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Development and characterization of microsatellite markers for *Solen grandis* using Illumina sequencing approach

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ABSTRACT. *Solen grandis* is an important economic and overexploited bivalve species. In order to perform its fine-scale genetic analyses, 105 pairs of microsatellites with polymorphism were identified through Illumina Hiseq platform and bioinformatic assembly technology in this study. The estimated fragment size ranged from 100 to 268 bp and the number of alleles per locus varied between 2 and 23. Observed and expected heterozygosities varied from 0.0667 to 1.0000 and 0.0966 to 0.9492, respectively. Fourteen loci deviated significantly from Hardy-Weinberg equilibrium after Bonferroni correction. These microsatellite markers developed in this study would be helpful for future genetic studies on *S. grandis* and closely related species.

Key words: *Solen grandis*; Microsatellite; Illumina sequencing

INTRODUCTION

*Solen grandi*s (Dunker, 1861), a bivalve mollusk of the family Solenidae, has become an important cultured species in China due to its high nutritive (Dai, 2002), high medical (Yang and Wang, 1996) and high economic value (Yuan et al., 2010). However, population decline caused by overfishing and deterioration of marine environment have limited the aquaculture industry. Under the consideration of restoration and promising strain selection, related genetic analyses should be performed. Microsatellite loci, also known as simple sequence repeats (SSR), are widely used for detecting genetic diversity, association mapping, genetic linkage mapping, and population and evolution analysis owing to their advantages such as hyper-variability, reproducibility, co-dominant inheritance, and extensive genomic coverage (Powell et al., 1996; Jiao et al., 2012). So far, some microsatellite markers have been isolated for *S. grandis* (Yuan et al., 2010; Jiang et al., 2016), but these do not satisfy the need of genetic studies and the development of its industry.

The purpose of this study was to use the next-generation sequencing (NGS) method (Illumina), which is more cost-effective and higher in throughput, to develop novel microsatellite markers for *S. grandis*. These new loci will greatly facilitate future genetic studies and brood stock management.

MATERIAL AND METHODS

Thirty wild *S. grandis* individuals were sampled successfully from Yantai in Shandong, China. Adductor muscles were collected and stored in ethanol, under -20°C condition. The modified phenol-chloroform protocol (Li et al., 2006) was used to extract genomic DNA. Genomic DNA quality and quantity were detected with the NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA) and 1.0% agarose gel.

Extracted DNA of one individual was randomly shotgun for specific fragments. The terminal of the fragments was repaired and 3' end was ligated with the A base. After combining with sequencing primers, approximately 170-bp fragments were purified, and then the library was constructed. The sequencing was performed on Illumina Hiseq 2000 platform.

The obtained sequence data were filtered and then subject to assembly. Microsatellite loci were identified using SSRHunter (Li and Wan, 2005). The criteria used in SSRHunter were as follows: five repeats for di-, tri-, tetra- and pentanucleotide repeats. Those loci with enough flanking sequence were used for primer design using PRIMER 5.0 (http://www.premierbiosoft.com/). Each pair of primers was tested on 30 *S. grandis* individuals. PCR amplifications were performed in a reaction mixture (10 μL) containing 50 ng genomic DNA, 1X PCR Buffer (Mg^{2+} plus), 0.2 mM of each dNTP, 0.25 U DNA ploymerase (TaKaRa) and 1 μM primer set. The thermo-cycling conditions were as follows: 3 min at 94°C, 35 cycles of 45 s at 94°C, 45 s at primer specific annealing, 45 s at 72°C and a final elongation of 5 min at 72°C. PCR products were genotyped by polyacrylamide gel and allele size was determined by a reference standard 10-bp ladder marker (Invitrogen).

Polymorphism information content (PIC) was calculated by program CERVUS 3.0 (Marshall et al., 1998). Number of alleles per locus (N_A), observed heterozygosities (H_O) and expected heterozygosities (H_E) were analyzed by the Microsatellite Analyzer software (Dieringer and Schlötterer, 2003). Deviations from Hardy-Weinberg equilibrium (HWE) and tests for linkage disequilibrium were performed by GENEPOP (Rousset, 2008).

