Application Note

INTEGRA

Implementing the Twist Human Core Exome Kit on the MIRO CANVAS

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] Variability in coverage can be minimized by ensuring that reagents are high quality, and by automating laboratory processes that have traditionally been performed manually.[3]

The Twist Human Core Exome Kit undergoes thorough quality control testing to ensure that all probes in the probe pools are present at the appropriate levels in order to limit wasted reads.[4] The uniformity of these reagents reduces costs and improves coverage of reads in singleplex and 8-plex pools.[4] Automating this kit on the MIRO CANVAS reduces the potential for contamination and variability.

The MIRO CANVAS also provides full walk-away automation, giving users the flexibility to perform other tasks while maintaining high quality results.

The MIRO CANVAS NGS prep system is a digital microfluidics platform that allows customized, 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] This application note describes the results that can be expected when using the Twist Human Core Exome Kit in protocols developed for the MIRO CANVAS. The resulting research use only libraries can then be sequenced using Illumina sequencing platforms.

Key benefits

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

Overview: How to implement the Twist Human Core Exome Kit on the MIRO CANVAS

MIRO CANVAS NGS prep system

Experimental set-up

The fully automated Twist Universal Library Prep protocol has been tested using 50 ng of high molecular weight DNA. DNA should be quantified using the Qubit™ dsDNA Broad Range Quantification Assay or similar before starting.[6] Fragmentation, end repair, adapter ligation, amplification and purification steps are all automated in this protocol. The Twist Fast Hybridization Target Enrichment 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 the 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 Twist Fast Hybridization Target Enrichment protocols are fully automated after reaction set-up.

Methods and results

Library preparation

The MIRO Universal Library Prep (Twist) protocol has been tested using 50 ng of NA12878* gDNA. Hybridization capture was then performed manually to assess the quality of the library preparation protocol alone on the MIRO CANVAS. Replicates of the libraries prepared on the MIRO CANVAS and manually (n=4, 8 in total) were sequenced on an Illumina NextSeq 500 or 550 platform, 75 Paired-End. Key metrics, such as depth of coverage and fold-80 scores, were comparable between the methods. Additionally, both methods yielded a similar percentage of duplicated reads and reads on target (**Figure 2**).

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

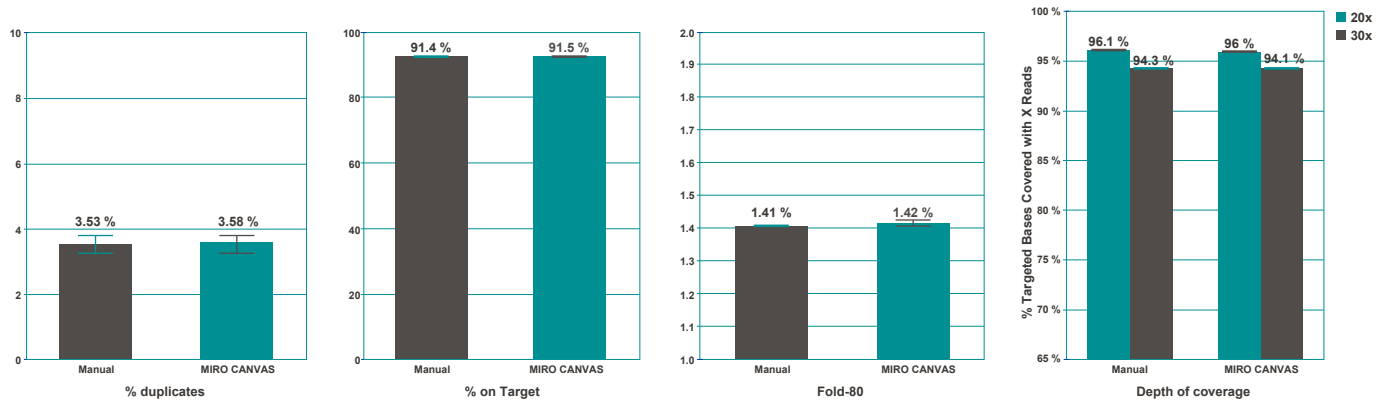

Figure 2: Multiple metrics were used to evaluate the sequencing data generated using both manual and Twist Universal Library Prep protocols. Hybridization capture was performed manually. All samples were subsampled to 150x raw sequencing coverage (70 M reads, 5.3 GB of data per sample).

Target enrichment

The Twist Fast Hybridization Target Enrichment protocol has been tested using inputs of 1500 ng of mixed DNA for 8-plex pools (multiplex, 187.5 ng per library) and 500 ng 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. Singleplex and 8-plex pools run on the MIRO CANVAS 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. Fold-80 scores were also similar between methods, with median scores varying by no more than 0.02 (**Figure 3**).

