Isolation and characterization of eight new microsatellite loci in the Norway lobster, 

*Nephrops norvegicus* (Linnaeus, 1758)

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Abstract

Eight new highly polymorphic microsatellite loci were developed for the Norway lobster, *Nephrops norvegicus*, and were analyzed for variability in samples collected at Icelandic and Scottish fishing grounds. The number of alleles per locus ranged from 10 (PLH4) to 48 (PLH33 and PLH35), and the observed heterozygosity ranged from 0.738 (PLH4) to 0.963 (PLH15). All loci were in Hardy–Weinberg equilibrium and no genetic linkage disequilibrium was detected.
The Norway lobster, *Nephrops norvegicus*, is a benthic decapod crustacean species of high commercial importance. It is widespread on the continental shelf and upper slope in the North-Eastern Atlantic Ocean, to the South of Portugal, and the Mediterranean Sea (Farmer 1975).

In the last decades, different molecular markers have been used for stock discrimination of exploited marine species. For the Norway lobster, mtDNA analyzes, allozymes, and microsatellite loci have revealed no or a low level of genetic divergence between the studied populations (Stamatis *et al.* 2004; Stamatis *et al.* 2006; Streiff *et al.* 2001). It is not known if the lack of differentiation obtained in these studies, is due to the small number of markers employed, or their resolution power. Consequently, it is evident that new polymorphic markers are required to gain a better insight into the population structure of the Norway lobster. Here, we report the isolation and characterization of eight polymorphic microsatellite loci for Norway lobster, tested on geographically distant populations, i.e. from Scotland and Iceland.

Genomic DNA was isolated from muscle tissue of one Norway lobster individual using Puregene DNA isolation kit (Gentra). The microsatellite development was performed according to our recent publications by using a microsatellite enrichment method (GT) (Skirnisdottir *et al.* 2008), and PCR-based isolation of microsatellite arrays (PIMA) (Jakobsdottir *et al.* 2006; Lunt *et al.* 1999). Sequencing and primer design were according to Jakobsdottir *et al.* (2006), but approximately 1000 clones were sequenced. Primers were designed from flanking sequences for 65 loci but eight passed all quality steps. The characterization of the eight new loci was based on 271 individuals (*N* = 182 Iceland and *N* = 89 Scotland). DNA isolation and polymerase chain reactions (PCR) were performed according to Skirnisdottir *et al.* (2008), using 0.075-0.16 µL (100 µM) of the labelled forward
primers and the same amount of reverse primers fitted with a GTTTCTT PIG-tail (Brownstein et al. 1996). PCR reactions were performed on a Tetrad2 Peltier (Bio-Rad) thermal cycler as follows: initial denaturation step of 4 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 50 s at 55 °C, 58 °C or 60 °C, 50 s at 72 °C, and a final elongation step of 7 min at 72 °C (Table 1). PCR products were analyzed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ size standard, and genotyped with GeneMapper 4.0 (Applied Biosystems). Observed ($H_0$), and expected heterozygosity ($H_E$) were estimated in GENETIX 4.05 (Belkhir et al. 2004), and varied from 0.738 (PLH4) to 0.963 (PLH15), and from 0.774 (PLH4) to 0.964 (PLH35), respectively (Table 1). The number of alleles per locus across all samples, ranged from 10 (PLH4) to 48 (PLH33 and PLH35) (Table 1). Genetic linkage disequilibrium statistics were calculated for all pairs of loci using GENEPOP’007 (Rousset 2008) and did not yield any significant values. Hardy-Weinberg expectations (HWE) were calculated using GENEPOP, and neither sample deviated from HWE (Iceland = 0.570 and Scotland = 0.242). MICRO-CHECKER (van Oosterhout et al. 2004) did not detect any null alleles for the microsatellites developed. Because some microsatellite loci have recently been suggested to be under the influence of selection, selection tests for all loci using the $F_{ST}$ outlier methods (Beaumont & Nichols 1996) implemented in LOSITAN (Antao et al. 2008), were performed. A total of 95,000 simulations were used for the calculation of the neutral mean $F_{ST}$, using the 95% and 99% confidence intervals. None of the microsatellite loci were outliers at the 95% or at the 99% level.

The new polymorphic microsatellite loci show no evidence of linkage disequilibrium, and do not appear to be influenced by selection, supporting the assumption that they are inherited in a simple Mendelian manner. They are suitable for population genetics, genetic monitoring, and kinship studies.
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Table 1. Characteristics of eight novel microsatellite loci, based on 271 samples of Norway lobster (*Nephrops norvegicus*) collected in Icelandic and Scottish fishing grounds. Allele ranges (*A*), number of alleles (*n*A*), expected heterozygosities (*H*E*), observed heterozygosities (*H*O) and GenBank accession numbers (AccN) are provided.

<table>
<thead>
<tr>
<th>Name</th>
<th>Repeats motif</th>
<th>Annealing °C</th>
<th>Primer Pair (5'→3')</th>
<th><em>A</em></th>
<th><em>n</em>A</th>
<th><em>H</em>E</th>
<th><em>H</em>O</th>
<th>AccN</th>
</tr>
</thead>
</table>
| PLH4  | GAT           | 55           | F: TTGTACGGTACTTGTAGTGTAGG
R: ATGCTGATCCAATCATAAACAAG | 93-132 | 10   | 0.774 | 0.718 | GU559883 |
| PLH5  | GT            | 55           | F: CTATTACTCAAGATGCTTACG TG
R: CAAAGGCTTAAACAGGCTGCTG | 276-300 | 12   | 0.858 | 0.867 | GU559884 |
| PLH12 | GTCT          | 60           | F: GAGGCTGGTCATGCTGTC
R: TCAGTGACCATTTG CATAAAGGGT | 324-424 | 28   | 0.831 | 0.828 | GU559885 |
| PLH15 | GA            | 60           | F: CAATGGCAAGAAGTGAATTG C
R: CACGTCGTTGACTCATTC | 87-159 | 37   | 0.946 | 0.963 | GU559886 |
| PLH31 | GACA          | 58           | F: ATTTCTAATTAAGGCTGACAG
R: TAAACAGACAGTGCTCCTCCTC | 341-445 | 27   | 0.877 | 0.867 | GU559887 |
| PLH33 | CA            | 60           | F: GAATTCTTACGATGCTACAAGGG
R: TGATATACGTGCTACTGCTGG | 146-242 | 48   | 0.960 | 0.903 | GU559888 |
| PLH35 | AC            | 58           | F: TAGATTACCCAGGCGAGAA
R: GAGAGCTGGACCGTAACAC | 122-234 | 48   | 0.964 | 0.943 | GU559889 |
| PLH46 | CA            | 60           | F: CCGGTGGATGTGCTTATACG
R: CCCATCTTGGTGATATGCGG | 245-317 | 34   | 0.936 | 0.956 | GU559890 |