Automating the Illumina Nextera® Rapid Capture Enrichment Protocol with the Tecan Freedom EVO NGS Workstation*

Contributed by Piotr Mieczkowski, Ph.D., Research Assistant Professor, and Director of the High-Throughput Sequencing Facility, Department of Genetics, University of North Carolina, Chapel Hill.

Introduction

The Illumina Nextera Rapid Capture Exome and Expanded Exome kits provide a fast and simple, all-in-one library preparation and hybridization-based enrichment process for identifying coding variants in targeted resequencing studies. Next-generation sequencing (NGS) platforms and automated liquid handling workstations enable high-throughput sample processing, allowing researchers to generate sequence data from DNA more efficiently. This application note describes an Illumina-qualified† automated protocol developed for parallel processing of up to 48 samples using Nextera Rapid Capture kits with the Tecan Freedom EVO NGS workstation (Figure 1). The robot's innovative air liquid displacement technology delivers highly reproducible Nextera Rapid Capture library preparation and exome enrichment with minimal user intervention.

Experimental Design

To compare data quality between different processing methods, same-source human DNA samples from the Coriell biobank[1] were processed using both manual and automated Nextera Rapid Capture methods. The data contained four sets of trios, pooled at 12-plex before enrichment. Sequencing-ready libraries were prepared from 48 samples (4 separate pools, 12 samples per pool) and then enriched using both Nextera Rapid Capture automated and manual protocols. To obtain sequencing data, a single pool from each method was selected. Each pool was run across two Rapid flow cells on an Illumina HiSeq® 2500 System and the data analyzed using the HiSeq Analysis Software[2] (version 0.9) and enrichment analysis workflow.

Automated liquid handling steps for dilution, normalization, and library preparation were executed by the Tecan Freedom EVO NGS workstation. The worktable includes three Inheco heat blocks with exchangeable plate adapters (384, 96, and 96 deep well) for thermal incubation. A 96-position magnetic plate was utilized for the magnetic bead separation steps. The worktable also includes several free-position shelves for tip box storage. The robotic manipulation arm provides easy transfer of labware during magnetic separation and sample processing steps.

Analysis and Results

To demonstrate data correlation between automated and manual library preparation methods, a single sample from one of the control trios was selected to compare the number of called indels and single nucleotide variants (SNVs) (Figure 2). The control data demonstrate that the number of unique indels and SNVs called by both methods is very low, indicating high correlation between the data sets. Similar results were observed for the other sequenced samples.

Furthermore, the sequencing results indicate that the automated Nextera Rapid Capture protocol designed for use with the Tecan Freedom EVO NGS workstation provides an effective, robust, and streamlined workflow for library preparation and exome enrichment. When compared side-by-side to manual processing, the automated method produces comparable sequencing data on the HiSeq 2500 System (Table 1), enabling higher-throughput processing without sacrificing data quality.

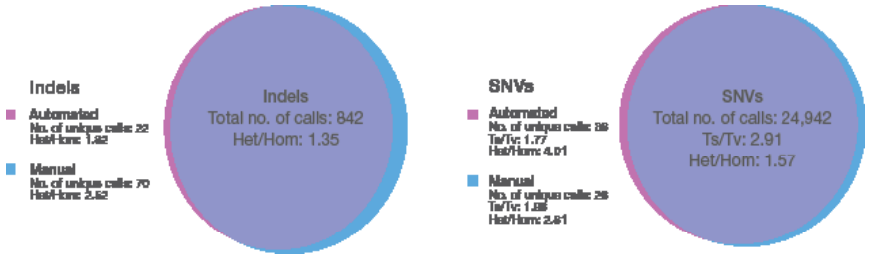
Conclusion

The automation-friendly workflow of the Nextera Rapid Capture protocol and the Tecan Freedom EVO NGS workstation provide a faster, more efficient solution for library preparation and exome enrichment. Using the automated Nextera Rapid Capture protocol, library preparation can be completed with minimal hands-on time in just a day and a half, generating sequencing data results the following day.

Figure 1: Tecan Freedom EVO NGS Workstation

In this figure, the Tecan Freedom EVO NGS workstation is set up to execute Illumina library preparation methods including Nextera Rapid Capture Exome.

Figure 2: Data Correlation between Automated and Manual Library Preparation Methods



Data generated by Nextera Rapid Capture Exome automated and manual library preparation methods at UNC were compared by selecting a single control sample (NA12878) from the 12-plex pooled libraries described in the experimental design section. The number of unique variants (indels and SNVs) and the corresponding transition/transversion and heterozygous/homozygous ratios for the unique calls identified by each method are indicated to the left of each Venn diagram. As demonstrated, both methods call a very low number of unique indels and SNVs, indicating high correlation between the data sets. Similar results were observed for other sequenced samples (data not shown).

Table 1: Performance Metrics Compared between Manual and Automated Nextera Rapid Capture Methods

Processing Method	Sample Plexity*	Estimated Insert Size (Median Bases)†	Number of Reads Aligned to the Target‡	Mean Target Coverage§	Duplication**	Target Bases at 10x††	Target Bases at 20x‡‡	Total SNVs Called§§	Total Indels Called***
Automated	12	175	54,399,184	102.96	8.32%	96.09%	89.99%	23,476	748
UNC (Manual)	12	163	104,949,959	118.20	17.05%	95.95%	90.08%	23,407	743
Illumina (Reference)	12	206	71,394,650	140.40	11.10%	97.90%	94.50%	24,968	894

* Number of pooled samples sequenced

† Median base size of the insert fragments

‡ Total number of reads that uniquely align to regions defined by the target manifest

§ Average number of regions covered in the target manifest file

** Percent of duplicate reads due to possible PCR artifacts generated during amplification †† Percent of target bases covered by at least 10 bases

‡‡ Percent of target bases covered by at least 20 bases

§§ Number of SNVs that contain the PASS filter in the target regions

*** Number of indels that contain the PASS filter in the target regions

HiSeq 2500 System performance metrics indicate comparable results between manual and automated (using the Tecan Freedom EVO NGS workstation) Nextera Rapid Capture library preparation methods.

Learn More

To obtain the Nextera Rapid Capture automation method for the Tecan Freedom EVO NGS workstation* discussed in this application note, visit www.tecan.com/ngs.

For questions regarding this application note, send inquiries to NGSPrep@tecan.com.

To learn more about the Nextera Rapid Capture Exome and Expanded Exome kits, visit www.illumina.com/nrc.

References

1. Coriell biobank (www.coriell.org/research-services/biobanking)
2. HiSeq Analysis Software (support.illumina.com/sequencing/sequencing_software/hiseq-analysis-software.ilmn)

* The application described in this document is provided as-is, is not an Illumina product, and is not accompanied by any rights or warranties. Customers using or adapting this application should obtain any licenses required and materials from authorized vendors. While customer feedback is welcomed, this application is not supported by Illumina Technical Support, Field Application Scientists, or Field Engineers.

† "Illumina-qualified" indicates that analysis by Illumina has shown that libraries prepared with the method perform comparably to those prepared manually.

Illumina • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

FOR RESEARCH USE ONLY

Pub. No. 770-2013-061 Current as of 11 November 2014

illumina®