RESULTS AND DISCUSSION

It is well known that the development of microsatellite markers using enrichment techniques requires a great amount of effort. The NGS provides a more cost-effective and higher throughput method to generate this useful marker (Ji et al., 2012). In this study, we used Illumina HiSeq 2000 platform to obtain 27,253.71 Mb clean data, which were assembled into 46,715 contigs (≥1 kb) and 46,877 scaffolds (≥1 kb), respectively.

From 250 loci we selected for microsatellite marker optimization, we found a total of 105 loci that were successfully amplified and proven to be polymorphic on 30 samples. It includes 50 dinucleotides, 43 trinucleotides, 10 tetranucleotides and 2 pentanucleotides (Table 1). The mean number of individuals successfully genotyped was 29.5, which indicates excellent amplification efficiency of these primers. N_A ranged from 2 to 23, with mean number of 8, and allele size varied between 100 and 268 bp. H_O and H_E ranged from 0.0667 to 1.0000 and 0.0966 to 0.9492, respectively. Fourteen loci significantly deviated from HWE after sequential Bonferroni correction (P < 0.05/105), probably because of null alleles or excessive heterozygosity. No linkage disequilibrium between each of loci was found. In addition, PIC values ranged from 0.090 to 0.929. According to Botstein et al. (1980), 103 loci of our study were highly or reasonably informative (PIC > 0.25).

Table 1. Microsatellite markers and their properties developed for *Solen grandis*.

