Turk. J. Fish.& Aquat. Sci. 19(11), 969-975
http://doi.org/10.4194/1303-2712-v19_11_08

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Mapping and Validation of QTLs Associated with Growth Trait in a F_1 Family of Freshwater Sleeper *Odontobutis potamophila*

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Article History
Received 08 January 2017
Accepted 20 November 2018
First Online 27 November 2018

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Keywords
Odontobutis potamophila
SLAF-Seq
SNP marker
QTL validation
Growth traits

Abstract

In the present study, 43 quantitative trait loci (QTL) associated with body length and body weight were identified based on the high density genetic map of *O. potamophila* composed of 6,311 SLAF markers. The phenotypic variance explained by these QTLs (Expl.%) were ranging from 11.9% to 15.4% and the threshold of logarithm of odds (LOD) score is 3. The 43 QTLs from *O. potamophila* were validated by Sanger sequencing. In total, 10 QTLs were validated as the true QTL associated with body length and body weight for the fish. It shows that the growth traits of *O. potamophila* were likely controlled by 10 QTLs distributed on three genetic linkage groups (LG4, LG6 and LG16). We found marker24165 was both associated with body length and associated with body weight, which will be useful in marker-assisted selection studies for the fish. Moreover, the 10 QTLs associated with growth traits will be used for identification of fast growth family or strain of *O. potamophila* in the future.

Introduction

According to the development of genotyping of Next generation sequencing (NGS) technology, restriction site associated DNA sequencing (RAD-Seq) and specific locus amplified fragment sequencing (SLAF-Seq) have been developed to screen genetic markers on large-scale for genetic mapping at low cost (Liu *et al*., 2014). The application of RAD-Seq and ddRAD-Seq has been reported in the localization of quantitative trait loci (QTL) associated with growth trait of fish such as Turbot *Scophthalmus maximus* L (Wang *et al*., 2015) and Asian seabass *Lates calcarifer* (Wang *et al*., 2015). Recently, the QTL associated with growth trait have been applied in aquatic animals based on high density linkage map constructed using SLAF-Seq, such as Pacific white shrimp *Litopenaeus vannamei* (Yu *et al*., 2015), Chinese mitten crab *Eriocheir sinensis* (Qiu *et al*., 2017), Swimming crab *Portunus trituberculatus* (Lv *et al*., 2017), Pikeperch *Sander lucioperca* (Guo *et al*., 2018) and Sea urchin *Strongylocentrotus intermedius* (Chang *et al*., 2018).

The freshwater sleeper *Odontobutis potamophila* is an economic valuable fish and relies mainly on wild resources. It is widely distributed in the middle and lower regions of the Yangtze River, Qiantang River in China. Because of the rapid development of the aquaculture industry in recent years, there is an urgent need to cultivate superior species with grow faster and resistant varieties strain through improvements in selective breeding (Zhang *et al*., 2017). At the present day, we developed and validated 33 SNP markers in *O. potamophila* from transcriptomic sequencing and a high-density SNP-based genetic map for *O. potamophila* has constructed with SLAF-Seq technology and HighMap software (Zhang *et al*., 2015; Zhang *et al*., 2017).

In this study, the high density genetic map of *O. potamophila* was established by SLAF sequencing. SNP markers were developed from QTL localization

associated with the growth traits of the fish, and the QTLs associated with body length and body weight could be used to identify fast- and slow-growing family of fish of

O. potamophila, which will provide useful molecular markers for marker-assisted selection of the species in the future.

Materials and Methods

Sample Collection and Preparation

Both families of fish used in the construction of mapping population were from different geographical *O. potamophila* population (female fish were collected from the Dangtu cultured population in Anhui Province, China (31º25'37N, 118º42'59E), male fish were collected from the Qiantang River wild population in Zhejiang Province, China (29º31'14N, 119º24'1E)). The experiments were proceeded on *O. potamophila* that is not regarded as species under second class state protection in China, therefore the specific permission of capture was not required. This study was approved by the Ethics Committee of Experimental Animals at Nanjing Normal University (Approval number: SYXK (Jiangsu) 2015-0028). F_1 progenys were obtained through the mating of single female and single male adult *O. potamophila*. The thousands of progeny from this mating were hatched in April 2014 at the same time, each offspring individuals were bred to August 2014 in the same water environment, and tail fin tissues of each *O. potamophila* were stored in 95% EtOH at -20 °C. One suitable mapping group was selected from two cultivated F_1 groups. Totally 113 *O. potamophila* individuals were collected, including two parents of one female (body length: 95.0 mm, body weight: 20.7 g) and one male (body length: 110.0 mm, body weight: 30.8 g) and 111 individuals of F_1 hybrid (body length from 31.69 mm to 78.39 mm, body weight from 0.71 g to 13.76 g).

