Introduction

Illumina library sample preparation for Next Generation Sequencing (NGS) is a long and complicated process that can also require precise fragment size selection through agarose gel separations in order to obtain high-quality sequencing results.

Beckman Coulter has released SPRIworks chemistry for low-throughput using the SPRI-TE Nuclease Acid Extractor and in now developing chemistry for high-throughput (HT) automation using Biomek® FX® liquid handlers for Illumina compatible NGS library sample preparation. The HT solution can produce up to 96 libraries in a 96-well format with minimum hands-on time and user-friendly Reversible Immobilization (SPRI) paramagnetic beads to offer various fragment sizes selection (150-350 bp, 250-450 bp, 350-750 bp, and 150 bp and up).

Downstream processes, including PCR reaction setup and cleanup, qPCR setup, and normalization, have been automated to create a suite of Biomek methods. This suite of automation methods has a single user interface (UI) to allow for easy selection of options.

SPRIworks HT Methods Suite

The SPRIworks HT Methods Suite offers the user the ability to run three different Biomek methods to prepare samples for loading onto the Illumina C-Bot. The first method covers the three steps of the sample preparation: library construction, size selection, and PCR setup without requiring any user intervention. The second method covers PCR Clean-Up using Ampure XP and qPCR setup using Kapa Biosystems Library Quanti KIT for Illumina. The first module offers the ability to perform library construction and size selection together, selection will then be applied to the insert size.

Automated Size Selection

Size Selection has been completely automated to allow for a walk-away solution that is reproducible. Four size selection ranges are available in the User Interface: No Selection, small, medium, and large. If electing to perform library construction and size selection together, selection will occur between each reaction. This format allows the user to save time and will allow selection to apply to the insert size. Downstream chip analysis of the samples will reflect the selected size range plus the size of the adaptors combined. Size selection can also be performed as a stand-alone method after library construction is completed. Selection will then be applied to samples already ligated to adaptors. Downstream chip analysis will reflect the size range selected.

The tables below show results of analyzer chip data for each size range option. Samples were processed through library construction using integrated size selection and reflect the selected size range including the size of the adaptors.

Library Construction

The starting input is clean fragmented gDNA supplied by the user. The user may choose their preferred method of fragmentation and purification. (*Agencourt Ampure XP is the recommended purification process with validated Biomek methods available to use)

PCR Clean-Up & qPCR Set-up

The second method performs PCR clean-up using Ampure XP and prepares a 96-well qPCR plate using Kapa Biosystems’ Kapa Illumina GA I Library Quantification Kit. Users can select to run both processes in one method run to save time.

Conclusions

• SPRIworks offers a complete package of chemistry and automated methods
  • Low-throughput and High-throughput automation solutions available
  • Chemistry and automation support from one company
  • Automated size selection saves time and is reproducible
  • User Interface allows for quick customization of various options such as:
    • Number of samples processed up to 96 per run.
    • Size selection ranges assigned to each well
    • On-the-fly decisions for Normalization and Pooling.

Normalization and Pooling

The last method in the suite allows the user to normalize and pool samples. Users can elect to run both processes together or separately. The Normalization method uses the raw qPCR data to calculate quantity and to give the users the ability to make on-the-fly decisions regarding normalization. The Normalization method pop-up an Excel® sheet that gives the quantities of each sample, and allows users to determine the final volume and concentration of the normalized plate. It also enables error handling to allow wells that do not meet the input volume or concentration to be skipped, or to continue normalization of low wells. Finally, it gives quick feedback on %CVs so outliers can be identified.