Locus	Primer sequence (5'-3')	Repeat motif	Fragment size (bp)	Ta (ºC)	N_A	H_O	H_E	PIC	P value	GenBank accession No.
DZC341	F:ACCTGCCGATAATGAGTGTTTCAT R:AAAGGGAGTAAGTCCTGGGTTCAC	CA$_{(8)}$	124-158	59	5	0.6333	0.6944	0.764	0.0373	KX530218
DZC334	F:CTTTAAAGGTCACACCCGATGTCT R:GCTGAAGTAGGTGTTTGATTTCATTG	CAT$_{(8)}$	134-198	59	12	0.6333	0.8333	0.887	0.0588	KX530219
DZC242	F:CCCTGTAAATGCCAATGAATATTTTA R:CCCTGTTTATATTTTGAATTGTGTATGA	ATTC$_{(4)}$	160-172	59	10	0.6667	0.8650	0.614	0.0060	KX530220
DZC236	F:GGGTTTAGTTTAACGATTGAAAAGGG R:TTGAAAACAGTTATGGTGGTGATGA	TCC$_{(7)}$	130-138	59	10	0.5667	0.8073	0.682	0.1903	KX530221
DZC239	F:AGTAAATTTCCCTATTCGTGGGCT R:GAGCACAATACACAACAGGTCGTT	AC$_{(13)}$	116-138	59	21	0.8333	0.9367	0.826	0.0173	KX530222
DZC156	F:GTGATGGCACTCTGTTTTTCTACTTAAA R:TAGGGCATTCTCAAAGAGATGGAG	TC$_{(18)}$	122-154	59	8	0.9000	0.8006	0.850	0.0592	KX530223
DZC143	F:TGACTAAAACTCACGATCGAAAGAAC R:AGCATGCCACCGAATATCTTTTA	CA$_{(22)}$	134-172	59	13	0.7000	0.8977	0.899	0.0128	KX530224
DZC159	F:AGAAAACAACAACAGACGGACGAT R:CTGCAGTGTGGCAGCACTATATCT	ATAG$_{(12)}$	100-126	59	13	0.7667	0.8921	0.786	0.0002*	KX530225
DZC153	F:GGTATGCATCATACATCGGATACCT R:GCACCAGAACAACAAACAAATGAG	TG$_{(18)}$	130-176	59	13	0.8000	0.9068	0.928	0.0668	KX530226
DZC400	F:GAATACACATTCGTGGGTTGTTGA R:TAGAGGACAAGAGTGGTCTCCACC	AG$_{(6)}$	180-224	59	14	0.9667	0.9249	0.903	0.8156	KX530227
DZC116	F:TTTTTCGAATGCTATCACTGTTTCC R:AAGCCTATGCACAGTTTCTTTTGC	TA$_{(10)}$	112-134	57	10	0.9000	0.8599	0.769	0.9892	KX530228
DZC177	F:CACATAAAGCTTCCAAAATAGGGG R:GCAAAACGGTAAACGCTTTCATAC	AC$_{(8)}$	146-184	57	11	0.7667	0.8887	0.929	1.0000	KX530229
DZC163	F:CGTATCTTGTCTTGTGGAATACGTG R:AAAATGTTGCTAACCCATTTTTGC	AC$_{(19)}$	120-152	55	7	0.5000	0.7605	0.909	0.3534	KX530230
DZC234	F:CACGAAAAGACGATGTCGAAACTA R:TTTAAGGGGTGTGTCAAATTACGC	AG$_{(20)}$	128-150	60	13	0.8000	0.8989	0.817	0.1543	KX530231
DZC350	F:TGGTCTAAATGCATTGTTTTCTGTG R:CCGCACCAACTAGTCTTCTGACTT	AAAT$_{(5)}$	112-130	59	18	1.0000	0.9316	0.794	0.6834	KX530232
DZC240	F:GGGTACGGATCGTAACTGATCTCTC R:AATAAACGAAAAACCGAGTGCAAA	AC$_{(16)}$	110-138	63	8	0.6000	0.7588	0.879	0.0000*	KX530233
DZC395	F:CGAGTTATATGCATAATTTAGCTGTGC R:AATGCTCTCAACATTCAACTCACAA	AC$_{(7)}$	142-168	63	13	0.2667	0.9056	0.875	0.0153	KX530234
DZC283	F:CCTTCGTTTTCAAACACTTAGGAGA R:GAGTACACCGGATGATTAAATGGG	TG$_{(13)}$	106-128	61	12	0.7333	0.8644	0.882	0.1010	KX530235
DZC381	F:TTCGCCACTTTTCAATGACATCTA R:TTGGGACAAATGCACTCTATCATC	AG$_{(13)}$	114-140	63	9	0.7333	0.8407	0.866	0.0008	KX530236
DZC288	F:TAAAGCGCGTTCTAATGGTTCTTC R:AACAACACATTGCAACAATAACTGC	AGT$_{(10)}$	150-168	57	13	0.6000	0.9068	0.873	0.0000*	KX530237
DZC292	F:AAGTACATGCGTGGATTCAGAAGG R:GACAACGACGAATCGAATACCTG	CA$_{(18)}$	140-170	64	12	0.7000	0.8915	0.903	0.0500	KX530238
DZC328	F:CCGTTTAAAAATAAATCCAACGCA R:CAGGCAAGTAAAAATAGCTGGATGA	AC$_{(15)}$	130-160	55	4	0.1667	0.4164	0.902	0.6560	KX530239
DZC216	F:CTTCACACAGCACCATCAACATC R:CTGAGAATGTTGCTGAGGCATACT	ACA$_{(9)}$	130-134	60	6	0.3333	0.6017	0.792	0.0027	KX530240

Table 1. Continued.

