Quantifying MicroGEM nucleic acid extracts

MicroGEM International PLC

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Introduction

MicroGEM's Temperature Controlled Extraction method (TCE) utilises the robust activity of MicroGEM's thermophilic protease, *prep*GEM, to extract nucleic acids from a multitude of samples. MicroGEM's TCE methodology does not introduce downstream inhibitors into the sample, such as ionic detergents like SDS, but instead uses the combination of heat and protease activity to extract nucleic acids in a single-tube, by simply adjusting the temperature. As MicroGEM's TCE does not require the use of harsh detergents to extract nucleic acids, and the extraction buffers are compatible with downstream analysis, extracted DNA/RNA generally does not need to be purified before analysis can take place.

For this reason, MicroGEM is able to extract nucleic acids quickly and simply with an unparalleled DNA/RNA recovery and a reduced risk of contamination. In some cases the extracts need to be quantified to ensure the correct amount of DNA/RNA is used as a template for the next step.

In this application note, we look at some common methods for nucleic acid quantification (UVspectrophotometry, florescent-dye and qPCR) and showthe most accurate methods when working with MicroGEM extracts.

Materials and Methods

Extraction

DNA was extracted from human buccal swabs, plant tissue, and bacteria. A buccal sample was prepared using two cotton swabs and suspending the cells in 600 µl of water. The bacterial sample was added directly to the extraction mix using a sterile loop. A leaf sample was crushed onto a *phyto*GEM storage card and two 3 mm punches were taken. Each type of sample was extracted with the PDQeX kits as listed below, following the recommended procedures:

PDQeX *phyto*GEM (Plant)	
10µl	**GREEN+** Buffer
2µl	*prep*GEM
10µl	*Histosolv*
10µl	Enhancer
68µl	DNA-free water

PDQeX *prep*GEM Bacteria	
10µl	**GREEN+** Buffer
2µl	*prep*GEM
10µl	Lysozyme
87µl	DNA-free water

PDQeX *prep*GEM Universal	
10µl	**BLUE** Buffer
2µl	*prep*GEM
68µl	DNA-free water
20µl	Buccal Suspension

Quantification

qPCR was carried out on an Applied Biosystems Quant Studio™ 5 instrument (CA, USA) using SYBR Green chemistry from Bioline (London, UK). Primers used are listed in Table 1. Quantification was performed by comparing the Cq values of the samples to a standard curve.

Type of Sample	Targeted gene	Primer sequences (F & R)
Human Buccal	BRCA	TGAACACCACTGAGAAGCGT GCTGTAATGAGCTGGCATGA
Bacteria	16S rRNA gene	GGTTAAGTGCCGCAACGAG TGACGTCKTCCCCKCCTTCCT
Citrus	Alcohol dehydrogenase	TGACAGAGGCGTCATGCTTAAC TGAGGTTCCAACGAAATGATAAATA

Table 1: *List of the primers used for the qPCRs*

For spectrophotometry, a DeNovix DS-11 FX Spectrophotometer was used to measure A260, A230, and A280. The device was blanked with the sample-specific extraction mix, and 2 μl of the extracted DNA was loaded onto the device for reading.

For fluorometric assays, the kits used were DeNovix® Broad Range (DeNovix), DeNovix® High Sensitivity (DeNovix, USA), iQuant™ High Sensitivity (Genecopoeia, USA), Quant iT™ PicoGreen® (Thermo Fisher Scientific, NZ) and Qubit™ High Sensitivity (Thermo Fisher Scientific, NZ) kits. Recommended procedures were followed and a standard curve using four DNA standards was made for each dye kit. Measurements for the standard curve and sample DNA were taken with a BioTek FLx800™ Fluorometer (Biotek, USA). Quantities of the DNA samples were determined by comparing the fluorescent reading to the standard curve.

Results

Absorbance-based DNA quantification with DeNovix

The DeNovix DS-11 FX was first blanked with 2 μl of 1x Buffer and absorbance was measured for buccal, plant and bacterial samples with the DeNovix DS-11 FX (Figure 1A). The DeNovix DS-11 FX was then blanked with water and 2 μl of each buffer was measured (Figure 1B).

