



# Normalization of DNA and RNA Samples with PIPETMAX® Normalization Assistant

Application Note TRANS0715

*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.

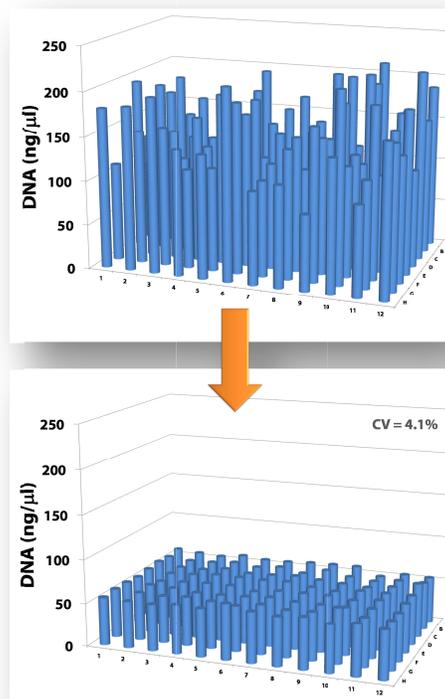


Figure 2. Bovine DNA samples were normalized using PIPETMAX Normalization Assistant. Graphs show the concentration of each sample before (top) and after (bottom) normalization.



## Materials & 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 268 equipped with multichannel pipette heads (MAX8x20 and MAX8x200). Manual liquid handling utilized Gilson PIPETMAN equipment. Gilson 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, Slovenia.

## 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.

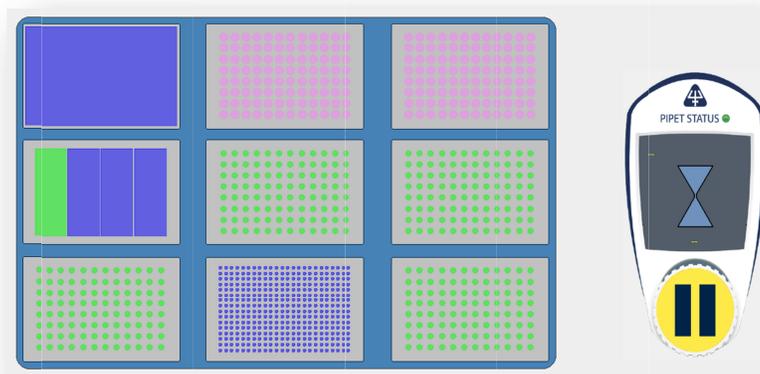


Figure 3. Screenshot from TRILUTION micro running a Normalization Assistant protocol. The PIPETMAX tray holds up to nine bed elements, providing flexibility in experimental design.

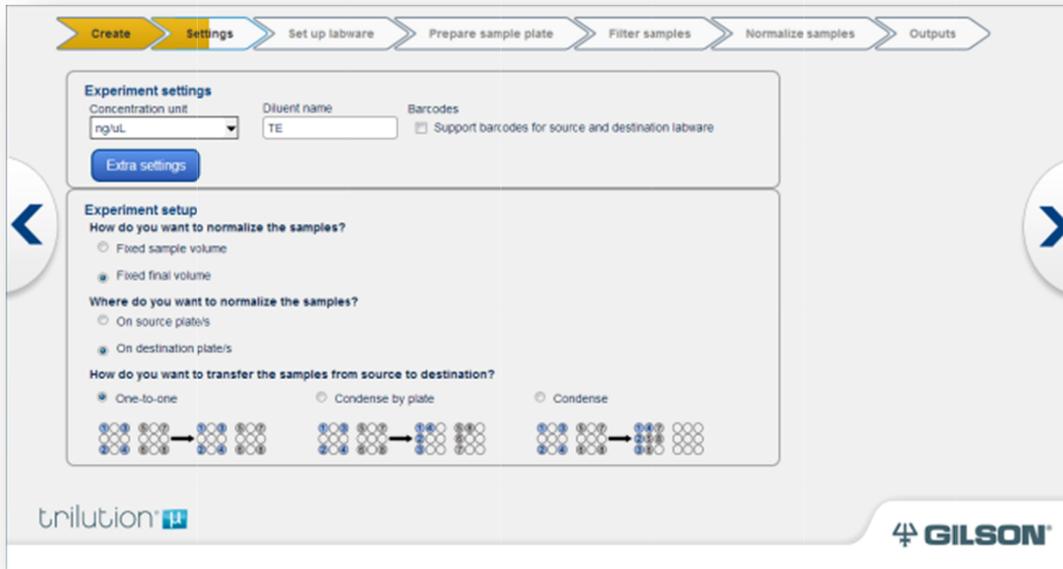


Figure 5. PIPETMAX® Normalization Assistant screenshot illustrating options for normalization scenarios

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, Figure 5).

### 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 6 shows the concen-

trations of the samples before and after normalization with PIPETMAX Normalization Assistant. The coefficient of variance (CV) was 1.8%.

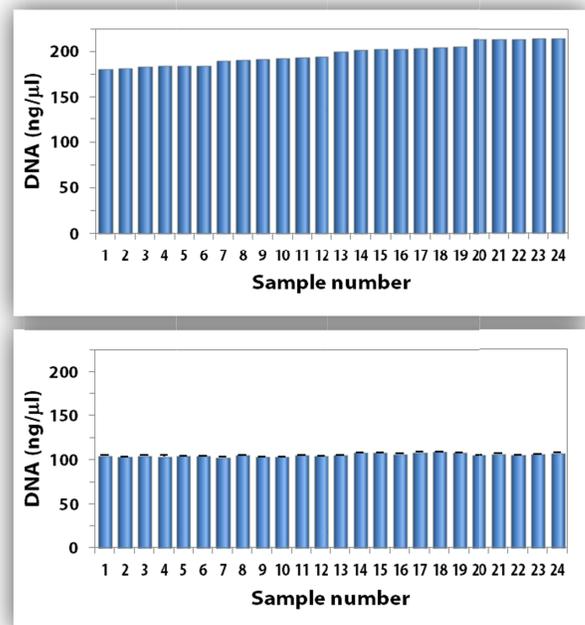


Figure 6. Human cDNA samples were normalized using PIPETMAX Normalization Assistant. Graphs show the concentration of each sample before (top) and after (bottom) normalization. Error bars in the bottom panel show standard deviation for triplicate measurements of concentration.

