Enabling library QC in under six minutes

NuQuant is a novel method for measuring the molar concentration of NGS libraries. Quantification methods such as qPCR require multiple manipulation steps, which can introduce sample variability. NuQuant directly measures the number of sequenceable molecules that are in your library, simplifying and speeding up NGS library quantification.

Why use NuQuant?

1. **Save money**: integrated library QC without the need for qPCR
2. **Save time**: simple fluorescence assay to determine library molarity
3. **Get more consistent results**: no serial dilutions of samples that introduce variability
4. **All inclusive**: NuQuant comes included in the Celero™ DNA-Seq (PCR workflow only) and Universal Plus mRNA-Seq library preparation kits

Features

- Direct measurement of library molar concentration for faster library quantification and pooling
- Labels each library molecule equivalently regardless of length for accurate quantification
- Commonly used wavelengths of 650/670 nm allow the use of plate readers without sample loss, simplifying high throughput quantification
- More reproducible than qPCR-based quantification methods for sample pooling
- NuQuant is compatible with other quantification methods

Technical details

- NuQuant apps (for Qubit 2.0, 3.0 and 4) are available on GitHub for download
- Compatible with most common benchtop fluorometers
- Included with Celero DNA-Seq PCR workflow and Universal Plus mRNA-Seq

Figure 1: Library quantification workflow with NuQuant. After the final bead purification step of the Celero DNA-Seq or Universal Plus mRNA-Seq with NuQuant library preparation kits, the libraries and standards are diluted in the NuQuant buffer. The standards and libraries are assayed on fluorometers such as 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 instruments.
Figure 2: Comparison of library quantification methods. Costs based on published list prices for Qubit BR DNA assay, Agilent HS DNA Bioanalyzer Chip, KAPA qPCR Library Quantification kit, and assume 11 libraries run per chip (Bioanalyzer) or 30 libraries per kit (qPCR, KAPA’s recommended 96 well protocol).

Figure 3: Strong correlation between NuQuant molarity and read numbers. Two users prepared eight libraries each from 10 ng of genomic DNA sheared to 200 or 300 bp using a Covaris system and amplified for 10 or 13 cycles to produce both lower and higher library concentrations. Purified libraries were quantified in duplicate by NuQuant, then equal volumes of all libraries were pooled and sequenced on one lane of an Illumina sequencer. Correlation between the measured molarity and number of reads for each samples indicates the accuracy of NuQuant quantification.

Figure 4: NuQuant has the lowest variability for library quantification. A set of eight libraries was distributed to six different users at multiple institutions. Users were asked to use NuQuant and their own preferred method of library quantification (number denoted by n). For each library, and each quantification method, the percent coefficient of variation (% CV) of molar concentration was calculated. NuQuant produced the lowest variation, followed by qPCR. Bioanalyzer gave the highest variation.