Enabling the Twist Human Core Exome Kit on Miro Canvas®

Data from a demonstrated protocol

Key points

- Library preparation and hybridization capture using Twist Bioscience's Human Core Exome Kit are automated on Miro Canvas
- These protocols have been developed using 50ng DNA input for library prep and 1500ng for hybridization capture
- Depth of coverage, quality scores, and other key metrics are comparable between manually prepared libraries and those run on Miro Canvas
- Automating library preparation and hybridization capture on Miro Canvas reduces the amount of hands-on time by >85%

Introduction

Whole exome sequencing (WES) provides a unique opportunity to dive deeply into the coding regions of the genome. It plays an important role in generating data for research, and in some cases clinical applications[1]. Strong WES analyses rely on even coverage of these regions, and ultimately on high quality capture reactions[2]. Potential to minimize many sources of variability exists in automating laboratory processes[3] that have traditionally been done at the benchtop, as well as in ensuring that reagents are high quality.

The Twist Human Core Exome kit undergoes thorough QC testing to ensure that all probes in the probe pools are present at the appropriate level in order to limit wasted reads[4]. The uniformity of these reagents reduces cost and improves coverage of reads in single-plex and 8-plex pools[4]. By automating the use of these reagents, such as on the Miro Canvas, potential for contamination and variability in procedure are minimized. Miro Canvas also gives users flexibility to utilize the true "walk-away" automation and perform other tasks in the meantime, while still maintaining high quality results.

Miro Canvas is a digital microfluidics (DMF) platform that allows custom low-throughput workflow automation for complex protocols such as NGS library preparation and hybridization capture. The system is compatible with a wide range of reagents[5], and as such, kits from both Miroculus and other reagent suppliers can be used. This application note describes the results that can be expected when using the Twist Bioscience Human Core Exome kit in protocols developed for Miro Canvas. The resulting research-use-only libraries can then be sequenced using Illumina sequencing platforms.

Workflow optimization

The fully-automated Miro Universal Library Prep (Twist) protocol has been tested using 50ng of high molecular weight DNA. Before beginning, DNA should be quantified using the Qubit dsDNA Broad Range Quantification Assay or similar[6]. Fragmentation, end repair, adapter ligation, amplification, and purification steps are all automated in this protocol. The Miro Human Exome Hyb Capture (Twist) protocol has been tested using single samples and 8-plex pools, and the volume of each sample library used depends on their respective concentrations[7]. Minimal hands-on sample preparation is required at the beginning of this protocol, leaving most steps automated on Miro Canvas including hybridizing probes with pools, binding targets to beads, post-capture amplification, and purification.

Figure 1. Experimental workflows. Both the Miro Universal Library Prep and Miro Human Exome Hyb Capture protocols are fully automated after reaction setup.

Results

Library Preparation automated on Miro Canvas produces comparable depth of coverage, quality scores, and metrics

The Miro Universal Library Prep (Twist) protocol has been tested using 50ng of NA12878* gDNA. To assess the quality of the library preparation protocol alone, libraries prepared on the Miro Canvas underwent manual hybridization capture. Four replicates from both libraries prepared on Canvas and libraries prepared manually, 8 total, were sequenced on a NextSeq High Output 75PE platform. Key metrics, such as depth of coverage and Fold 80 scores, were comparable between the two methods (Fig. 2). Additionally, percent of reads on target and percent of duplicated reads were similar between methods (Fig. 2).