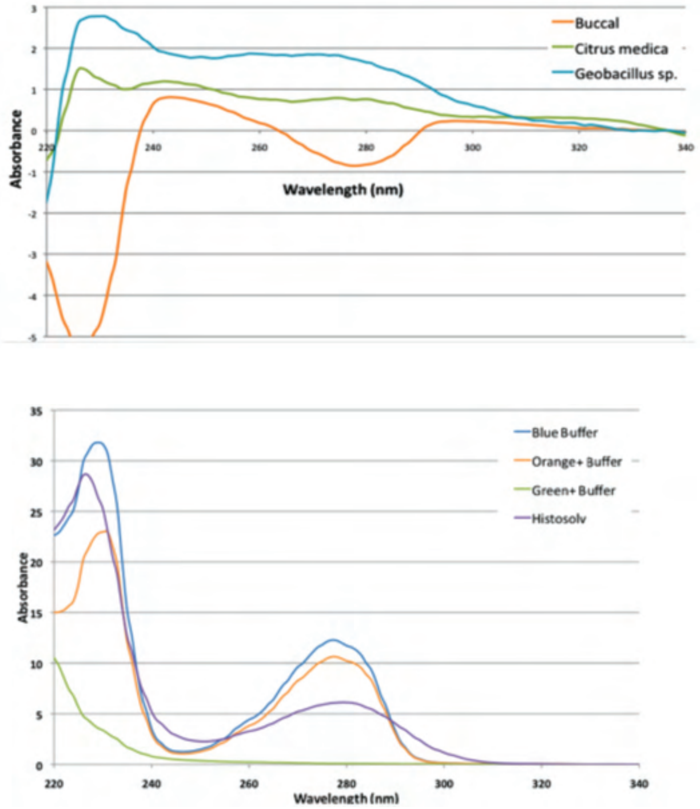
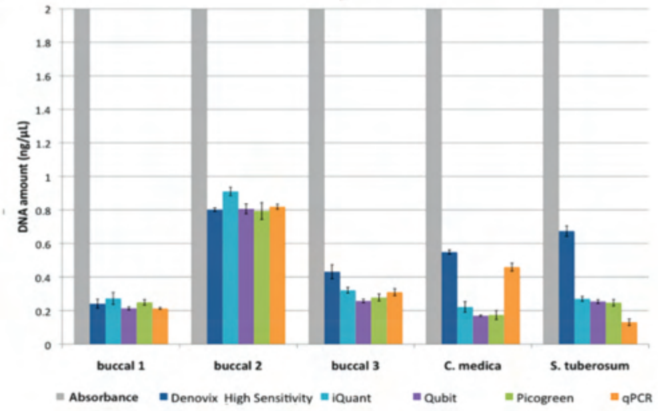


Figure 1A: *DNA absorbance plots from buccal, plant and bacterial extracts with DeNovix DS-11.* ***1B:*** *DeNovix DS-11 absorbance plots of ZyGEM buffers*

DNA quantification by Fluorometer and qPCR

Samples were assessed with fluorometric and qPCR assays and the absorbance-based estimations from the DS-11 were included for comparison.



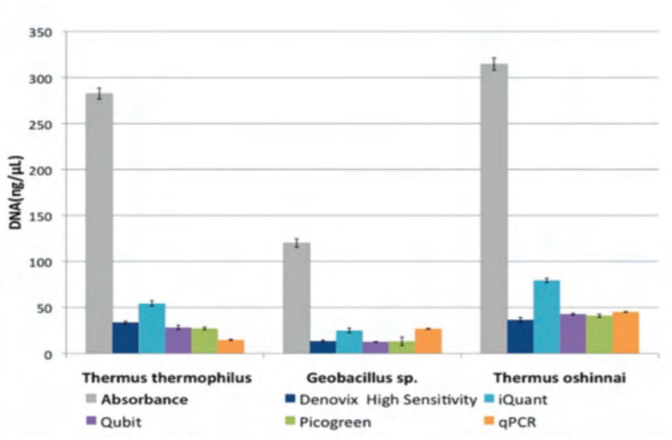


Figure 2A: *DNA concentration of buccal and plant extracts with DeNovix DS-11, fluorometric assays and qPCR.* ***2B:*** *DNA concentration of bacterial extracts with DeNovix DS-11, fluorometric assays and qPCR. Error bars were generated from triplicate experiments.*

Discussion

Results showed that fluorometric and qPCR assays obtained similar values whereas UV-spectrophotometry dramatically overestimated the amount of DNA.

