PIPETMAX® Normalization Assistant: Automated Normalization of DNA and RNA Samples

APPLICATION BENEFITS

Quantitation and normalization of nucleic acids is critical, as the concentration affects the outcome of downstream procedures; however, manual nucleic acid normalization is an error-prone procedure that requires tedious calculations and constant readjustment of pipettes. Manual data entry is also a challenge for the effective recordkeeping and sample tracking required for traceability.

SOLUTIONS

Normalization Assistant allows everyone in your lab to create fully-automated DNA or RNA normalization protocols on PIPETMAX®. Dilution and sample volumes are automatically calculated and are documented in run reports. Sample information can be imported and exported to save time and prevent errors. Normalization Assistant brings effortless traceability and reliability to your DNA or RNA normalization sample prep while performing all of your normalization sample prep for you.

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ABSTRACT

Normalization of the concentration of nucleic acid samples is a crucial yet tedious process that requires constant readjustment of the volume of the pipetting device. PIPETMAX® Normalization Assistant automates this process: calculations are carried out by the software, eliminating arithmetic errors, and pipetting volumes are adjusted automatically. Users can import and export sample information and reports, improving the traceability of the normalization workflow. In this application note, we show three examples of nucleic acids that were normalized using Normalization Assistant: genomic DNA, cDNA, and RNA.

INTRODUCTION

Isolation and purification of nucleic acids from cells, tissues, and fluids is a key step in many biological experiments and diagnostic protocols. Quantitation and normalization of nucleic acids is critical, as the concentration affects the outcome of downstream procedures. Gilson has developed PIPETMAX Normalization Assistant to automate the normalization process. PIPETMAX Normalization Assistant (Figure 1) is compatible with all quantitation methods and can normalize both DNA and RNA samples.

Figure 1
PIPETMAX® Normalization Assistant

⁜ GILSON®

MATERIALS AND METHODS

Nucleic Acid Quantitation

PIPETMAX® Normalization Assistant is compatible with any quantitation method in which the concentration data are provided as a numeric value, including Agilent BioAnalyzer; fluorescence detection methods such as Qubit, Ribogreen, SYBR green; and spectrophotometric determination of absorbance at 260 nm, including Nanodrop. Quantitation data in this application note were generated using a BMG Clariostar, Thermo Nanodrop, or Agilent BioAnalyzer. Use of the Clariostar plate reader was generously provided by BMG.

Liquid Handling

Automated liquid handling was carried out using a Gilson PIPETMAX equipped with multichannel pipette heads (MAX8x20 and MAX8x200). Manual liquid handling utilized Gilson PIPETMAN® equipment. Gilson PIPETMAN DIAMOND tips were used for all experiments.

Software

PIPETMAX Normalization Assistant is a software package that runs on Gilson TRILUTION® micro 2.0. A selection of labware is preloaded in the software; labware geometry definition files were edited using the Labware Creator utility when required.

Nucleic Acid Samples

Nucleic acids used in this study were from mammalian sources (bovine or human). Genomic DNA was from a commercial source. RNA isolation and cDNA synthesis were carried out using standard methods and were a generous gift from the National Institute of Biology in Slovenia.
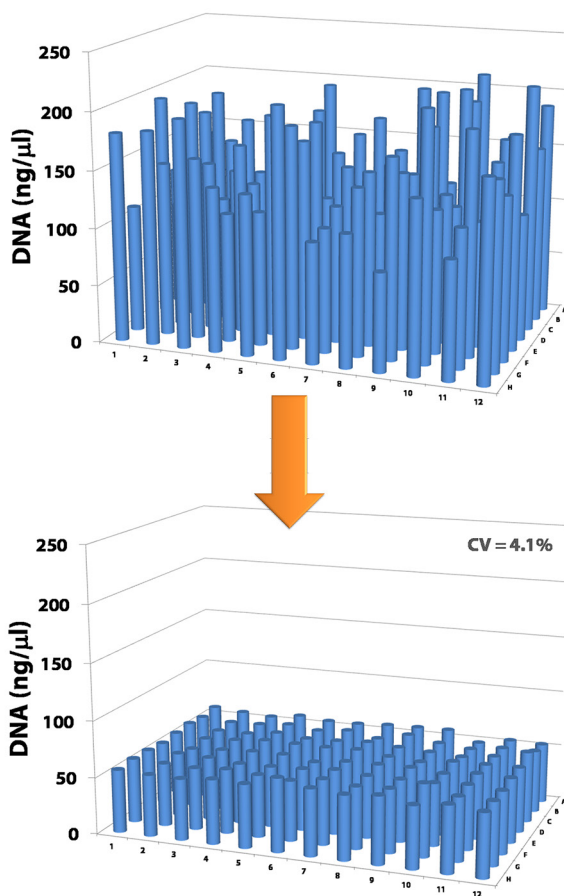
Figure 2
Bovine DNA samples were normalized using PIPETMAX® Normalization Assistant. Graphs show the concentration of each sample before (top) and after (bottom) normalization.

RESULTS AND DISCUSSION

PIPETMAX® Normalization Assistant is a user-friendly software package that prompts users to provide sample position, starting concentration and volume —either by uploading a file or entering information manually—then automatically calculates how much sample and diluent are required to achieve the desired concentration and volume. Reports are generated that track samples, experimental parameters, reagents, and labware used, improving the traceability of the normalization process.

