Application Note: 07006

Comparison of Automated and Manual Setup of the PicoGreen® DNA Quantitation Assay on the Thermo Scientific Matrix® Hydra® DT and the Thermo Scientific Matrix PlateMate® 2x2

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Key Words

- Thermo Scientific Matrix Hydra DT
- Thermo Scientific Matrix PlateMate 2x2
- Thermo Scientific Matrix D.A.R.T.s Tips
- Quant-iT PicoGreen dsDNA Assay Kit

Abstract

Determining the quantity of double stranded DNA (dsDNA) in a sample is a vital procedure used in many laboratories. Assays used to collect this information can be tedious, especially when trying to conserve samples and reagents by reducing the total sample volume. The Thermo Scientific Matrix Hydra DT and the Thermo Scientific Matrix PlateMate 2x2, both versatile bench top automated liquid handlers, are used in this experiment to increase efficiency and reproducibility of the setup of the PicoGreen dsDNA quantitation assay. Additionally, the automation equipment was programmed to aspirate and dispense lower volumes of reagents and samples used in the assay in order to reduce waste and still achieve comparable results. In this experiment we followed the instructions of the Quant-iT™ PicoGreen dsDNA Assay Kit to compare setup on the Matrix Hydra DT and Matrix PlateMate 2x2 to manual setup and then reduced the total volume from 210 µl to 105 µl on both instruments. The total volume was further reduced to 52.5 µl using the Matrix Hydra DT. Our results indicate that the Matrix Hydra DT and Matrix PlateMate 2x2 efficiently and comparably perform the assay setup as well as successfully decrease the amount of reagent and sample volume necessary to generate reliable results.

Introduction

Quantitation of dsDNA is a routine procedure performed in laboratories where applications include protocols for DNA fragment purification, cDNA library synthesis and measurement of reaction end products and, most commonly, polymerase chain reactions (PCR)[1]. The quantity of dsDNA was traditionally determined by using a spectrophotometer to measure the absorbance of a sample at 260 nm. This method encounters problems because other compounds found in DNA samples affect the readings. Alternative methods of quantifying DNA, such as gel analysis, are not only time consuming, but employ the use of carcinogenic chemicals. The alternative to these tedious, time consuming methods is to use the dsDNA specific dye, PicoGreen. Studies have shown that dsDNA quantitation by this dye, when compared to other methods, more closely represents the amount of DNA visualized in quantitative gel arrays[1]. While PicoGreen has simplified and standardized dsDNA quantification assays, this study applies fluorescent dye technology to an automated platform in order to save valuable time, reduce workload, conserve samples and increase the precision and accuracy of this high-throughput method.

Figure 1: Matrix Hydra DT and Matrix PlateMate 2x2.

The Matrix Hydra DT (see Figure 1) is a compact, versatile bench top instrument, capable of automating many laboratory liquid handling applications. The instrument's small footprint allows it to conveniently fit into standard biological safety cabinets or onto bench tops where space is limited. The Matrix Hydra DT can access 96-, 384-, and 1536-well plates, reagent reservoirs and other accessories which can be configured for specific laboratory uses. Available in either 96- or 384-channel format, the Matrix Hydra DT has a combined volume range of 0.5 µl to 300 µl. The 100 µl unit has a volume range of 0.5 µl to 100 µl, depending on the tips it is equipped with.

The Matrix PlateMate 2x2 (see Figure 1) can perform many of the same functions as the Matrix Hydra DT, while offering more deck positions. The Matrix PlateMate 2x2 operates with interchangeable positive displacement and air displacement pipetting heads, which gives the user a choice of 96- or 384-channel configuration and a dynamic volume range. Thermo Scientific Matrix D.A.R.T.s® (Disposable Automation Research Tips) tips, used with the Matrix Hydra DT and Matrix PlateMate 2x2, eliminate cross-contamination and the possibility of liquid carry-over.

The Matrix Hydra DT is programmed via on-board keypad controls or the PC-based ControlMate® software interface. Matrix PlateMate is controlled via ControlMate software interface only. In this study, the 100 µl, 96-

channel, Matrix Hydra DT and the Matrix PlateMate 2x2 with 5-300 µl air displacement pipetting head, are equipped with 1-100 µl Matrix D.A.R.T.s tips and used to compare the automated setup to the manual setup of the Quant-iT PicoGreen dsDNA Assay Kit.

