



## Short communication

## Magnetic beads, a particularly effective novel method for extraction of NGS-ready DNA from macroalgae

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## ABSTRACT

Next Generation Sequencing (NGS) technologies allow for the generation of robust information on the genetic diversity of organisms at the individual, species and higher taxon levels. Indeed, the number of single nucleotide polymorphisms (SNPs) detected using next-generation sequencing is order of magnitudes higher than SNPs and/or alleles detected using traditional barcoding or microsatellites. However, the amount and quality of DNA required for next-generation sequencing is greater as compared with PCR-based methods. Such high DNA amount and quality requirements can be a hindrance when working on species that are rich in polyphenols and polysaccharides, such as macroalgae (seaweeds). Various protocols, based on column-based DNA extraction kits and CTAB/Phenol:Chloroform, are available to tackle this issue. However, those protocols are usually costly and/or time-consuming and may not be reliable due to variations in the polyphenols/polysaccharide content between individuals of the same species. Here, we report the successful use of a magnetic-beads-based protocol for efficient, reliable, fast and simultaneous DNA extraction of several macroalgae species. DNA extracted from macroalgae using this method is of high quality and purity, allowing successful library preparation for next generation sequencing. We generated Genotype-By-Sequencing (GBS) data for 12 *Ulva* spp. (Sea Lettuce; Ulvophyceae, Ulvaceae) individuals and were able to generate a robust phylogenetic tree that we compared with traditional barcoding methods.

## 1. Introduction

In recent years, the widespread use of next-generation sequencing (NGS) technologies has led to the increasing use of restriction site-associated DNA sequencing (RAD-seq) for various genetic studies involving ecological assessment [1], genetic characterisation [2] and GWAS (Genome-Wide Association Study) [3] analyses. RAD-Seq is an umbrella term for techniques using DNA restriction enzymes and subsequent fragment size selection to sequence a reduced representation of the genome of interest [4]. The major advantage of RAD-Seq methods is the potential for multiplexing a large number of samples in a single library and/or sequencing lane, drastically reducing the cost of sequencing per sample [5].

Sanger sequencing methods such as barcoding [6] or microsatellite analysis [7], typically involving a simple PCR, require a very low DNA input (from picograms to a few nanograms), can work on partially degraded DNA, and the presence of contaminants such as polysaccharides and polyphenols can generally be mitigated by diluting the DNA 40–500 folds before performing the PCR [8,9]. RAD-Seq however,

requires a larger starting DNA input (50–500 ng of DNA in a relatively low volume, typically 50 µL), is sensitive to DNA degradation, and requires high-molecular-weight DNA free of contaminants to perform reliably [10]. Hence, obtaining high yields of high purity DNA represents a major challenge when extracting samples for RAD-Seq and more generally NGS-based sequencing. DNA extraction from macroalgal samples has proven difficult over the years, due to the presence of high amount of polysaccharides and phenolics [11,12] that can inhibit downstream enzymatic reactions [13]. To reduce contaminants co-purification together with DNA, various methods have been published. The most common ones involve the use of column-based DNA extraction kits (QIAGEN DNeasy, MASHREY-NAGEL NucleoSpin, MOBIO PowerPlant Pro), CTAB/Phenol:Chloroform, or a combination of both [9,11,12,14,15]. However, all of those methods have drawbacks. First, column based kits are expensive, and efforts to remove contaminating polysaccharides often lead to reduced yield. Second, CTAB-based and CTAB combined with kits are time consuming and involve the use of toxic chemicals such as phenol and chloroform. In addition, population-based genetics or genetic marker(s) discovery require DNA extractions

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from several hundred individuals, making the use of 96-well plate DNA extraction protocols a prerequisite for time-efficient extractions on a large number of samples in a minimal time.

