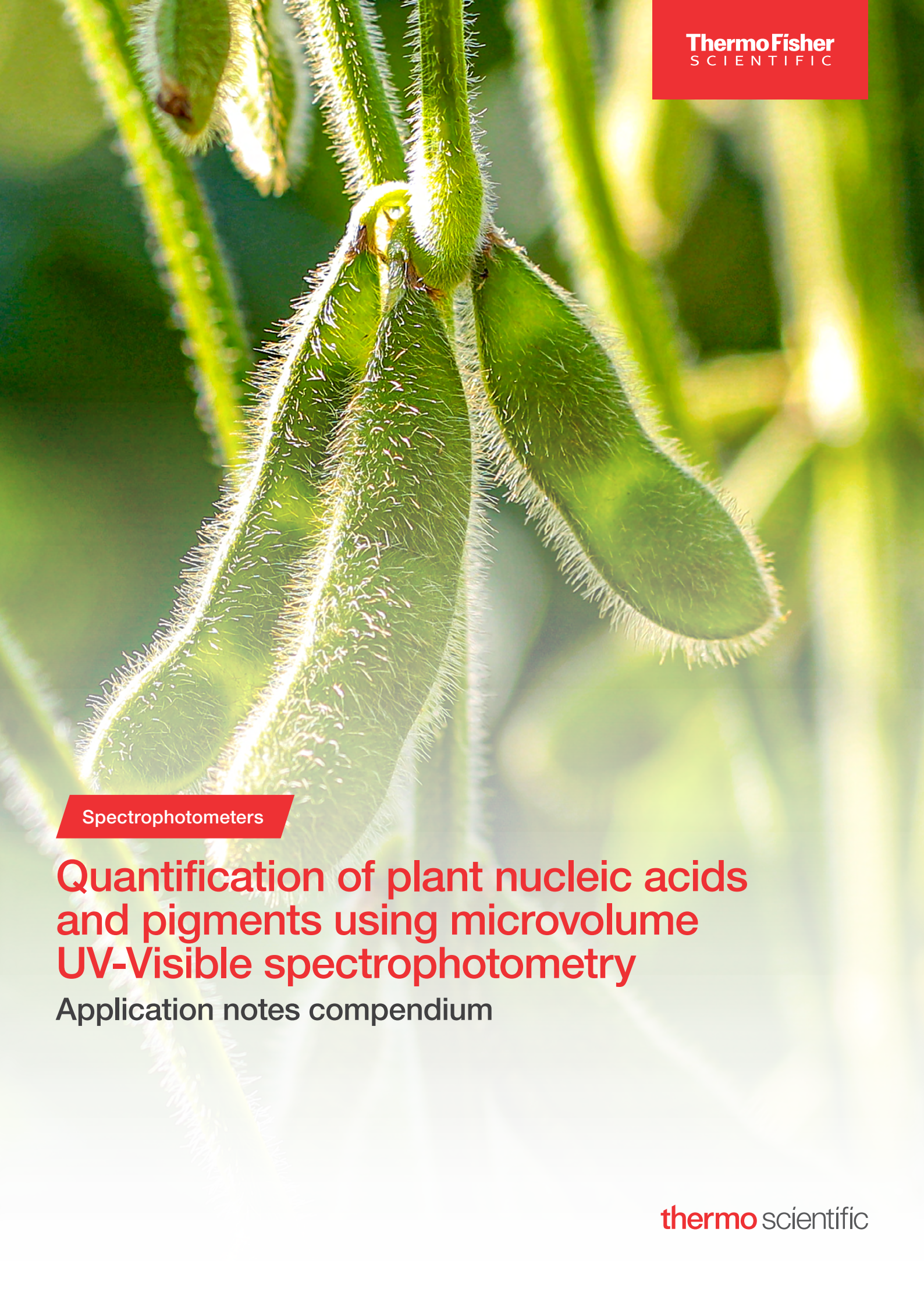

ThermoFisher SCIENTIFIC

Spectrophotometers

Quantification of plant nucleic acids and pigments using microvolume UV-Visible spectrophotometry

Application notes compendium

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Introduction

Spectrophotometric measurement of nucleic acids is common, both to assess purity and to quantify DNA extracted from plant matter. Spectrophotometry can also be used to measure the absorbance of light by plants' chlorophyll molecules. In this compendium of application notes, we provide examples of how the Themo Scientific™ NanoDrop™ One/One^{C} and Thermo Scientific™ NanoDrop™ Eight UV-Visible Spectrophotometers can be used to quantify nucleic acids and pigments extracted from plant sources as diverse as soybeans, cucumbers, spinach and macroalgae.

Researchers who wish to quantify the amounts of chlorophyll a and chlorophyll b within plants will find that the NanoDrop One/One^{C} spectrophotometer can utilize a user-defined custom method to measure the pigments. This could be beneficial to fields ranging from plant biology to environmental science or disease prevention and medical drug discovery.

Studies that deal with topics of social and environmental importance, like GMOs in the food supply or the ability of seaweed to adapt to ocean warming and acidification, are also included herein. NanoDrop instruments were used to analyze extracted soybean DNA to assess the nucleic acid purity and identify any contaminants present prior to GMO testing with qPCR. Microvolume analysis also was able to determine the concentration and purity of macroalgae dsDNA with accuracy.

In a study of how extraction buffer composition affects the polysaccharide content of the purified nucleic acids, microvolume analysis was able to show that contamination levels are dependent on both the plant species used and the salt content of the extraction buffer.

Microvolume UV-Vis analysis provided by NanoDrop spectrophotometers is a useful technique for obtaining accurate concentration and purity data about plant-derived nucleic acids and pigments, as the four studies contained in this compendium demonstrate.

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NanoDrop One/One^{C} Microvolume UV-Vis Spectrophotometer.

NanoDrop Eight Microvolume UV-Vis Spectrophotometer.

Quantify chlorophyll *a* and chlorophyll *b* with a custom method

Using the NanoDrop One Spectrophotometer

Abstract

Scientists can accurately quantify chlorophyll *a* and chlorophyll *b* on the Thermo Scientific™ NanoDrop™ One/One^{C} Microvolume UV-Vis Spectrophotometer using a user-defined custom method.

Introduction

Chlorophyll *a* is the principal pigment that converts light energy to chemical energy, and chlorophyll *b* is the accessory photosynthetic pigment that transfers light it absorbs to chlorophyll *a*. Chlorophyll *a* is found in all plants, green algae, and cyanobacteria, and chlorophyll *b* is found in plants and green algae. Chlorophyll quantitation is valuable in a vast array of disciplines including but not limited to plant biology, environmental science, ecotoxicology, disease prevention, and medical drug discovery.

