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High throughput genomic DNA purification, PCR and fragments analysis reaction setup on microalgae using Macherey-Nagel Nucleospin 96 Plant Kit* and Beckman Coulter's Biomek® FX* Laboratory Automation Workstation equipped with ORCA® robotic arm

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ABSTRACT

The informations included in this paper describes the utilization of the liquid handler Biomek FX Laboratory Automated Workstation, equipped with ORCA® robotic arm, in a integrated fully automated protocol for the purification of genomic DNA from microalgae, (using Macherey Nagel Nucleospin 96 Plant Kit) PCR reactions and fragments analysis samples plates setup. Using this system, genomic DNA is purified by vacuum filtration, the protocol uses a combination of CTAB extraction and subsequent binding to a special silica membrane. The detergent CTAB (cetyl trimethyl ammonium bromide) is able to remove polysaccharides and other contaminants, while DNA remains in solution. The resulting clear lysate is mixed with a special buffer in order to adjust binding conditions for the NucleoSpin® Plant membrane. After loading this mixture onto the plate, contaminants are washed away with washing buffers. The genomic DNA can finally be eluted in a low salt elution buffer. A specific protocol was designed and validated in SAMI® software to use the kit on the automated core system. The following system components for the purification of genomic DNA, PCR and fragments analysis reactions setup will be described:

• The automated Core System workstation: Biomek FX & ORCA
• The software and method to drive the workstation

Representative fragments analysis data, obtained using as template the genomic DNA purified using this system, on a Beckman Coulter's CEQ 2000XL DNA Analysis System will be shown.

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INTRODUCTION

Methodology for genomic DNA extraction using Macherey Nagel Nucleospin 96 Plant reagents was developed for the Biomek FX, an automated liquid handler from Beckman Coulter Inc. equipped with ORCA Robotic arm, PCR machine and Plates Carousel, (Fig. 1). This platform, in our configuration, provides sufficient deck space to process one 96 wells samples plate for genomic DNA purification at a time, up to a maximum of twelve plates without user interaction after initial setup. Furthermore, the Biomek Software allows for user-defined variables to be included in the method. Using our settings, a 96-well plate can be processed in approximately 50 minutes using only the liquid handler; using the complete core system (BFX, ORCA and Carousel) two 96-well plates can be processed in approximately 95 minutes, four in 180 minutes up to 12 plates, unattended, in less then 530 minutes.
The results presented here demonstrate that the quantity and quality of genomic DNA purified in HT (High Throughput) using Macherey Nagel Nucleospin 96 Plant Kit reagents on the Biomek FX is suitable for PCR and fragments analysis reactions analyzed on the CEQ2000XL DNA Analysis System (Beckman Coulter).

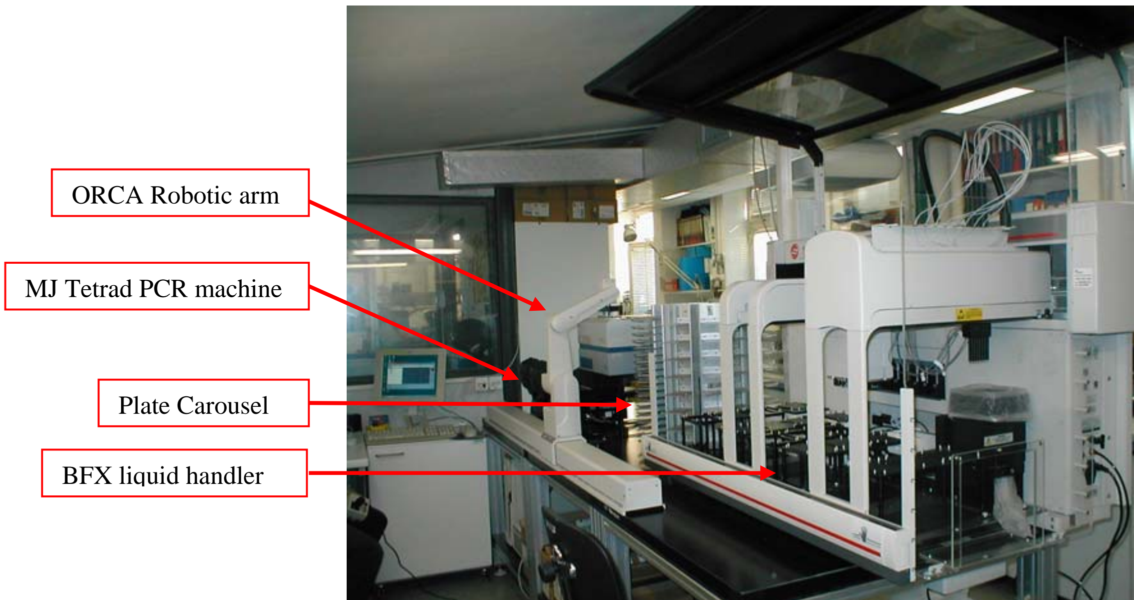

Fig. 1) The automated Core System workstation: Biomek FX, plates carousel, PCR & ORCA

