DNA Extraction Methods for Macroalgal *Ulva* Species

Shennel Gelin, Kristen Dostie, Matthew Bresemen, Amy Battocletti, Carol Thornber, J.D. Swanson
Department of Biology and Biomedical Sciences, Salve Regina University, Newport, RI & *Department of Biological Science*, University of Rhode Island, Kingston, RI

Abstract

Macroalgal *Ulva* is a bloom-forming algae that can create negative environmental and economical impacts in the Narragansett Bay, RI. In order to better understand these harmful environmental impacts, we intend to use microsatellite markers to DNA fingerprint different *Ulva* clones and determine the genetic structure of *Ulva* populations. To determine the best method to extract DNA from Ulva cells we investigated different methods of disrupting cell walls. One method involved grinding the sample with a mortar and pestle using liquid nitrogen and the other method involved grinding the sample using a PCT shredder. Using both disruption methods, two different extraction processes were tested and compared to determine which process yielded a higher concentration and purer quality of DNA. The first method tested was a QIAGEN DNeasy Plant Mini Kit in which a step-by-step protocol was followed to isolate DNA from the samples. The second method that was used was a phenol/chloroform extraction which uses CTAB buffer. After testing both methods of disruption and both methods of extraction, we checked the samples on a 1% agarose gel in 1X SB buffer in order to compare the bands and see which methods were more efficient. In addition the DNA samples from both extraction methods were quantified using a spectrophotometer. It was found that both disruption methods produced similar yields and the phenol/chloroform method yielded higher concentrations of DNA. We expect to extract DNA from about 480 samples by the end of summer and use the DNA to identify microsatellite regions within the genome.

Methods

Field Collection:
Biweekly field collections were conducted at three sites in Narragansett Bay: Sandy Point, Oakland Beach and Chepiwanoxet. 40 samples from each site were collected from both sub tidal and intertidal regions and brought to University of Rhode Island for species identification and to Salve Regina University for DNA Extraction. Once the sample arrived at Salve, they were flash-frozen with liquid Nitrogen and kept in a -80 ºC freezer. After the samples were identified, DNA was extracted from the samples of *U.rigida* and *U.compressa*. The DNA was extracted comparing two methods of tissue disruption and two methods of extraction.

Figure 1: *Ulva rigida*

Figure 2: *Ulva compressa*

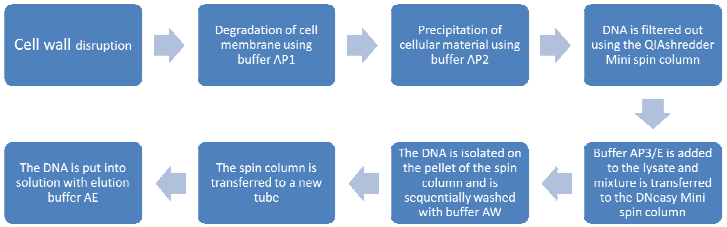
Tissue Disruption Methods:
Mortar and Pestle
Initially the mortar and pestle are chilled with liquid nitrogen. The frozen sample is added to the mortar and liquid nitrogen is poured over it. Next, the sample is crushed to a fine powder using the pestle and is scraped into a microcentrifuge tube filled with extraction buffer
PCT Shredder
First, the DNA sample is inserted into the shredder Pulse Tube and a buffer is added to the tube. The tube is then capped and placed into a holder. Next, the PCT Shredder drill is fitted onto the cap and the drill grinds the sample into the buffer and the homogenate is poured into a microcentrifuge tube.

Figure 3: Mortar and Pestle

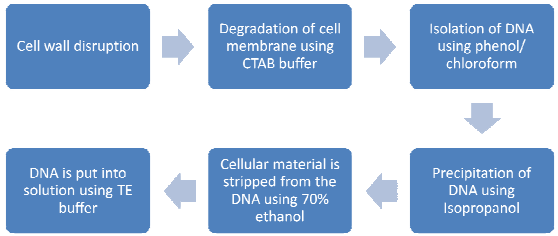
vs.

