Application Note

dsDNA Quantification by Fluorescence
For Automated NGS Library QC

Reliable DNA quantification is a critical factor in next-generation sequencing (NGS) workflows, directly influencing library normalization, pooling accuracy, and overall sequencing performance. After library preparation, precise determination of double-stranded DNA (dsDNA) concentration is required to ensure optimal loading of sequencing platforms and balanced representation of multiplexed samples. Fluorescence-based quantification methods offer high specificity for dsDNA and superior sensitivity compared to absorbance-based techniques, making them ideally suited for library quality control applications.

This application note introduces an automated on-deck fluorescence quantification workflow with the eviFluor Duo Fluorometer integrated into liquid handling systems. The performance is evaluated in terms of accuracy and repeatability, demonstrating its suitability for reliable library quantification in automated NGS sample preparation environments.

NGS Library Preparation and Quality Control

NGS workflows involve multiple steps in which nucleic acid samples are transformed into sequencing-ready DNA libraries (see Figure 1).

Library quality control (QC) includes the assessment of library concentration, fragment size distribution, and overall library integrity prior to normalization and pooling. It is essential to prevent issues such as overloading (leading to poor cluster quality), underloading (resulting in low data output), or uneven library representation that can cause biased sequencing.

While UV absorbance remains the preferred method for rapid post-extraction quantification and purity assessment, fluorescence-based methods are most commonly applied for final library quality control. Due to their high sensitivity and specificity for double-stranded DNA, fluorescence measurements provide more accurate concentration data and reduce the risk of overestimation compared to absorbance-based approaches. While qPCR-based methods can provide information on amplifiable library molecules, fluorescence-based quantification offers a simpler and faster approach to routine library QC.

Principle of Fluorescence-Based Quantification

Fluorescence-based quantification relies on the use of dyes that selectively bind to double-stranded DNA. These dyes exhibit minimal interaction with RNA, single-stranded DNA, and free nucleotides, enabling specific detection of the target molecule. In solution, the unbound dye shows only low fluorescence. Upon binding to dsDNA, however, the fluorescence signal increases significantly. The resulting fluorescence intensity, measured in relative fluorescence units (RFU), is directly proportional to the amount of dsDNA present in the sample.

In contrast to UV absorbance measurements, fluorescence-based quantification requires the use of a calibration curve generated from standards with known dsDNA concentrations. The fluorescence signal obtained from the sample is compared to these standards to determine the dsDNA concentration. Because the signal originates specifically from dye-bound dsDNA, the measurement is largely unaffected by common contaminants such as proteins, salts, or free nucleotides.

Figure 1: NGS sample preparation workflow

Together, these characteristics enable highly sensitive and selective quantification of double-stranded DNA, making fluorescence-based methods well suited for accurate library quantification in NGS workflows.

Manual Versus Automated Workflow

Despite advances in automation, library QC is still commonly performed manually using benchtop fluorometers or microplate readers. While widely used, these methods introduce several challenges:

- Manual pipetting: increases hands-on time and the risk of operator-induced errors
- High reagent consumption: requires large volumes of dye/working solution
- Manual data transfer: adds workload and risk of transcription errors
- Limited integration into automated workflows: requires dedicated bench space and creates bottle-necks in medium- to high-throughput applications

To address these limitations, the eviFluor Duo Fluorometer provides an automated, on-deck, cuvette-based workflow that integrates seamlessly with liquid handling systems.

Figure 2: eviFluor Duo Fluorometer

Integrating fluorescence measurement directly into the liquid handling workflow offers the following key advantages:

- Precision and reproducibility: Automation removes manual pipetting variability and ensures consistent sample handling
- Walk-away processing: Once samples are loaded on the liquid handler, the process runs fully hands-free for up to 288 samples (3 x 96 wells)
- Reduced reagent consumption: Optimized on-deck workflows minimize the required volumes of dye and working solution by a factor of five
- Data integrity: Automated digital data capture provides traceability, reduces errors and supports downstream analytics
- Space-saving design: A single SBS deck position is required, enabling seamless integration into automated workflows

Performance Data

Measurement Workflow

The following study demonstrates fluorescence measurements performed on the eviFluor Duo Fluorometer, integrated into a liquid handler, and compares them with a benchtop fluorometer, using the Qubit™ 1X dsDNA High Sensitivity (HS) Assay Kit.

Automated Workflow With eviFluor Duo Fluorometer

For measurements with the eviFluor Duo Fluorometer integrated into a liquid handling system, 2 µL of each sample is dispensed into a 96-well mixing plate with the first two wells containing 2 µL of assay standards.