MATERIALS AND METHODS

Samples (i. e. *Posidonia oceanica; Caulerpa racemosa*) was collected and immediately cleaned and stored (~2 cm of stolon) in eppendorf tube with silica gel. Before starting the extraction procedure samples are homogenized using a Mixer/Mills and then transferred in a new tube adding immediately the first extraction buffer according to the procedures described in the Kit's user manual. Genomic DNA was purified on a Biomek FX dual bridge (Fig. 1). Hardware needed on this platform includes a *gripper tool* and a *vacuum manifold*. Used labwares are removed from the deck of the instrument by ORCA robotic arm. The collar and manifold have been adapted to allow for elution into a standard microtiter plate. The Spacer Collar is used to stack the Plant Binding plate, the safe-spacer plate and, finally, the recovery plate (Fig. 5). After the samples have been homogenized, the DNA it's extracted with lysis buffers containing chaotropic salts, denaturing agents and detergents. The standard isolation ensures lysis of plant material with CTAB lysis buffers specially developed for plants. Lysis mixtures is cleared by filtration (300 sec. 20 InHg) in order to remove polysaccharides, contaminations and residual cellular debris. The clear supernatant is mixed with binding buffer and ethanol to create conditions for optimal binding to the silica membrane and loaded on binding plate that contains 96 independent columns. After washing with two different buffers, DNA can be eluted in low salt buffer and is ready-to-use for downstream applications (Fig 2a).

Fig. 2a) The genomic DNA extraction method on the Biomek FX platform

The *gripper tool* provides for automated disassembly and reassembly of the stack (Fig. 5). A random subset of genomic DNA extracted is gel controlled (gel electophoresys on a 1% agarose gel; TBE 0,5X with ethydium bromide) and then the PCR reactions are prepared, in automation, with 0.5/1 μl DNA; 0.2/0.5 [pmol/μl] each oligo; taq [0.075u/μl] in a total volume of 20μl. Some reactions are randomly gel controlled (gel electophoresys on a 1% agarose gel; TBE 0,5X with ethydium bromide) and opportunely combined in multiplex in the fragments analysis samples plate assembled, in automation, with: 0.4/1.0 μl PCR samples; 0.4 μl size standard and formammide up to a total volume of 20 μl (Fig 2b).

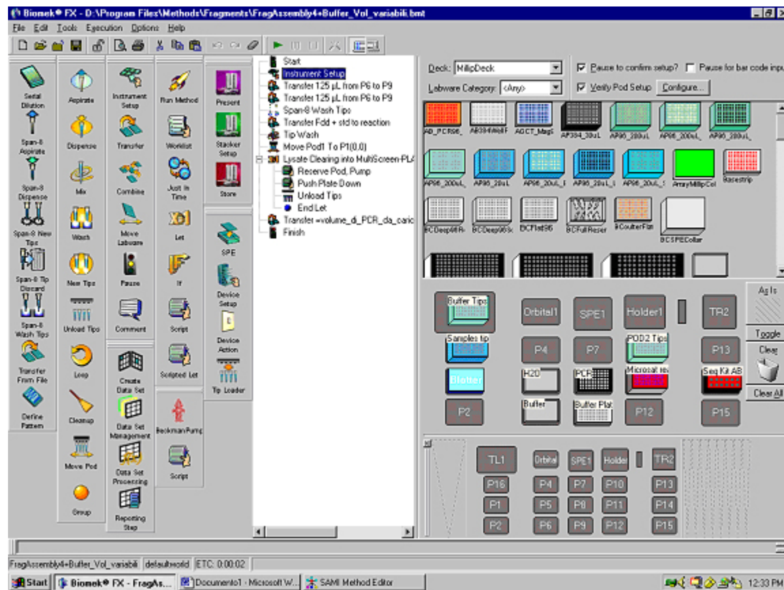

Fig. 2b) The fragment analysis plate setup method on the Biomek FX platform

The fragments analysis plates are analyzed on a Beckman Coulter CEQ2000XL DNA Analyzer. The Biomek FX protocol is edited and controlled via Biomek Software, a common software architecture among Beckman Coulter's Laboratory Automation Workstations. Software features include user-defined variables, log protocol control, and sample tracking. The whole integrated core system (BFX, ORCA and Carousel) is controlled via SAMI Software (Beckman Coulter) that allow us to build method to process up to 12 unattended plates. Example view of the Biomek method (Fig. 2a; 2b) and SAMI method (Fig. 3) are shown:

Fig. 3) The genomic DNA extraction, PCR reactions and fragments analysis plate setup protocol, integrated on SAMI platform

The genomic DNA extraction method on the Biomek FX purify a single 96-well plate of samples at a time with a final elution volume of 100µL. The method has been optimized to purify genomic DNA ready to use in PCR and fragments analysis reactions. Steps such as binding condition setup, wash and vacuum times have been optimized to deliver consistent results without contamination. The starting deck instrument setup for genomic DNA purifications is shown below (Fig. 4). A vacuum filtration manifold is fixed to the deck on the SPE site; using the *gripper tool*, the holder is accessed for the assembly and disassembly of the collar and plates (Fig. 5).

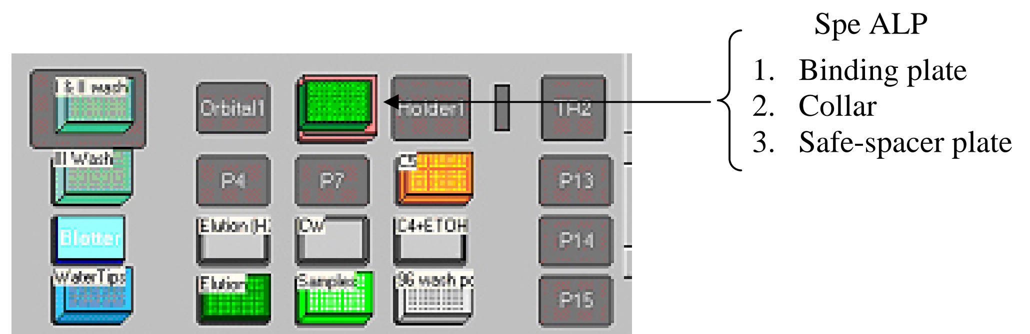

Fig. 4) The starting instrument setup for genomic DNA extraction method on Biomek FX

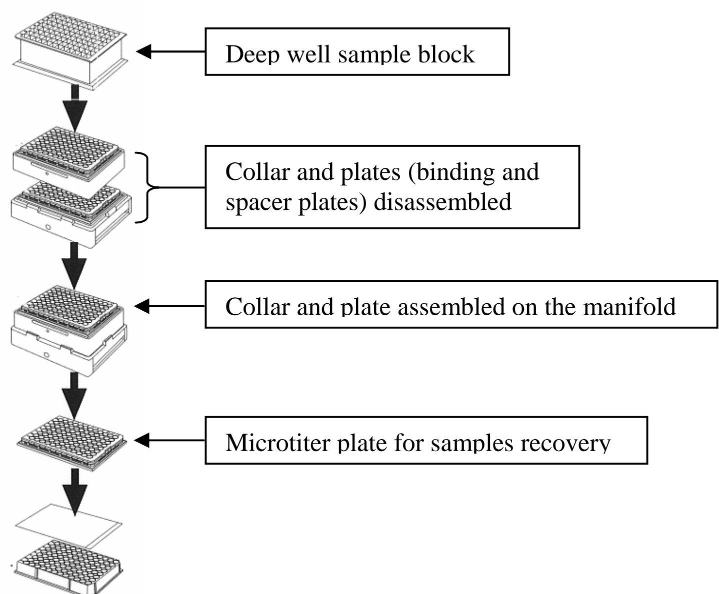

Fig. 5) Schematic view of the plates used in the extraction protocol and the assembly scheme of collar and plates.

RESULTS

Quantity and quality of recovered genomic DNA were determined by gel electophoresys and PCR.

