

NuQuant[®]

Application Note



**A SIMPLE AND ACCURATE METHOD TO QUANTIFY MOLAR CONCENTRATIONS
OF NGS LIBRARIES FOR ILLUMINA SEQUENCERS**



INTRODUCTION

Accurately determining the number of sequenceable molecules after library preparation is a crucial step for a successful Illumina NGS run. Poor quantification of NGS libraries can lead to run failures due to flow cell over-clustering, or increased sequencing costs due to inefficient sequencing from under-clustering of the flow cell. Accurate library quantification is especially crucial for new high capacity sequencers such as the Illumina NovaSeq™ that can generate 10 billion reads, enabling multiplexing of over 300 mRNA-Seq samples (assuming 30 million reads per sample). Errors in multiplexing due to inaccurate library quantification can lead to variability in the number of reads per sample, resulting in insufficient data for some samples and may require expensive resequencing of underrepresented samples.

NuQuant is a novel library quantification method, which accurately measures molar library concentration without the need for separate fragment size analysis. This proprietary method uses a fluorescent label which is incorporated into the library molecules during library preparation. NuQuant is independent of library size, allowing library molarity to be directly measured using fluorometers such as the Qubit™ (Invitrogen) or standard plate readers. The simplicity of library quantification with NuQuant, and how this workflow streamlines the process of pooling libraries for sequencing on Illumina instruments, is highlighted in Figure 1.

This application note highlights the accuracy of library quantification with NuQuant against a range of libraries with different insert sizes and distributions, and shows how it compares to typical methods using quantitative PCR (qPCR) and Bioanalyzer® (Agilent) systems.

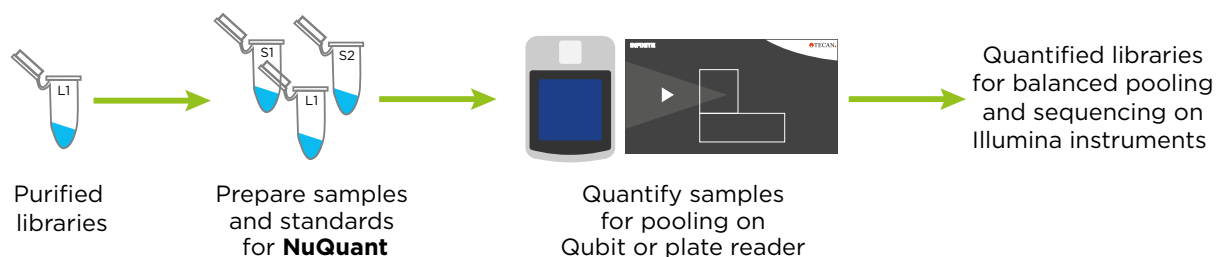


Figure 1: Library quantification workflow with NuQuant. After final purification of libraries using Celero™ DNA-Seq or Universal Plus mRNA-Seq with NuQuant kits, the libraries and standards are diluted in the NuQuant buffer. The standards and libraries are assayed on the Qubit or other compatible fluorescence plate readers. The molar concentration of each library is provided for use in pooling. The pooled libraries are ready for sequencing on Illumina NGS platforms.

Library quantification accuracy with NuQuant compared to Bioanalyzer and qPCR.

The most commonly used methods for measuring library concentration include fluorometry, microfluidic electrophoresis and qPCR. Library quantification using fluorometry (eg. Qubit) and microfluidic electrophoresis (eg. Bioanalyzer) is highly unreliable, as these methods measure total nucleic acid concentrations, which include both sequenceable and non-sequenceable molecules. This results in variation of the library concentration for samples that are not homogenous [1]. qPCR is currently the most accurate method, as it only quantifies sequenceable molecules. However, qPCR is time-consuming and requires additional steps – such as diluting the library (typically 10 thousand to one million-fold), preparing standards, and running the qPCR reactions – all of which can introduce variability [2]. In addition, qPCR uses expensive reagent kits and plastic consumables. Both qPCR and Qubit mass measurements require a separate fragment size analysis step in order to calculate the molar concentration of libraries, adding time and cost to these quantification methods.

The accuracy of library quantification with NuQuant compared to Bioanalyzer and qPCR is shown in Figure 2. In this study, eight libraries were distributed to multiple sites for library quantification. Four of the sites consisted of external users where they compared library quantification with NuQuant to either qPCR or Bioanalyzer. As shown in Figure 2, NuQuant had the lowest variation across all users, followed by qPCR. Bioanalyzer quantification showed the highest variation.



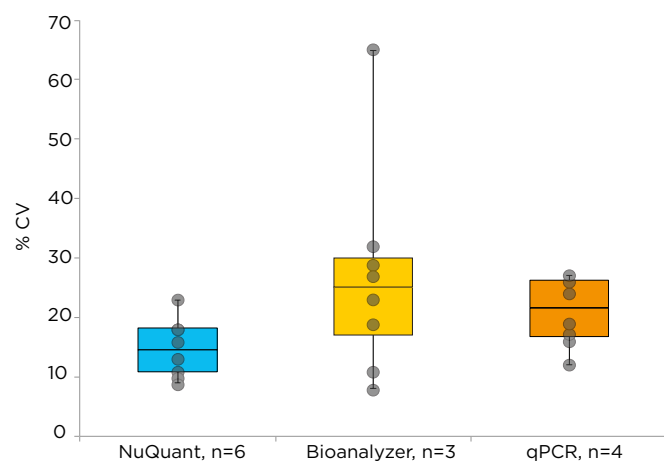


Figure 2: A set of eight libraries was distributed to six different users at multiple institutions. For each library, and each quantification method, the percent coefficient of variation (% CV) of molar concentration was calculated.

NuQuant library quantification and correlation to cluster density.

