Automating Roche's KAPA HyperCap Workflow on a Biomek i7 Hybrid Automated Workstation

Introduction

The Roche's KAPA HyperCap Workflow allows the user to enrich DNA sequencing libraries previously prepared utilizing the Roche KAPA HyperPrep or Roche KAPA HyperPlus library preparation kits with KAPA HyperCap Enrichment Probes. KAPA HyperExome Enrichment probes can provide insights into a whole human exome while custom panel designs can be utilized to tailor the enrichment process to a smaller subset of genes of interest.

In this application note, we demonstrate the automated performance of Roche's KAPA HyperCap Workflow on the Biomek i7 Hybrid Automated Workstation. The workflow can process up to 96 library pools, allowing for single-plex or multiplex enrichment of DNASeq libraries.

The automated method on the Biomek i7 Hybrid Automated Workstation provides:

- Reduced hands-on time and increased throughput
- Reduction in potential pipetting errors
- Standardized workflow for improved results
- Quick implementation with ready-to-implement methods
- Knowledgeable support from Roche and Beckman Coulter Life Sciences

Figure 1. Biomek i7 Hybrid Automated Workstation.

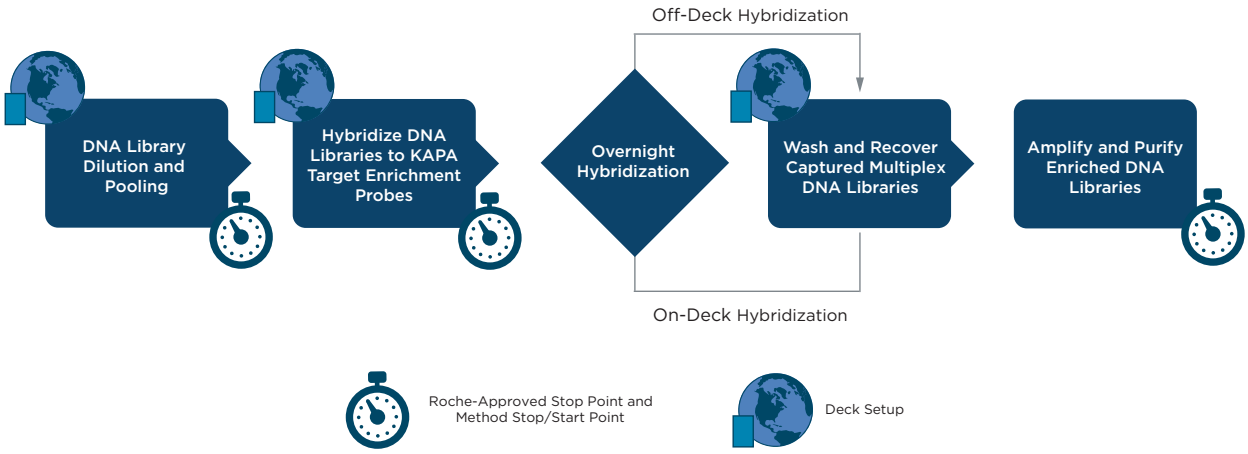

Figure 2. Automated KAPA HyperCap Workflow.

Timings

Timing estimates for enriching 96 libraries in 12 eight-plex enrichment pools are presented below. Timing estimates do not include reagent thawing, library QC, or the overnight hybridization (minimum 16 hours).

Action	Automated Library Pooling	Automated Hybridization Setup	Automated Target Enrichment	Total
Biomek Setup	10 min	20 min	20 min	50 min
Biomek Run Time	24 min	50 min	3 hr, 41 min	4 hr, 55 min

Table 1. Timing estimates.