Here, we report the use of a modified magnetic beads protocol (using MASHEREY-NAGEL NucleoMag Plant kit) for cheap, efficient and rapid purification of DNA from several macroalgal species. We found that magnetic beads produce the highest yield and purity, and are relatively cheap compared with column-based commercial kits. Moreover, the 96-well format allows for simultaneous purification of a large number of samples quickly. Then we used the DNA extracted from 12 strains of *Ulva* spp. to generate RAD-seq libraries, sequenced them, and used them to generate a phylogenetic tree that we compared to barcode sequencing-based phylogenetic tree.

## 2. Materials and methods

### 2.1. Algae materials

Intertidal laminar *Ulva* individuals were collected from several sites in Ireland (Supplementary Table 1), transported from sampling sites in a cooler box in bags filled with seawater. The Netherlands samples originate from the Eastern Scheldt and were shipped to Ireland in Falcon tubes filled with seawater. Upon arrival to the lab, all *Ulva* samples were rinsed with distilled water, blotted on tissue paper, and immediately frozen in liquid nitrogen. Prior to further use, samples were stored at  $-80^{\circ}\text{C}$ . Samples of the kelp *Saccharina latissima* (Phaeophyceae, Laminariaceae) meristem ( $\sim 3 \times 3$  cm in size), also collected throughout Ireland, were treated in the same way. The other macroalgae samples belong to 10 species, identified morphologically, and consist of six brown algae: three *Fucus* species (*Fucus vesiculosus*, *F. spiralis* and *F. serratus*); the commercially important (for biostimulant production) *Ascophyllum nodosum* [16], the kelp *Laminaria digitata* [17], and *Pelvetia canaliculata*. Four red algae species were also included: *Gracilaria gracilis*, important in food-grade agar production [18], *Phycodrys rubens*, a mixture of *Chondrus crispus* and *Mastocarpus stellatus*, producers of carrageenans [19], and the *Ascophyllum* epiphyte *Vertebrata lanosa* [20]. Fronds from several individuals from each species were harvested from a sheltered shore at the Claddagh, Galway (Supplementary Table 1), pooled and processed in the laboratory as described above. After freezing, all samples were freeze dried, and ground to a fine powder using a ball mill (QIAGEN TissueLyser II).

### 2.2. DNA extraction protocols

All *Ulva* spp. and *Saccharina latissima* DNA extractions were performed on sample aliquots of 7 mg dried powder and 200  $\mu\text{L}$  elution volume, to allow for comparison between DNA extraction methods.

DNA was extracted using NucleoMag Plant kit (744,400.1) following the manufacturer's instructions, with some modifications: the lysis of samples was performed for 2 h at  $56^{\circ}\text{C}$  with the addition of 20  $\mu\text{L}$  of 1 mg/mL of proteinase K (Sigma-Aldrich P6556) and 3  $\mu\text{L}$  of RNase A (provided). After lysis, samples were centrifugated for 15 min at  $4^{\circ}\text{C}$  (instead of room temperature), and the supernatant was used for the rest of the DNA extraction. The magnetic rack used was Invitrogen™ Magnetic Stand-96 (AM10027), and Sigma-Aldrich (BR701354-24EA) deep well plates.

QIAGEN DNeasy PowerPlant Pro (13400-50) and QIAGEN DNeasy Plant Mini Kit (69106) were used as per the manufacturer's instructions, with the addition of 20  $\mu\text{L}$  of 1 mg/mL of proteinase K at the lysis stage.

The CTAB method was adapted from [21]. Lysis was performed using 600  $\mu\text{L}$  of CTAB buffer (100 mM Tris-HCl pH 7.5, 50 mM EDTA pH 7.5, 2% CTAB, 2 M NaCl, 2% PVP and 1%  $\beta$ -Mercaptoethanol added just before use), 20  $\mu\text{L}$  of 1 mg/mL of proteinase K, 3  $\mu\text{L}$  RNase A, and incubated for 2 h at  $56^{\circ}\text{C}$ . After lysis, 200  $\mu\text{L}$  of 3 M Potassium Acetate pH 4.8 was added to the samples and incubated for 30 min on ice. After incubation, the samples were centrifugated at 13,000 g for 30 min at

$4^{\circ}\text{C}$ , and the supernatant (avoiding top white layer containing polysaccharides) extracted twice with an equal volume of Phenol:Chloroform:Isoamylalcohol (25:24:1), and once with an equal volume of Chloroform:Isoamylalcohol (24:1). DNA was precipitated with 1/10 volume Sodium Acetate pH 5.2 and 2.5 volumes 100% Ethanol for 20 min at  $-80^{\circ}\text{C}$ . The pellet was washed twice with 75% Ethanol and resuspended with 200  $\mu\text{L}$  of water. DNA yield and quality was assessed with UV absorbance using an IMPLEN Spectrophotometer.