MicroGEM's TCE method challenges the dogma of nucleic acid extraction by extracting DNA/RNA ready for PCR, RT-qPCR, NGS etc without the need for purification steps and therefore maximising speed, simplicity and nucleic acid recovery.

The removal of the unnecessary purification steps means that the nucleic acid extracts remain in a solution of MicroGEM buffer and amino-acids, resulting from *prep*GEM's protease activity.

Aromatic ring-structures in MicroGEM's buffer and amino acids, such as phenylalanine, absorb UV light during UV-spectrophotometry and therefore skew the data resulting in an inaccurate and overestimated concentration.

Whilst affecting UV-spectrophotometry, these compounds do not impair downstream analysis.

In summary, as well as qPCR, fluorometric assays provided the most accurate values for quantifying DNA, especially the assays employing high sensitivity dyes. Of the dyes evaluated in this study, the dyes Denovix, PicoGreen, Qubit and iQuant High Sensitivity Kit were found to be most the compatible with MicroGEM extracts.

These methods are therefore recommended for quantifying MicroGEM extracts.

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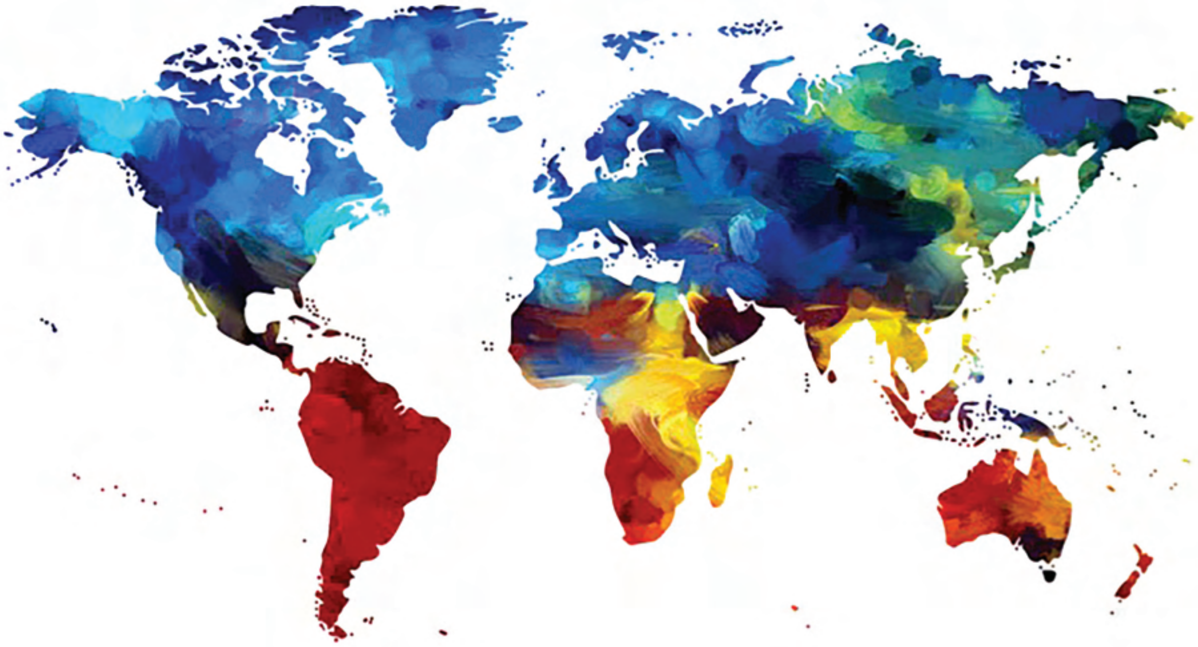

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At MicroGEM, our goal is to democratize molecular biology, enabling a broader spectrum of users to both employ and benefit from molecular techniques. The first step is the simplification of sample preparation. Our temperature-driven, single-tube process simplifies and reduces the number of steps for traditional nucleic acid extraction, resulting in high-quality extracts with reduced contamination and high yields - all in minutes, not hours.

MicroGEM