ddPCR® Quantification and Quality Control of Ion Torrent Sequencing Libraries for Optimal Loading and Multiplex Balancing onto the Ion PGM

Introduction

A ddPCR method was developed to accurately quantify and quality control Ion Torrent AmpliSeq sequencing libraries. A high-throughput assay protocol was designed to test the universal region of Ion Torrent’s adapter sequence. ddPCR 1-D plots allow assessment of the quality of the libraries generated, as adapter dimer and potential chimeric species are in populations distinct from those of the correctly constructed libraries. Accurate ddPCR quantifications allow the user to load the optimal amount of libraries such that passing percent-templated Ion Sphere particles (ISPs) are achieved, and maximal useable sequencing reads are generated while minimizing iterated polyclonal reads. ddPCR quantifications using KAPA Bioscience’s kit yielded failed percent-templated ISPs, which caused low natural library reads, high iterated polyclonal reads, and highly variable reads between multiplexed libraries. Performing “equalization” per Life Technologies’ protocol caused up to a fourfold difference in reads.

Materials and Methods

Genomic DNA (Coriell) AmpliSeq library preparation manual (Life Technologies cat #IMAN0006735), AmpliSeq library kit 2.0 (Life Technologies cat #4475345), Ion AmpliSeq Cancer Hotspot panel v2, AmpliSeq library equalizer kit (Life Technologies cat #4482296), Library quantification kit for Bio-Rad® iCycler™ thermal cycler with 96 deep wells (Bio-Rad cat #185-1197), CFX96 Deep Well™ optical reaction module (Bio-Rad cat #184-4096), ddPCR™ NGS library quantitation kit for Ion Torrent DNA sequencing libraries were constructed from eight individual Coriell DNAs using the Ion AmpliSeq Cancer Hotspot panel library kit, and Ion Xpress barcode adapters. Libraries were prepared for ddPCR or qPCR quantification or “equalization” using Life Technologies’ kit. An attempt was made to normalize the eight libraries to an 8 pM total concentration using ddPCR- or qPCR-determined concentrations or “equalization,” and then pooled. For ddPCR, input concentrations were titrated from 2–100 pM in search of an optimum sequencing load amount. The pooled libraries were templated on Ion Sphere particles (ISPs) and enriched using the Ion One Touch DL systems. After loading onto Ion 314 chips, the libraries were sequenced on the Ion PGM.

Library Preparation

Step 1
Amplify with Ion AmpliSeq cancer panel primers.

Step 2
Partial digestion.

Step 3
Ligate adapters.

Step 4
Quantify and quality control with ddPCR.

Fig. 1. Library preparation workflow.

Results

Fig. 2. ddPCR workflow. A, ddPCR: final library reads; B, ddPCR: filtered polyclonal reads; C, “equalized”: filtered polyclonal reads; D, KAPA: final library reads; E, ddPCR: Ion OneTouch DL system; F, Ion Xpress barcode adapters.

Conclusions

- ddPCR accurately quantifies Ion Torrent sequencing libraries
- ddPCR allows for a quality control assessment of the libraries
- If the ddPCR QC shows signs that cannot be explained, a researcher might choose to perform a sequencing run, saving time and money
- KAPA qPCR generated secondary products that yielded inaccurate library concentration determinations. This caused failed template ISPs, low read counts, high polyclonality, and highly variable reads
- The Equalizer kit caused reads to be up to 4-fold variable for an 8-plex
- A load range of 4–8 pM, and optimally 6 pM, per ddPCR was found
- The 8-plex of barcoded libraries was exceptionally balanced per ddPCR
- Maximal and minimal polyclonal reads were established when using ddPCR, which saves significant time and money when sequencing

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