Accurate quantification of Illumina® NGS libraries using Clarity™ digital PCR system

I. Overview

The advent of next-generation sequencing (NGS) has enabled rapid and cost-effective generation of genome-scale sequence data with unprecedented resolution and accuracy. For Illumina® sequencing platforms, DNA libraries are clonally amplified to generate clusters prior to sequencing on a flow cell[1]. To obtain high-quality sequence data, loading the flow cell with an appropriate amount of library DNA is crucial for generating clusters at an optimal density. Loading an insufficient quantity of DNA results in low cluster density and hence reduces sequencing yield. On the other hand, an overabundance of library can lead to poor data quality due to suboptimal cluster resolution. Accurate quantification of libraries prior to loading of flow cells is therefore a key aspect in the Illumina® NGS workflow.

Standard methods for quantifying NGS libraries (e.g. spectrophotometric and fluorometric analysis) account for total DNA content and are non-specific for adapter-bound library fragments. This consequently compromises accurate target quantification. While quantitative PCR provides a more accurate alternative for library quantification by detecting only adapter-ligated sequences, it requires the generation of a standard curve which can be costly and time-consuming. In contrast, digital PCR is a new technology for rapid and accurate library quantification without the need for a standard curve[2,3].

Here, we demonstrate that digital PCR using the Clarity™ system can be integrated into the Illumina® NGS workflow for accurate quantification of sequencing libraries. Using EvaGreen® dye and primers targeting the P5 and P7 flow cell sequences, the Clarity™ digital PCR system is well-suited for quantifying Illumina® NGS libraries irrespective of the library type.

FEATURES

- **Easily incorporated** into the Illumina® NGS library construction workflow
- **Accurate quantification** of any Illumina® NGS library flanked by the P5 and P7 flow cell sequences
- **Cost-effective** and **versatile** using EvaGreen®-based assay

Clarity™ Digital PCR Reader

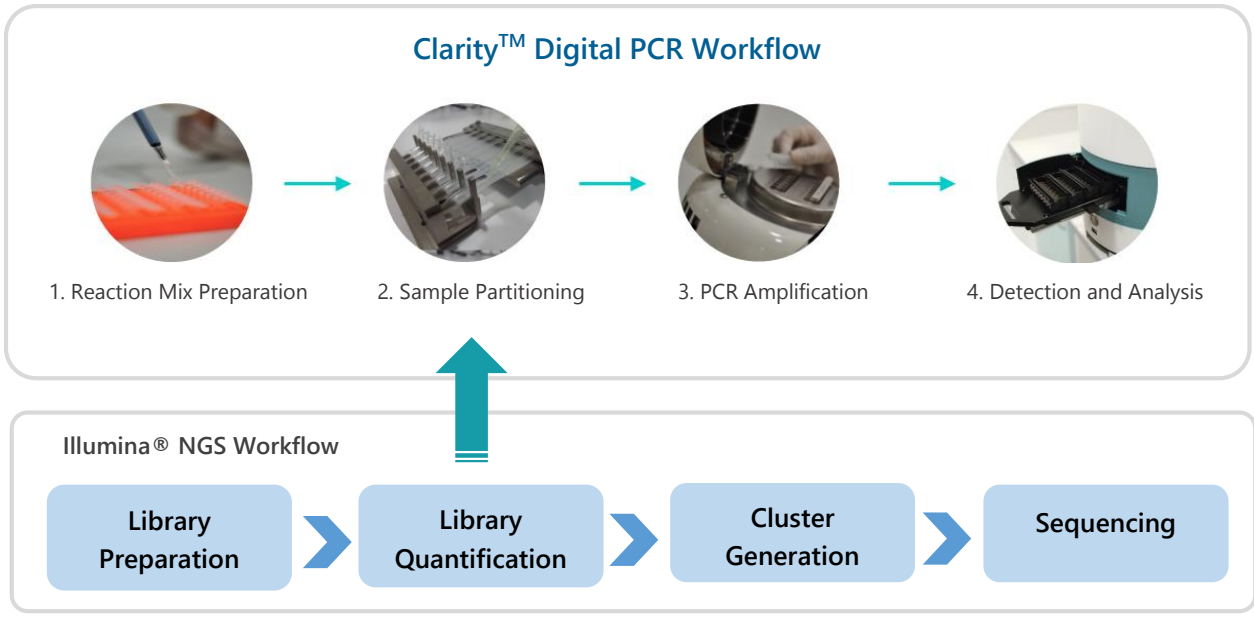

Figure 1. Illumina® NGS library quantification workflow using Clarity™ digital PCR system. The Clarity™ system provides a simple and fast platform to quantitate NGS libraries via digital PCR. The workflow involves four key steps: (1) reaction mix preparation, (2) sample partitioning, (3) PCR amplification and, (4) detection and analysis. Results are provided in copies/µL of reaction which can be easily converted to molar concentrations.