Materials:

1. Quant-iT PicoGreen dsDNA Assay Kit
   a. BR reagent (Component A) 1.0 ml 200x in DMSO
   b. Quant-iT dsDNA BR buffer (Component B) 250 ml
   c. DNA standards (Component C) set of 8, 500 µl each (0, 5, 10, 20, 40, 60, 80, 100 ng/µl)
2. Thermo Scientific Matrix Hydra DT (Item no. 1096-DT-100)
3. Thermo Scientific Matrix PlateMate 2x2 (Item no. 301-10005)
4. Tecan Genios Reader
5. ControlMate Software v1.3.38
6. Thermo Scientific Matrix D.A.R.T.s Tips, 100 µl (Item no. 5526)
7. Thermo Scientific Matrix 8-Channel Serial Dilution D.A.R.T.s Tip Magazine, 300 µl (Item no. 501-30020)
8. Thermo Scientific Matrix 96-Well Polystyrene Microplate, Black, Flat Bottom (Item no. 4939)
9. Thermo Scientific Matrix 384-Well Polystyrene Microplate, Black, Flat Bottom (Item no. 4327)
10. Thermo Scientific Matrix 96-Well Polypropylene Microplate, U Bottom (Item no. 4917)
11. Thermo Scientific Matrix Disposable Automation Reservoirs, 96-Channel, 125 ml (Item no. 1064-05-5)
12. Single and Multichannel Thermo Scientific Matrix Electronic Pipettes, 125 µl (Item nos. 1021 & 2001)
13. Distilled Water

Methods:

1. 100 µl of BR reagent was mixed with 20 ml of BR buffer and placed into the reagent reservoir
2. 100 µl sample stocks were prepared by performing 1:1 dilutions of genomic DNA until four dilutions were complete
3. A stock plate was made by pipetting 100 µl of each standard (first column) and sample (second column) into the first two columns of a 96-well polypropylene microplate
4. The filled reagent reservoir was placed into the second stage position on the Matrix Hydra DT
5. A black, flat bottom 96-well microplate was placed into the first position of the Matrix Hydra DT stage
6. The Matrix D.A.R.T.s tip serial dilution magazine was placed into the Matrix Hydra DT
7. ControlMate software was used to program the assay setup (see Figure 2):
   a. The plate was filled with BR solution:
      i. The Matrix Hydra DT deck moved to the second position and aspirated 100 µl of BR solution
      ii. The deck moved to position one and the Matrix Hydra DT dispensed into columns 1 and 2 of the 96-well plate
      iii. This was repeated twice more to fill columns 3-6 with BR solution
   b. At the ControlMate "tip change" command, the user inserted a fresh magazine of tips arranged for the first two columns only
   c. During the tip change, the user also replaced the reagent reservoir in position two with the 96-well stock plate containing standards and samples
   d. The following addition of standards and samples were made to the assay plate:
      i. In order to ensure that all of the sample would be dispensed into the assay plate, a preliminary air gap was aspirated by moving to the top of the plate in deck position two and aspirating 25 µl of air
      ii. From position two, the Matrix Hydra DT aspirated 10 µl of standard or sample with a 15 µl air gap
      iii. The Matrix Hydra DT moved to position one and dispensed all of the liquid in the tips into the first two columns of the 96-well plate
      iv. This was repeated two more times to fill columns 3-6 with standards or samples

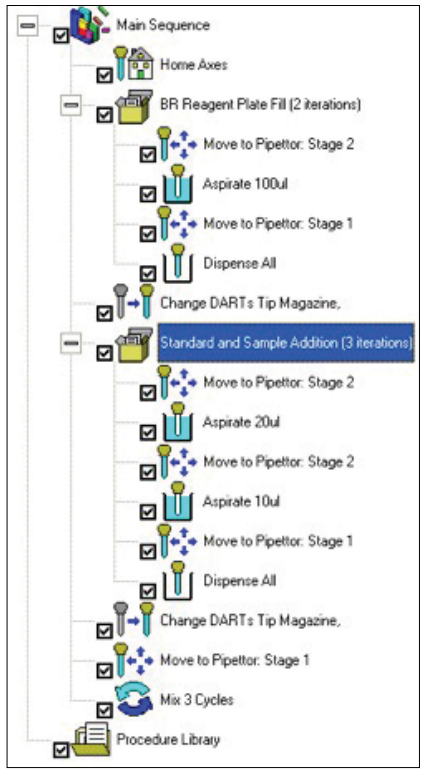

Figure 2: The ControlMate protocol used to automate each assay. Modifications were made to this program depending on plate format (96- or 384-well), automation equipment used (Matrix Hydra DT or Matrix PlateMate 2x2) and the volume of liquid used for each assay. Files which contain the exact protocol used for each assay are available upon request.

