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 CARD® (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® Tag-Seq HV kit enables detection of low-frequency alleles and has the ability to differentiate 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 automated Encompass Optimum™ workstation and microfluidic Rheonix CARD® 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.

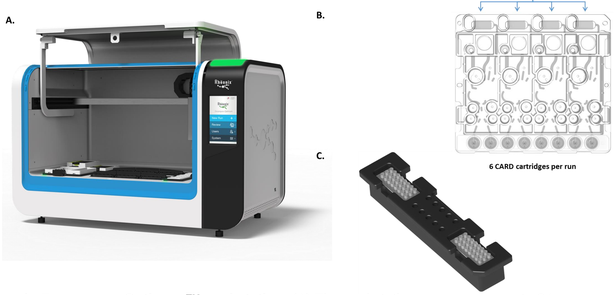


Figure 1. Rheonix Encompass Optimum™ workstation. (A) The workstation can process up to 24 raw samples or gDNA with minimal or no user intervention, depending on the application. (B) Robotic technology delivers samples and reagents to the Rheonix CARD® cartridge, which is a microfluidic device that processes four individual samples. (C) Encompass index and library rack positioned on the deck of the workstation will hold two 24-well PCR plates, one containing the sequencing indexes and the other the final libraries for sequencing.

Materials and Methods

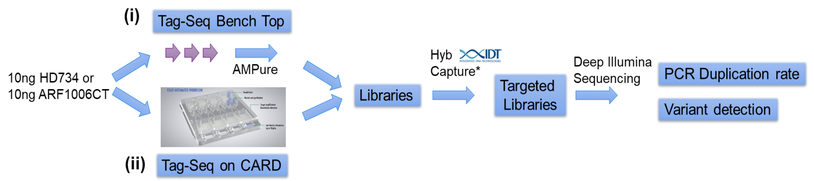
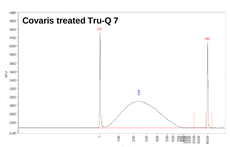


Figure 2. Experimental design. Comparison of the performance of manual (benchtop) library preparation (i) with Rheonix (CARD) automated prepared libraries (ii) using 10 ng of two input DNA samples, a Covaris-treated Horizon Tru-Q 7 reference standard and an Accuref Quan-Plex™ EGFR patient-like ctDNA. For this application the samples were manually loaded onto the Rheonix CARD® cartridge. *Hybridization capture was carried out using IDT xGen® Pan-Cancer Panel and targeted enrichment of libraries was completed. Libraries were sequenced on the Illumina NextSeq platform.

Results

A



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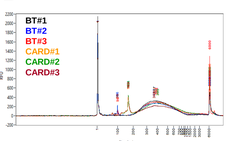


C

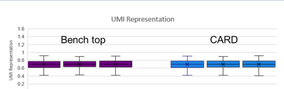
Sample		Lib. conc. (ng/µl)	Lib. yield (ng)	Avg. size (bp)	Peak size (bp)	Molar (nM)
Bench Top	1	74.4	3720	407	367	277
	2	39.5	1975	414	363	145
	3	54.0	2700	416	388	197
CARD	1	15.8	869	420	365	57
	2	23.1	1224	440	395	80
	3	24.8	1265	434	363	87

Figure 3. ThruPLEX Tag-Seq HV with Tru-Q 7 Library Quality Metrics. Fragment analysis for (A) Covaris-treated Tru-Q 7 gDNA and (B) manual and Rheonix prepared libraries. ~160 bp adapter dimer 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.

A

Library prep.	Reads	% Reads mapped	% Duplicate	% reads on or near baits	Avg. bait coverage before dedup	Avg. Coverage after dedup by UMI
Bench Top	13M	99%	69%	92%	1,405	262
	10M	99%	65%	92%	1,096	200
	13M	99%	70%	92%	1,397	259
CARD	13M	99%	74%	92%	1,380	218
	13M	99%	70%	92%	1,250	225

B



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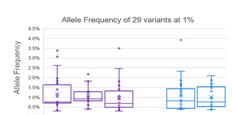

Figure 4. ThruPLEX Tag-Seq HV with Tru-Q 7 Sequencing Quality Metrics. One automated prepared library was removed from analysis due to low read depth. (A and B) All other samples were comparable to manually prepared libraries. (C) All libraries generated by manual and automated methods allowed the detection of low allele variants (1%) HD734.

Results

A

Sample		Lib. conc. (ng/µl)	Lib. yield (ng)	Avg. size (bp)	Peak size (bp)	Molar (nM)
Bench Top	1	106.0	5300	297	305	541
	2	56.2	2810	292	302	292
	3	108.0	5400	292	306	560
CARD	1	22.1	1216	302	310	111
	2	31.3	1534	296	306	160
	3	23.1	1224	296	305	118

B

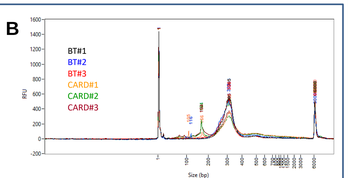

Figure 5. ThruPLEX Tag-Seq HV with Accuref EGFR ctDNA 1% Library Quality Metrics. (A) While concentrations were lower for automated prepared libraries, all concentrations and yields met library requirements. (B) Bioanalyzer analysis indicated that the average size and peak size for both library types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

A

Library prep.	Reads	% Reads mapped	% Duplicate	% reads on or near baits	Avg. bait coverage before dedup	Avg. Coverage after dedup by UMI
Bench Top	13M	99%	68%	93%	1,519	366
	13M	98%	63%	93%	1,500	347
	13M	99%	62%	93%	1,522	366
CARD	13M	99%	68%	92%	1,506	346
	11M	99%	60%	92%	1,287	304
	13M	99%	69%	92%	1,512	349

B

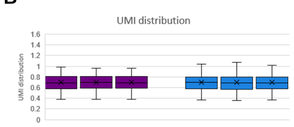

Figure 6. ThruPLEX Tag-Seq HV with Accuref EGFR ctDNA 1% Sequence Quality Metrics. Automated and manually prepared libraries were comparable. (A) Sequence reads mapped (%), duplicates (%) and average coverage were similar for both library preparation types. (B) Moreover, the even distribution of the 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 (1%). Missed variants were present in the same regions and were missed by both manual and automated prepared methods (data not shown).

Discussion

Automation of the Takara Bio ThruPLEX® Tag-Seq HV kit on the Rheonix Encompass Optimum™ workstation successfully produced sequence-ready libraries comparable to those prepared manually on benchtop. Evaluation of sequencing quality metrics such as % reads mapped, coverage, % duplicates and % reads on or near baits demonstrated that automated and manually prepared libraries were equivalent. An input of 10 ng was sufficient to produce high-quality, individually tagged DNA fragments with UMIs and unique dual indices (UDI), which allowed detection of low-frequency alleles (1%) and rare variants. The even representation of UMIs was demonstrated by their tight distribution around the theoretical average of 0.7% for both libraries prepared using benchtop or automated methods. A larger input volume is required for higher complexity libraries.

Conclusion

The automated process significantly reduces hands-on time and total time-to-results, can produce unique molecular tagged libraries for the templates of rare variant detection per run and is a cost-effective solution to increased sample throughput.

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