

PerfeCra[®] NGS Quantification Kits

Accurate library quantification for next gen sequencing

PerfeCra NGS Quantifications kits use real-time PCR with prediluted standards for the convenient and accurate quantification of NGS libraries for the Ion Torrent or Illumina NGS platforms.



Quanta
BIOSCIENCES™

FEATURES AND BENEFITS

- Accurate and sensitive method for NGS library quantification
- Stabilized, prediluted standards for convenient use
- Consistency across a broad range of samples
- Sensitivity of detection to quantify low concentration libraries
- Quantification for either Illumina or Ion Torrent

Accurate quantification of the number of amplifiable library molecules is the most critical step in the NGS workflow in obtaining high quality read data with next-generation sequencing technologies. The PerfeCra NGS Library Quantification Kit uses real-time PCR to specifically quantify library molecules that possess the appropriate adapter tag at each end. These are the suitable template molecules for either emulsion PCR used for the Ion Torrent platform or Bridge PCR used for Illumina NGS platforms.

PerfeCra NGS Quantification kits simplify the library quantification process by providing stabilized, pre-diluted standards, pre-qualified primer sets, and an optimized dilution buffer for your NGS library samples. This minimizes pipetting errors and ensures reproducible and precise qPCR results, even with dilute samples. The robust qPCR performance of PerfeCra SYBR Green SuperMix provides accurate quantification of NGS libraries with varying fragment sizes or GC content. Kits are available to support all major qPCR instrument platforms.

Ion Torrent

The Ion Torrent DNA standard produces a 183-bp amplicon (51.9% GC) using primers that target the "A" and "trP1" adaptor sequences:

Ion Torrent forward: 5'-CCA TCT CAT CCC TGC GTG TC-3'

Ion Torrent reverse: 5'-CCT CTC TAT GGG CAG TCG GTG AT-3'

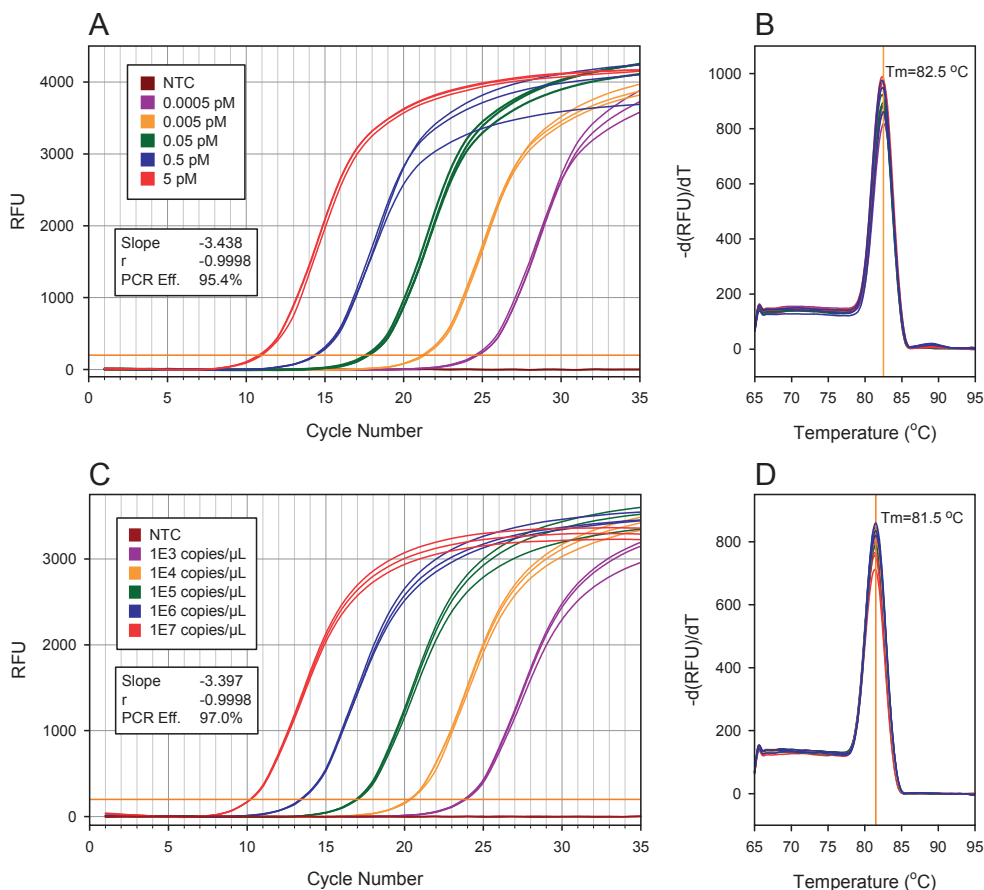
Illumina

The DNA standard for Illumina NGS platforms generates a 426-bp amplicon (48.8% GC). Primer sequences correspond to the "P5" and "P7" primer sequences for Illumina sequencing libraries:

Illumina forward primer: 5' AAT GAT ACG GCG ACC ACC GA 3'

Illumina reverse primer: 5'-CAA GCA GAA GAC GGC ATA CGA-3'

A common problem with some NGS library quantification protocols is the use of DNA standards that are too concentrated and generate qPCR data that are outside of the linear dynamic range for many qPCR instruments. Improper baseline settings result in compressions between the highest concentrated DNA standards that in turn give rise to inflated PCR efficiencies and inaccurate library quantification results. The NGS DNA standards supplied with the PerfeCra NGS Library quantification kits have been carefully selected to avoid these artifacts and produce NGS library standard curves with exceptionally high linear regression correlation coefficients.



PerfeCra NGS library Quantification Kit performance data. qPCR amplification of each of the five supplied DNA standards for Illumina NGS libraries (panel A) or Ion Torrent libraries (panel C) were carried out with the supplied primer sets (300 nM final concentration) and PerfeCra SYBR Green SuperMix in 20- μ L reaction volumes on a Bio-Rad CFX-96. Reactions were incubated for 5 min at 95°C followed by 35 cycles of: 95°C, 10s; 60°C, 20s; 45s, 72°C. Real-time fluorescence data was collected and analyzed at completion of the 72°C extension step. After completion of PCR, a dissociation (melt) curve was performed to verify amplification of a single specific product (panels B and D).

NGS Library Quantification

kit components:

- NGS Primer Mix (10 μ M ea.)
- Pre-diluted standard DNAs
- Dilution Buffer for NGS library samples
- Bundled with one of the following:
 - PerfeCra SYBR Green SuperMix
 - PerfeCra SYBR Green SuperMix ROX
 - PerfeCra SYBR Green SuperMix Low ROX
 - PerfeCra SYBR Green SuperMix Low ROX

ORDERING INFORMATION

PRODUCT

PRODUCT	VWR Cat. No.	Quanta Cat. No.
PerfeCra NGS Quantification Kit - Ion Torrent	xxxxx-xxx	95151-500
PerfeCra NGS Quantification Kit - Ion Torrent/ROX	xxxxx-xxx	95152-500
PerfeCra NGS Quantification Kit - Ion Torrent/Low ROX	xxxxx-xxx	95153-500
PerfeCra NGS Quantification Kit - Illumina	xxxxx-xxx	95154-500
PerfeCra NGS Quantification Kit - Illumina/ROX	xxxxx-xxx	95155-500
PerfeCra NGS Quantification Kit - Illumina/Low ROX	xxxxx-xxx	95156-500

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