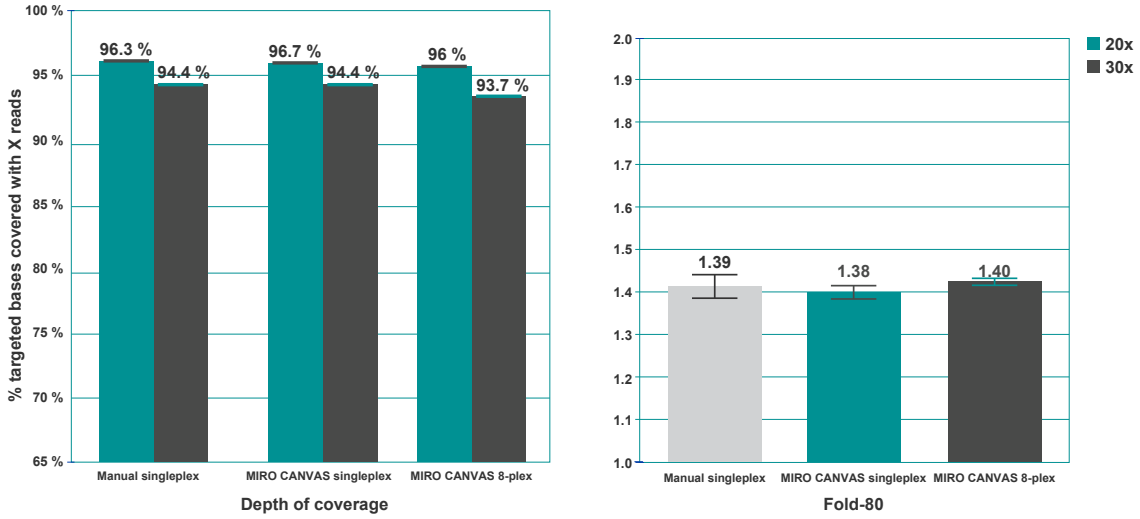

Figure 3: There was no significant difference between manual singleplex, MIRO CANVAS singleplex, and MIRO CANVAS 8-plex coverage of targets and fold-80 scores. All samples were subsampled to 150x raw sequencing coverage (70 M reads, 5.3 GB of data per sample).

Additional sequencing was completed using 2 x 8-plex pools, one prepared manually and the other on the MIRO CANVAS. Targets covered at 30x and fold-80 scores for each pool were comparable (**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 the MIRO CANVAS (**Figure 4**).

Table 1: Key metrics were comparable for 8-plex pools enriched manually and on the MIRO CANVAS. All samples were subsampled to 150x raw sequencing coverage (70 M reads, 5.3 GB of data per sample).

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	571 M	443 M
30x coverage	93 %	93.70 %
Zero coverage	1.10 %	1.30 %
AT dropout	6.88	5.71
GC dropout	0.64	0.74
Median insert size	248	200

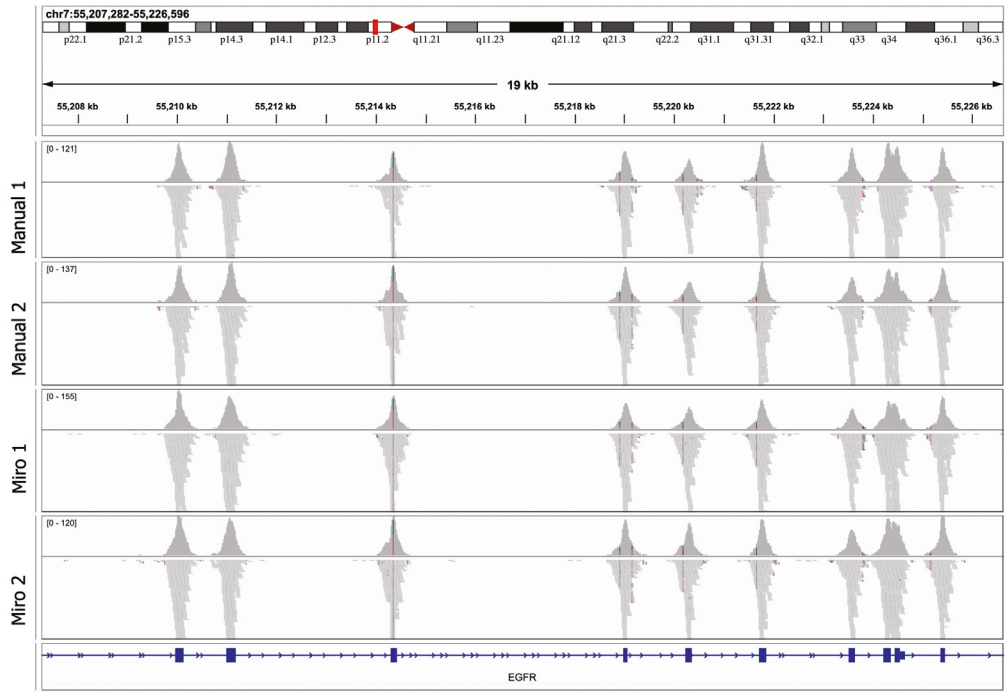

Figure 4: Representative IGV browser tracks of EGFR exons 2-10 from core exome-enriched libraries prepared manually and on the MIRO CANVAS.

Time savings

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 3 hours of hands-on time, while the Twist Universal Library Prep requires about 15 minutes to set up for a fully automated run of 3 hours and 20 minutes.

The Twist Fast Hybridization Target Enrichment 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 the MIRO CANVAS, taking about 5.5 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 7.5 hours.

Conclusion

The MIRO CANVAS is an advanced digital microfluidics platform that can be used to automate library preparation and hybridization capture with the Twist Bioscience Human Core Exome Kit. Both the Twist Universal Library Prep and Twist Fast Hybridization Target Enrichment 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

1. Rabbani B *et al*. The promise of whole-exome sequencing in medical genetics. *J Hum Genet* 2014; 59: 5–15. https://doi.org/10.1038/jhg.2013.114
2. Lelieveld SH *et al*. Comparison of exome and genome sequencing technologies for the complete capture of protein-coding regions. *Hum Mutat* 2015; 36: 815-822. https://doi.org/10.1002/humu.22813
3. Holland I and Davies JA. Automation in the life science research laboratory. *Front Bioeng Biotechnol* 2020; 8: 571777. https://doi.org/10.3389/fbioe.2020.571777
4. