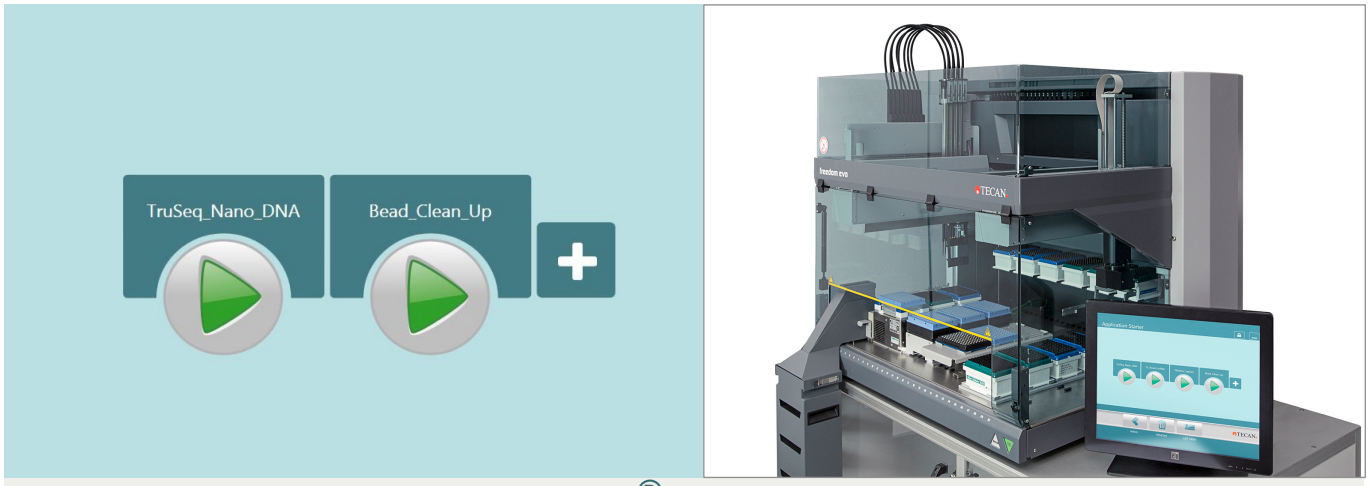

Automated Illumina TruSeq® Nano DNA sample preparation

Reproducible NGS library preparation from low quantities of DNA using the TruSeq Nano DNA Sample Preparation Kit on the Freedom EVO® NGS workstation

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Introduction

The TruSeq Nano DNA Sample Preparation Kit enables efficient investigation of samples where the amount of DNA available is limited. This low-input method generates excellent quality data from as little at 100 ng of DNA, allowing the study of samples with restricted DNA availability, for example from tumors, and helping to preserve precious sample material for use in future studies.

The automated workflow includes simple bead-based size selection, avoiding the sample losses typically associated with gel-based selection, and can be completed in less than a day with minimal hands-on time. In addition, the protocol enables a variety of read lengths to be selected, meeting the ever-increasing read lengths of Illumina sequencing instruments. This application note describes an automated-protocol for parallel processing of up to 48 samples using TruSeq Nano DNA Sample Preparation Kits on the Freedom EVO NGS workstation (Figure 1). The user-friendly TouchTools™ interface guides the operator through automation set-up to deliver highly reproducible, sequencing-ready DNA libraries for single read, paired-end and indexed sequencing with minimal user intervention.

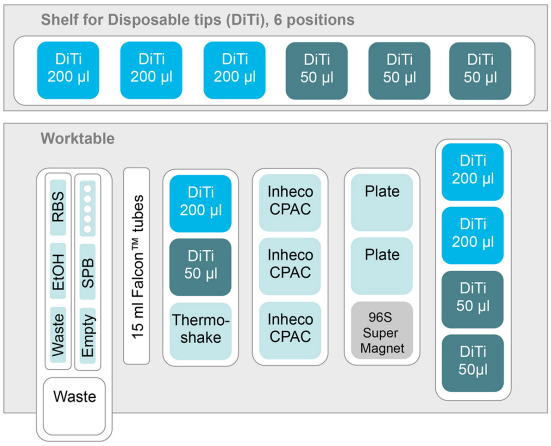


Figure 1: Deck layout of the Freedom EVO NGS workstation. The Tecan Freedom EVO NGS workstation is set-up for Illumina library preparation, including TruSeq Nano DNA sample preparation. The worktable includes three thermal devices (Inheco CPAC to keep reagents cool and provide optimal conditions for the enzymatic steps. A heated shaker (Inheco Thermoshake), a 96-position magnetic plate separator (Alpaqua 96S Super Magnet Plate) and a Robotic Manipulator Arm are provided for efficient bead clean-up steps. In addition, the compact worktable provides storage space for up to 12 tip boxes, allowing longer unattended runs.

Experimental design

Automated liquid handling steps for bead clean-up, end repair, size selection, A-tailing, adapter ligation and PCR enrichment preparation were performed on the Freedom EVO NGS workstation using 24 same-source samples (NIST NA12878)[1]. DNA input was increased from 100 to 200 ng per sample to improve both assay performance and the quality of the libraries produced. The protocol included the option of an insert size around 350 bp. Quality control of the constructed libraries was performed using a PerkinElmer LabChip® GX system (Figure 2). From a total of 24 libraries, 12 were pooled and sequenced in two flow cells (four lanes) of an Illumina HiSeq® 2500 System.

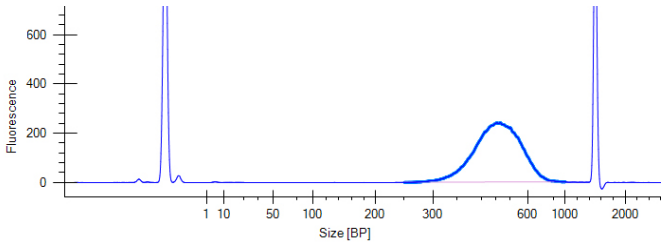


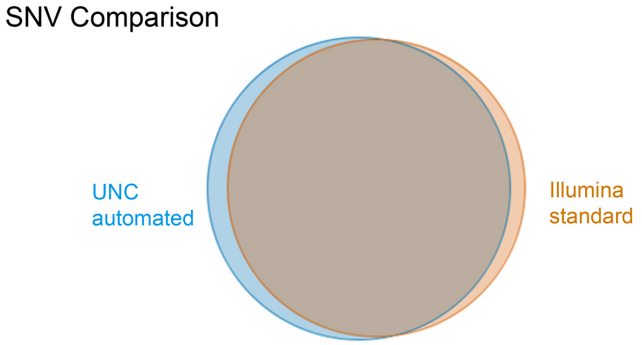
Figure 2: DNA size distribution of libraries constructed show the expected size range for a 350pb insert plus 130pb adapters and show no visible sign for adapter dimer formation.

Analysis and results

Sequencing data was analyzed using the HiSeq Analysis Software WGS protocol (BWA/GATK)[2]. To demonstrate the quality of the data generated, the results were compared with the Illumina 'gold standard' NIST NA12878 reference material, sequenced to 50x coverage. The Illumina gold standard data shows low numbers of unique indels and single nucleotide variations (SNVs) in the tested data set, indicating a high correlation between the standard library and libraries constructed using the automated protocol (Figure 3). Quality control results from the analysis of the sequenced samples demonstrate the reliability of the automated TruSeq Nano DNA protocol (Table 1).

Input ID	Variant type	Total VCF	Total Platinum	ComparedSites	Precision	Recall
UNC automated	SNV	3'301'081	3'346'428	3'062'755	0.99609	0.91694
Illumina gold standard	SNV	3'176'618	3'346'428	3'014'302	0.99845	0.902

Table 1: Comparison of Precision Recall values for SNV between UNC automated library preparationand Illumina Gold Standard. This data shows that the precision and recall value of the automated method and Illumina Gold Standard values are equivalent.



	Unique to UNC automated	Common	Unique to Illumina standard
Total Count	308'700	3'090'151	184'082
Percent	9.08%		5.62%
Novelty	-	-	-

Figure 3: Comparison of SNVs from the automated library preparation method with the Illumina reference data. Data from the TruSeq Nano DNA automated library preparation method was compared with Illumina reference data by selecting a single control – NA12878 – and analyzing sequencing data from 12 samples. The automated method and manually prepared Illumina reference data have a very low number of unique SNVs, indicating high correlation between the data sets.

Summary

The automation-friendly workflow of the Illumina TruSeq Nano DNA protocol, combined with the Freedom EVO NGS workstation, provides a faster, more efficient solution for library preparation. The TouchTools user interface guides users effortlessly through each step of the workflow, reducing training needs, minimizing the risk of manual errors and increasing the reproducibility of the process. The automated protocol requires minimal hands-on time and can be completed in just over half a day.

Learn more

To obtain the automated TruSeq Nano DNA protocol for the Freedom EVO NGS workstation discussed in this application note, contact your Tecan sales representative, visit www.tecan.com/NGS, or contact NGSprep@tecan.com.

To learn more about TruSeq Nano DNA Library Prep kits, visit www.illumina.com/products/truseq-nano-dna-sample-prep-kit.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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