

JetSeq[™] Library Quantification Kit

Library

Dilution buffer

Workflow for the JetSeq Library Quantification Assay A set of six pre-diluted DNA Standards (representing a 10-fold dilution series), as well as appropriately diluted Library samples, are amplified by qPCR, using the JetSeq FAST qPCR Mix and primers based on the Illumina P5 and P7 sequences. The average Ct value for each DNA Standard is plotted against

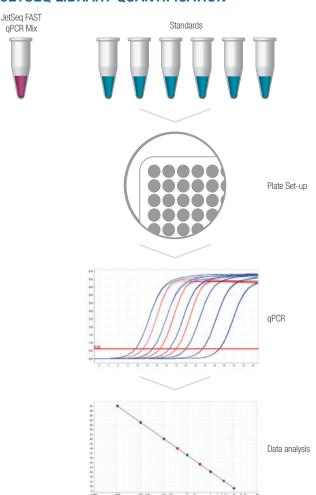
log₁₀(concentration in pM) to generate a standard curve, where the diluted library samples concentration can be calculated.

Powering NGS

- Accurate: qPCR-based assay for quantification of only adapter-ligated library molecules, thereby enabling optimal flow cell loading for maximum data yield and quality
- Sensitive: reliable quantification of even low-yield libraries, ideal for both PCR and PCR-free library preparation methods
- Fast: delivers accurate assay results in as little as 90 minutes, thereby reducing time to results
- Convenient: contains a series of six pre-diluted DNA standards for rapid, simple standard curve development
- Economical: contains sufficient reagents and standards to quantify eighteen individual libraries on separate plates

The JetSeq[™] Library Quantification Kit is designed to eliminate the need for time-consuming and expensive titrations and provides an accurate, inexpensive method of quantifying the number of amplifiable library molecules loaded onto a flow cell on Illumina® instruments.

JETSEQ LIBRARY QUANTIFICATION





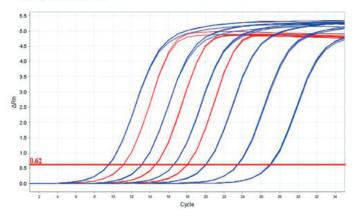
IMPROVED SEQUENCING DATA

Accurate quantification of the number of amplifiable library molecules loading into a flow cell is one of the most critical steps in the next-generation sequencing (NGS) workflow in obtaining high-quality read data. Loading an insufficient amount of amplifiable library DNA will result in low cluster density and reduced sequencing yield, whereas overabundance of amplifiable library DNA may increase cluster density and result in poor quality data.

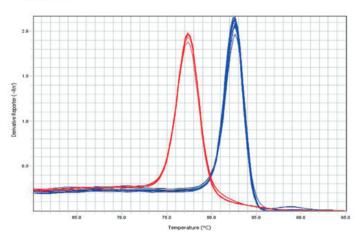
Standard methods of NGS library quantification, by electrophoresis or spectrophotometry, have low sensitivity, are non-specific for adapter-bound DNA and typically require a large amount of library sample for analysis. The JetSeq Library Quantification Kit, by contrast, uses qPCR to quantify only DNA molecules with adapters at both ends, which are the only amplifiable molecules during bridge PCR on the Illumina platform.

The JetSeq Library Quantification Kit therefore provides highly accurate quantification of adapter-ligated, sequence-ready library DNA molecules. In addition, the higher sensitivity of qPCR allows quantification of libraries at very low concentrations, even below the detection threshold of conventional spectrophotometric methods.

A. Amplification curve



B. Melt curve



C. Standard curve

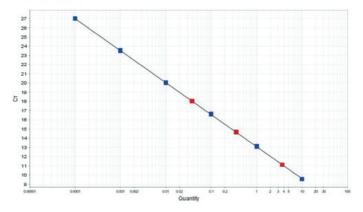


Fig. 1 JetSeq Library Quantification Kit Performance.

A) qPCR amplification of each of the six supplied DNA standards (blue) and a tenfold serial dilution of the diluted Illumina NGS library (red) were carried out with the supplied primer sets in triplicate. B) A melt curve was performed to verify amplification of a single specific product for both standards and library even at the lowest concentrations. C) From the resulting standard curve a size adjusted library concentration was then determined. The amplification plots demonstrate a limit of detection of 0.0001 pM (100 aM).

LIBRARY QUANTIFICATION STRATEGY

The JetSeq Library Quantification Kit, is an optimized, robust SYBR® Green based qPCR kit that provides accurate quantification of NGS libraries for sequencing on Ilumina® platforms. The JetSeq Library Quantification Kit contains six pre-diluted standards (10 pM to 100 aM) to minimize pipetting errors, a pre-qualified P5 and P7 Illumina adapter sequence primer mix to ensure reproducible and precise qPCR results and an optimized dilution buffer for the NGS library samples to ensure that library samples fall within the detection range of the assay. Quantification is achieved by interpolation from a standard curve generated using the six standards (Fig. 1). The JetSeq Library Quantification Kit undergoes strict quality control to ensure reliable lot-to-lot consistency with minimal variability, to ensure consistent performance (Fig. 2), thereby allowing more consistent cluster density across multiple libraries and over an extended period of time.

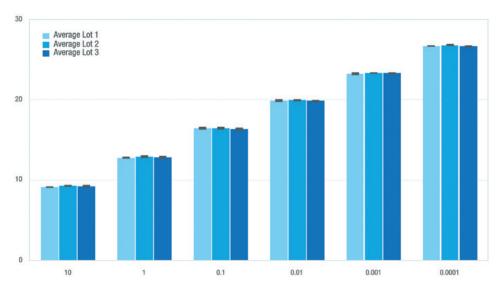


Fig. 2 JetSeq Library Quantification Kit lot-to-lot variation.

Three different lots of the JetSeq Library Quantification Kit were compared by generating an amplification plot using the standards from each kit and the Mic Personal gPCR Cycler. The results illustrate that the JetSeg Library Quantification Kit delivers exceptional accuracy in library quantification.

KIT CAPACITY

This JetSeq Library Quantification Kit will allow the quantification of up to 18 DNA libraries on separate 96 or 384-well plates and up to a maximum of 62 libraries on six 96-well plates or 76 libraries on two 384-well plates. The pre-diluted standards and a straight forward protocol also makes the JetSeq Library Quantification Kit ideal for automation and high-throughput applications.

Ordering Information

JetSeq™ Kit	Size	Cat. #
JetSeq Hi-ROX Library Quantification Kit	500 Reactions	BIO-68028
JetSeq Lo-ROX Library Quantification Kit	500 Reactions	BIO-68029

Contact information:

Global

E: info@meridianlifescience.com Toll free: +1 800 327 6299 Australia

E: info.au@meridianlifescience.com Tel: +61 (0)2 9209 4180





