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PRIMER NOTE

Characterization of SSRs from the American lobster, *Homarus americanus*

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Abstract

The American lobster (*Homarus americanus*), a commercially important benthic marine crustacean, is widely distributed along the continental shelf of the western North Atlantic. The population substructure of this species remains poorly understood despite its economic value. Informative markers are required to clarify relationships between local populations. To this end, we developed eight polymorphic short sequence repeats (SSR) for the American lobster, which were derived from expressed sequence tags. Additionally, we tested four SSRs previously identified for the Norway lobster (*Nephrops norvegicus* L.) for cross-species utility; only one of these showed polymorphism.

Keywords: American lobster, EST, Homarus, microsatellite, SSR

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Studies using enzyme, mitochondrial and nuclear DNA-based markers have thus far shown limited resolution between lobster populations on the East coast of the USA (Tam & Kornfield 1996; Harding *et al*. 1997; Jones *et al*. 2003). Recently, Crivello and collaborators (Crivello *et al*. 2005) used microsatellite markers to show small but significant genetic differentiation (up to 4%) between local populations of American lobster in Long Island Sound and the Hudson Canyon (200 km apart). Despite this finding, our overall understanding of the genetic substructure and recruitment processes of this major marine food resource remains incomplete. A more comprehensive understanding of American lobster population structure will provide a better foundation for sound resource management in the future.

Design of simple sequence repeats (SSR), also known as microsatellites, through data mining of sequences submitted to public expressed sequence tag (EST) databases has been widely used in the study of plant genomics (Scott *et al*. 2000) and more recently applied to animal systems (Roberts *et al*. 2005). Advantages of this method include the ease of identification of repeats, the absence of bias towards enriched repeats, and the ability to identify new quantitative trait loci. In this study, American lobster ESTs were downloaded from the National Center for Biotechnology Information (NCBI) and screened for SSRs. The ESTs characterized were from a normalized, multiple tissue cDNA library (Towle & Smith 2006). Repeats were identified using the simple sequence identification tool www.gramene.org/db/searches/ssrtool (Temnykh *et al*. 2001). Fifteen primer pairs flanking SSRs with a minimum of five tandem repeats and 15-bp length were designed using PRIMER3 software (Rozen & Skaletsky 1998). In addition, primers were tested for four SSRs previously identified in the related species, the Norway lobster (*Nephrops norvegicus* L) (Streiff *et al*. 2001).

Genomic DNA isolated from 29 Gulf of Maine individuals using a Chelex (Bio-Rad) extraction technique (Walsh *et al*. 1991) was tested for amplification and polymorphism at each locus. The polymerase chain reaction (PCR) was carried out according to the following specifications: initial denaturation for 8 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 1 min at 50–60 °C, dependent on the specific primer set used (Table 1), and 1 min at 72 °C with a final extension step of 10 min at 72 °C. The total reaction volume was 25 μL and contained 0.4 μM of both forward and reverse primer (IDT), 0.25 μM of each dNTP (Roche), 2.5 μL 10× RED *Taq* Polymerase Buffer (Sigma), 0.25 U Red *Taq*

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Table 1 Simple sequence repeats (SSR) in the American lobster (*Homarus americanus*)

Locus (Accession no.)	Primers	T_{an}	SSR	Size (bp)	N_a	N_i	H_O	H_E	F_{IS}	P_{HW}
LOBP1 (AF221991)	F: ACTGTCCGGTCAGGTTGAGA R: TCAGTCAAGAGATTGGGGAG	52	$(CT)_{30}$	148	4	28	0.500	0.487	–0.027	0.5167
LOBP2 (CN952278)	F: GACTATGGGTCACGGCGAAAG R: AAACGAGGTCTGTGTGGTGGG	60	$(GGA)_8$	153	5	29	0.759	0.777	0.025	0.4833
LOBP3 (CN949888)	F: AGGTAGGTGGTTATGCTGGGTTG R: CCAAGATAGTCAGGCAGTCAGTCA	55	$(CCT)_6$	111	4	28	0.286	0.314	0.091	0.4111
LOBP4 (CN952085)	F: GGGAGGAGATGATGATGTTGTGC R: CGAGGTAGCCAGTGAGGATGGTAA	50	$(GAG)_5$	131	2	26	0.385	0.483	0.206	0.2778
LOBP6 (CN951408)	F: GCTCTCAAAGCTTTTGCGTATT R: AAGAGCTGCGTAGGTATGTTGA	52	$(CA)_5AA(CA)_{10}$	149	6	28	0.893	0.797	–0.122	0.1167
LOBP7 (CN950936)	F: GAGGGTTCTTCCTCCTACGG R: ATGCCCCTTTCAAAATCACA	50	$(TG)_{11}$	161	3	27	0.148	0.352	0.583	0.0056
LOBP13 (CN853871)	F: GTAGTTCCTCGGTCCGTCAG R: CAGGAGGACCCATGAGAGAG	49	$(CCT)_5$	225	3	20	0.222	0.208	–0.071	0.8556
LOBP14 (CN853819)	F: CAGTAGGGCATTTGAGAGGTTTCTGTG R: TCTGAAATGGACGGCGGGTAGAAA	50	$(AT)_{22}$	373	3	20	0.895	0.607	–0.493	0.0056
LOBP15 (CN853494)	F: GCAGTTTCGGTTCGGTTATC R: TTGAAAAAGTTGACCTTTACACAAT	50	$(TG)_{12}$	226	3	20	0.647	0.540	–0.205	0.1778

T_{an}, annealing temperature; N_a, number of alleles; N_i, number of individuals assayed; H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; F_{IS} values; P_{HW}, probability that genotype proportions conform to Hardy–Weinberg equilibrium. The adjusted value for significance (5%) following Bonferroni corrections is 0.00556. Primers for locus LOBP1 were developed by Streiff *et al*. (2001) for *Nephrops norvegicus*.

DNA polymerase (Sigma) and 1.0 μL of chelexed DNA template. All products were electrophoresed on Spreadex EL-400 S-50 gels (Elchrom Scientific), using the SEA 2000™ advanced submerged gel electrophoresis apparatus (Elchrom Scientific). Gels were run at 120 V and 990 mA for 20–25 min and visualized by staining with SYBR gold. Alleles were scored visually. Expected and observed heterozygosities were calculated in GENETIX (Belkhir *et al*. 1997); F_{IS} values and linkage disequilibrium tests were calculated in FSTAT (Goudet 1995; Belkhir *et al*. 1997). MICRO-CHECKER was used to check for the possible presence of null alleles (van Oosterhout *et al*. 2004).

Eight polymorphic SSRs were identified from among the ESTs of the American lobster. A ninth polymorphic SSR for American lobster was identified from among four SSRs originally reported in the Norway lobster (AF221991). Of these polymorphic loci, four included trinucleotide repeats, four included uninterrupted dinucleotide repeats, and one was an interrupted dinucleotide repeat. The number of alleles ranged from two to six. Expected heterozygosities ranged from 0.21 to 0.80 while observed heterozygosities ranged from 0.15 to 0.89. The observed heterozygosities at loci LOBP7 and LOBP14 were significantly lower than expected, however, MICRO-CHECKER found possible evidence for null alleles at locus LOBP7 only. There is no significant linkage disequilibrium among any of the loci.

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