The flexible system is compatible with a wide range of reagents and consumables. Select from a list of pre-installed labware including tubes, microplates and reservoirs—or use the utility to define a custom piece of labware to meet your experimental requirements. Thermal blocks and coolers for temperature control are available as an option. Place your labware and reagents onto the liquid handler bed as guided by the wizard-style software, then start the run and walk away.

Figure 3
Screenshot from TRILUTION® micro running a Normalization Assistant protocol. The PIPETMAX® tray holds up to nine bed elements, providing flexibility in experimental design.
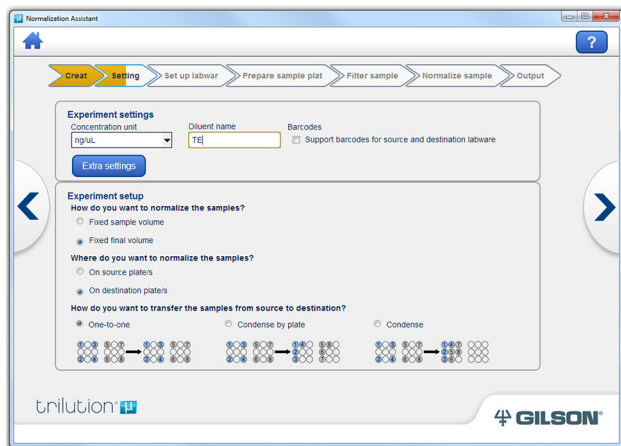
Figure 4
PIPETMAX® Normalization Assistant screenshot illustrating options for normalization scenarios.

The software includes several features designed to enhance your workflow. If desired, user-provided data can be used to filter and eliminate isolates that do not meet minimum quality and concentration criteria. Samples can be normalized either on the source plate(s) or destination plate(s). Samples from multiple source plates can also be condensed into a single destination plate during the normalization process (Figure 3 and Figure 4).

NORMALIZATION OF DNA

Example 1
Genomic DNA from calf thymus was normalized in a 96 well UV-transparent microplate, using the 'fixed final volume on destination plate' scenario (Figure 2).

Example 2
Twenty-four cDNA samples were normalized using PIPETMAX® Normalization Assistant. The normalization scenario for this experiment was 'fixed sample volume on source plate.' Nucleic acids were quantitated using Nanodrop readings. Figure 5 shows the concentrations of the samples before and after normalization with PIPETMAX Normalization Assistant. The coefficient of variance (CV) was 1.8%.
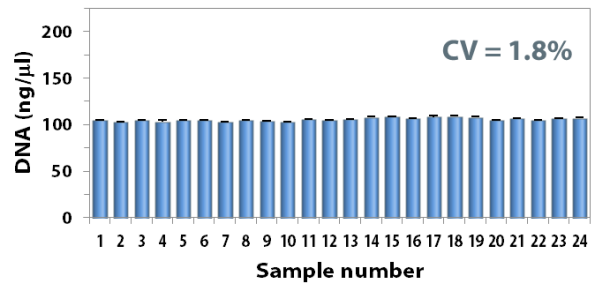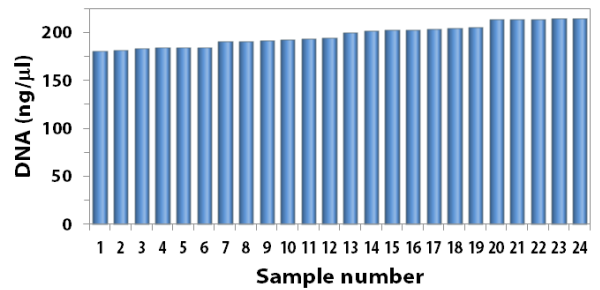
Figure 5
Human cDNA samples were normalized using PIPETMAX® Normalization Assistant. Graphs show the concentration of each sample before (top) and after (bottom) normalization. Error bars in the bottom panel show standard deviation for triplicate measurements of concentration.

Normalization of RNA

Twenty-four RNA samples in triplicate were normalized using PIPETMAX Normalization Assistant and the 'fixed sample volume on source' scenario. RNAse-free labware and sterile filter tips were used. The coefficient of variance (CV) was 1.6%. RNA integrity was maintained throughout the normalization process (Figure 6). No degradation was observed.

In conclusion, PIPETMAX Normalization Assistant couples the quality you've come to expect from PIPETMAN with a robotic stage and easy-to-use software. Whether you're new to automation or experienced with robotic liquid handlers, PIPETMAX Normalization Assistant fits into your experimental workflow and gives you time to focus on science instead of repetitive tasks.

Figure 6

Agilent BioAnalyzer data are shown for one representative RNA sample before (top) and after (bottom) normalization. Abbreviations: RIN, RNA Integrity Number; FU, fluorescence units; nt, length in nucleotides.

SUMMARY

- PIPETMAX® Normalization Assistant prepares nucleic acid samples (DNA or RNA) with normalized concentration for downstream processes and is compatible with any quantitation method.

- Flexible workflow allows user to upload sample information including sample name, concentration, starting volume, position, quality, and more, minimizing manual data input. The software automatically calculates the volumes of sample and diluent required.

- Supports a wide range of labware, including tubes, reservoirs, and microplates. Samples can be normalized on the source or destination plate. Multiple source plates can be condensed into one destination plate if desired.

- Automated liquid handling reduces user errors and repetitive tasks, freeing up researchers' time.

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