SLAF Library Preparation and Sequencing

Genomic DNA was extracted from fins using the Easy Pure Marine Animal Genomic DNA Kit (TRANS, Beijing TransGen Biotech Co., Ltd. Beijing, China) according to the manufacturer's protocol. The construction of a specific-locus amplified fragment (SLAF) library after *Rsa* Ⅰ (New England Biolabs, NEB, USA) digestion of genomic DNA and Illumina sequencing were as described previously (Sun *et al*., 2013).

Sequence Data Grouping, Genotyping and Linkage Map Construction

Low-quality reads (quality score<20e) were filtered out, all SLAF pair-end reads with clear index information were clustered as detected by BLAT (Kent, 2002) (−tileSize = 10 -stepSize = 5). Sequences with the identity of over 90% were grouped in one SLAF locus as described previously (Sun *et al*., 2013). Alleles of each SLAF locus were then defined. All polymorphic SLAF loci were genotyped using the consistency of SNP loci in the parental and offspring. Genotype scoring was performed through a Bayesian approach (Sun *et al*., 2013). A posteriori conditional probability was counted. Genotyping quality score was used to select the qualified markers (Zhang *et al*., 2015). High-quality SLAF markers for the genetic mapping were filtered by following original standards: (1) average sequence depths should be >10-fold in parents; (2) SNP number greater than 3; (3) completely homozygous parents; (4) degree of completion of progeny lower than 85%; (5) serious partial separation ($P<0.01$).

HighMap software was used to order SLAF markers and correct genotyping errors within linkage groups (LGs) (Liu *et al*., 2014). Based on marker locations in chromosomes, marker loci were partitioned into LGs. The markers of modified logarithm of odds (MLOD) value is greater than 5 were selected for label of mapped onto the genetic map. The Gibbs sampling, spatial sampling and simulated annealing algorithms were integrated to precede an iterative procedure of marker ordering (Jansen, de Jong, & van Ooijen, 2001; Van Ooijen, 2011). According to parental contribution of genotypes, mistake-adjusting strategy of SMOOTH was then proceeded and a k-nearest neighbor algorithm was applied to input missing genotypes and skewed markers were added into this map through applying a multipoint method of maximum likelihood (Van, Stam, Visser, & van Eck, 2005; Huang *et al*., 2012; Zhang *et al*., 2013). The sex-specific maps were constructed using markers, while the consensus map was established by integrating the parental maps (Van Ooijen, 2011). Kosambi mapping function was estimated by Map distances (Kosambi, 1943).

QTL Analysis Using High-Density Genetic Map

The QTL analysis was proceeded using interval mapping method of MapQTL 6.0 software. The threshold of logarithm of odds (LOD) score for significance (P = 0.05) was determined using 1,000 permutations. The phenotypic variance explained by each QTL (Expl.%) was calculated in MapQTL6.0 software.

QTL Validation

Totally 20 *O. potamophila* individuals were genotyped for each SLAF tag of the QTL for the validation by using Sanger sequencing. These samples were collected from the same F_1 progeny population of *O. potamophila* used in the construction of linkage map (10 big individuals and 10 small individuals): big individual of F_1 progeny (body length from 63.15 mm to 78.39 mm, body weight from 6.38 g to 13.76 g); small individual of F_1 progeny (body length from 31.69 mm to 38.51 mm, body weight from 0.71 g to 1.43 g). Genomic

DNA was extracted from fins, PCR was conducted by primers designed using Primer 5 software, and PCR products were subjected to Sanger sequencing on an ABI 3730 Genetic Analyzer to confirm their SNP genotypes.

Results

SLAF Genotyping and Linkage Map

After applying several criteria filters, 6,716 SLAF markers were used for the construction of the first genetic map of *O. potamophila* in our previous study (Zhang *et al*., 2017). Finally, 6,311 of these 6,716 markers were mapped onto the genetic map in 22 linkage groups spanning 3953.42 cM with an average inter-marker distance of 0.63 cM after linkage analysis. In total, 9,468 SNP loci were detected among 6,311 SLAF markers on the final map and the different SNP numbers in each linkage group (LG1-LG22) were investigated. The number of SNP markers per chromosome ranged from 267(LG12) to 630(LG15). On average, each LG contained 430 SNP markers.

Data Analysis and QTL Mapping

The 43 QTLs for body length and 3 QTLs for body weight were identified based on the high-density genetic map (3 QTLs in 43 QTLs for body length were also associated with body weight). 10 markers between 23.69-29.36 cM are responsible for body length in LG4. 1 marker between 118.84-119.01 cM and 2 markers between 120.93-121.06 cM are responsible for body length and body weight in LG6. 15 markers between 191.57-192.58 cM and 15 markers between 192.71-194.31 cM are responsible for body length in LG16 (Table 1). The 43 QTLs were mapped to LG4, LG6 and LG16. The phenotypic variance explained by these QTLs (Expl.%) ranging from 11.9% to 15.4% and the threshold of logarithm of odds (LOD) score was 3 (Figure 1).

Horizontal coordinate correspond to genetic markers distributed on genetic linkage groups(LG). Vertical coordinate correspond to the LOD and the Expl.%. Blue represents Lod, red represents Expl.%.

Results of QTL Validation

Totally 43 QTLs associated with body length and body weight of *O. potamophila* were validated from SLAF sequencing. Finally, 10 of 43 QTLs were successfully validated by using Sanger sequencing. In total, 10 QTLs were validated as the true QTL associated with body length and body weight for the fish, because each SNP markers appearence frequency were remarkable defferenced between 10 large sample and 10 small sample of F_1 family in *O. potamophila*. 13 SNP loci were detected in10 QTLs. In 10 QTLs, only Marker24165 was QTL associated with both body length and body weight. Marker4494 had two SNP loci, and Marker24165 had three SNP loci (Table 2**)**. Marker68115, Marker123134, Marker4317 and Marker20952 were located in the region of 23.69 -29.36 cM in LG4. Marker24165 was located in the region of 120.93-121.06 cM in LG6. Marker159548, Marker44271, Marker4494, Marker37011 and Marker53123 were located in the region of 191.57-192.58 cM in LG16. Marker159548, Marker44271, Marker4494, Marker37011 and Marker53123 belonged to one hyplotype.

Discussion

The high density genetic map of Asian seabass *Lates calcarifer* was constructed with 3,321 SNPs generated by ddRAD-Seq in a F_2 family. Based on the high density genetic map, one genome-wide significant and five suggestive QTL for growth traits were detected in 6 LGs. A candidate gene, *ACOX*1 within the significant QTL on LG5 was differentially expressed between fast- and slow-growing Asian seabass (Wang *et al*., 2015). The high density consensus genetic linkage map of a turbot *Scophthalmus maximus* L. family was constructed through SNPs generated by the RAD-Seq with the restriction enzyme, *Pst*I. A total of 14 QTLs associated with body length, body weight in second growth periods were detected in 10 LGs (Wang *et al*., 2015). 11 QTLs for body length and 7 QTLs for body weight were detected based on the high density linkage map constructed using SLAF-Seq of Pacific white shrimp *Litopenaeus vannamei*, which located at different linkage groups. In 17 QTLs, only one marker (Marker7605) was found to be significant for both growth traits, and it will be useful in marker-assisted selection studies for Pacific white shrimp (Yu *et al*., 2015). A second generation SNP and SSR integrated linkage map of Chinese mitten crab *Eriocheir sinensis* was constructed. The final integrated linkage map included 17,680 SNPs generated by SLAF-Seq and 629 SSR on the 73 LGs. Based on the high density genetic map, 3 significant growth-related QTLs were QTL mapping localized to a 1.2 cM region in

Table 1. The basic information associated with growth traits for body length and body weight

Name of growth trait	LOD threshold	Linkage Group ID	Start (cM)	End (cM)	Marker Number
Body length	3.00	LG4	23.69	29.36	10
Body length (body weight)	3.00	LG6	118.84	119.01	1
Body length (body weight)	3.00	LG6	120.93	121.06	2
Body length	3.00	LG16	191.57	192.58	15
Body length	3.00	LG16	192.71	194.31	15

Table 2. 10 QTLs associated with body length and 1 QTL associated with body weight from *O. potamophila* validated by Sanger sequencing

Marker ID	Linkage Group ID	Primers	Type of SNP loci and appearence number of SNPs in two population
Marker68115	LG4	F: GTGTCCACTTCACCAAGAC R: TCATCTGGACATGTCCTA	L: 8 A/G S: 1 A/G
Marker123134	LG4	F: CCGGTTGTTCAATCAATGTTC R: CGGAAATAGCTAAACATGG	L: 2 A/G S: 8 A/G
Marker4317	LG4	F: CTGTCCCTTTATGGAGTATG R: ACCACGACACTGCTGATCAC	L: 8 A/G S: 2 A/G
Marker20952	LG4	F: TGGTTTACAGACTCCTGATC R: GTTGCTGTTTACTTGCTACAG	L: 8 C/T S: 3 C/T
Marker24165-1	LG6	F: CACTATTGCCACACGGAGG R: CATAGCCATGGAGCCTTAAG	L: 1 A/T S: 7 A/T
Marker24165-2			L: 9 C/T S: 3 C/T
Marker24165-3			L: 7 A/T S: 2 A/T
Marker159548	LG16	F: TTAGTATTTGTATCAATTACTATATATGACTGAGACAT R: CTTGGCAGTTTCCACAGAAAGA	L: 2 C/T S: 5 C/T
Marker44271	LG16	F: AATGAGTCCACTCGACTGTAGTAAAA R: TCAGTGGGGGCATACGTC	L: 2 C/T S: 5 C/T
Marker4494-1	LG16	F: GTTCCTTTAAGGGGAGGGGTG R: TGATTCATTAATGTTGAATTCCCTCA	L: 2 A/G S: 5 A/G
Marker4494-2			L: 2 A/G S: 5 A/G
Marker37011	LG16	F: CCCCCCAGATCAGATGATCATAT R: TTCCTCACAGTAGAGGATCGATTCT	L: 2 C/T S: 5 C/T
Marker53123	LG16	F: CATGGTTCGACATGATAAAC R: CACAATTCTCTGCTTTTGGC	L: 2 C/T S: 5 C/T

L: Large sample of F_1 family in *O. potamophila.*
S: Small sample of F_1 family in *O. potamophila.*

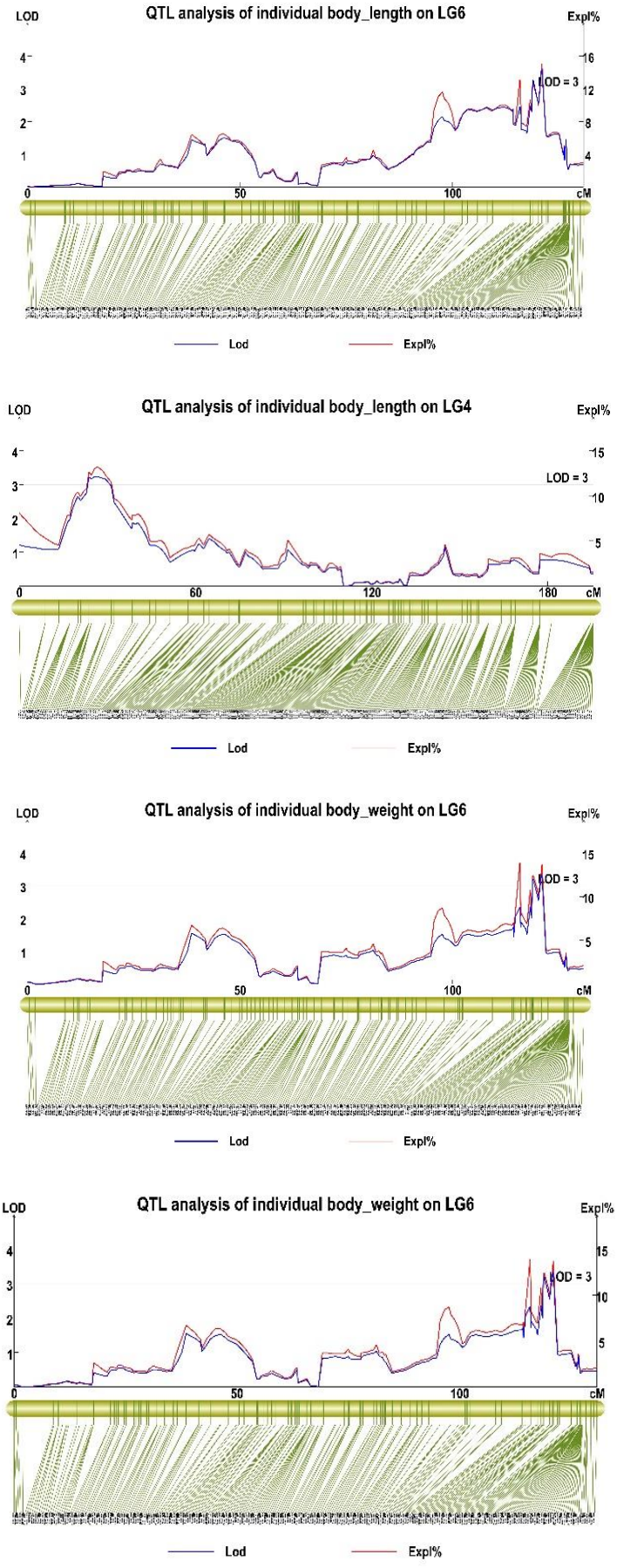

Figure 1. The Distribution of 43 QTLs for body length and body weight.

LG53(Qiu *et al*., 2017). The first high density genetic linkage map of pikeperch (*Sander lucioperca*) using SLAF-Seq was constructed. The final integrated linkage map consisted of 8,159 SLAFs in 24 LGs. Based on the high density genetic map, 5 QTLs for body length and 3 QTLs for body weight were detected (Guo *et al*.,2018). In our study, based on the high density genetic map of *O. potamophila* composed 6,311 SLAF markers, the QTLs for 43 body length and 3 body weight were identified. Ten QTLs of *O. potamophila* were validated by using Sanger sequencing. The growth traits of *O. Potamophila* are likely controlled by 10 QTLs distributed on three genetic linkage group (LG4, LG6 and LG16) (see Table 2**)**. Only marker24165 was both QTL associated with body length and body weight. Our results are consistent with QTLs related to growth trait of turbot (Wang *et al*., 2015), Pacific white shrimp (Yu *et al*., 2015) and pikeperch (Guo *et al*.,2018).

SLAF-Seq is measured by sequencing the paired-ends of the sequence-specific restriction fragment length. However, RAD-Seq is measured by randomly broken genomic DNA with restriction enzymes. Due to the selection of fragment length without random break, SLAF-Seq with better repeatability is considered than RAD-Seq. SLAF-Seq is a useful technology to develop chromosome-specific molecular markers with the characteristics of high success rates, specificity, stability and low cost. Furthermore, it can be applied for the development of a controlled number of SNP markers and the construction of high-density linkage map of fish without a reference genome sequence.

These 10 QTLs associated with body length and body weight of *O. potamophila* were first reported from validated data by SLAF-Seq in fish. Therefore, our study will provide a useful approach to construct high density linkage map and identify QTL associated with the growth traits of fish without reference genome sequence, which will be useful for the molecular genetic breeding of fish.

Acknowledgments

This work was supported by the National Spark Program Project (2013GA690166), Project Foundation of the Academic Program Development of Jiangsu Higher Education Institution (PAPD) and the Research and Development Program of Jiangsu Province (BE2013441).

References

Chang, Y.Q., Ding, J., Xu, Y.H., Li, D., Zhang, W.J., Li, L., & Song, J. (2018) SLAF-based high-density genetic map construction and QTL mapping for major economic traits in sea urchin *Stronglocentrotus intermedius*. *Scientific reports*, 8(1), 820. https://doi.org/10.1038/s41598-017-18768-y

Guo, J.Q., Li, C.J., Teng, T., Shen, F.F., Chen, Y.N., Wang, Y.F., ... Ling, Q.F. (2018) Construction of the first high-density genetic linkage map of pikeperch (*Sander lucioperca*) using specific length amplified fragment (SLAF) sequencing and QTL analysis of growth-related traits. *Aquaculture*, 497, 299-305. https://doi.org/10.1016/j.aquaculture.2018.07.047

Huang, X.H., Zhao, Y., Wei, X.H., Li, C.Y., Wang, A.H., Zhao, Q., ... Han, B. (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nature genetics*, 44(1), 32–39. https://doi.org/10.1038/ng.1018

Jansen, J., de Jong, A.G., & van Ooijen, J.W. (2001) Constructing dense genetic linkage maps. *Theoretical and applied genetics*, 102(6-7), 1113–1122. https://doi.org/10.1007/s001220000489

Kosambi, D.D. (1943) The estimation of map distances from recombination values. *Annals of Human Genetics*, 12(1),172–175. http://dx.doi.org/10.1111/j.1469-1809.1943.tb02321.x

Kent, W.J. (2002) BLAT - the BLAST-like alignment tool. *Genome Research*, 12(4), 656–664. https://doi.org/10.1101/gr.229202

Liu, D.Y., Ma, C.X., Hong, W.G., Huang, L., Liu, M., Liu, H., ... Zheng, H.K. (2014) Construction and analysis of high-density linkage map using high-throughput sequencing data. *PLoS One*, 9(6), e98855. http://dx.plos.org/10.1371/journal.pone.0098855

Lv, J.J., Gao, B.Q., Liu, P., Li, J., Meng, X.L. (2017) Linkage mapping aided by de novo genome and transcriptome assembly in *Portunus trituberculatus*: applications in growth-related QTL and gene identification. *Scientific reports*, 7(1), 7874. https://doi.org/10.1038/s41598-017-08256-8

Qiu, G.F., Xiong, L.W., Han, Z.K., Liu, Z.Q., Feng, J.B., Wu, X.G., ... Chen, L. A second generation SNP and SSR integrated linkage map and QTL mapping for the Chinese mitten crab *Eriocheir sinensis*. *Scientific reports*, 7, 39826. https://doi.org/10.1038/srep39826

Sun, X.W., Liu, D.Y., Zhang, X.F., Li, W.B., Liu, H., Hong, W.G., ... Zheng, H.K. (2013) Slaf-seq: an efficient method of large-scale de novo SNP discovery and genotyping using high-throughput sequencing. *PLoS One*, 8(3), e58700. http://dx.plos.org/10.1371/journal.pone.0058700

Van Ooijen, J.W. (2011) Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. *Genetics research*, 93(5), 343–349. https://doi.org/10.1017/S0016672311000279

Van, Os.H., Stam, P., & Visser, R.G. (2005) SMOOTH: a statistical method for successful removal of genotyping errors from high-density genetic linkage data. *Theoretical and Applied Genetics*, 112(1), 187–194. https://dx.doi.org/10.1007/s00122-005-0124-y

Wang, L., Wan, Z.Y., Bai, B., Huang, S.Q., Chua, E., Lee, M., ... Yue, G.H. (2015) Construction of a high-density linkage map and fine mapping of QTL for growth in Asian seabass. *Scientific reports*, 5, 16358. http://dx.doi.org/10.1038/srep16358

Wang, W.J., Hu, Y.L., Ma, Y., Xu, L.Y., Guan, J.T., & Kong, J. (2015) High-density genetic linkage mapping in turbot (*Scophthalmus maximus* L.) based on SNP markers and major sex- and growth- related regions detection. *PLoS One*, 10(3), e0120410. http://dx.plos.org/10.1371/journal.pone.0120410

Yu, Y., Zhang, X.J., Yuan, J.B., Li, F.H., Chen, X.H., Zhao, Y.Z., ... Xiang, J.H. (2015) Genome survey and high-density genetic map construction provide genomic and genetic resources for the Pacific White Shrimp *Litopenaeus*

vannamei. *Scientific reports*, 5, 15612. http://dx.doi.org/10.1038/srep15612

Zhang, H.W., Yin, S.W., Zhang, L.J., Hou, X.Y., Wang, Y.Y., & Zhang, G.S. (2015) Development and validation of single nucleotide polymorphism markers in *Odontobutis potamophila* from transcriptomic sequencing. *Genetics and Molecular Research*, 14 (1), 2080–2085. http://dx.doi.org/10.4238/2015.March.20.18

Zhang, H.W., Huang, L., Yin, S.W., Zhang, G.S., Ding, Y.D., & Zang, X. (2017) High-density SNP-based genetic map for *Odontobutis potamophila. Animal Genetics*, 48(1), 126-127. http://dx.doi.org/10.1111/age.12471

Zhang, J., Zhang, Q.X., Cheng, T.R., Yang, W.R., Pan, H.T., Zhong, J.J., ... Liu, E.Z. (2015) High-density genetic map construction and identification of a locus controlling weeping trait in an ornamental woody plant (*Prunus mume* Sieb. et Zucc). *DNA Research*, 22(3), 183–191. https://doi.org/10.1093/dnares/dsv003

Zhang, Y.X., Wang, L.H., Xin, H.G., Li, D.H., Ma, C.X., Ding, X., ... Zhang, X.R. (2013) Construction of a high-density genetic map for sesame based on large scale marker development by specific length amplified fragment (SLAF) sequencing. *BMC Plant Biology*, 13, 141. https://doi.org/10.1186/1471-2229-13-141