High-throughput target enrichment preparation of NGS libraries on the Biomek i7 Hybrid Workstation

Elisa Vega[1], Alejandro Quiroz Zarate[1], Marsha McMakin[1], Rachel Kasinskas[1], *Jonathan Nowacki[1], Sean Chien[2], Zach Smith[3]
[1]Roche Diagnostics Corporation, Wilmington, MA
[2]Roche Diagnostics Solutions, Pleasanton, CA
[3]Beckman Coulter Life Sciences, Indianapolis, IN
*Author no longer at Roche

INTRODUCTION

Whole-genome sequencing (WGS) with next-generation sequencing (NGS) technology has driven a wide diversity of investigations and applications in human health. However, WGS remains a high-cost method and often does not provide either the precision or depth of information required to investigate complex diseases, or to detect rare or low-frequency genetic variants. Enrichment of specific genomic regions through NGS target enrichment offers a more economic path to deeper, more precise sequencing by focusing sequencing reads on only the desired regions.

The KAPA HyperCap workflow is a single workflow that yields target-enrinched libraries by combining:

- KAPA DNA Library Preparation Kits (either **KAPA HyperPrep Kit**, which uses mechanically fragmented input DNA or **KAPA HyperPlus Kit**, which includes enzymatic fragmentation and uses intact input DNA), and
- **KAPA HyperCap Target Enrichment probes** for solution- based hybridization (including panels for the human exome, many diseases and biological pathways, and custom panels).

This study demonstrates the successful automation of the KAPA HyperCap Workflow v3.2 on the Beckman Coulter Biomek i7 Hybrid Workstation using the **KAPA HyperPlus Kit** and the **KAPA HyperExome** human exome probe panel. Thus, this new automated method offers the capability of high-throughput target enrichment with the minimal variability that is an important benefit of automation.

AUTOMATED KAPA HYPERCAP WORKFLOW

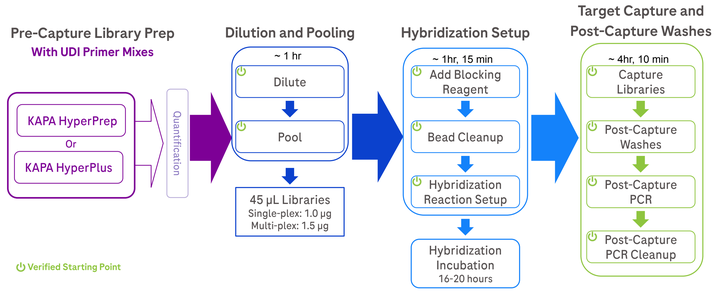


Figure 1. KAPA HyperCap Workflow v3.2 on Biomek i7 Hybrid Workstation. The overall workflow is structured as four separate methods (shown in purple, dark blue, light blue, and green) to allow flexibility in sample preparation planning. This diagram shows the KAPA HyperCap Workflow v3.2, starting with library preparation (purple) using either KAPA HyperPrep Kit or KAPA HyperPlus Kit with KAPA UDI Primer Mixes, followed by subsequent target enrichment and cleanup. The provided time estimates are based on 96-sample runs.

METHODS

Library Preparation and Target Enrichment on Beckman Coulter's Biomek i7 Hybrid Workstation:

- Libraries (n=96) were generated with the KAPA HyperPlus Kit, which includes an enzymatic fragmentation step, using 100 ng input of intact NA12878 human gDNA (workflow step in purple) .
- The libraries were normalized and multiplexed into twelve capture pools of eight libraries each (8-plex); libraries were pooled by column (workflow step in dark blue).
- Twelve enriched multiplexed capture library pools were prepared following the standard KAPA HyperCap workflow v3.2 (workflow step in light blue and green).
- Pre-capture libraries and post-capture library pools were quantified with Qubit Flex and fragment distribution was assessed on the Agilent 2100 BioAnalyzer.

Sequencing and Analysis:

- Two 8-plex capture pools with libraries from the middle and last column of library preparation were sequenced on NextSeq 500 with high-output flow cells, generating 75bp paired-end reads; pool selection was geared towards demonstrating the absence of plate/edge effects.
- The sequencing data for each of the libraries was down-sampled to 30M paired-end reads for target enrichment analysis.