Locus	Primer sequence (5'-3')	Repeat motif	Fragment size (bp)	Ta (°C)	N_A	H_O	H_E	PIC	P value	GenBank accession No.
DZC330	F:CCGTTTAAAAATAAATCCAACGCA R:CAGGCAAGTAAAAATAGCTGGATGA	ACAT(5)	128-188	55	2	0.5667	0.4130	0.868	0.7952	KX530241
DZC275	F:GGCATCAATATTTTGCTGAATAGC R:AGATTTAGGTTACACACATGAGAGAGG	CT(9)	140-160	55	3	0.9667	0.5407	0.845	0.0038	KX530242
DZC245	F:CCAATTTTCGAATGTTTGGATGAT R:TTAAAATTGGATGCATATCGAGGG	AC(16)	138-166	55	9	0.5667	0.5853	0.882	0.0000*	KX530243
DZC183	F:CATGCATTAAAGTACATATGGAAACAGA R:TTCGACATTGAGAAAGTGTTGTGA	AC(18)	122-148	54	4	0.1333	0.5435	0.828	0.8712	KX530244
DZC189	F:TTCCTCGCAATATGCCTAAAGATA R:TGGAAGAAGTCTATTATTCATACATGGG	AG(9)	148-182	54	5	0.3000	0.5073	0.861	0.0788	KX530245
DZC294	F:GTTTGTAGTGCTCCGACCCTGAAT R:TGGCCCCTACTCAAATTTGTTTTT	GGTA(4)	166-186	57	5	0.6333	0.6062	0.826	0.7881	KX530246
DZC263	F:CCTTGAGCTTGTCTGTCTCTTTCC R:AAAACTCTAATATGGCACCCCGTT	ATC(9)	132-160	56	2	0.3000	0.3045	0.736	0.0000*	KX530247
DZC308	F:TCTGGAATGTAGAACCAATATCTCTCA R:AAAAATAAGAAATGGAGAGTGGGC	TC(20)	194-228	57	8	0.7667	0.7085	0.893	0.0737	KX530248
DZC314	F:GTCCAAACCGAAGCAGAGGTATC R:GCATAAGATGCGAGGAACATTTTT	TTTA(4)	142-184	57	5	0.1667	0.7768	0.929	0.0153	KX530249
DZC445	F:TGATGGTACATCAGCGTAGAGCTAAA R:CAAGGGTTGGCAAAATTCAAGTAA	AG(21)	140-176	57	7	0.7000	0.7266	0.899	0.0062	KX530250
DZC323	F:ATTAATTTCTTCTGCTGCGTCCAA R:TATTGAGAAGGCAGAGAATGGAGC	TCC(6)	144-156	57	6	0.6000	0.7311	0.382	0.0101	KX530251
DZC279	F:CATCGTAAATGACACGATTTTGGA R:GCAATATGGTTGAAATATTGCCGT	TTG(5)	140-150	57	5	0.3000	0.4045	0.263	0.0004*	KX530252
DZC391	F:TTTCGTATGGTTTCTTGCACTTGA R:GTCTATGGGAACACCGGTTGTAAA	ATC(5)	140-156	57	6	0.2667	0.3305	0.711	0.0141	KX530253
DZC410	F:GGTCAAAGCAATGTACTGGCTACC R:TTTGCGAAATTAACAACTCAAAGACA	GTT(7)	106-154	56	6	0.3667	0.6198	0.742	0.0028	KX530254
DZC225	F:TAGCTGAATCAAATTCTGCCATGT R:ATTGGTTCCTGGAATTTTGTGAGT	GT(12)	134-176	52	6	0.6333	0.6463	0.856	0.0026	KX530255
