

1 **Characterization of EST derived SSRs from the bay scallop, *Argopecten irradians***

2 Steven Roberts, Christina Romano, and Gabriele Gerlach

3 Marine Biological Laboratory, Woods Hole, MA 02543

4

5 Abstract

6 Interest in bay scallop conservation has resulted in organized stock enhancement
7 efforts and increased attention to fisheries management issues. Genetic markers can
8 facilitate the monitoring of enhancement efforts, characterization of wild populations, and
9 optimize hatchery practices. We have identified eight polymorphic simple sequence
10 repeat markers including one dinucleotide repeat, six trinucleotide repeats and one
11 compound dinucleotide repeat, in expressed sequence tags generated from multiple bay
12 scallop cDNA libraries. The numbers of alleles range from 2 – 5. The expected and
13 observed heterozygosities range from 0.093-0.720 and 0.095-0.600, respectively.

14

15 The bay scallop (*Argopecten irradians*) is found in the western North Atlantic
16 along the majority of the United States coast. The bay scallop is a hermaphroditic bivalve
17 mollusk with an enlarged, single adductor muscle that is able to quickly clasp both valves
18 together for propulsion. Commercial landings of bay scallops in the United States peaked
19 at over 1200 metric tons in 1982, corresponding to more than 11 million dollars (US) of
20 revenue (NMFS 2004). Over the past twenty years populations have experienced
21 significant declines that have been attributed to several factors including habitat loss and
22 pressure from commercial and recreational fisheries. One approach to enhance bay
23 scallop populations involves spawning wild broodstock in a hatchery and releasing

24 offspring into the wild (Arnold 2001). The aim of this work was to identify genetic
25 markers that could be used in evaluating stock enhancement efforts. These same markers
26 will also aid in characterizing population dynamics of native populations and assist
27 aquaculture operations efficiently manage stocks.

28 In order to identify genetic markers, bay scallop expressed sequence tags (ESTs)
29 were screened for simple sequence repeats (SSRs). This approach has been used
30 successfully across several taxa, and is prevalent in the field of plant genomics (i.e.
31 Kantety et al 2002). To date, we have generated and submitted 2089 cDNAs to NCBI's
32 EST database from the bay scallop (Roberts et al. unpublished, Roberts and Goetz 2003).
33 These ESTs are from developing larvae as well as from adductor muscle and gonad
34 tissue. Simple sequence repeats were identified using the Simple Sequence Identification
35 Tool (<http://www.gramene.org/db/searches/ssrtool>) (Temnykh et al. 2001). Primers
36 flanking SSRs with a minimum of 5 tandem repeats and 14 bps were designed using
37 Macvector 7.2 (Accelrys) or by visual inspection.

38 DNA was isolated from mantle tissue of bay scallops collected from Cape Cod,
39 MA utilizing a Chelex (BioRad) extraction technique (Walsh et al, 1991). The PCR was
40 carried out under the following conditions: initial denaturation for 3 min at 94°C followed
41 by 30-40 cycles of 1 min at 94°C, 1 min at 52-58 °C according to the specific primer set
42 used (Table 1) and 1 min at 72°C with a final extension step of 7 min at 72°C. PCRs
43 were carried out in a total volume of 18 µl containing 0.2 µM each of forward and
44 reverse primers, 0.2 µM dNTPs (Invitrogen), 1.8 µl 10X AmpliTaq Buffer containing 15
45 mM MgCl₂ (Applied Biosystems), 0.36 U AmpliTaq DNA Polymerase (Applied
46 Biosystems), and 0.8 µl DNA template.

47 Electrophoresis of the PCR fragments was performed using the SEA 2000TM
48 advanced submerged gel electrophoresis apparatus (Elchrom Scientific AG, Switzerland).
49 Products and a 10 bp DNA Ladder (Invitrogen) were separated on Spreadex EL-400 or
50 EL-600 S-100 gels (Elchrom Scientific AG, Switzerland) at 95-150 V and 998 mA for
51 30-45 minutes at 55°C; and visualized by staining with SYBR Gold. Alleles were scored
52 manually by visual inspection. The data were first analyzed using Micro-checker (van
53 Oosterhout, et al. 2004) for the presence of genotyping errors. Expected and observed
54 heterozygosities, and Fis values were calculated using FSTAT version 2.93 (Goudet
55 1995) (Table 1). Pairwise tests for linkage disequilibrium were also performed using
56 FSTAT. Sequential Bonferroni corrections were applied for all multiple tests.