Spectrophotometry is a common method used to measure the absorbance of light by the chlorophyll molecules. The NanoDrop One/One^{C} UV-Vis Spectrophotometer can be used to measure the absorbance of chlorophyll. Chlorophyll *a* and chlorophyll *b* absorb light at slightly different wavelengths. Chlorophyll *a* absorbs light at 433 nm and 666 nm and chlorophyll *b* absorbs light at 462 nm and 650 nm. The NanoDrop One/One^{C} UV-Vis application can be used to observe the spectrum of each chlorophyll *a* and chlorophyll *b* and identify major absorbance peaks (Figure 1). With this information, a user-defined custom method including user-defined formulas can be created to measure the absorbance and determine the concentration of chlorophyll.

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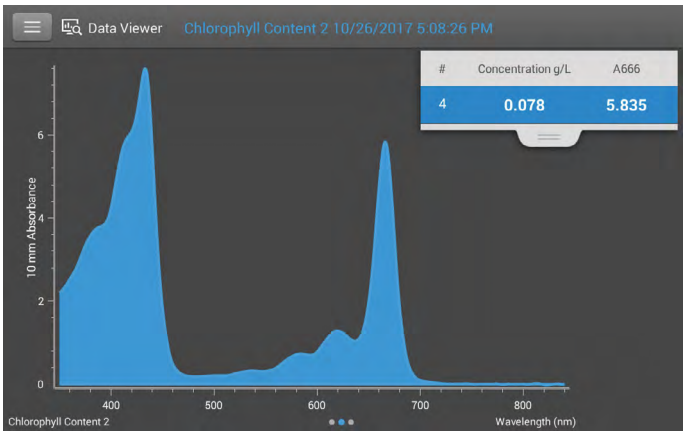
chlorophyll *a*



chlorophyll *b*

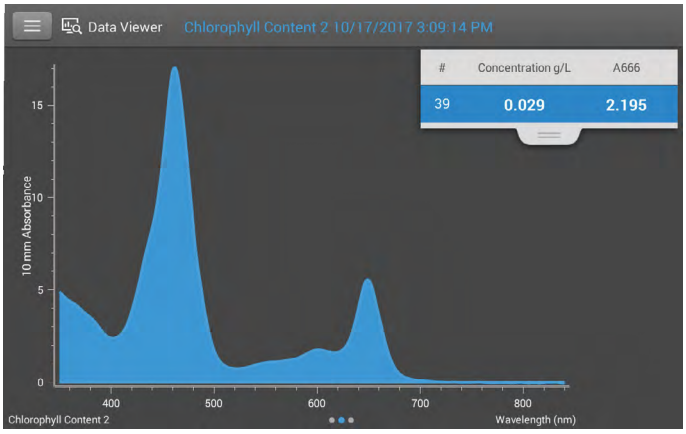


Figure 1. Absorbance spectrum of chlorophyll *a* and chlorophyll *b*. Chlorophyll *a* displayed major absorbance peaks at 433 nm and 666 nm (top). Chlorophyll *b* displayed major absorbance peaks at 462 nm and 650 nm (bottom).

Methods

Sample Preparation

Various solvents can be used to extract chlorophyll *a* and chlorophyll *b*. Porra et al. (1989) discuss assays of chlorophyll *a* and chlorophyll *b* suspended in various solvents including dimethylformamide, methanol, and 80% aqueous acetone. Barnes et al. (1991) describe the use of dimethyl sulfoxide (DMSO) for the extraction and determination of chlorophyll *a* and chlorophyll *b*. Based on the findings of Barnes et al., and the low toxicity and low vapor pressure of DMSO, we determined DMSO was an appropriate solvent for chlorophyll *a* and chlorophyll *b* measurements on the NanoDrop One/One^C pedestal.

Pure chlorophyll *a* from spinach (Sigma-Aldrich® Product # C5753) and pure chlorophyll *b* from spinach (Sigma-Aldrich Product # C5878) were each dissolved in 100% DMSO. Half-fold serial dilutions for each suspension were prepared using DMSO. The samples were stored in amber tubes at –20°C until measured.

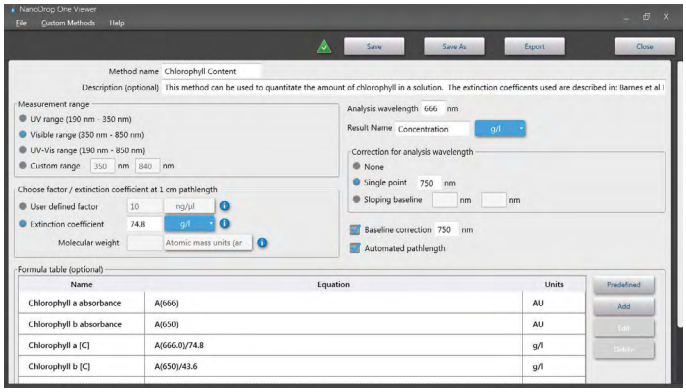


Figure 2. Chlorophyll Content custom method created to quantify chlorophyll *a* and chlorophyll *b* samples suspended in 100% DMSO.

Chlorophyll Quantification Custom Method

The NanoDrop One/One^C PC Viewer Custom Method application allows a user to specify how to calculate and report results. The Custom Method application was used to create a user-defined custom method to quantify chlorophyll *a* and chlorophyll *b*.

For this study, the following custom method parameters were used:

Wavelength range	Visible (350–850 nm)
Extinction coefficient	74.8 g/L
Analysis wavelength	666 nm
Correction for analysis wavelength	750 nm
Baseline correction	750 nm
Automated pathlength	On

Custom formulas were entered in the Formula table to report the absorbance at the analysis wavelength for chlorophyll *a* and chlorophyll *b*, and to calculate the concentration of pure chlorophyll *a* and pure chlorophyll *b*. Additional formulas were added to report the concentration of chlorophyll *a*, chlorophyll *b*, and chlorophyll *a*+*b* in samples containing a mixture of chlorophyll *a* and chlorophyll *b*. The formulas were taken from Barnes et al. (1991) and the peak locations were determined in the UV-Vis application (Figure 1).

The custom method was loaded in the Custom Method application on the NanoDrop One/One^C local control. The custom method was run to measure each chlorophyll *a* and chlorophyll *b* serial dilution in triplicate.

Note: For this study, the NanoDrop One/One^C UV-Vis application was used to identify the major peaks of chlorophyll *a* and chlorophyll *b* to determine the analysis

wavelength. If you are using a solvent other than DMSO, we recommend using the UV-Vis module to identify the maximum peaks for your samples, and modifying the custom method accordingly.

Custom Method Download

1. Navigate to www.thermofisher.com/nanodrop
2. On the left, select "NanoDrop Software Download"
3. Choose the "NanoDrop One/One^{C}" tab
4. Select "Local Control Software Download Instructions"
5. Scroll to "How to add a NanoDrop One/One^{C} Custom Method file" and click on "Chlorophyll Content Method"
6. Unzip the custom method file and copy the .method file to a USB device and then follow the online "Instructions for uploading a Custom Method to the instrument from a USB device".

Performance Data

Each chlorophyll *a* and chlorophyll *b* dilution was measured using the user-defined custom method described above. Each sample was measured in triplicate to assess reproducibility (Tables 1 and 2). Standard deviation values in all cases were below 0.002 Abs (10 mm equivalent).

