Utilizing the Rheonix NGS OnePrep™ Solution to automate the Takara Bio ThruPLEX® Tag-Seq HV library preparation kit

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Abstract
The fully automated Rheonix NGS OnePrep™ solution streamlined next generation sequencing (NGS) library preparation for the new Takara Bio ThruPLEX® Tag-Seq HV kit for Pan-Cancer targeted sequencing. Here we demonstrate how molecularly tagged, sample indexed, sequence-ready libraries were produced using the Encompass Optimum™ workstation and microfluidic Rheonix CARID® (Chemistry and Reagent Device) cartridge. Sequence data demonstrated that automated and manually prepared libraries were equivalent and allowed the detection of low (1%) allele frequency variants.

Introduction
As NGS is rapidly evolving, there is increasing demand to accurately detect low-frequency alleles and to discriminate between molecules. This is critical to the development of highly sensitive, NGS-based assays for use in research and clinical applications such as disease predisposition analyses, understanding disease mechanisms and targeted therapeutics, as well as cancer and developmental research. The newly launched Takara Bio ThruPLEX® tagged-HV kit enables detection of low-frequency alleles and has the ability to discriminate between molecules at high sensitivity and specificity, with 144 discrete unique molecular identifier (UMI) sequences used to “tag” each DNA molecule.

Automation of such a kit can offer increased sample throughput and thus reduce the bottleneck associated with library preparation. The Rheonix NGS OnePrep™ solution, which includes the Encompass Optimum™ workstation and microfluidic Rheonix CARID cartridge (Figure 1) was used to automate the ThruPLEX®-Tag-Seq HV kit for the purpose of Pan-Cancer targeted sequencing. Manual and automated prepared libraries were compared, and library and sequencing quality metrics were evaluated.

Materials and Methods

Results

Discussion

The automated process significantly reduces hands-on time and total time-to-results, which could produce unique molecular identifiers (UMIs) and unique index sets of rare variant detection per run and is a cost-effective solution to increased sample throughput.

Figure 1. Rheonix Encompass Optimum™ workstation. (A) The workstation can process up to 24 raw samples or gDNA with minimal or no user intervention, depending on the application. (B) Robust technology delivers samples and reagents to the Rheonix CARID® cartridge, which is a microfluidic device that processes four individual samples. (C) Encompass index and library preparation will hold 24 wells of PCR plates, containing the sequencing indices and the forward the flow libraries for sequencing.

Figure 2. Experimental design. Comparison of the performance of manual (benchtop) library preparation with Rheonix (CARID) automated prepared libraries (A) using 10 ng of raw DNA samples and a CFX96-FAST™ real-time PCR system. For this, five samples were tested on the ThruPLEX CARID, and targeted areas of genomes were sequenced. (A) Genes were sequenced on the Encompass NextSeq platform.

Figure 3. ThruPLEX Tag-Seq HV with TruQ-Q Library Quality Metrics. (A) Fragment distribution for (A) Creactive protein-DQ-Q gDNA and (B) manual and Rheonix prepared libraries. 110 bp adapter design was present in automated prepared samples. (C) However, peak/average library sizes were acceptable. All DNA yields met the minimum input requirement of greater than 500 ng.

Figure 4. ThruPLEX Tag-Seq HV with TruQ-Q Sequencing Quality Metrics. One automated prepared library was removed from analysis due to low read depth. In A and B all other samples were comparable to manually prepared libraries. (C) All libraries generated by manual and automated methods allowed the detection of low allele variants (1%) HD754.

Figure 5. ThruPLEX Tag-Seq HV with Accuref EGFR ctDNA 1% Library Quality Metrics. (A) While concentrations were lower for automated libraries, all concentrations and peaks met library requirements. (B) Bisakaryon analysis indicated that the average size and peak size for both library types was comparable.

Figure 6. ThruPLEX Tag-Seq HV with Accuref EGFR ctDNA 1% Sequence Quality Metrics. Automated and manually prepared libraries were comparable. (A) Sequence reads mapped (%), duplicates (%), and average coverage were similar for both library preparation types. (B) Moreover, the even distribution of the 144 UMIs was equivalent for both the benchtop and automated generated libraries. All libraries generated by both library preparation methods allowed the detection of low allele variants (%). Missing variants were present in the same regions and were missing by both manual and automated prepared methods (data not shown).