<u>*Gel electophoresys.*</u>

Quantity and quality of recovered genomic DNA was tested by gel electophoresys. On a 1% agarose gel (TBA 0,5X with ethydium bromide) 5µl of recovered DNA was loaded using λII as marker. A gel picture is shown below. (Fig.6)

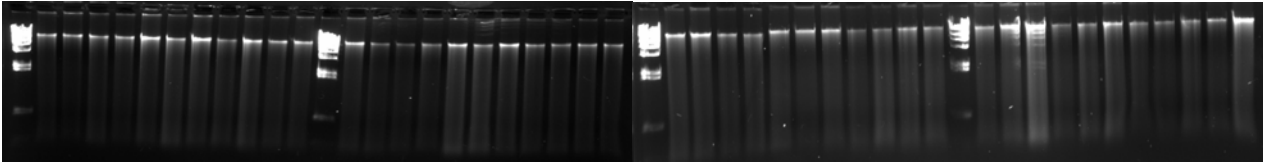

Fig. 6) Gel electophoresys of 5µl genomic DNA extracted

<u>*PCR assay to detect cross contamination*</u>

Starting 96 deep wells sample block were assembled sorting algal samples and negative controls (same volume of water) into adjacent wells such that cross-contamination could be assessed using a PCR-based assay. Samples obtained were used in a PCR amplification reaction. All algal samples were easily amplified and no product was observed in wells originally loaded with water, indicating that contamination of adjacent wells did not occur during processing (Fig. 7).

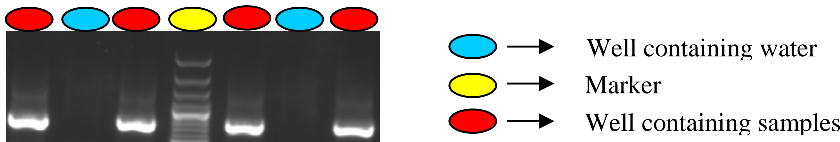

Fig. 7) Agarose electophoresys of PCR product from samples test deep wells block (algal samples sorted with negative control)

Fragments Analysis

Fragments analysis samples, assembled as previously described, are successfully analyzed on a Beckman Coulter's CEQ 2000XL DNA Analysis System (Fig. 8). The average of reaction automatically analyzed is ~85% calculated over a samples data-set of 1920 multiplex reactions.

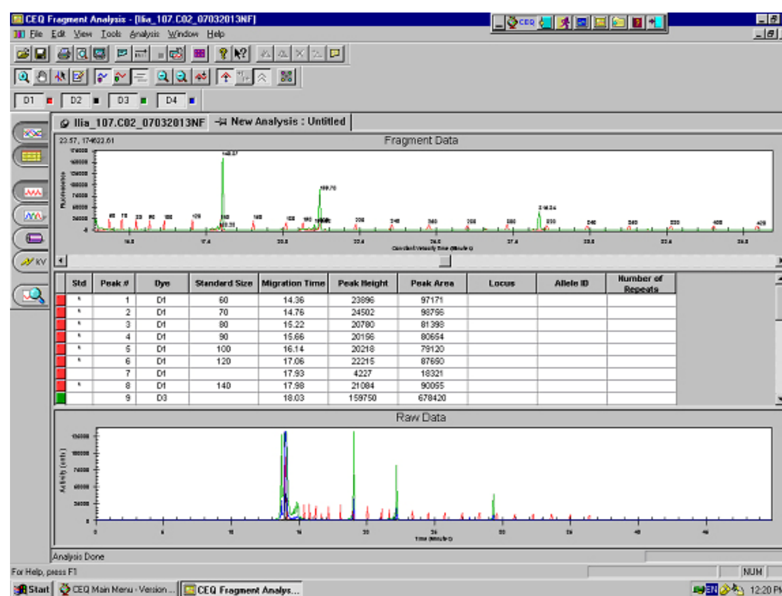

Fig. 8) Fragments analysis reaction result on Beckman Coulter's CEQ 2000XL DNA Analysis System.

CONCLUSIONS

The data obtained demonstrates that the Biomek FX Laboratory Automated Workstation, equipped with ORCA® robotic arm is a suitable platform for the high throughput genomic DNA extraction from vegetal samples using Macherey Nagel Nucleospin 96 Plant Kit reagents, setup of PCR reactions and assembly of fragments analysis samples plates. The three steps are combined in a single, fully automated, protocol.

The method and system described here:

- Generated high quality genomic DNA suitable for downstream application like PCR and fragments analysis
- Are able to setup PCR reactions sorting different oligo pairs and different DNA templates.
- Are able to assembly fragments analysis samples plates combining different PCR products.
- Are able to manage high number of samples in complete, but flexible, automation and generate a complete samples tracking from the sources plates to the fragments analysis samples plates.

Furthermore the introduced automated workstation is extremely flexible allowing us to minimize the costs and to adjust the protocols just "on-demands", making the automated solution accessible for many different research groups.

Acknowledgments
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