8. Using ControlMate software, the program was modified to decrease the reagent and standard or sample volumes to 100 µl and 5 µl respectively, as well as added 50 µl of reagent and 2.5 µl of sample to the plate. For the smaller volume dispenses, 96-channel, 100 µl Matrix D.A.R.T.s tips were configured to occupy the first two columns of the tip magazine. This assay was performed using a 384-well microplate in place of the 96-well microplate. In the above protocol, where the reagent, standard or samples were added to columns 1 and 2, 3 and 4 and 5 and 6 in the 384-plate configuration, this is replaced by quadrants 1, 2 and 3. The protocol was otherwise the same for both plate configurations
9. The plates were read by the Tecan Genios reader and analyzed using XFluor4 software
10. This procedure was repeated using a similar ControlMate program modified for the Matrix PlateMate 2x2 to perform the automated setup of the assay (Both the 200 µl BR reagent/10 µl standard and 100 µl BR reagent/5 µl standard assays were performed)
11. This procedure was repeated using calibrated Matrix single and multichannel electronic pipettes to perform the assay manually

Results:

Comparison of Standard Curves Using 200 µl BR Reagent and 10 µl of Standards

The correlation coefficient, R^2, denotes the percentage of variation between the dependent variables (in this study, concentration of DNA) and the independent predictor variables (in this study, fluorescence)[2]. In order to compare the manual assay setup to setups using the Matrix Hydra DT and Matrix PlateMate 2x2, we compared the R^2 values of each standard curve generated. For the recommended kit assay volume (200 µl BR reagent and 10 µl of standard), the Matrix Hydra DT and Matrix PlateMate 2x2 setups produced comparable R^2 values to the manual setup (see Figure 3).

Comparison of Standard Curves Using 100 µl BR Reagent and 5 µl of Standards

In order to conserve valuable reagents and samples, a program was generated to determine if the volumes of reagent and samples could be decreased by 50% and still receive comparable R^2 values. We reduced the amount of BR reagent to 100 µl and the standard/sample volume to 5 µl. Both automated setups produced highly correlative standard curves which were comparable to the curve produced by the manual setup (see Figure 4).

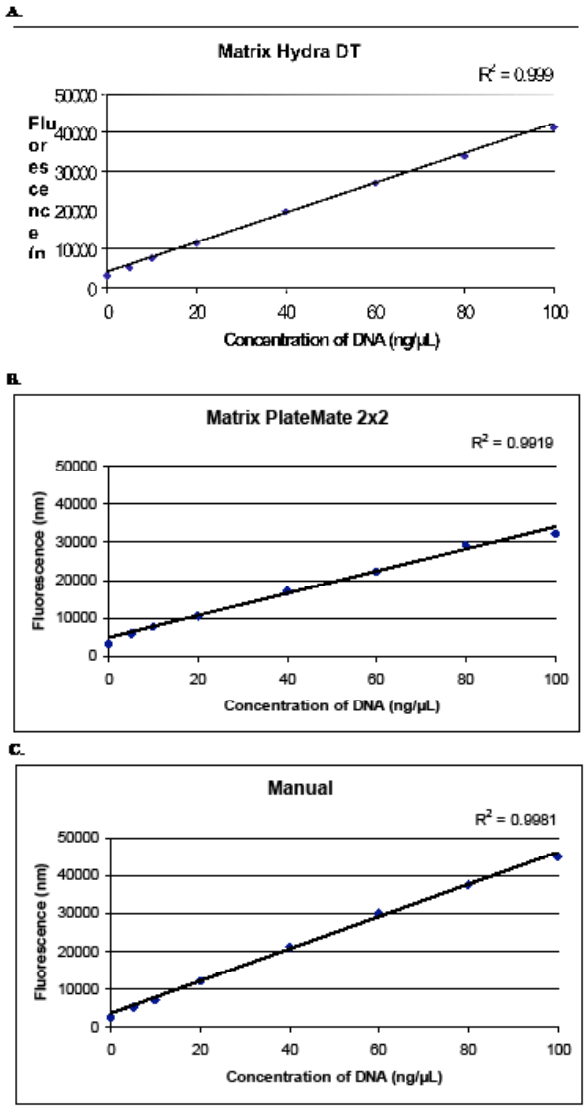

Figure 3: Standard curves generated with 200 µl BR solution and 10 µl of standard stock solutions (0, 5, 10, 20, 40, 60, 80, 100 ng/µl).

Comparison of Standard Curves Using 50 µl BR Reagent and 2.5 µl of Standards

The volume range of the Matrix Hydra DT allowed us to further reduce the final volume of the setup to 50 µl of BR reagent and 2.5 µl of standard or sample. The R^2 value produced by the automated setup at this volume level was larger than the value produced by the manual setup. Therefore, indicating a higher degree of correlation in the standard curve resulting from the automated setup. This assay successfully reduced the total volume of reagents and samples necessary by 75% (see Figure 5).