Subsequently, 38 µL of Qubit™ 1X working solution are added. Sample and reagent are mixed in the plate by pipette mixing to ensure homogeneous mixing, followed by an incubation time of 2 minutes.

Following incubation, 10 µL of the prepared solution are aspirated, starting with the two standards first to generate the calibration curve. A cuvette is then picked up with the pipette tip and transferred to the eviFluor Duo detection unit. The system first records a baseline measurement and subsequently measures the empty cuvette to correct for optical background and instrument noise. The sample is then dispensed into the cuvette, and the fluorescence emission signal is measured.

Manual Workflow With Benchtop Fluorometer

For the benchtop fluorometer, standards and samples are prepared according to the manufacturer's instructions, using 190 µL of Qubit 1X working solution, and 10 µL of standards or samples respectively.

Results and performance data

Figure 3 shows measurements performed with lambda dsDNA on the eviFluor Duo fluorometer and a benchtop reference fluorometer. Eight replicates of each sample were measured. The results demonstrate excellent agreement between the eviFluor Duo Fluorometer and the reference fluorometer (R^2>0.999). In addition, the eviFluor Duo exhibited consistently low coefficients of variation (CV), indicating high measurement precision (see Figure 4).

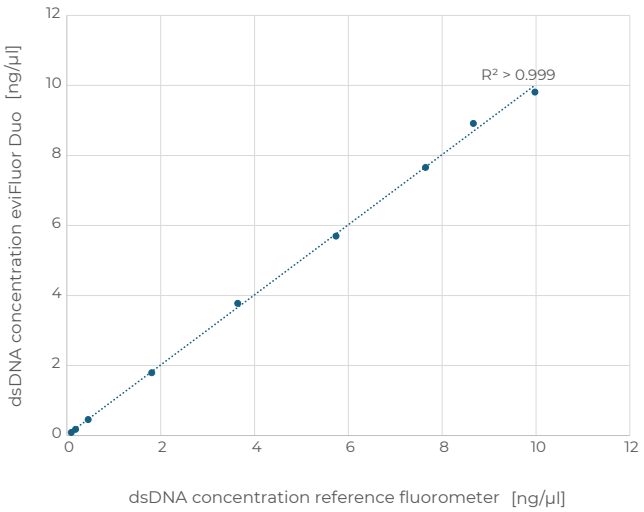

Figure 3: Correlation of dsDNA concentration measurements of lambda DNA using Qubit 1X dsDNA HS kit: eviFluor Duo Fluorometer versus reference benchtop fluorometer

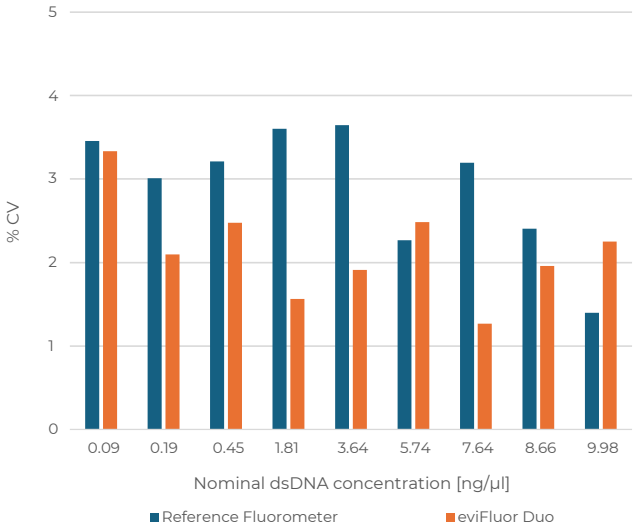

Figure 4: Coefficient of variation (CV) of dsDNA concentration

Summary and Conclusion

An automated fluorescence-based workflow enables accurate and reproducible quantification of double-stranded DNA (dsDNA) for NGS library quality control. The eviFluor Duo Fluorometer, integrated into a liquid handling system, performs fully automated, on-deck measurements with minimal hands-on time.

Measurements using lambda dsDNA and a Qubit™ 1X dsDNA HS assay kit show excellent agreement with a benchtop reference fluorometer (R^2>0.999) while consistently low coefficients of variation confirm high precision.

Direct integration of fluorescence quantification into the automated workflow reduces manual handling, lowers reagent consumption, and improves data integrity. High sensitivity and specificity for dsDNA make the eviFluor Duo Fluorometer a robust and scalable solution for reliable NGS library quantification.

Contact: info@hseag.com / Subject to technical changes

For more information, consult
www.on-deck-fluorometer.com