# Genomic DNA Normalization Using Omega Bio-tek's Mag-BIND® EquiPure gDNA Normalization Kit

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## Introduction

DNA normalization is a crucial step for any next-generation sequencing or PCR-based analysis workflows. Quantification and normalization of DNA is traditionally done either by UB absorbance or fluorescent nucleic acid stain-based methods requiring tedious processes of quantification, computation, and concentration adjustment. Omega Bio-tek's Mag-BIND® EquiPure gDNA Normalization Kit offers a simple and rapid solution for normalization eliminating the quantification and concentration adjustment, saving both time and resources. The kit uses proprietary Mag-BIND® normalization beads, which have limited capacity and allow only a certain amount of input DNA to be captured. It follows a bind-wash-elute procedure to deliver equal quantities of DNA ready for various downstream applications without further quantification. In this technical article, we discuss the normalization results obtained from 3 variable inputs of genomic DNA. We further tested the experimental protocol on a larger sample set of 51 and results obtained are also reported here.

## Materials & Methods

DNA was extracted from whole blood using Omega Bio-tek's Mag-BIND® Blood & Tissue DNA HDQ 96 Kit (M6399). Normalization studies were done in triplicate on 50 µL of DNA at 56 ng/µL, 132 ng/µL, and 242 ng/µL concentrations using Omega Bio-tek's Mag-BIND® EquiPure gDNA Normalization Kit (M6423) following the recommended protocol outlined in the manual and eluted in 25 µL of elution buffer to arrive at a final normalized product. The same protocol was followed for a sample set of 51 to test for validity and reproducibility. Each sample was an independent extraction isolated using Omega Bio-tek's Mag-BIND® Blood & Tissue DNA HDQ 96 Kit (M6399). All DNA samples were quantified using Promega's QuantiFluor® dsDNA system.

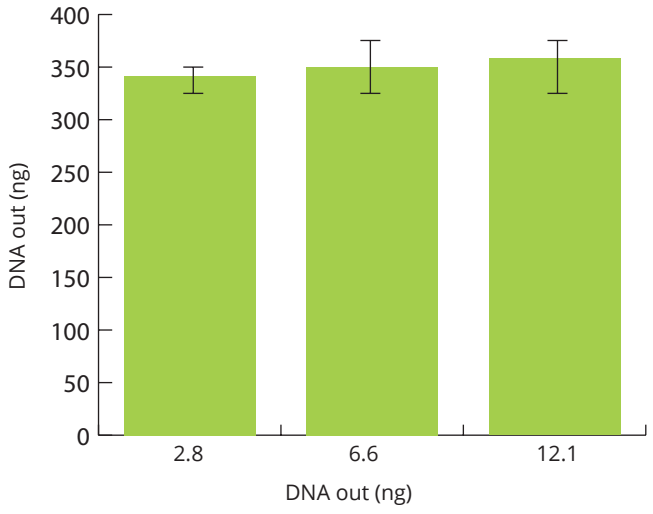



**Figure 1.** Genomic DNA normalization obtained from triplicate experiments with DNA inputs of 2.8 µg, 6.6 µg, and 12.1 µg.

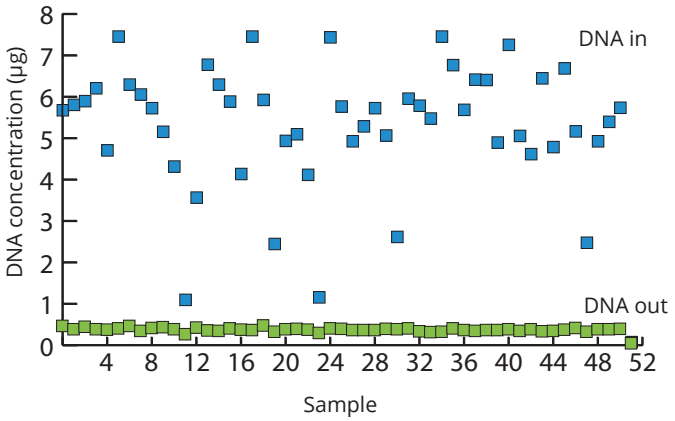



**Figure 2.** Genomic DNA normalization from 51 independent samples.

## Results

Figure 1 shows the normalized DNA amount obtained from genomic DNA inputs of 2.8 µg, 6.6 µg, and 12.1 µg using the Mag-BIND® EquiPure gDNA Normalization Kit. DNA consistently normalized to ~350 ng (± 8.33 ng) for the input amounts tested. After validating the protocol, it was verified on a sample set of 51, which is more representative of a real-life experimental scenario. The results of this study are as shown in Figure 2. The input DNA ranged from 1.1 µg to 7.46 µg. After normalization, the DNA was eluted in 25 µL of elution buffer and the average out was 373 µg with a co-efficient of variation of 11.23% in the data. If we take into account only those input DNA amounts greater than 2 µg, we were able to acheive less than 10% variability in the normalized DNA amount. We also estimated the time required for genomic DNA normalization of 96 samples using Thermo Scientific's NanoDrop™ 2000 and PicoGreen® compared to that of the time required using Mag-BIND® EquiPure gDNA Normalization Kit (M6423) (Figure 3). The entire normalization process was achieved in 30 minutes using the Mag-BIND® EquiPure gDNA Normalization Kit and took ~70 minutes with PicoGreen® when quantified on a plate reader and ~180 minutes using NanoDrop™. It puts into perspective the significant time savings achievable with the kit over traditional methods.

## Discussion

Based on our experimental findings, we recommend an input DNA amount of 2 µg or greater to obtain approximately 350 ng of normalized DNA out with less than 10% variability in the output amount. Typically, different extraction techniques yield varying sizes of isolated genomic DNA, which can cause differences in normalized amount and degree of variability in the data. For best results, magnetic bead-based purification methods and salt-out precipitation methods are recommended over silica spin columns. Silica spin columns tend to shear gDNA in a wider fragment size range increasing the variation in the normalized product. It is highly recommended to quantify 10% of the samples to find the accurate normalization range for your data.

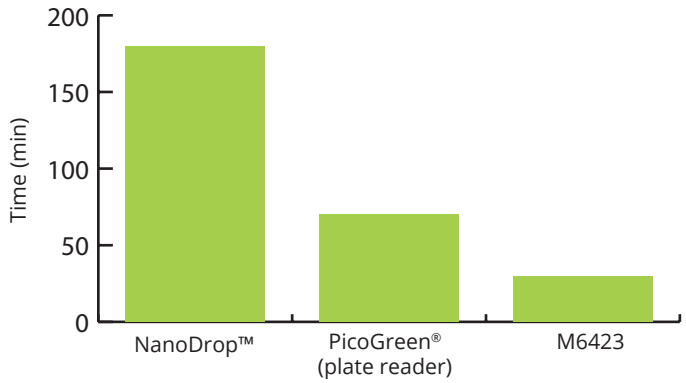
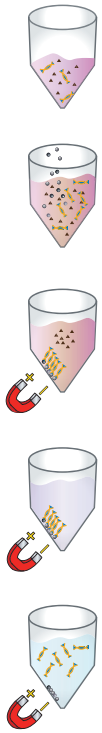



**Figure 3.** Comparison of time required for gDNA normalization of 96 samples using different quantification methods. Time is estimated based on our experiments.

The Mag-BIND® EquiPure gDNA Normalization Kit can be easily integrated into most high throughput application workflows and is fully automatable on various liquid handling platforms such as Hamilton Microlab® STAR™, Tecan Evo®, Caliper SciClone, and Beckman Coulter Biomek® instruments.

## Illustrated Protocol



Transfer up to 50 µl gDNA

Add binding buffer and magnetic particles

Magnetize and remove supernatant

Wash twice with SPM Buffer

Dry

Elute DNA and transfer

## Product Information

| Description | Product No. | Preps |
|---|---|---|
| Mag-BIND® EquiPure gDNA Normalization Kit | M6423-00 | 1 x 96 |
| | M6423-01 | 4 x 96 |
| Mag-BIND® Blood & Tissue DNA HDQ 96 Kit | M6399-00 | 1 x 96 |
| | M6399-01 | 4 x 96 |

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