Chlorophyll *a*	A(666) (n=3)	Conc. (g/L) (n=3)	Std. dev.
Sample 1	12.096	0.162	0.001
Sample 2	5.841	0.078	0.000
Sample 3	3.270	0.044	0.000
Sample 4	1.705	0.023	0.000

Table 1. Chlorophyll *a* content. The table displays calculated results for dilutions of chlorophyll *a* measured in triplicate.

Chlorophyll *b*	A(650) (n=3)	Conc. (g/L) (n=3)	Std. dev.
Sample 1	10.342	0.237	0.001
Sample 2	5.455	0.125	0.002
Sample 3	2.852	0.065	0.001
Sample 4	1.474	0.034	0.000

Table 2. Chlorophyll *b* content. The table displays calculated results for dilutions of chlorophyll *b* measured in triplicate.

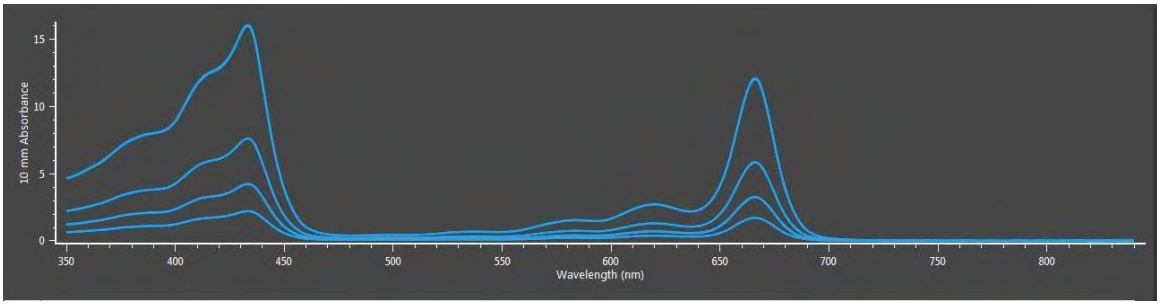


Figure 3. Custom method absorbance spectrum for each sample measurement of Chlorophyll *a* from spinach, Sigma-Aldrich Product # C5753, suspended in 100% DMSO. Chlorophyll *a* displayed major absorbance peaks at 433 nm and 666 nm.

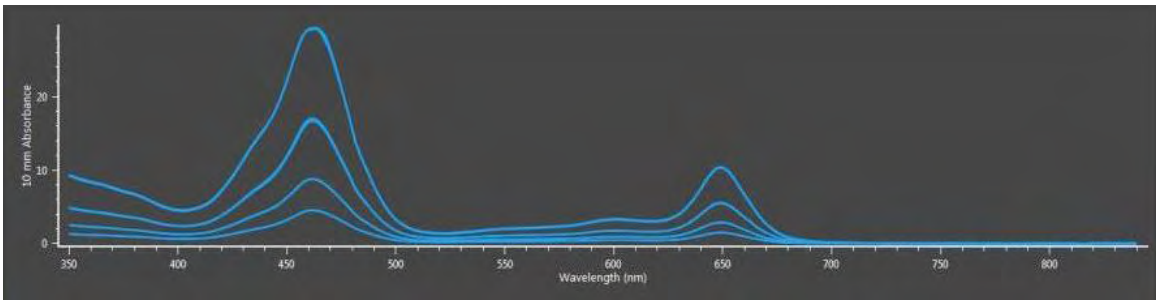


Figure 4. Custom method absorbance spectrum for each sample measurement of Chlorophyll *b* from spinach, Sigma-Aldrich Product # C5878, suspended in 100% DMSO. Chlorophyll *b* displayed major absorbance peaks at 462 nm and 650 nm.

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Conclusion

This study demonstrates the NanoDrop One/One^{C} UV-Vis Microvolume Spectrophotometer can be used to accurately quantify chlorophyll *a* and chlorophyll *b* using a user-defined custom method. The low standard deviation indicates there was a high agreement between replicate measurements. This validates the NanoDrop One/One^{C} Spectrophotometer can quantify samples accurately and reproducibly. The ability to measure chlorophyll *a* and *b* using a custom method serves as a valuable tool for research and the advancement of science.

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T141 Rev. 28 Feb 2018

Application note

Quantifying soy DNA extracts for downstream GMO detection

NanoDrop Eight Spectrophotometer

Introduction

The controversy of grocery products containing genetically modified organisms (GMO) has heightened the demand for analytical testing in the food and beverage industry.[1,2] GMOs, or transgenic crops, contain DNA that was modified through bioengineering to introduce a desired trait that is not naturally occurring in the species.[1] Globally, soybeans stand as the most popular transgenic crop, where herbicide tolerance is the most desired trait, and pest resistance follows close behind.[3] Current legislation for regulating GMO-based products varies between countries, indicating the need for worldwide, reliable testing procedures.[2] Qualitative PCR is implemented first to screen for the presence or absence of common regulatory elements used in transgenic (GMO) crops. Quantitative PCR is then performed, detecting more specific targets and providing a quantitative analysis on the target copy number.[4] Completing a qualitative assessment prior to quantitative PCR saves time and resources by eliminating targets that were defined as absent. In this note, a qualitative assessment was performed to determine the presence or absence of common regulatory elements.

Transgenic crop production

The most common DNA construct regulatory elements that enable transgenic crop production are the cauliflower mosaic virus (CaMV) 35S promoter (P35S), the *Agrobacterium tumefaciens* nopaline synthase terminator (TNOS), and the figwort mosaic virus (FMV) 34S promoter (P34S).[5,6,7] The CaMV 35S RNA gene promoter sequence is well characterized and functions by facilitating transcription of the desired transgene.[5,8] The TNOS sequence contains the polyadenylation site that serves to terminate transcription.[6] The 34S promoter sequence from FMV shows a relatively high percent homology compared to the P35S sequence from CaMV, specifically in transcription initiation sites. Variations in the FMV enhancer sequence, along with the location relative to the promoter, may serve as drawbacks compared to CaMV.[7] However, designing a dual promoter vector with P35S and P34S has advantages for the efficient expression of two transgenes compared to incorporating one promoter in a bicistronic vector.[7,9]

There are two popular methods for producing transgenic crops with the designated DNA construct: particle bombardment (Figure 1A) and transformation via *Agrobacterium* (Figure 1B).[1] With the particle bombardment method, the DNA construct is bound to particles which are accelerated at high velocity into plant tissues or cells to the nucleus. The DNA construct releases from the particles and integrates into the plant DNA via homologous recombination.[10,11] After the bombardment, the cells are transferred to a selective media based on the chosen selection agent in the DNA construct, and the plant regenerates until it is a fully grown transgenic plant.[10,12] With the *Agrobacterium* method, *Agrobacterium* naturally infects plants and uses its own tumor-inducing (Ti) plasmid to integrate transfer DNA (T-DNA) into the plant genome.[10] When producing transgenic plants, the desired trait is inserted into the Ti plasmid, reintroduced to the *Agrobacterium*, and infects the plant. T-DNA containing the transgene integrates into the plant DNA, and cells are selected and regenerated as described with the particle bombardment method.[10,12]

