Aquacult. Res.*, 48: 531–536

Qiu, X.M., X.Y. Meng, X.L. Guo, J. Wang and X.L. Wang, 2013. New polymorphic microsatellite markers for Chinese shrimp (*Fenneropenaeus chinensis*) from sequence database. *Conserv. Genet. Resour*., 5: 461–463

Thapa, N., H.L. Byung and I.S. Kim, 2007. TGFBIp/betaig-h3 protein: a versatile matrix molecule induced by TGF-beta. *Intl. J. Biochem. Cell Biol*., 39: 2183–2194

Wang, Y., X. Wang, X. Meng, H. Wang, Z. Jiang and X. Qiu, 2014. Identification of two SNPs in myostatin (MSTN) gene of *Takifugu rubripes* and their association with growth traits. *Mol. Cell Mol. Cell. Prob.,* 28: 200–203

Xu, D.X., L.N. Sun, S.L. Liu, L.B. Zhang and H.S. Yang, 2014. Polymorphisms of heat shock protein 90 (HSP 90) in the sea cucumber *Apostichopus japonicus* and their association with heat-resistance. *Fish Shellf. Immunol*., 41: 428–436

Yu, J.K., T.M. Dake, S. Singh, D. Benscher, W. Li, B. Gill and M.E. Sorrells, 2004. Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. *Genome,* 47: 805–818

Zhou, X., J. Cui, S. Liu, D. Kong, H. Sun, C. Gu, H. Wang, X. Qiu, Y. Chang, Z. Liu and X. Wang, 2016a. Comparative transcriptome analysis of papilla and skin in the sea cucumber, *Apostichopus japonicus*. *Peer J*., 4: e1779

Zhou, X., H. Wang, J. Cui, X. Qiu, Y. Chang and X. Wang, 2016b. Transcriptome analysis of tube foot and large scale marker discovery in sea cucumber, *Apostichopus japonicas*. *Compar. Biochem. Physiol. D Genom. Proteom.,* 20: 41–49

Zhou, Z.C., Y. Dong, H.J. Sun, A.F. Yang, Z. Chen, S. Gao, J.W. Jiang, X.Y. Guan, B. Jiang and B. Wang, 2014. Transcriptome sequencing of sea cucumber (*Apostichopus japonicus*) and the identification of gene-associated markers. *Mol. Ecol. Resour*., 14: 127–138

Zhu, C.K., L. Cheng, J.G. Tong and X.M. Yu, 2012. Development and characterization of new single nucleotide polymorphism markers from expressed sequence tags in common carp (*Cyprinus carpio*). *Intl. J. Mol. Sci.,* 13: 7343–7353

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