Experiment Design

To demonstrate the automation of Roche's KAPA HyperCap Workflow on a Biomek i7 Hybrid Automated Workstation, 96 libraries were first prepared using the Roche KAPA HyperPlus automated method on the Biomek i7 Hybrid workstation. Inputs were 100 ng human genomic DNA NA12878 (Coriell Institute) for each technical replicate. KAPA UDI Primers were utilized during the course of library preparation. Following library preparation, the libraries were quantified using Qubit (Thermo Fisher) and assayed on a 2100 Bioanalyzer (Agilent) to determine fragment size. Pre-enrichment libraries were pooled into twelve 8-plex pools with a total pool mass of 1.5 µg per pool as detailed in Roche's KAPA HyperCap Workflow protocol. Library pools were enriched using the automation method for Roche's KAPA HyperCap Workflow on a Biomek i7 Hybrid Automated Workstation in conjunction with the HyperExome v1 Panel.* Overnight hybridization was performed on the Biomek i7 workstation for 16 hours. Following enrichment, the enriched library pools were quantified using Qubit (Thermo Fisher) and assayed on a 2100 Bioanalyzer (Agilent) to determine fragment size prior to sequencing. Sequencing was performed on two enriched library pools on an Illumina NextSeq 500 sequencer using a 2x75 paired sequencing run with a High Output v2.5 sequencing kit. Bioinformatics analysis was performed at Roche using an in-house bioinformatics pipeline. A single-plex enrichment pool was prepared manually and sequenced to provide comparative data on the efficacy of the automated method.

Results

Pre-enrichment library yields were consistent across all replicates (n = 96, average total yield of 3,449 ng) and no dropouts were observed. Pre-enrichment library sizes were also consistent (n = 11, average size 371 bp). Following enrichment, enriched library pool yields (n = 12, average total yield 483.5 ng) and enriched library pool fragment sizes (n = 12, average 381 bp) were consistent across all library pools and no dropouts were observed.

Two automated 8-plex enriched library pools and one manual single-plex enriched library pool were sequenced on an Illumina NextSeq 500 sequencer. Libraries were down-sampled to 30 million paired reads per library. Bioinformatics analysis showed an equivalent Fold 80 Base Penalty of 1.5 for both manual and automated libraries. Mean target coverage was slightly higher for the manual enriched library (54.4 X coverage) compared to the automated enriched libraries (51.6 X coverage). On target non-duplicate reads were again slightly higher for the manual enriched library (77.2%) compared to the automated enriched libraries (74.48%). Bases covered at 30 X, 40 X, and 50 X read depth were similar across manual and automated libraries.