RESULTS

Library Preparation with KAPA HyperPlus Kit

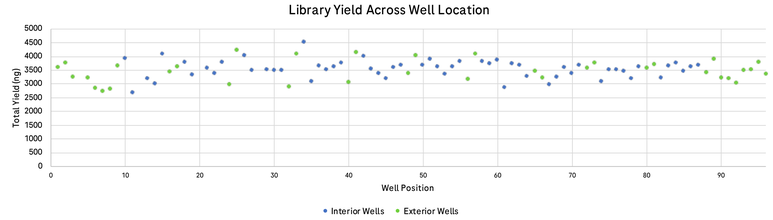


Figure 2. Library yield was similar across all 96 wells. The graph shows the pre-capture library yields generated from 100ng input of intact NA12878 human gDNA on Beckman Coulter's Biomek i7 Hybrid Workstation. A comparison of the exterior (green) and interior (blue) position wells demonstrates comparable performance, minimal variation and a coefficient of variation (CV) of 7.3%. Well A01 in a 96 well plate is designated as position 1 and well H12 as position 96, this sequential order is followed in column fashion for all other wells (down then across; starting at top left corner of plate in landscape position).

Pre-capture and Post-capture Library Quality Metrics (prior to sequencing) are Comparable

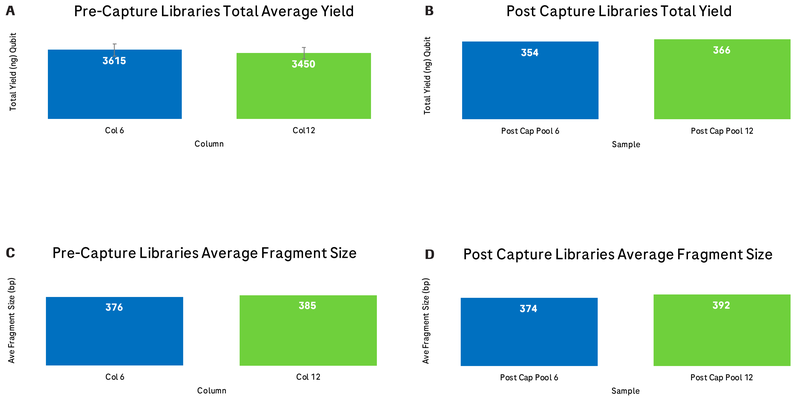
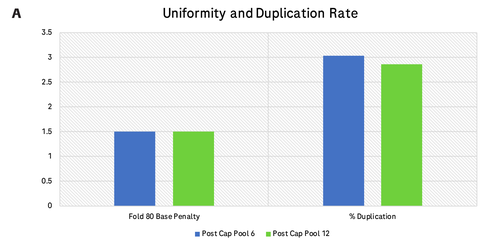


Figure 3. Comparison of pre-capture and post-capture pre-sequencing library metrics. Graphs A and B above show the comparison of library yields in the middle of the plate (column 6) and the end of the plate (column 12) for both pre-capture and post capture. In the pre-capture the average of column 6 and 12 are taken and compared to each other to demonstrate comparable library yields across the plate. In the post-capture the columns were pooled and Post Cap Pool 6 represents Column 6 and Post Cap Pool 12 represents column 12. The yields demonstrate consistency and comparability throughout the KAPA HyperCap Workflow v3.2. Graphs C & D show the comparison of average library fragment sizes pre and post capture. The consistency in library fragment sizes from pre to post capture demonstrates decreased variation and consistent sample processing throughout the KAPA HyperCap Workflow v3.2.

SEQUENCING QUALITY METRICS AND TARGET ENRICHMENT ANALYSIS

This Automated Method Provides Successful Enrichment of Target Regions with High-Quality Sequencing Libraries



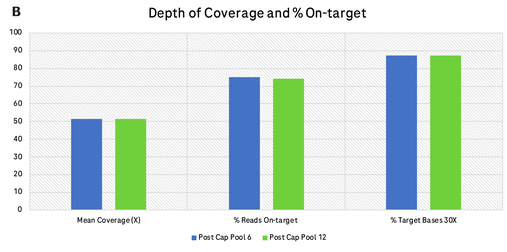


Figure 4. Uniformity, low duplication rates and depth of coverage for target regions of interest. The sequencing data analysis demonstrates even representation of regions of interest complimented with low duplication rates (A) indicating the generation of high-quality and diverse libraries. Target enrichment analysis demonstrates high on-target rates and deep coverage, as shown in (B), indicating that the regions of interest were present in each of the libraries with significant coverage.

CONCLUSION

Automation of the **KAPA HyperCap workflow on the Biomek i7 Hybrid workstation** generated high-quality, target-enriched sequencing libraries with **KAPA HyperPlus Kit** and the **KAPA HyperExome** probe panel.

The library quality metrics and sequencing data demonstrate:

- Generation of pre-capture libraries with consistent yields within an acceptable range
- Comparable fragment distributions for pre- and post- capture libraries
- Generation of high-quality target-enriched libraries that yield low duplication rates, high on-target rates, and uniform depth of coverage across the target regions.

Overall, the automation of KAPA HyperCap on the Biomek i7 Hybrid Workstation offers a solution with efficient generation of high-quality, target-enriched sequencing libraries with a range of throughput capabilities.

Data on file.
Project: Biomek i7 HyperCap

MC-US-14350 A794 01/24