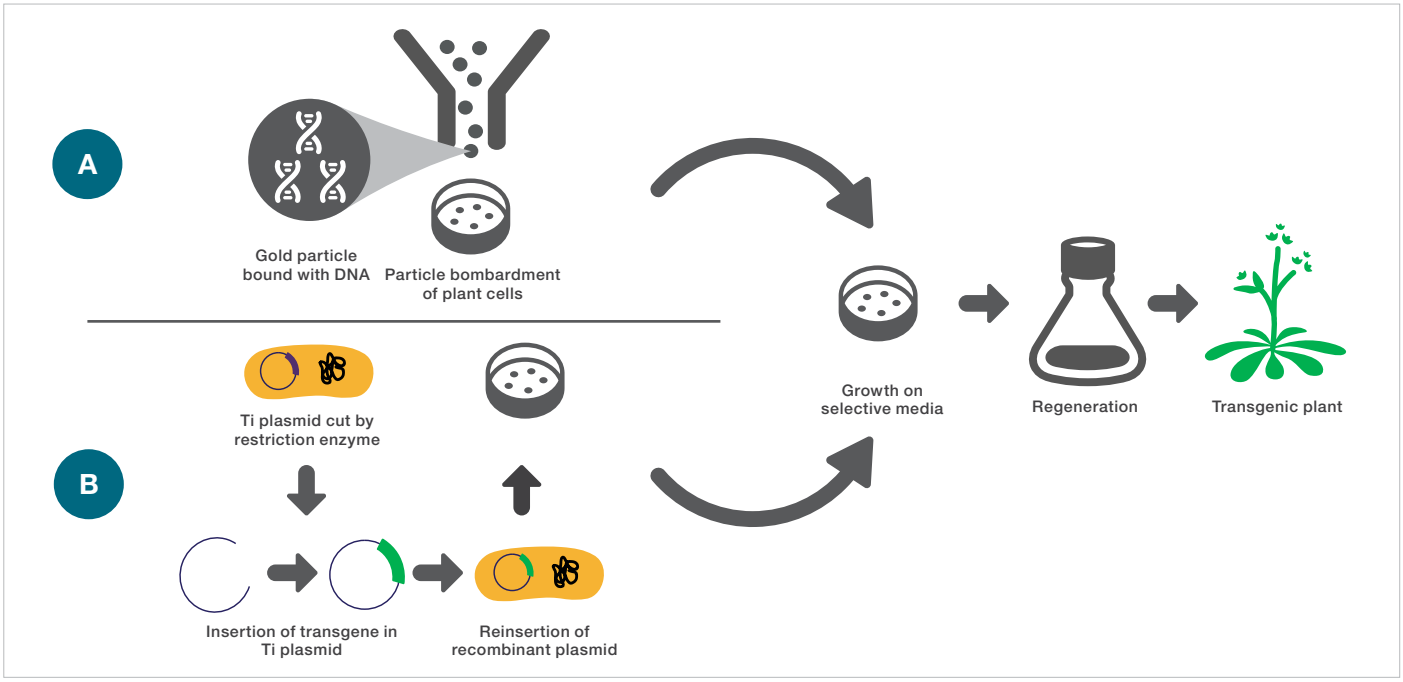

Figure 1: Transgenic crop production methods. A) Particle bombardment transformation. B) *Agrobacterium* transformation.

GMO detection via qPCR

GMO detection has become simpler through multiplex qPCR assays where the targets are P35S/CaMV, TNOS/A. *tumefaciens*, and P34S/FMV. Because these regulatory elements are naturally found in their corresponding virus or bacteria, it is common to experience false GMO-positive reactions if the plant has been naturally infected.[8] Multiplex qPCR allows simultaneous detection of the regulatory elements and the virus or bacteria to eliminate false-positive results.

PCR is a sensitive assay that requires template DNA to be a specific amount and optimal purity to ensure a successful and reproducible reaction.[13] To determine the quantity and quality of template DNA, the Thermo Scientific™ NanoDrop™ Eight Microvolume UV-Vis Spectrophotometer calculates concentration and identifies contaminants from nucleic acid extractions. Through chemometric algorithms, the Thermo Scientific™ Acclaro™ Sample Intelligence Technology provides valuable information on sample contaminants, improving necessary sample clean-up efficiencies (Figure 2). To further simplify sample processing upstream of PCR, the NanoDrop Eight instrument includes eight microvolume sample pedestals for high-throughput protocols.

Common PCR inhibitors are residual extraction materials such as protein and phenol,[13] which are identified with the Acclaro technology built into the NanoDrop Eight software. When extracting nucleic acids from food sources, proteins are abundant and frequently co-extracted if proper extraction technique is not followed. Contaminating proteins are known to inhibit the DNA polymerase in PCR, reducing PCR efficiency.[14] Identifying a contaminant with the NanoDrop Eight software allows scientists to make simple modifications to extraction protocols without the need for extensive troubleshooting when PCR efficiency is poor.

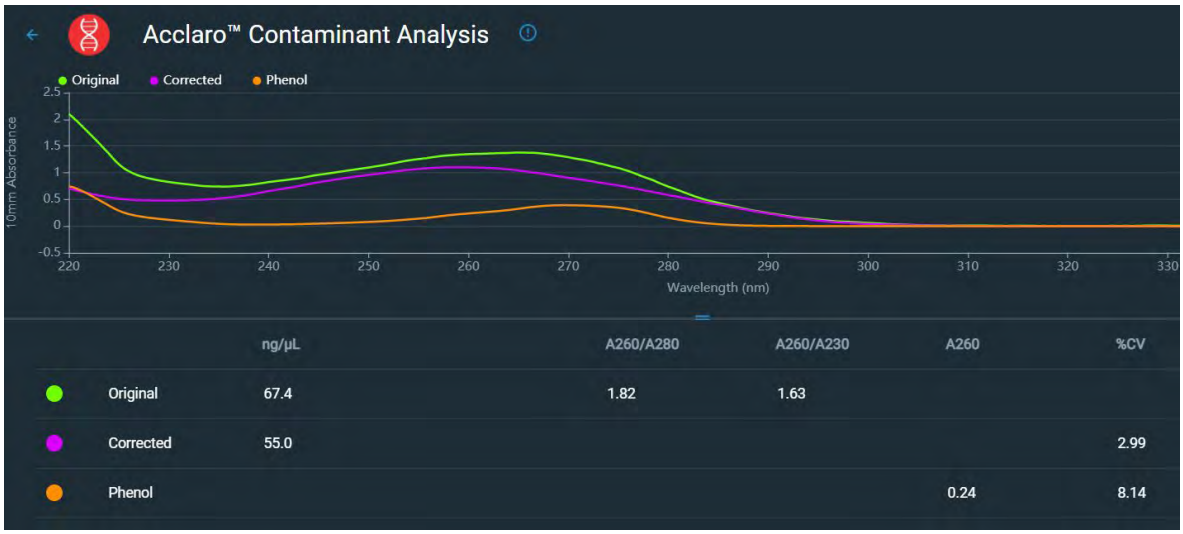

Figure 2: The Acclaro technology built into the NanoDrop Eight operating software. Phenol was identified as a contaminant, and its absorbance contribution was reported. A corrected DNA concentration was calculated, and the corresponding spectrum is displayed in purple.