II. Clarity™ digital PCR system accurately quantifies DNA fragments flanked by the Illumina® P5 and P7 sequences

The accuracy and precision of Clarity™ digital PCR system in quantifying Illumina® NGS libraries were validated using a set of DNA standards[4] flanked by the P5 and P7 flow cell sequence motifs (KAPA Biosystems; Catalogue No. KK4903; Table 1 and Figure 2). The use of an EvaGreen® dye-based assay provides a cost-effective and versatile platform for accurate quantification of Illumina® NGS libraries irrespective of the library preparation method.

Table 1. Quantification of the Illumina® NGS standards using Clarity™ digital PCR system. The EvaGreen® dye-based digital PCR assay was designed to amplify the 452bp library fragment using primers that target the P5 (5'-AATGATACGGCGACCACCGA-3') and P7 (5'-CAAGCAGAAGACGGCATACGA-3') sequences. Analyses were performed on Standards 4, 5, 6, and their respective two-fold dilutions (marked with an asterisk), whose expected copies per microliter of reaction fall within the dynamic range of the system. Results shown are representative of three independent experiments performed in triplicates.

DNA Standard	Expected Concentration (copies/µL)	Measured Concentration # (copies /µL)	Relative Uncertainty Δ (%)
4	1205	1547	2.80
4/2*	603	790	1.39
5	121	148	2.37
5/2*	60	74	3.99
6	12	15	2.38
6/2*	6	8	4.94

 # Corresponds to the mean of each set of triplicate;
Δ Calculated by dividing standard deviation with the mean of each set of triplicate

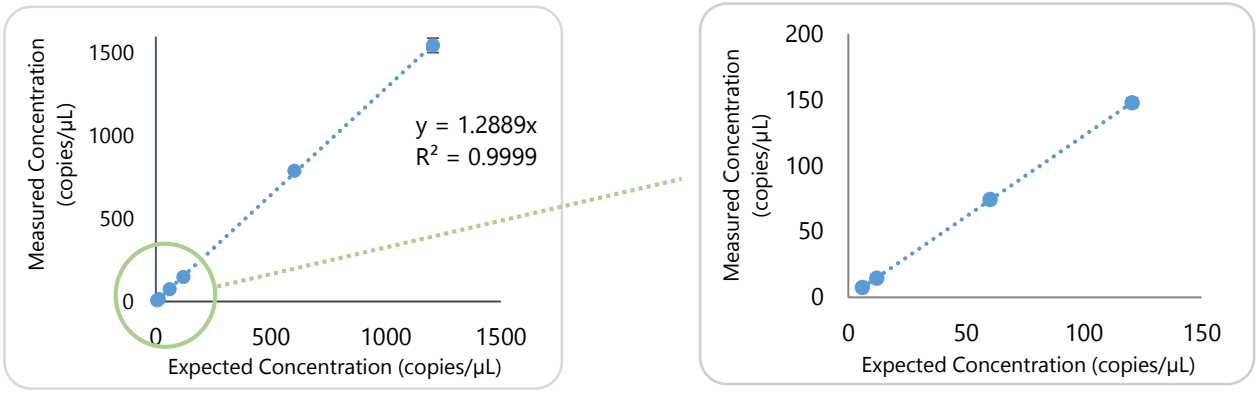

Figure 2. Graphical representation of the results obtained in Table 1. Error bars denote standard deviations of each set of triplicates.

III. Conclusion

Clarity™ digital PCR system supports accurate quantification of the Illumina® NGS libraries and thus maximizes success in generation of optimal cluster densities to yield high-quality sequencing data. Further investigations are currently on-going to validate the correlation between library concentrations quantified by Clarity™ system and cluster densities.

References:

[1] Illumina (2015) An Introduction to Next-Generation Sequencing Technology.
[2] Huggett JF, Cowen S and Foy CA. (2015) Considerations for digital PCR as an accurate molecular diagnostic tool. Clin Chem. 61, 79-88.
[3] Baker M. (2012) Digital PCR hits its stride. Nature Methods 9, 541–544.
[4] KAPA Biosystems (2014) KAPA Library Quantification Technical Guide (KAPA Library Quantification Kits for Illumina® platforms)

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