DZC417	F:GGGGTTTGGCAATTACATTAAGAG R:TACTTCCCACGTCGTCTGTTATGA	ACC(4)	153-168	59	6	0.5000	0.5328	0.090	1.0000	KX530256
DZC397	F:AACGTTTTCCTCACTTGAAGTTGC R:AGTTTGTCCTCGCTTTACAGATCG	TG(16)	104-130	59	5	0.5333	0.6780	0.891	0.7227	KX530257
DZC398	F:GCTCATATGTTGCATTTTTGGACA R:CTTGCAGCATAGGTGCATTAAAAA	TG(7)	100-122	57	5	0.2667	0.4672	0.816	0.0022	KX530258
DZC435	F:CGTTACAACAGGGTCGTATCACTG R:AAAAGCTGATAAGGAAAGGCAAGG	TC(7)	140-166	59	4	0.4667	0.5249	0.809	0.0021	KX530259
DZC439	F:TGGTTCACCTTGATAACATTTCCA R:ACAGGGTCTGTCGAAGATCTGAAC	CT(8)	106-130	57	5	0.2667	0.7350	0.873	0.2359	KX530260
DZC446	F:AGACAGGTGTACGTTCCTTTCCTG R:AATGGAAGACAACTTCCGAACCTT	AC(14)	124-164	59	3	0.4667	0.5249	0.910	1.0000	KX530261
DZC337	F:AAGGGAATAGTTTGATGCATTTGG R:AGATTATCCAGTTTCGGTGGATTC	TTG(4)	160-178	58	4	0.4667	0.5390	0.719	0.0950	KX530262
DZC201	F:TGCAATGTACAAAGGAAAACAAAAA R:TAAAACCCAAACTAGAGCATTGGC	AAC(5)	118-150	58	2	0.4000	0.5085	0.881	0.0000*	KX530263
DZC126	F:AAGAAGGCTTATCTTCGCTCTTGA R:AGGCAGATAAATGAGCTAGTCGGT	TG(16)	100-114	58	7	0.2667	0.7921	0.645	1.0000	KX530264
DZC372	F:ATGCGTTTTCCTCTTCTATCTCGT R:AAAGTCAAGGTCATCAAAATGACATAC	TC(7)	136-160	60	8	0.2333	0.2780	0.806	0.9491	KX530265
DZC421	F:GATTCATGAAAAGCCATCCGATAC R:TCTTCGAAAAACAGACGAGCTAAAA	CA(16)	134-148	58	5	0.2333	0.4260	0.847	0.2115	KX530266
DZC169	F:ACAATGAATGCGTATAATGTAAACGTG R:AAAAATCGTAAGCTGGACTGTTGG	TC(15)	110-136	55	6	0.2667	0.4627	0.805	0.0008	KX530267
DZC425	F:TACAGCCTCTCTCATTCTCCCAAT R:AGACTTGACCTCGAAATTCAATGC	CT(6)	130-146	63	5	0.0667	0.4910	0.745	0.0018	KX530268
DZC345	F:GCGTTTAAAGGATACACATTTCGC R:ACGGTTAAATGAGCCATAACTCCA	AAT(10)	124-148	61	6	0.3667	0.7124	0.865	0.0031	KX530269
DZC247	F:GCGGGTGAGCAGACTAATGTTAAA R:GGCAATACCCTTTTGTCATGTGAT	TTGT(5)	140-146	59	11	0.6667	0.7531	0.346	0.1438	KX530270