*NA12878 DNA was obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research

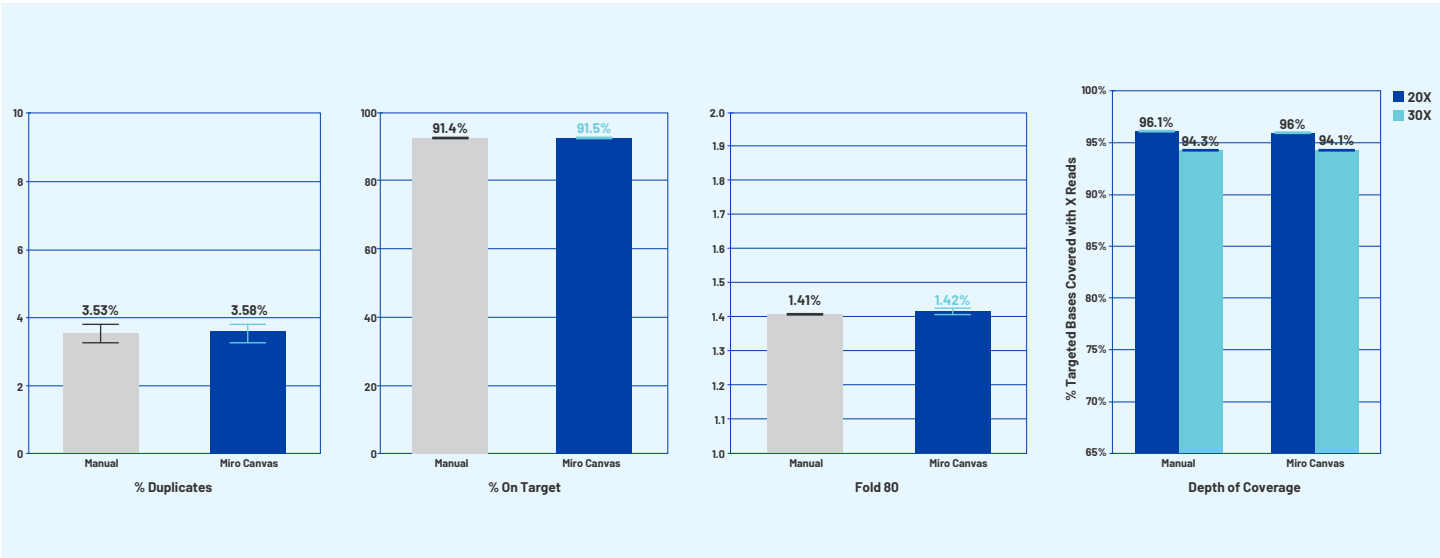

Figure 2. Multiple metrics used to evaluate sequence data generated using both manual and Miro Universal Library Prep protocols. Hybridization capture was performed manually. All samples are subsampled to 150x raw sequencing coverage (70M reads, 5.3Gb of data per sample).

Target enrichment using benchtop and Miro Canvas protocols showed comparable results after sequencing

The Miro Human Exome Hyb Capture (Twist) has been tested using inputs of both 1,500ng of mixed DNA for 8-plex pools (multiplex, 187.5ng per library), and 500ng for individual libraries (singleplex). Samples run on the Miro Canvas for hybridization capture previously underwent manual library preparation. Sequencing was performed using the NextSeq High Output 75PE platform. Samples that were run on Miro Canvas, both singleplex and 8-plex pools, were compared to singleplex samples that had been enriched manually. Coverage of target bases at 20x and 30x was comparable between manual and automated protocols for single samples and 8-plex pools (Fig 3). Fold 80 scores were also similar between treatments, with median scores varying no more than 0.02 (Fig 3).

METRIC	MANUAL 8-PLEX	MIRO CANVAS 8-PLEX
Fold-80	1.37	1.4
Off-bait	7%	9.30%
Mean Coverage	58.4	72.4
Hs-Library Size	571M	443M
30x Cov	93%	93.70%
Zero Cov	1.10%	1.30%
AT Dropout	6.88	5.71
GC Dropout	0.64	0.74
Median Insert Size	248	200

Table 1. Key metrics are comparable for manually and Miro Canvas enriched 8-plex pools. All samples are subsampled to 150x raw sequencing coverage (70M reads, 5.3Gb of data per sample).

Additional sequencing was completed using two 8-plex pools, one prepared manually and the other on the Miro Canvas. For these pools, targets covered at 30x and the fold 80 scores were comparable between both (Table 1). Reads for these runs were assessed using the Miro Canvas Integrative Genomics Viewer (IGV). The IGV outputs displayed confident mapping of target genes across multiple exons from pools enriched using Miro Canvas (Fig 4).