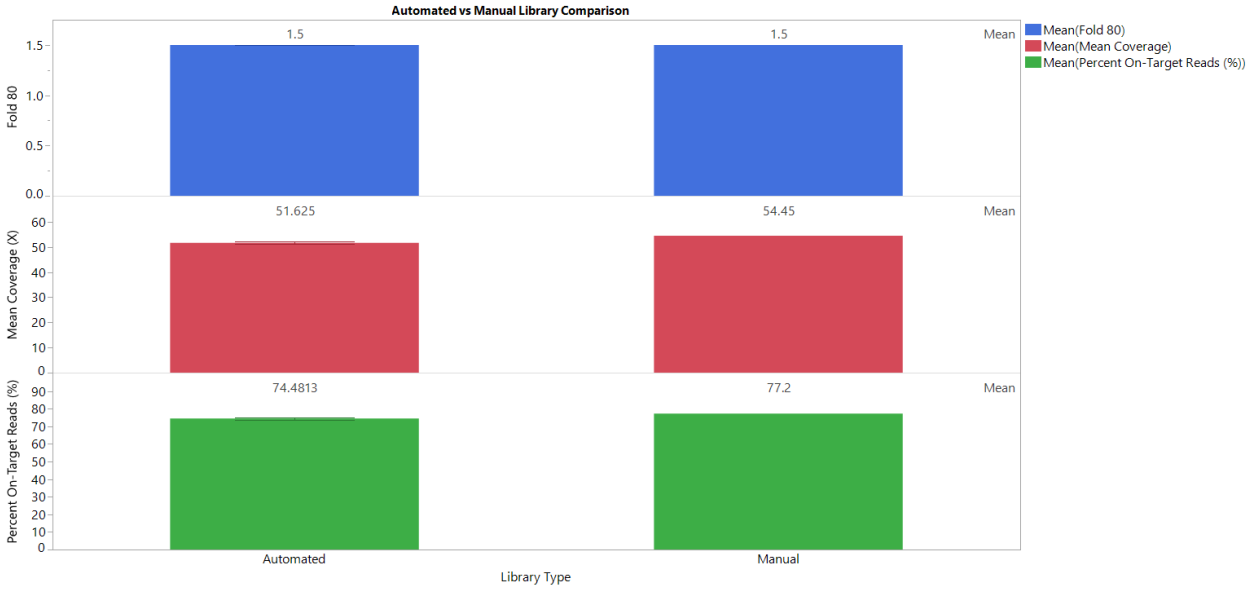

Figure 3. Automated vs Manual Library Comparison.

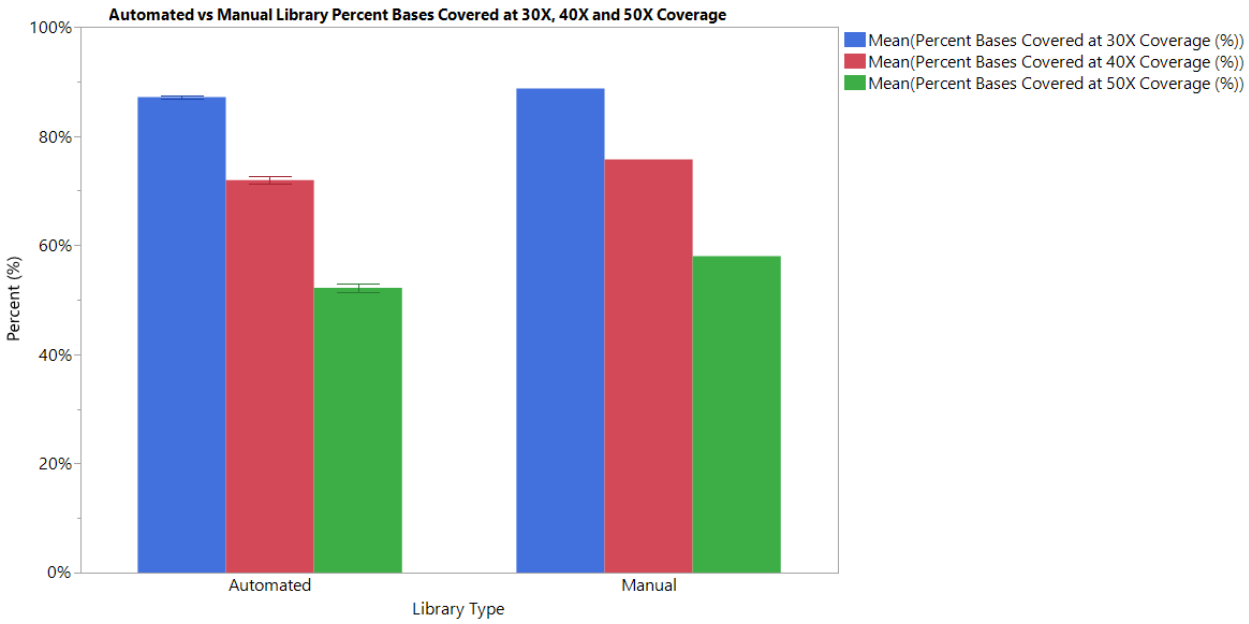

Figure 4. Automated vs Manual Library Base Coverage Depth.

Conclusion

With this data, we conclude that libraries enriched with Roche's KAPA HyperCap Workflow automated on the Biomek i7 Hybrid Automated Workstation show equivalent performance to libraries constructed manually.

* KAPA HyperExome V1 is now replaced by KAPA HyperExome V2, which is the latest Roche WES design, and delivers coverage of recent versions of ACMGv3.1, RefSeq, CCDS, ClinVar, Ensembl, and COSMIC genomic databases.

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