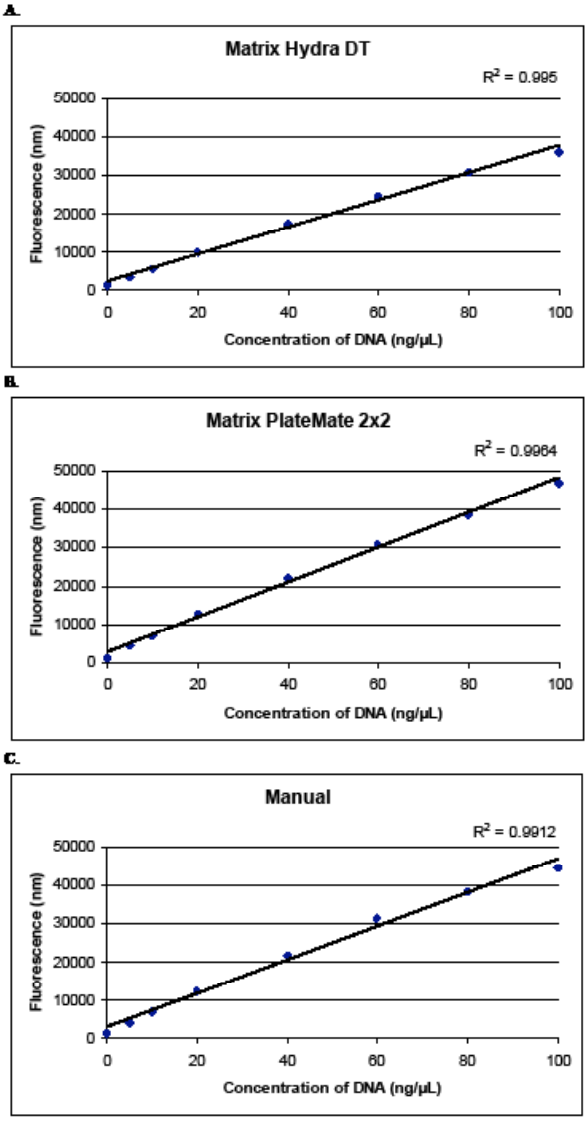

Figure 4: Standard curves generated with 100 µl BR solution and 5 µl of standard stock solutions (0, 5, 10, 20, 40, 60, 80, 100 ng/ µl).

Conclusion:

The Matrix Hydra DT and Matrix PlateMate 2x2 are bench top liquid handlers which have the capability to automate assays to improve efficiency and reproducibility. We have demonstrated that these instruments can successfully and comparably partially automate the setup of the PicoGreen Quant-iT dsDNA Quantitation Kit. In an effort to save expensive reagents and samples, we have also demonstrated that automated setups yield similar results while using 50% less reagent and sample. Automated setup was more successful than manual setups when the reagent and sample volumes were reduced by 75%.

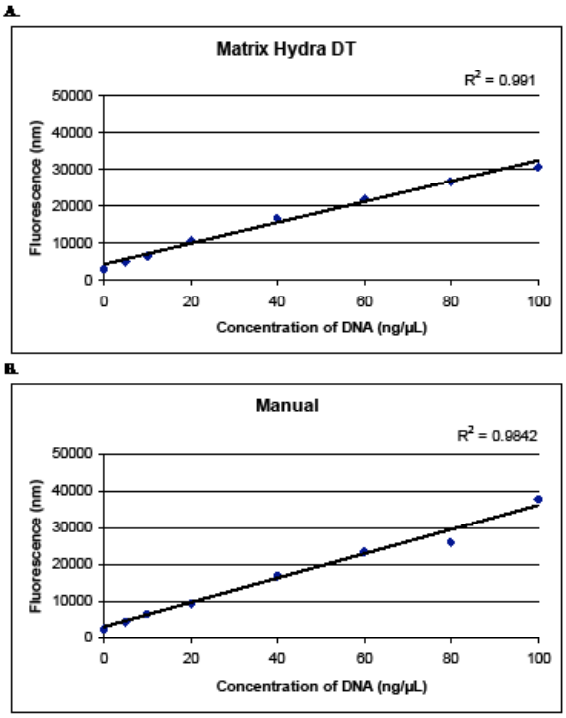

Figure 5: Standard curves generated with 50 µl BR solution and 2.5 µl of standard stock solutions (0, 5, 10, 20, 40, 60, 80, 100 ng/µl).

Our experiments indicate that using the Matrix Hydra DT and Matrix PlateMate 2x2 for automated assay setup successfully allows for conservation of valuable reagents and samples without compromising assay integrity.

Reference:

1. Ahn, S.J., Costa, J., Emanuel, J.R. PicoGreen® quantitation of DNA: effective evaluation of samples pre- or post-PCR. Nucleic Acids Research. 1996, 24:2623-2625.
2. Moore, D., McCabe. Introduction to the practice of statistics. 1993 W.H. Freeman and Company, New York, 854.

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