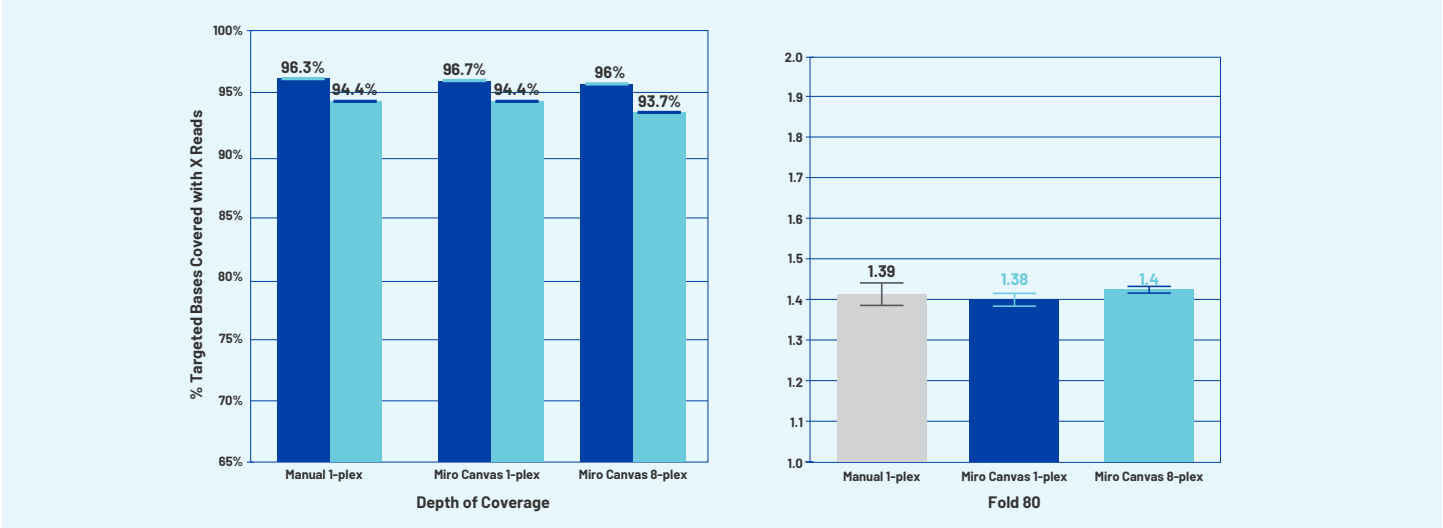

Figure 3. Coverage of targets and fold 80 scores did not differ significantly between manual singleplex, Miro Canvas singleplex, and Miro Canvas 8-plex. All samples are subsampled to 150x raw sequencing coverage (70M reads, 5.3Gb of data per sample).

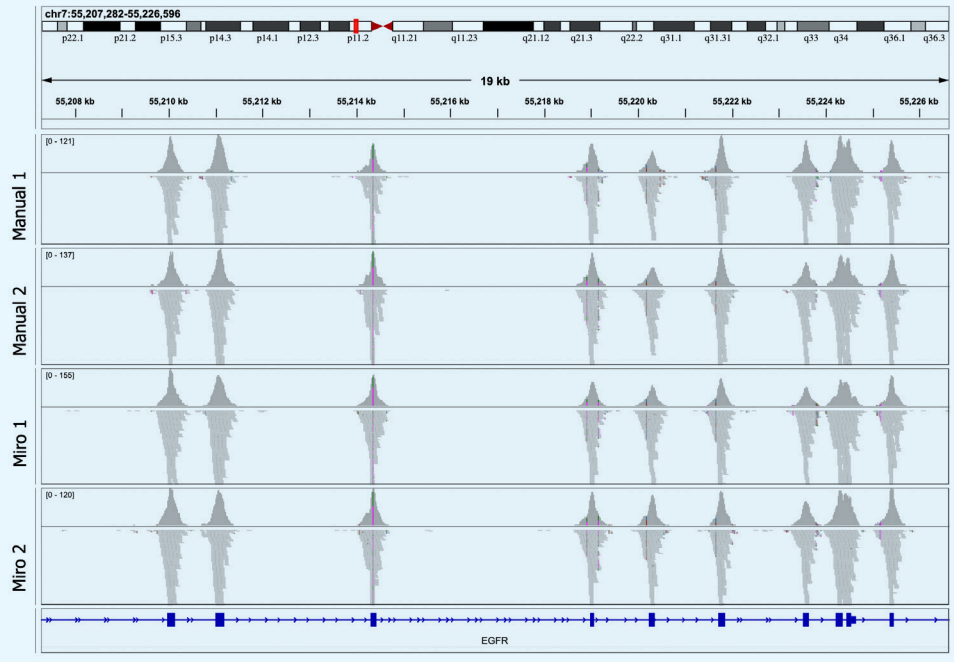

Figure 4. Representative IGV browser tracks of EGFR exons 2-10 from manually prepared and Miro Canvas core exome enriched libraries

Utilizing Miro Canvas for library preparation, hybridization, and capture significantly decreased hands-on time

While the overall time taken between the library preparation and hybridization capture protocols does not vary significantly between manual preparation and Miro Canvas automation, the hands-on time is far less for the automated applications. Manual library preparation requires about three hours of hands-on time, while the Miro Universal Library Prep (Twist) equires about 15 minutes to set up for a three hour and twenty minute fully-automated run. The Miro Human Exome Hyb Capture (Twist) protocol begins with several steps that cannot be automated, such as preparing pools and hybridization mix, and therefore requires about an hour of benchtop work. The steps that follow are fully automated on Miro Canvas, taking about five and a half hours to complete. This hour of hands-on time pales in comparison to the time taken by the manual protocol, which requires as much as seven and a half hours of hand-on time.

Summary

Miro Canvas is an advanced DMF platform that can be used to automate library preparation and hybridization capture with the Twist Bioscience Human Core Exome kit. Both the Miro Universal Library Prep (Twist) and Miro Human Exome Hyb Capture (Twist) protocols are fully-automated from fragmentation to elution and hybridization to elution, respectively. These automated protocols and their manual counterparts yield comparable results, but the greatly reduced hands-on time required by the Miro Canvas makes it a valuable tool for any laboratory.

References

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Legal Disclaimers

Revision History

Version
1.0

Revisions
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