DZC112	F:TAGCCAATGAAAGACTGCGTAATG R:TGACTGCACAAAATCCAGTGAAAT	TTG(7)	104-114	59	17	0.7000	0.8802	0.507	0.0453	KX530271
DZC333	F:CGGGTAAAACACTTATATCACTCGC R:CGTCCTTATTTGCTGTACTTTCTTCA	AGAT(17)	120-130	59	7	0.4333	0.5955	0.378	0.0005	KX530272
DZC246	F:TATATGGTCGATATGACGCAAAGC R:CCCACTCCCACTGAAACTTAACAT	CCAA(6)	102-118	59	17	0.7667	0.9181	0.367	0.0495	KX530273
DZC171	F:GAAACACGACGCCATTATAAATCTG R:TCTGGATCTTGAAATGTAGTAAATCCG	AC(15)	150-162	59	16	0.9333	0.9198	0.535	0.0005	KX530274
DZC191	F:GCAAAATATGCAAAATTATCGTGA R:CAATGTATTACTGAAGCAGCCAAG	TTG(17)	196-208	59	19	1.0000	0.9492	0.309	0.0318	KX530275
DZC202	F:TCTTCTGATGAAGTAACATTTGCG R:CATTTTGATCATTTTCGTACACACA	TGTC(5)	152-168	59	16	0.9000	0.9311	0.576	0.0134	KX530276
DZC162	F:TTAAATTGAAGTTTAGAAATACGGCACA R:GTCAGACAAGCTGGATGTCGAGT	AG(10)	130-136	59	13	0.6667	0.8740	0.324	1.0000	KX530277
DZC115	F:AGTTCTTGTCACTGCCCAATTTTA R:TCCACTTTATATTTACGTCATTGTGTTT	TC(11)	114-122	59	11	0.7667	0.8480	0.421	0.3994	KX530278
DZC140	F:TGAGTATCTTCAACCAACTCCATTCA R:GCAGTAAACCACCCAAAAGCATAA	TC(19)	108-126	59	9	0.8667	0.8305	0.570	0.0791	KX530279
DZC218	F:TCCAGGTGATACTGAAGCTATGGA R:GGCAGATGGTGCAGTAGAATGTTA	GAT(4)	156-170	60	14	0.8000	0.8927	0.745	0.0080	KX530280
DZC335	F:CTGCAGTGTGGCAGCACTATATCT R:CCATTACATAAGGTCTTGGGTTCAA	CAA(6)	128-134	59	13	0.5667	0.8932	0.375	0.6103	KX530281
DZC356	F:GAGGTCACTGTGAACATCACGTTT R:GGATTTCATTCGTGAAAAGTTAGGG	CA(15)	140-160	59	13	0.7333	0.9169	0.530	0.0773	KX530282
DZC429	F:GTGATTGCGACCGAGTCATATTG R:AGTCTGCTCGGAAGGAAAAAGAAA	GT(11)	140-146	59	8	0.5000	0.8299	0.611	0.0259	KX530283
DZC408	F:ACTTTTGTATGTCATGGTCTGCGA R:TGTGACTGACAGCAGAGGTACTCA	TAG(6)	138-150	59	14	0.9000	0.8847	0.449	0.0043	KX530284
DZC110	F:TATAAGGGCATGGGGCATAGTTTA R:AGATAATGAAATCATAATAGCAAATGCG	AG(12)	146-166	57	11	0.7333	0.8678	0.489	0.0016	KX530285
DZC152	F:TTATGGTGCTTTTTGTGGTGTTTG R:AAAACCATGCAGTGAGATGACCTT	TAA(5)	150-156	54	11	0.9333	0.8582	0.255	0.6735	KX530286

Table 1. Continued.

Locus	Primer sequence (5'-3')	Repeat motif	Fragment size (bp)	Ta (ºC)	N_A	H_O	H_E	PIC	P value	GenBank accession No.
DZC166	F:TTCAGCAACATCACAAGCAATACA R:CATCATCACCATTATCTGTGCCAT	$ATG_{(10)}$	150-168	54	11	0.4333	0.7678	0.694	0.1329	KX530287
DZC108	F:GAGCCACAAGGTCTTCCTTACAAA R:ACATTTTCAAAGCTAGGAAGTGGG	$TTG_{(7)}$	140-148	60	15	0.8667	0.9119	0.729	0.0010	KX530288
DZC192	F:AGACGATTTTCAGAAGTCAAACGC R:CAGGCTTGACGTCACATAACTTTG	$AT_{(10)}$	126-138	60	23	0.8667	0.9480	0.671	0.0362	KX530289
DZC119	F:TGGCATGGAGAGTTTTGAGTAATA R:CAGAAGCGCTTATCAATCTTCTTT	$TTG_{(23)}$	120-130	55	17	0.7667	0.9220	0.679	0.0190	KX530290
DZC120	F:GTTTGAAAAGAATTACAGGCAGCA R:ATGCATTACTTCATCGTTGAGCAG	$AC_{(13)}$	152-158	55	7	0.1667	0.4051	0.362	0.0054	KX530291
DZC117	F:CGATGAGTATGTTCACGATTGCAT R:TGCATGATGATAAATAATGCGGAC	$GA_{(12)}$	120-138	55	5	0.1667	0.2763	0.752	0.0140	KX530292
DZC204	F:CTGTCCCACCAATAACCAAACTCT R:ACATTTTACCAAGCTGGAAACGAA	$TCTG_{(4)}$	118-128	54	12	0.6667	0.8847	0.381	0.0058	KX530293
DZC227	F:ATGCTAAACAAGTCGAACCACCTT R:AAATATTGCCGAAGCGACTGTAAA	$TGACT_{(5)}$	100-114	54	2	0.1000	0.0966	0.258	0.0798	KX530294
DZC250	F:CTCATGCCACCATTAGTCATATCG R:ACATGAAGGAAGGAAGAGTACGGA	$TC_{(13)}$	130-144	60	13	0.9667	0.9119	0.538	0.0107	KX530295
DZC220	F:GGCAAAATACCGTTTAATGCGTAA R:TTAACAGGGGTAATTTGTTTTGCG	$TGTAT_{(5)}$	112-122	54	11	0.4667	0.8497	0.606	0.0746	KX530296
DZC211	F:TGCAAACATGTAAGCTGTCAAACTT R:TGCATGATATTTAATGGAAATTGGAA	$AAC_{(6)}$	142-154	54	8	0.6667	0.8446	0.495	0.0418	KX530297
DZC255	F:AAATATTCAGACGATGGAAAAAGGAA R:TTAACGCCGAAGGTATGTGAACTT	$AG_{(11)}$	100-108	60	7	1.0000	0.7051	0.617	0.0721	KX530298
DZC379	F:CGATCCTTTTGTCGACTGACTCTT R:TAAGGAAGGACAGAATCCCACATC	$TTC_{(8)}$	144-158	60	11	0.9333	0.8412	0.618	0.0005	KX530299
DZC371	F:AAACCCACCTTGGTCGATTTTAG R:GAGAAAGTGAGCCAATGTATTGATAGC	$TGT_{(5)}$	148-156	64	2	0.3333	0.4520	0.431	0.0191	KX530300
DZC302	F:AATCTGGATCAGACATCAGTCAAAA R:TGAATGTCAAACGCATAGTTTTAGC	$GT_{(13)}$	236-250	55	5	0.5000	0.5655	0.427	0.0094	KX530301
DZC309	F:AGGGATATGGTAATGATGGTTGGA R:GCAGCGGCAGCATAATGTATC	$TGA_{(14)}$	138-150	61	7	0.3667	0.3842	0.675	0.0000*	KX530302
DZC348	F:ACGTAGCCGCTTAAAAGTTATGCC R:TTCGGATCCGTAGCCTATTACGTT	$TG_{(11)}$	200-214	64	5	0.2667	0.3011	0.499	0.0000*	KX530303
DZC274	F:TGACGGAATAAGTGTGCTTGATGT R:AACCAATGAATCAACAACTCCCAG	$GAT_{(8)}$	148-156	55	7	0.6333	0.5910	0.399	0.3246	KX530304
DZC355	F:CCTCCTCGCCTCATTATCATTATC R:CAAGTATTGGATGATCCCCTCTGT	$TCA_{(23)}$	140-154	60	7	0.5667	0.7701	0.305	0.0204	KX530305
DZC276	F:TTTCTGTGTCACCTGTTTAAGGCA R:TTTGGAACATATGTGTCACCTGCT	$TCTG_{(8)}$	106-112	60	2	0.5000	0.5079	0.421	0.2671	KX530306
DZC431	F:AACAATCAATCATGCAGAAAGCAA R:TATTAGAATAGCGCCACCTCCAAC	$CG_{(7)}$	152-158	55	4	0.7667	0.5938	0.375	0.2036	KX530307
