**ELECTRONIC SUPPLEMENTARY MATERIALS**

The following information accompanies the article:

**Isolation and characterization of microsatellite markers for the red alga *Porphyra umbilicalis***

Elena Varela-Álvarez (\*)1, Ana C. Balau1, Cristina Paulino1, Estibaliz Berecibar2, Gareth A. Pearson1 and Ester A. Serrão1

1CCMAR-CIMAR, Centro de Ciências do Mar, Laboratório Associado, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

2Task Group for the Extension of the Continental Shelf (EMEPC), Rua Costa Pinto 165, Paço de Arcos 2770-047, Apartado 1139, Portugal.

**EXPERIMENTAL: MICROSATELLITE LIBRARY DEVELOPMENT**

Four microsatellite enriched libraries were constructed using different probe mixes as in Varela-Álvarez *et al*. 2017. For Library 1, we used mix 4 in Glenn and Schable (2005): (AAAT)8(AACT)8(AAGT)8(ACAT)8(AGAT)8; for Library 2, a motif mix was created based using nucleotide repeats that were complementary repeats to those in other *Porphyra* spp. microsatellites: *P. haitanensis* (Zuo *et al.* 2007) and *P. yezoensis*, (Kong *et al.* 2009): (CT12, GT12 CA12 ATT12), (recently transferred to the genus *Pyropia,* Sutherland *et al.* 2011); for Library 3 all the motifs in library 1 and 2 were mixed. All these three libraries were constructed using the protocol described by Glenn and Schable (2005). For Library 4: 5´biotionylated and 3’CT15-23 dcc were used following the enrichment protocol by Billote *et al.* 1999. In detail, DNA was digested with restriction Enzyme *Rsa* I (New England Biolabs) and simultaneously ligated to double-stranded SuperSNX linkers (SuperSNX24 forward 5′-GTTTAAGGCCTAGCTAGCAGCAGAATC-3’ and SuperSNX24 reverse 5′-GATTCTGCTAGCTAGGCCTTAAACAAAA-3’). Linker-ligated DNA was denatured and hybridized to biotinylated microsatellite oligonucleotide mixes, which were then captured on magnetic streptavidin beads with biotinylated probes (Magne-sphere, Promega, Madison, WI). Unhybridized DNA was washed away and the remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the forward SuperSNX24 as a primer, and cloned into pGEM-T Easy vector (Promega, Madison, WI) and transformed into DH5a competent cells. A total of 792 positive clones were transferred to microplates containing LB/Ampicillin (100 μg/μL), incubated (4 h, 37°C), diluted in ultrapure water (Sigma), and heated (10 min) to provoke cell lysis. This solution was used as DNA template for PCR with standard SP6 and T7 primer amplification to detect clones with inserts. A total of 192 positive clones were isolated and sent to MACROGEN (Seoul, Korea) for sequencing. Sequences from both strands were assembled in Codon Code Aligner 3.7.1.