57 Primers were designed for 29 SSRs, of which 8 were not easily amplified, 13
58 were monomorphic, and 8 were polymorphic. The eight polymorphic SSRs identified
59 include one dinucleotide repeat, six trinucleotide repeats and one compound dinucleotide
60 repeat. The numbers of alleles range from 2 – 5. The expected and observed
61 heterozygosities range from 0.093-0.720 and 0.095-0.600, respectively. The reduced
62 heterozygosity and departure from Hardy-Weinberg equilibrium at locus GL23, suggests
63 the presence of null alleles. Further, tests for null alleles using Micro-checker were only
64 significant at GL23. There is no linkage disequilibrium between any loci, including two
65 pairs of loci located within single ESTs (GP340:GP63 and C1831:C1832). The p-values
66 resulting from tests of linkage disequilibrium for G340-GP63 and C1831-C1832 are
67 0.21071 and 0.42857 respectively; the adjusted p-value is 0.001786. Using NCBI
68 BLASTn analysis, two sequences were identified in GenBank (accession numbers
69 AY485259 and AY496639) having homology with loci G340 and GP63.

70

71 Acknowledgements

72 This project was supported by National Research Initiative Competitive Grant no. 2003-
73 35206-12834 from the USDA Cooperative State Research, Education, and Extension
74 Service and by a grant from the County of Barnstable Massachusetts, USA (both to
75 SBR).

76

77 References

78 Arnold, WS. 2001 Bivalve enhancement and restoration strategies in Florida, U.S.A.
79 *Hydrobiologia*, 465(1-3): 7-19

80

81 Goudet J (1995) FSTAT version1.2: a computer program to calculate Fstatistics. *Journal*
82 *of Heredity*, 86: 485-486. Version 2.9.3 available from
83 <http://www.unil.ch/izea/software/fstat.html>.

84

85 Kantety RV, La Rota M, Matthews DE, Sorrells ME. (2002) Data mining for simple
86 sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and
87 wheat. *Plant Molecular Biology*. 48(5-6):501-510

88

89 National Marine Fisheries Service. 2004. Annual Commercial Landing Statistics.
90 [Online]. http://www.st.nmfs.gov/st1/commercial/landings/annual_landings.html

91

92 Roberts SB. and Goetz, F.W. 2003. Expressed sequence tags analysis of genes expressed
93 in the bay scallop, *Argopecten irradians*. (Short Report) Biological Bulletin, 205: 227-
94 228.

95

96 Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001)
97 Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L):
98 frequency, length variation, transposon associations, and genetic marker potential.
99 Genome Research, 11: 1441-1452.

100

101 van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker:
102 software for identifying and correcting genotyping errors in mirosatellite data. Molecular
103 Ecology Notes, 4: 535-538.

104

105 Walsh PS, Metzger DA, and Higuchi R. 1991. Chelex 100 as a medium for simple
106 extraction of DNA for PCR-based typing from forensic material. Biotechniques, 10: 506-
107 513.

Locus (Accession #)	Primers	T _{an}	SSR	Size (bp)	N _a	N _i	H _o	H _e	P _{HW}
M26 (CV660848)	F: CACTTTCAGCAGATATTCTTGAGG R: TCCCATCCTCTCCTTCACAG	55	(GAT) ₁₀	120	5	44	0.591	0.656	0.1938
GL23 (CV828452)	F: ATAAAACAGGCAAAGAGGCAC R: TGCTTGGTGAATGGGGC	55	(CA) ₁₁	132	5	20	0.25	0.649	0.0063
S336 (CN783139)	F: GCGGAGGCAGATTCTTTCTTTTC R: GGTCGTGGATTGTAAGCATTGTC	54	(CAG) ₅	128	4	23	0.478	0.608	0.1375
G340 (CN783297*)	F: CGCTTGTGTTTTACGAGGAGAAGG R: TGACGGGGTGTGATGTCTGACC	53	(GAT) ₅	114	5	47	0.575	0.642	0.1625
GP63 (CK484125*)	F: AACTTTTCCCTCATCGTGCACC R: CAGTCACAACATCAACCTGCC	54	(CAG) ₅	223	4	20	0.600	0.522	0.2250
N391 (CN782436)	F: TCATCGCCTCCACCTTCAG R: GATCACACTTTGATTTGCCTACG	58	(AG) ₁₄ A(AG) ₅	243	4	22	0.591	0.720	0.0938
C1831 (CK484157)	F: CGAGTATCAATAGCCGAATCTAAGC R: CCGTAGTTAGATCTCTGTTGGTAG	52	(GGC) ₅	122	2	21	0.095	0.093	0.9625
C1832 (CK484157)	F: CAGTTATGGATCAGGCGGTAGAAG R: GCGAGCGAGTACAACCTTAAAACAC	55	(GTG) ₆	122	2	23	0.217	0.198	0.7813

Table 1: EST-SSRs in the bay scallop (*Argopecten irradians*)

T_{an}, annealing temperature; N_a, number of alleles; N_i, number of individuals assayed; H_o, observed heterozygosity; H_e, unbiased expected heterozygosity; P_{HW}, probability that genotype proportions conform to Hardy-Weinberg equilibrium. The adjusted value for significance (5%) following Bonferroni corrections is 0.00625. Asterisk indicates that multiple bay scallop ESTs contain given loci. The accession number for the sequences each loci was originally characterized is listed.