Experimental procedures

DNA extraction with CTAB

Soy DNA was isolated from commercial tofu, soy milk, edamame, and soybeans with a modified protocol from Doyle and Doyle (1987) using the ionic detergent cetyltrimethylammonium bromide (CTAB). A stock of CTAB extraction buffer was made by adding 1.4 M NaCl and 20 mM EDTA to CTAB buffer (Promega, MC1411). Soybeans were soaked in dH_2O overnight. A 700 µL aliquot of CTAB extraction buffer was added to 200 mg of tofu, edamame, and soybeans and 200 µL of soy milk. Solid samples were pulverized twice via sonication for 30 seconds at 40% amplitude using a Branson Sonifier SFX150. All samples were incubated at 65°C for one hour with occasional vortexing and centrifuged at 16,000 x g for 5 minutes. One volume phenol:chloroform:isoamyl alcohol (25:24:1 v/v) (Invitrogen, 15593031) was added to the supernatant, and the sample was vortexed and centrifuged at 16,000 x g for 5 minutes. The aqueous layer was treated with 10 µL RNase A (Thermo Scientific, EN0531) at 37°C for 30 minutes. One volume chloroform:isoamyl alcohol (24:1 v/v) (Sigma, 25666) was subsequently added, vortexed, and centrifuged at 16,000 x g for 5 minutes. The aqueous layer was mixed with 2/3 volume isopropanol and 0.08 volume sodium acetate and incubated for one hour at room temperature to precipitate DNA. The DNA was pelleted by centrifuging at 16,000 x g for 5 minutes, and the pellet was washed with ice-cold 70% ethanol and centrifuged twice, removing the supernatant after each spin. The pellet was dried at room temperature for 10 minutes and dissolved in 50 µL Tris-EDTA (TE) pH 8.0. Samples were measured in replicates of four using the eight-channel mode on the NanoDrop Eight instrument.

qPCR with GMO screening kit

The Thermo Scientific™ TaqMan™ GMO Screening Kit was designed to test for the presence or absence of GMOs in DNA extracts from food and feed samples. The kit includes a positive control and the necessary primers and probes for multiplex reactions targeting: P35S/CaMV, TNOS/A. *tumefaciens*, and P34S/FMV, as well as an endogenous plant control and internal positive control for identifying PCR inhibition. Tofu, soy milk, edamame, and soybean DNA samples were diluted to 20 ng/µL prior to loading on the qPCR plate. The qPCR was performed with an Applied Biosystems™ QuantStudio™ 6 Pro Real-Time PCR System with the cycling conditions outlined in the TaqMan GMO Screening Kit procedure. Results were analyzed using the Presence Absence Analysis qPCR application on the Thermo Fisher Connect Platform.

Results

Following the CTAB extraction method, DNA from tofu, soy milk, edamame, and soybeans was checked for concentration and purity with the NanoDrop Eight Spectrophotometer (Figure 3). The DNA was extracted in a high yield; all samples were close to 100 ng/µL. All samples exhibited excellent purity, evidenced by the A_{260}/A_{280} purity ratios falling between 1.85 and 1.90 and the absence of any Acclaro technology warnings. Incubating at cold temperatures during the isopropanol precipitation step was found to precipitate excess salts; thus, incubating at room temperature is recommended. Some DNA extraction protocols include RNase treatment after resuspending DNA in buffer post-precipitation, thus requiring a second DNA precipitation step.[15] The RNase treatment in this experiment was performed in between chloroform phase separations to remove contaminating enzymatic material and to prevent DNA loss from a second precipitation.

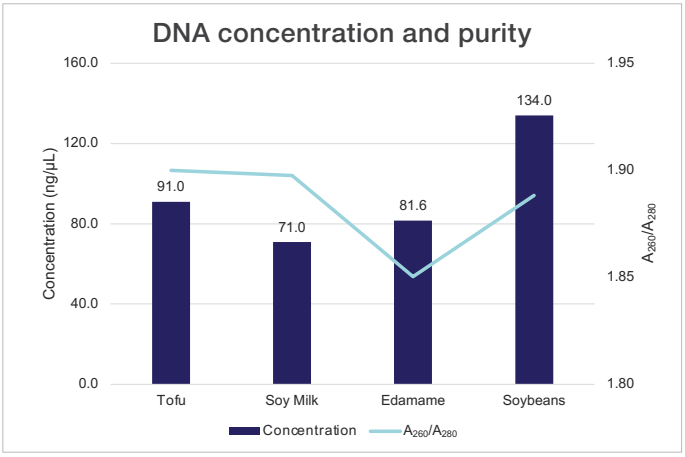

Figure 3: DNA concentration (ng/µL) and A_{260}/A_{280} purity ratio reported by the NanoDrop Eight instrument for DNA extracted using the CTAB method. Data displayed are averaged from four replicate measurements.

The results of the presence-absence qPCR are outlined in Table 1. All samples tested positive for the plant endogenous control as well as the internal positive control (IPC), indicating the samples were of plant origin and PCR inhibitors were not present. Tofu and soy milk DNA were negative for the presence of any transgenic material and natural infection from CaMV, A. *tumefaciens*, and FMV. The edamame and soybean DNA tested positive for P34S and FMV, indicating the natural presence of FMV and no transgenic material specific to this assay. The soybean DNA also tested positive for P35S and CaMV, indicating the natural presence of CaMV and no transgenic material specific to this assay.

Table 1: Results from the Presence Absence Analysis qPCR application on the Thermo Fisher Connect Platform after performing qPCR with the TaqMan GMO Screening Kit.

Duplex Assay	Component	Tofu	Soy milk	Edamame	Soybeans
P35S/CaMV	P35S	–	–	–	+
	CaMV	–	–	–	+
TNOS/A. *tumefaciens*	TNOS	–	–	–	–
	A. tumefaciens	–	–	–	–
P34S/FMV	P34S	–	–	+	+
	FMV	–	–	+	+
Plant/IPC	Plant	+	+	+	+
	IPC	+	+	+	+

For the edamame and soybeans, the next step would entail a qPCR relative quantification curve using a soy-specific endogenous control to further confirm these findings and quantify target copy numbers.[4] When performing quantification curves, it is crucial that samples are pure, which can be a challenge for highly processed food samples.[14] A slight change in the quantification cycle due to the presence of PCR inhibitors or an inaccurate template amount has a considerable effect on target quantification. Implementing the NanoDrop Eight instrument prior to performing the quantification curve will instill confidence in qPCR results.