### 2.3. PCR analysis and sanger sequencing

To test whether DNA extracted with magnetic beads, columns or CTAB was suitable for downstream applications, we used PCR to assess for the presence of inhibitors and whether the DNA is of sufficient quality and quantity. Most microsatellite or Sanger sequencing methods to investigate genetic diversity and/or phylogeny in macroalgae require a 1:50 to 1:500 fold dilution of the extracted DNA in order to obtain amplifiable DNA, due to the presence of various PCR inhibitors such as polysaccharides and polyphenols [8,9,22]. Hence, we performed PCRs on a dilution series of the DNA extracted from both macroalgae species, against the rubisco large subunit *rbcL* gene for *Ulva* spp. [9], and the microsatellite SLN510 for *Saccharina latissima* [7]. PCRs were performed in 25  $\mu\text{L}$  reaction volumes using MyTaq Red Mix (bioline), following the manufacturer's instructions, using 1  $\mu\text{L}$  of undiluted DNA, or DNA diluted 1:5, 1:10 and 1:100 folds with water. The PCR program consisted of  $95^{\circ}\text{C}$  for 3 min, followed by 35 cycles of  $95^{\circ}\text{C}$  for 30 s,  $58^{\circ}\text{C}$  for 30 s and  $72^{\circ}\text{C}$  for 30 s, and a final extension of 7 min at  $72^{\circ}\text{C}$ . The list of primers is available in Supplementary Table 2. For barcoding, PCR amplicons were sent to LGC Genomics GmbH (Germany) for Sanger sequencing using *rbcL* primers SHF1 and SHF4 [9].

### 2.4. RAD-Seq: genotyping by sequencing (GBS)

500 ng to 2  $\mu\text{g}$  of DNA extracted using magnetic beads from 12 *Ulva* individuals (6 individuals from Ireland and 6 individuals from the Netherlands) was sent to Novogene (Hong Kong) for GBS library construction and sequencing [23]. Libraries were constructed using MseI and EcoRI digestion, an insert size of  $\sim 300$  bp, and sequenced using Illumina HiSeq platform (150 bp paired end reads). After demultiplexing and adaptor trimming, paired-end reads were analysed using the pyrad toolkit [24], with de novo assembly method and 0.85 clustering threshold. After analysis, the phylogenetic trees were generated using RAxML [25], and visualised using Figtree (<http://tree.bio.ed.ac.uk/software/figtree/>). Clean reads (adaptors and barcodes removed) are available at Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) under the accession number SRP131215.

## 3. Results and discussion

### 3.1. Comparison of DNA extraction methods on *Ulva* spp. and *Saccharina latissima*

We selected two species of ecological and agronomical importance, the Sea Lettuce *Ulva* spp. and the kelp *Saccharina latissima*. Both species are cultivated [26–28], and efforts are ongoing to generate genetic markers and/or characterise the genetic diversity amongst these species, mostly in wild strains [7,14,29,30]. However, most of those studies rely on either Sanger sequencing or microsatellite markers to infer genetic diversity, and little to no genome-wide information is available on those species, possibly due to the difficulty of obtaining good quality DNA from macroalgae.

DNA from four individuals from laminar *Ulva* spp. and four individuals from *Saccharina latissima* was extracted using the four different protocols described in the method section. As shown in Fig. 1A, the NucleoMag protocol generated the highest yield, with an average of  $18 \text{ ng} \cdot \mu\text{L}^{-1}$  DNA, for both macroalgae species. The three other

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