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

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Introduction

The Illumina Nextera Rapid Capture Exome and Expanded Exome kits provide a fast, simple, all-in-one sample 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. Here we describe an 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 sample preparation and exome enrichment with minimal user intervention.

Experimental Design

To compare data quality results 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. A single pool from each method was then selected for sequencing in two flow cells (4 lanes) of an Illumina HiSeq® 2500 System.

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

The data was analyzed using the HiSeq Analysis Software[2] (version 0.9) and enrichment analysis workflow. The 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 HiSeq 2500 sequencing data (Table 1) and enables higher-throughput processing without sacrificing data quality.

Figure 1: Tecan Freedom EVO NGS Workstation

1 Eight-Tip Pipetting Arm, (AirLiHa)
2 Robotic Manipulator Arm (RoMa)
3 Temperature-controlled shaker
4 Temperature-controlled units

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

Conclusion

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

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	185	57,184,038	109.83	21.02%††	97.14%	92.56%	23,959	795
Manual	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

†† Higher duplicate rate is due to lower post-enrichment yield and is being optimized. Duplicate rate does not affect overall sequence quality or other performance metrics.

‡‡ 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 performance metrics indicate comparable results between manual and automated (using the Tecan Freedom EVO NGS workstation) Nextera Rapid Capture sample 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/products/nextera-rapid-capture-exome-kits.ilmn.

References

1. www.coriell.org/research-services/biobanking.
2. HiSeq analysis software, support.illumina.com/sequencing/sequencing_software/hiseq-analysis-software.ilmn.

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