Conclusions

When utilizing qPCR for GMO detection, data reliability is a top priority as food and beverage products must comply with government regulations on transgenic crops. Preparing DNA for analysis by qPCR requires excellent purity and the concentration to be known. With the NanoDrop Eight spectrophotometer, DNA quality and quantity of eight microvolume samples are determined simultaneously in less than twenty seconds. The Acclaro technology provides important sample contaminant information, ensuring a successful extraction protocol and subsequent qPCR run.

NanoDrop Eight Microvolume UV-Vis Spectrophotometer

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Application note

Macroalgae DNA quantification and contaminant characterization with the NanoDrop One Spectrophotometer

Introduction

In coastal marine environments, macroalgae, or seaweed, serve as a shelter or food source for a variety of aquatic life.[1-2] Macroalgae also play a role in carbon sequestration, which involves capturing and removing carbon from the atmosphere to reduce the effects of climate change. Estuaries, oceans, and other bodies of water containing macroalgae act as biological carbon sinks that quickly sequester and store atmospheric carbon.[2-3] A key question of concern today is whether macroalgae can survive and adapt to rising sea temperatures and ocean acidification.

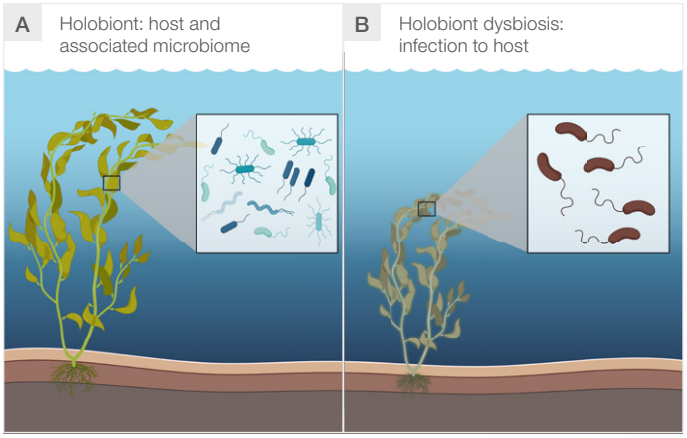

Figure 1. A) Holobiont consisting of seaweed and the associated microbiome in symbiosis. B) Dysbiosis of the holobiont causing an infection. Image created with BioRender.com.

The adaptability of macroalgae to the changing ocean environment depends largely on the seaweed-associated bacteria.[3-4] The host seaweed and the associated microbiome is called the holobiont, which describes the symbiosis as a functional unit (Figure 1A).[4-5] For example, bacteria depend on the macroalgae for polysaccharides as an energy source, while macroalgae depend on the bacteria for essential nutrients, such as vitamin B_{12}.[6-7] The seaweed-associated bacteria are also major players in seaweed morphogenesis.[4] When environmental conditions deviate from optimal, dysbiosis of the holobiont causes infections to the seaweed or atypical morphogenesis (Figure 1B).[4,8] However, the microbiome can adapt to protect the seaweed from the changing environment. Some marine bacteria, such as *Vibrio* spp., form a biofilm on the surface of macroalgae and serve as a protective barrier.[4]

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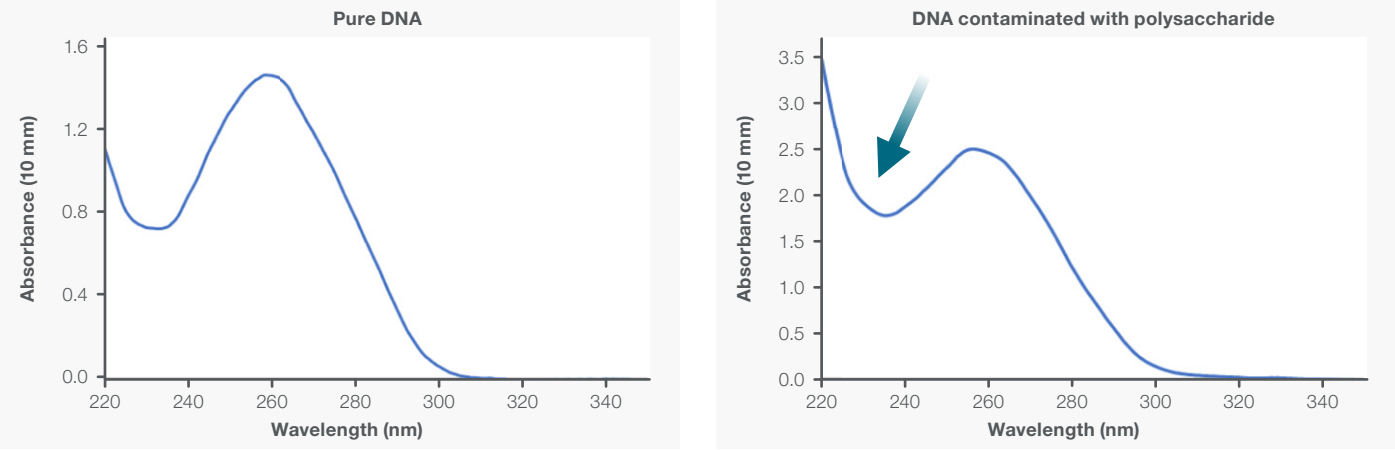

Figure 2. Polysaccharides and polyphenols absorb light at 230 nm, causing an absorbance increase in the typical nucleic acid trough. Image created with BioRender.com.

To better understand how macroalgae adapt to the changing environment, performing targeted qPCR or sequencing the genomes of bacteria associated with the holobiont can provide useful information. Polysaccharides and polyphenols are frequently co-extracted along with nucleic acids in macroalgae samples, some of which are known PCR inhibitors that inhibit the Taq polymerase or cross-link with the nucleic acid.[9-10] By applying a UV-Vis spectrophotometry checkpoint after extraction, the contamination can be visualized. An absorbance increase at 230 nm and a low A260/A230 purity ratio are indicative of polysaccharide or polyphenol contamination (Figure 2).[11] It is important to properly clean up the contamination to ensure a pure sample is used to prevent failed downstream reactions caused by inhibitors. This application note outlines extracting DNA from *Ulva* spp. and *Laminaria* spp. and performing a quality and quantity analysis with the Thermo Scientific™ NanoDrop™ One/One^{C} UV-Vis Microvolume Spectrophotometer in preparation for downstream qPCR or next-generation sequencing (NGS).

Experimental Procedures

Seaweed DNA and the associated bacterial DNA were isolated from *Ulva* spp. and *Laminaria* spp. (Carolina Biological Supply) using the Thermo Scientific™ MagMAX™ Plant DNA Isolation Kit (Cat. No. A32549), which is a magnetic bead-based purification kit optimized for nucleic acids with Invitrogen™ Dynabeads™ MyOne™ Carboxylic Acid beads. The nucleic acid extraction procedure was automated with the Thermo Scientific™ KingFisher™ Flex Purification System to enhance sample purity and to eliminate the hands-on requirement of tedious cetyltrimethylammonium bromide (CTAB) lysis and phenol, chloroform-based extraction procedures. Each extraction with the MagMAX isolation kit was performed per manufacturer's instructions, including the pre-processing steps, using 100 mg of plant tissue. Two samples of *Ulva* and *Laminaria* were not treated with RNase to illustrate the effect on the A260/A280 absorbance purity ratio. The *Laminaria* samples were treated with Polyvinylpyrrolidone, M.W. 40,000 (PVP40) (Thermo Scientific Chemicals, J62417.A1) to remove contaminating polysaccharides and polyphenols.

Additionally, one *Ulva* sample was extracted utilizing the CTAB (OPS Diagnostics, CEB 125-01) and phenol, chloroform-based method to compare purity results with the MagMAX isolation kit. After the extraction, DNA was quantified and qualified with the dsDNA application on the NanoDrop One spectrophotometer microvolume pedestal in replicates of 5 using a 2.0 µL sample volume for each replicate.

Sample	Concentration (ng/µL)	Standard Deviation (ng/µL)	A260/A280	A260/A230
***Ulva* with RNase**	47.02	0.38	1.88	1.24
***Ulva* without RNase**	290.68	1.14	2.15	2.45
***Ulva* CTAB extraction**	78.29	0.28	1.78	0.32
***Laminaria* with RNase**	53.22	0.36	1.95	1.81
***Laminaria* without RNase**	119.17	0.17	2.07	1.40

Table 1. DNA Concentration and purity results using the NanoDrop One spectrophotometer microvolume pedestal.

Results

The concentration and purity results of extracted dsDNA determined by the NanoDrop One spectrophotometer are shown in Table 1. The reported concentration standard deviations fall within the range of 0.17 – 1.14 ng/µL, detailing the reliability and accuracy of the NanoDrop One instrument in determining concentration.

The *Ulva* and *Laminaria* samples that were not treated with RNase have an inflated concentration in comparison to samples that were treated with RNase. Since the A260/A280 purity ratio of "pure" dsDNA is expected to be around 1.8 and around 2.0 for "pure" RNA, the higher-than-expected A260/A280 ratio is associated with RNA contamination in a dsDNA sample. This is due to the higher A260/A280 ratio of uracil in RNA (4.00) compared to thymine's lower ratio in DNA (1.47).[12] For successful qPCR or NGS, the dsDNA concentration must be accurate. The presence of RNA co-absorbing with dsDNA artificially increases the reported concentration and the sample is thus mistakenly overdiluted, affecting the downstream qPCR or NGS results. To combat the high A260/A280 ratio, adding RNase reduced the *Ulva* and *Laminaria* purity ratios from 2.15 to 1.88 and 2.07 to 1.95, respectively (Figure 3).

The generally accepted range for the A260/A230 purity ratio of "pure" dsDNA and RNA is 2.0-2.2. However, the lower cutoff is flexible depending on the downstream dilution factor and nature of the contaminant.[13] The *Ulva* dsDNA sample that was extracted using the CTAB-based method exhibited the lowest A260/A230 ratio of 0.32. The high viscosity of the sample and the absorbance increase at 230 nm represents polysaccharide contamination, which is detrimental to NGS library preparation and qPCR.[14] Figure 4 represents the purity improvement using the MagMax isolation kit as opposed to the traditional CTAB method. The addition of high-salt buffers and PVP40 aids in preventing co-precipitation of polyphenols and polysaccharides, as shown in the samples extracted using the MagMAX isolation kit (*Ulva* with/without RNase and *Laminaria* with/without RNase) with A260/A230 ratios ranging from 1.24 – 2.45.

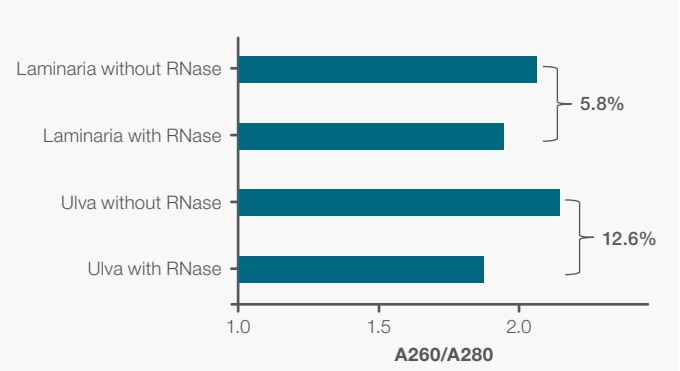

Figure 3. Observed increase in A260/A280 for DNA samples not treated with RNase. Percentage indicates percent change.

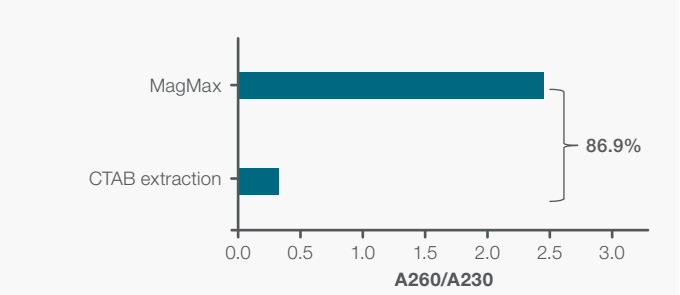

Figure 4. Purity improvement of A260/A230 using the MagMax isolation kit compared to the CTAB-based extraction method. Percentage indicates percent change.

Conclusions

Given the environmental impact of macroalgae, it is important to protect and maintain its health and longevity to ensure future carbon capture and habitat conservation. To obtain high-quality data from NGS or qPCR, the macroalgae dsDNA starting material must have a reliably determined concentration and purity. When extracting dsDNA from macroalgae, RNA, polyphenols, and polysaccharides are frequently co-extracted. Utilizing the MagMAX isolation kit along with RNase and PVP40 has been shown to reduce or remove the contaminating material compared to the CTAB and solvent-based extraction. The NanoDrop One spectrophotometer serves as a reliable tool for ensuring successful downstream assessments of the seaweed-associated microbiome and the adaptability to the changing ocean environment.

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Application note

Detection and Avoidance of Polysaccharides in Plant Nucleic Acid Extractions

NanoDrop Spectrophotometers

Introduction

Spectrophotometric measurement of nucleic acids is common, both to assess purity and to quantify DNA or RNA. By measuring the absorbance at 260 nm, it is possible to calculate nucleic acid concentration. Absorbance at other wavelengths, particularly 280 nm and 230 nm, may be used as a measure of the sample's purity by calculating the ratio of the absorbances at these wavelengths to the absorbance at 260 nm. Because proteins have an absorbance maximum at 280 nm, the A260/A280 ratio may be used to assess protein contamination: a ratio of less than 1.6 is commonly considered "poor", while ratios above 1.8 are considered "good". Similarly, because many polysaccharides absorb at or around 230 nm, the A260/A230 ratio may be used for their detection: ratios of 1.6 or less are considered "poor", while ratios above 1.9 are considered "good".

The A260/A230 ratio is of special importance for plant nucleic acid extractions because plants typically have high polysaccharide contents. In addition to cell wall material and sugar or starch content, plant cells contain a vast array of polysaccharides, which have been shown to inhibit the DNA polymerase in PCR and next-generation sequencing library preparation when co-precipitated with nucleic acids, preventing a successful reaction.[1,2]

Generally, plant nucleic acid extractions begin with tissue homogenization, either in buffer or using liquid nitrogen prior to adding buffer. The extraction buffer is a key component of the procedure, with each ingredient playing a specific role. The addition of sodium chloride to the buffer helps to prevent polysaccharides from precipitating following the addition of alcohol. Polysaccharides are therefore discarded in the supernatant following nucleic acid precipitation and centrifugation, resulting in DNA pellets with low polysaccharide contamination.[3,4]

In this study, the effect of salt concentration in extraction buffer on the polysaccharide content of the purified nucleic acids is demonstrated. By examining the A260/A230 purity ratio using a Thermo Scientific™ NanoDrop™ One/One^{C} Spectrophotometer, polysaccharide contamination was determined as being dependent on both the plant species used and the salt content of the extraction buffer. The NanoDrop One/One^{C} spectrophotometer uses a variable pathlength to determine concentration and purity using only 1-2 µL sample volumes.

Experimental Procedures

The DNA extraction method is a modification of the Invitrogen™ DNAzol™ Reagent protocol.[5] For isolation of RNA, 0.25 volumes of 10 M LiCl should be substituted for isopropanol in step 7. Nucleic acids were extracted from cucumber (Cucumis sativus) samples using the following procedure:

1. Two extraction buffers were formulated:
    - High salt buffer: DNAzol (Invitrogen, 10503027), 1% PVP-40, 1.5 M NaCl
    - Low salt buffer: DNAzol, 1% PVP-40, 0.5 M NaCl
2. Plant tissue (95 – 105 mg) was placed in a 1.5 mL microcentrifuge tube and mixed with 300 µL extraction buffer (High Salt and Low Salt buffer separately).
3. Samples were sonicated using a Branson Sonifier at 70% amplitude for 30 seconds to grind plant tissue and release cellular contents.
4. Chloroform:isoamyl alcohol solution (24:1, 300 µL) was added and samples were incubated at 25°C for 5 minutes.
5. Samples were vortexed at 12,000 rpm for 10 minutes and the top aqueous layers were transferred to fresh 1.5 mL microcentrifuge tubes.
6. Steps 4 and 5 were repeated.
7. Nucleic acids were precipitated using an equal volume of isopropanol and then incubated at room temperature for one hour.
8. Nucleic acids were pelleted by centrifugation at 12,000 rpm for 10 minutes.
9. Pellets were washed twice with 1.0 mL 70% ethanol and centrifuged at 12,000 rpm for 5 minutes.
10. Pellets were dried and resuspended in 30 µL tris-EDTA pH 8.0.
11. Nucleic acid concentrations and purity ratios were determined using 2.0 µL on the pedestal of a NanoDrop One^{C} spectrophotometer and the dsDNA application.

Results

There were no great differences in nucleic acid concentrations between the high salt and low salt buffers, but the A260/A230 purity ratios were markedly lower using the low salt buffer (Figure 1).

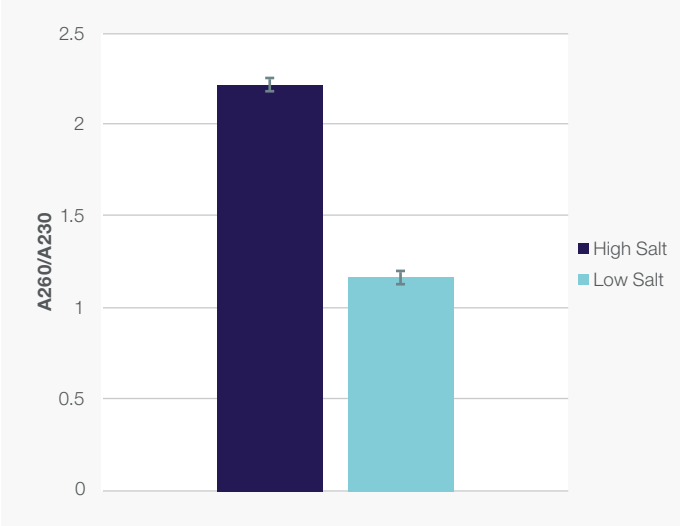

Figure 1. A260/A230 purity ratios of extracted nucleic acid samples from cucumber. Extractions were performed using both high salt buffer (1.5 M NaCl) and low salt buffer (0.5 M NaCl). For each bar, n=7; error bars represent ± standard deviation.

Examination of the absorbance spectra confirmed this observation (Figure 2). Although the absorbance peak for nucleic acids was consistently at 260 nm, the trough normally observed at 230 nm shifted to approximately 240 nm as a result of the increased sample absorbance at 230 nm using the low salt buffer for extraction.

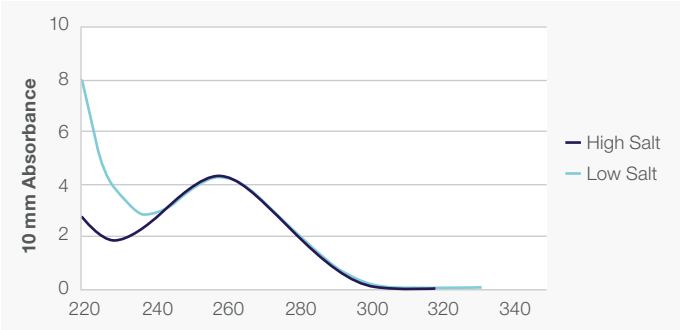

Figure 2. Absorbance spectra of cucumber nucleic acids extracted using high salt buffer (dark blue) and low salt buffer (light blue).

Conclusions

The presence of polysaccharides in extracted plant DNA is a common concern for plant molecular biologists as the polysaccharides inhibit reactions in PCR and NGS.[1,2] However, the presented data show that in many cases this can be averted with the use of increased salt concentrations in extraction buffer.[3,4]

The nucleic acid concentrations measured were approximately three times greater than what is measurable using a cuvette-based spectrophotometer. An advantage of microvolume quantification using NanoDrop spectrophotometers is the use of multiple pathlengths, automatically selecting the most appropriate. By doing so, the NanoDrop One/One^{C} spectrophotometer is capable of measuring dsDNA samples up to 27,500 ng/µL, where a cuvette-based spectrophotometer would require extensive sample dilution.

Although the automatic calculation of purity ratios by NanoDrop spectrophotometers is very convenient, it is important to view the sample spectra as other contaminants, such as phenol and guanidine, are also detectable. The large dynamic range and low volume requirements, coupled with the automatic calculation of purity ratios and display of spectra, make the NanoDrop One/One^{C} spectrophotometer an ideal instrument for the analysis of extracted nucleic acid samples.

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