DZC167	F:TGTTACGCATATGTTTTGTTTCGC R:GAAATATGATGATGAGCACTTTGGG	$TATG_{(12)}$	138-150	54	4	0.4333	0.6785	0.748	0.0000*	KX530308
DZC260	F:GTGATGAGGATTTAATGGGTGGAG R:CAACATTTGATTCTGGTTCAGTGC	$CCT_{(4)}$	100-110	59	4	0.3333	0.3915	0.321	0.0374	KX530309
DZC360	F:TACGGTCCGCCATTAATTGATTT R:GAACGCAAAGATAACCATCAGAGC	$TGT_{(10)}$	250-268	57	9	0.7333	0.7944	0.765	0.0000*	KX530310
DZC420	F:CCACTGTAACCCAGTTTTTCAACA R:CATCTCCTGAATGGATTTTTAACCA	$TGTT_{(5)}$	130-146	57	6	0.4000	0.6712	0.235	0.1020	KX530311
DZC444	F:GGCGAAGTATACATATACACGACAAGG R:AGGCACTCAAAATGGAAATCAAAC	$GT_{(14)}$	196-216	57	5	0.3333	0.5350	0.707	0.0224	KX530312
DZC128	F:AACAACAATTTCAACTGCTTCACAAA R:CACGGTTTCACGGATATCTTCTACA	$TAT_{(6)}$	180-194	59	5	0.2000	0.3266	0.479	0.0579	KX530313
DZC132	F:CAGCGTCTGGAAATACGTTAATATG R:TGAATGTAGACTATGCCTGGTATTGA	$TGGA_{(6)}$	146-168	58	4	0.3000	0.3226	0.267	0.1154	KX530314
DZC312	F:AATCTTAAAATTTGCCGCCTTCTG R:TCAAATATTCAAGACGATATCCATGAAA	$TG_{(18)}$	158-170	58	9	0.3667	0.8028	0.535	0.0000*	KX530315
DZC107	F:CTGATCCGTCGAAAGGGTAAAGTA R:TGTTAAAAGATTATCCATTTCATCATGG	$GAT_{(8)}$	150-162	58	5	0.2000	0.2475	0.398	0.0026	KX530316
DZC396	F:CGGTGTACTTGTGGATTGCATAGA R:TGAAAGAGATGAACGCAAAAATGA	$ATTC_{(5)}$	150-168	55	7	0.5333	0.7588	0.423	0.0011	KX530317
DZC380	F:TGTCCGAGCTATTAACATTATTTCCA R:CTTTTCTGCGAAGTTGTTGGTTTT	$TGT_{(9)}$	130-148	57	6	0.4000	0.5141	0.460	0.2446	KX530318
DZC244	F:GGTACCGTCAAGGATGATATCGAG R:TTCAAAATGGGTCAATCAATGCTA	$TC_{(12)}$	116-126	55	5	0.1333	0.5898	0.417	0.2550	KX530319
DZC424	F:AACAGGTGGCCTATATCCTTCGTA R:TTCATGCCTTTTATACAGCGTTTG	$TA_{(8)}$	116-124	63	3	0.5000	0.5237	0.456	0.0162	KX530320
DZC127	F:ACTCTTTGCATTCAAGCTCCACAC R:CATTGGAGTCGGTGCTGATAATTT	$GT_{(12)}$	210-230	60	5	0.4333	0.4480	0.559	0.0000*	KX530321
DZC419	F:GCGGATTGAATCTCATTACACACA R:CGAACAGAAAAATCGTTTCTAGGC	$CT_{(10)}$	120-136	63	8	0.3667	0.5955	0.657	0.0000*	KX530322

Ta = annealing temperature; N_A = number of alleles per locus; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphism information content; *significant deviations from Hardy-Weinberg equilibrium (P < 0.05/105).

Compared to the traditional enrichment techniques and Sanger sequencing method, the development of 105 loci using the Illumina pair-end shotgun sequencing approach was a success. With 105 polymorphic microsatellite loci now developed and characterized in this

paper, fine-scale genetic diversity and structure studies, as well as selection and mapping studies will be possible for *S. grandis*, which contribute to make reasonable recovery and management plans guiding aquaculture programs.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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