

APPLICATION NOTE

QIAseq Human Exome and QIAseq xHYB Actionable Exome Kits on Hamilton NGS STAR

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Introduction

Exome sequencing is becoming a widely used approach for mutation analysis in inherited disorders. QIAGEN and Hamilton have developed an automated protocol that can be used for both the QIAseq Human Exome and QIAseq xHYB Actionable Exome kits. The combination of the single-day workflow of the kits with automation and rapid analysis with the QIAGEN CLC LightSpeed module enables a fast, cost-effective workflow. This method is for users who process a large number of samples and require a standardized workflow that produces high-quality data with a fast turnaround time.

- A fast, single-day exome workflow combined with automation decreases library preparation time
- Automation enables high-throughput processing of samples
- An integrated exome workflow from sample to bioinformatics delivers high-quality data, quickly



Figure 1: NGS STAR with ODTC

As more clinical laboratories are performing exome sequencing, it is important to standardize workflows to improve reproducibility. Automation of library preparation reduces manual handling errors and minimizes batch-to-batch inconsistencies, which leads to more samples being processed with higher quality and decreased turnaround times.

Method Description

Extracted DNA samples are first processed with QIAseq FX DNA Library Kits in 96 reactions on the Hamilton NGS STAR (Fig. 1) to create the whole genome libraries. Up to 8 samples can be pooled per hybridization capture reaction, and the samples are subsequently dried in a Speed-Vac off-deck. Hybridization capture is performed to specifically target the key library fragments in the Exome panels. After incubation (30 min to 4 hours), the biotin-coupled xHYB Probes and their hybridized targets are captured on Streptavidin beads. Non-specific targets are washed away, and the captured targets are amplified using the On-Deck Thermal Cycler (ODTC). The amplified library is purified and QC'd to prepare for sequencing (Fig. 2). User interaction is only required for deck setup and off-deck sample drying.

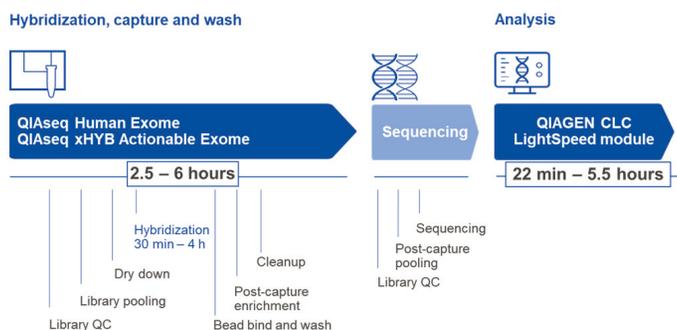


Figure 2: QIAseq Human Exome and QIAseq xHYB Actionable Exome Workflow



Automating QIAseq Exome kits library preparation

System Description

The system makes use of a standard NGS STAR deck layout (Fig. 3). The method was verified with the optional On-Deck Thermal Cycler (ODTC), enabling full walk-away PCR integration.

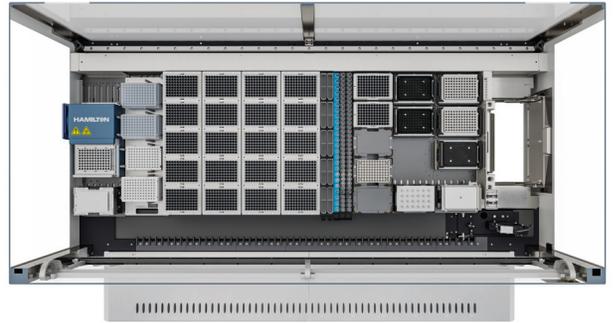


Figure 3: Deck Layout NGS STAR

Kit Description

The QIAseq Human Exome and QIAseq xHYB Actionable Exome use a hybridization capture-based target enrichment approach to specifically enrich exonic sequences of the human genome from indexed whole genome libraries. The QIAseq xHYB Actionable Exome Panel combines comprehensive detection (>10,000 genes) of disease-causing and disease-associated variants curated by HGMD with the compact target region design (12.3 Mb) of a traditional clinical exome panel. Both kits facilitate the highly uniform enrichment of target sequences from indexed whole-genome libraries, and the flexible workflow allows for the simultaneous hybridization of up to 8 indexed libraries in as little as 30 minutes. Hybrid capture-based target enrichment is compatible with various samples, including high-quality gDNA, cfDNA, formalin-compromised DNA, and physically sheared DNA.

Results

Whole genome library preparation was performed on 96 samples. A total of 48 Human Exome and 48 xHYB Actionable Exome panel libraries were processed and sequenced on Illumina NextSeq 550. Coverage uniformity (Fig. 4) was equally consistent among the replicate pools, irrespective of the total library input. QIAseq Human Exome and QIAseq xHYB Actionable Exome both produced very complete and uniformly represented capture libraries.

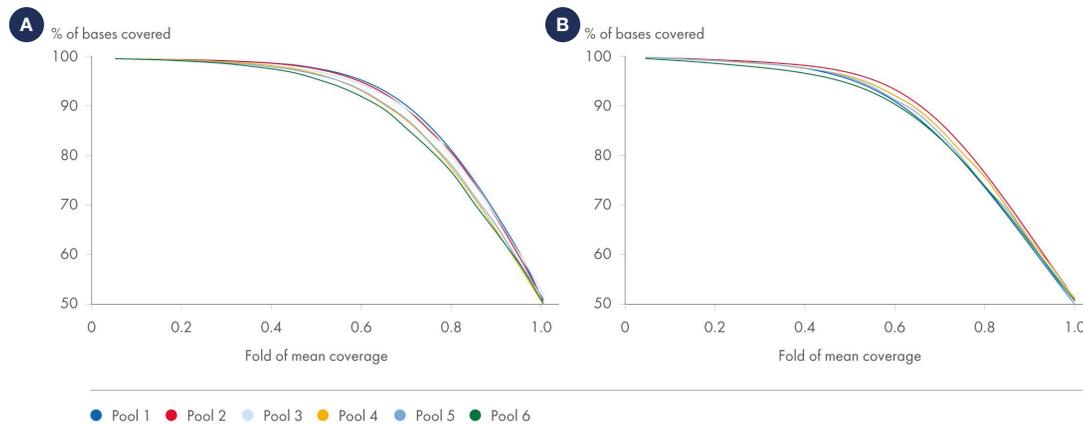


Figure 4: Target Base Coverage of QIAseq Human Exome (Fig. 4A) and QIAseq xHYB Actionable Exome (Fig. 4B).

Discussion / Summary

The automated protocol allows for the processing of 192 individual libraries in 24 pools (8 samples per pool). Manual and automated samples show comparable:

- Average target region coverage and required sequencing reads
- Coverage uniformity (irrespective of total library input)
- Coverage completeness
- Target specificity

Others

System Requirements	Part Number	Provider
NGS STAR	806600	Hamilton Bonaduz AG
ODTC	10147734	Hamilton Bonaduz AG
iSWAP Plate Handler	190220	Hamilton Bonaduz AG
QIAseq Human Exome Kit	333939	QIAGEN
QIAseq Human xHYB Actionable Exome	333375	QIAGEN

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