To determine the correlation between library concentration measured by NuQuant and total reads, DNA-Seq libraries were generated using the Celero DNA-Seq kit. 10 ng of human genomic DNA was fragmented to either 200 or 300 base pairs and amplified with 10 or 13 PCR cycles. Each library was generated in duplicate by two independent users for a total of 16 libraries. The 16 libraries were quantified by NuQuant and ranged in concentration from 144 to 3130 nM. The libraries were pooled at equal volumes regardless of molar concentration and sequenced on one lane of an Illumina sequencer. Total reads were plotted against concentration (nM) for each library (Figure 3). The number of sequencing reads for each sample in the pool directly correlated with the molar concentration of each sample. The strong correlation ($R = 0.97$) between the number of reads per sample and NuQuant molar concentration demonstrates the accuracy of NuQuant quantification. In addition, the NuQuant method accurately quantifies libraries independent of amplification cycle number or insert sizes.

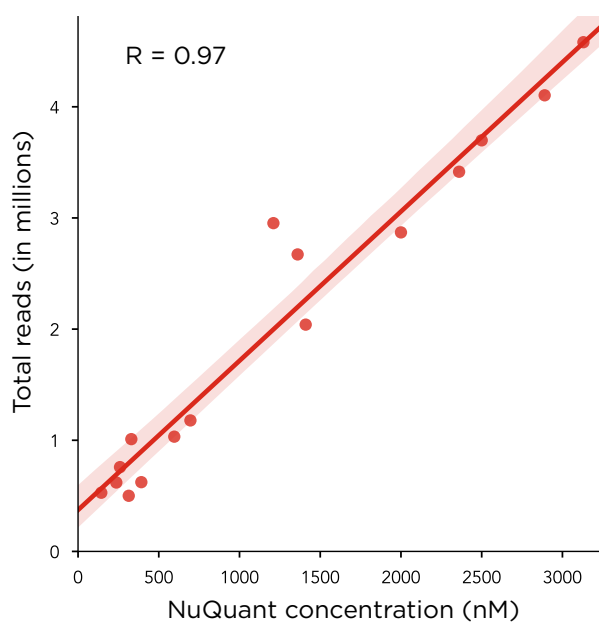


Figure 3: Strong correlation between NuQuant molarity and read numbers from Illumina sequencer. Each library condition (10 or 13 cycles, and 200 or 300 bp) was performed in duplicate for a total of 16 libraries.

Comparison with other NGS library quantification methods.

NuQuant is a fast, cost-effective method for NGS library quantification that eliminates the preparation time and cost associated with current methods. NuQuant is compatible with standard fluorometers, such as Qubit or fluorescence plate readers, eliminating the need for any special equipment.

The overall time and cost needed to measure molar concentration of NGS libraries by NuQuant is compared to other methods in Figure 4. Library quantification with NuQuant can be completed in approximately six minutes without the need for fragment size analysis, at a significantly lower cost than other commonly used methods.





Figure 4: Library quantification workflow with NuQuant compared to qPCR, Qubit and Bioanalyzer. Following the recommended workflow for reliable library quantification by KAPA® Biosystems (Roche) [3, 4], qPCR library quantification can take up to four hours, whilst Qubit and Bioanalyzer analysis can take over an hour for results. NuQuant takes approximately six minutes for library quantification using a simple fluorometric assay, without the need for a separate fragment size analysis. Library quantification with NuQuant is included with certain Tecan kits, eliminating the additional cost associated with library quantification.

CONCLUSION

Here we show the clear benefits of NuQuant, a novel method to accurately measure the molar concentration of NGS libraries. NuQuant speeds up NGS library quantification without the need for additional reagent kits or fragment size analysis. More importantly, library quantification using NuQuant results in lower variability compared to qPCR or Bioanalyzer. NuQuant is simple to use and allows users to combine samples, achieving highly uniform read numbers on Illumina NGS sequencing platforms.

The following DNA and RNA library preparation kits come standard with NuQuant.

REFERENCES

- 1) Robin, JD, et al. Comparison of DNA Quantification Methods for Next Generation Sequencing. *Scientific Reports*, 2016, 6, 24067.
- 2) Ruiz-Villalba, A, et al. Amplification of nonspecific products in quantitative polymerase chain reactions (qPCR). *Biomolecular Detection and Quantification*, 2017, 14, 7-18.
- 3) Kapa Library quantification kits. Next-generation DNA Sequencing meets Next-generation qPCR. SS104001, A179, 4/16
- 4) Kapa Library quantification technical guide. Kapa Library quantification kits for Illumina platforms. v1.14

ORDERING INFORMATION

Product name	No. of reactions	Part no.
Celero DNA-Seq	8, 24	0360-08, 0360-24
Celero DNA-Seq Metaplex	96	0360A-96
Celero DNA-Seq + UDI	96	0360A-UDI
Universal Plus mRNA-Seq with NuQuant	24, 96	0520-24, 0520-A01
Universal Plus mRNA-Seq with NuQuant, human globin AnyDeplete®	24, 96	0521-24, 0521-A01
Universal RNA-Seq with NuQuant, human AnyDeplete	32, 96	0530-32, 0530-A01
Universal RNA-Seq with NuQuant, mouse AnyDeplete	32, 96	0531-32, 0531-A01
Universal RNA-Seq with NuQuant, <i>Drosophila</i> AnyDeplete	32, 96	0532-32, 0532-A01
Universal RNA-Seq with NuQuant + UDI, human AnyDeplete	96	0537-A01
Universal RNA-Seq with NuQuant + UDI, mouse AnyDeplete	96	0538-A01
Universal RNA-Seq with NuQuant + UDI, <i>Drosophila</i> AnyDeplete	96	0539-A01



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