Streamlining Next Generation Sequencing quality management using high throughput fluorometric DNA/RNA quantification

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- **FLUOstar® Omega microplate reader quantifies nucleic acids for NGS applications**
- **Fluorescent nucleic acid quantification performed in high throughput**
- **Integrate quantification in the automatic workflow of NGS sample preparation**

Introduction

Next generation sequencing (NGS) technology and applications are rapidly evolving leading to highly increased sampling frequency. Accurate fluorometric quantification of nucleic acids is one of the key qualification parameter in gating samples through the preparation workflow. Given the high and diverse sample number this represents a major challenge (Figure 1).

Here we show that combining Qubit assays with the FLUOstar® Omega multi-mode plate reader is ideally suited for high-throughput sample quantification that directly impacts quality of deep sequencing experiments. The assay is fast, precise and straightforward and can be easily included in automated library preparation systems.

Figure 1 represents a typical, quality controlled NGS workflow. Depending on the assay (WGS, ChIP-Seq, RNA-Seq etc.) DNA or RNA analytes are fluorometrically quantified and quality, together with integrity parameters, is evaluated. In a second step, sequencing libraries are constructed and successful preparation validated using fluorometric DNA quantification in combination with capillary electrophoresis. Prior sequencing, libraries are pooled to facilitate multiplex sequencing and pools are loaded on a sequencing instrument for analysis. Percentage of DNA amount of each library in a pool as well as total amount of DNA loaded on a sequencing instrument directly influences quality of each sequencing experiment and sequencing run, making precise quantification at any step of the workflow fundamental.

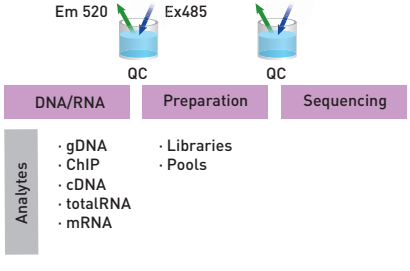

Fig. 1: Quality management in NGS workflows using fluorometric nucleic acid quantification. Fluorometric quantification gates samples between different steps of the workflow (analytes: gDNA: genomic DNA, ChIP: Chromatin immunoprecipitation, cDNA: complementary DNA, mRNA: messenger RNA, QC: quality control).

Assay Principle

For specific and sensitive fluorometric quantification of a wide range of samples Qubit assay kits have become gold-standard in NGS applications. The nucleic acid quantification kits provide fluorophores that specifically bind dsDNA or RNA and subsequently enhance their fluorescence. The fluorescence intensity correlates with the amount of dsDNA or RNA and is used to calculate the nucleic acid concentration. Qubit chemistry is typically detected directly in a sample tube on a Qubit fluorometer. Therefore, Qubit cannot be integrated into an automatic workflow. Transfer of the reaction to a microplate opens the possibility to use the Qubit fluorescent dyes for automated nucleic acid quantification.

Materials & Methods

- Qubit dsDNA HS Assay (ThermoScientific, Q32854))
- Qubit RNA HS Assay (ThermoScientific, Q32855)
- Qubit RNA BR Assay (ThermoScientific, Q10210)
- Qubit Assay tubes (ThermoScientific, Q32856)
- 96-well plates, black, F-bottom (Greiner bio-one, 655076)
- Qubit® single tube fluorometer (ThermoScientific)
- FLUOstar® Omega (BMG LABTECH)

Experimental procedure

DNA/RNA samples were diluted with the respective Qubit®working solution either in Qubit® assay tubes or into 96-well microplates. Measurement volume was for all assays 200 µl including a 10 µl standard and 2 µl or 1 µl sample. In microplates samples and Qubit working solution were mixed by pipetting using a 300 µl 8-channel pipette. After 2 minutes incubation, fluorescence intensity was detected using the FLUOstar Omega multi-mode plate reader (BMG labtech) or the Qubit® single tube fluorometer. In case of the FLUOstar Omega data was exported as standard excel.

Instrument settings

Optic settings	Fluorescence intensity, top optic, endpoint		
	Filters	DNA	Ex: 485 Em: 520 Gain: 1100
		RNA	Ex: 630-10 Em: 670-10 Gain: 2600
General settings	Number of flashes	20	
	Settling time	0.5 s	

Results & Discussion

DNA sequencing libraries and total RNA samples were quantified using the FLUOstar Omega microplate reader or the Qubit® single tube fluorometer. Plate reader measurements were conducted in duplicate and standard

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deviation of technical replicates automatically calculated using the MARS analysis software package. For both, DNA and RNA quantification, measurements agree very well between the different fluorescence detection devices (Figure 2). The FLUOstar Omega enables simple replicate analysis thus enhancing measurement precision. Quantification assays can easily be carried out using multichannel pipetting to increase throughput and enable seamless integration into liquid handling systems.

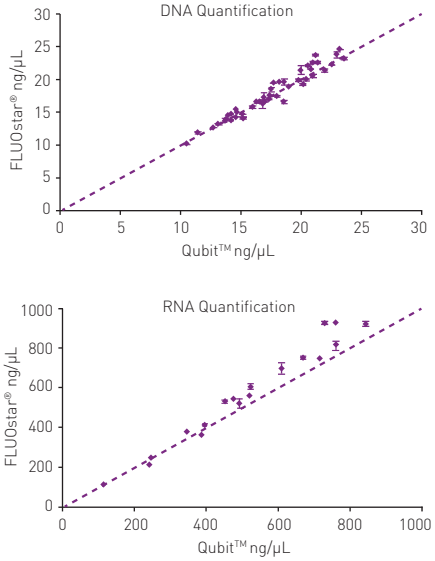

Fig. 2: DNA and RNA fluorometric quantification using the FLUOstar Omega multi-mode plate reader in comparison to the Qubit® single tube fluorometer.

Scarce samples (low volume, low concentration) are typical challenges for accurate quantification. We compared quantification of rare (low input/low volume) RNA samples on both the FLUOstar Omega multi-mode plate reader and the Qubit® single tube fluorometer. While the Qubit instrument only measured concentration in a fraction of the analyzed samples the plate reader detected RNA concentration in all measured samples (sample volume for measurement: 1 µl) demonstrating superior sensitivity.

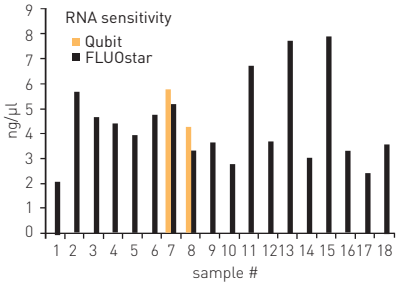

Fig. 3: RNA detection sensitivity on the FLUOstar Omega multi-mode plate reader or the Qubit® single tube fluorometer.

Conclusion

The NGS sample preparation laboratories are conducting nucleic acid quantification assays on a daily basis but technology that provides adequate throughput and precision remains a challenge.

Here we show that combination of Qubit assays with the FLUOstar Omega multi-mode microplate reader is ideally suited to measure a wide range of nucleic acids and improves compared to the single tube fluorometer on sensitivity, precision and speed. Especially large laboratories that perform automated high throughput sample preparation will profit from this technology.