Figure 4: PCT Shredder

QIAGEN DNeasy Plant Mini Kit:



Phenol/ Chloroform Extraction:



DNA Analysis:
The analysis of the DNA extractions involved running a 1% agarose gel in 1xSB buffer. A standard 1KB plus DNA ladder was used as a reference to estimate the size of the DNA. The DNA samples were also quantified and their purity was measured using a spectrophotometer.

Results

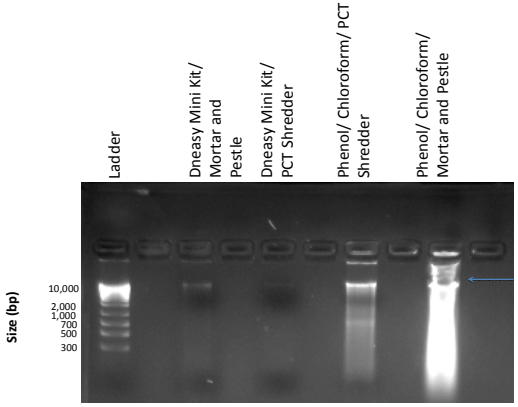


Figure 5: DNA sample run on a 1% agarose gel at 250 volts for 10 minutes.

Spectrophotometer Readings

	Concentration (ng/µL)				Purity (260/280)			
	DNeasy Mini Kit	S.D	Phenol/ Chloroform	S.D	DNeasy Mini Kit	S.D	Phenol/ Chloroform	S.D
Mortar & Pestle	41.6	45.0	333	403	2.36	0.416	1.82	0.303
PCT Shredder	28.2	24.4	211	228	2.33	0.534	1.76	0.226

Figure 6: The average spectrophotometer readings of DNA samples using all four methods of extraction.

DNeasy Plant Mini Kit Spectra

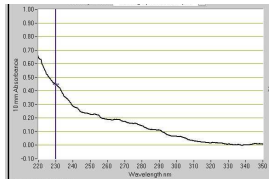


Figure 7: Absorption spectra of DNA sample extracted using the DNeasy Plant Mini Kit

Phenol/ Chloroform Spectra

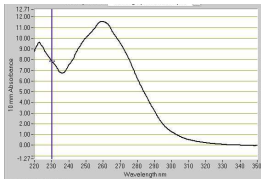


Figure 8: Absorption spectra of DNA sample extracted using the Phenol/ Chloroform method.

Discussion

When comparing the two disruption methods, the gel electrophoresis analysis, showed bands of similar intensity. Also, the spectrophotometer analysis showed that there were very little differences between the concentration and purity of DNA using both methods. However, when comparing the two extraction methods there were significant differences between them. In the gel electrophoresis analysis, the DNA bands produced by the phenol/ chloroform extraction appeared brighter than the DNA bands produced by the DNeasy Plant Mini Kit extraction. Also, the spectrophotometer showed that the phenol/chloroform extraction method yielded a higher concentration and purer quality of DNA than the DNeasy Plant Mini Kit method.

From the results, it was concluded that the best method of extraction was using the phenol/ chloroform method with either the mortar and pestle or the PCT Shredder for the cell wall disruption step. This method yielded a higher concentration of DNA and is more cost effective than using the DNeasy Plant Mini kit. Also, between both disruption methods the mortar and pestle was cheaper, but using the PCT Shredder lowered the risk of contamination by previous DNA samples because new tubes were used for each samples and the mortar and pestle had to be sterilized between samples.

References

Thornber, C., & Swanson, J.-D. (2011). STAC Proposal. University of Rhode Island.

Acknowledgements

The authors would like to thank RI STAC for funding this project as well as our collaborators Dr. Carol Thornber and students at the University of Rhode Island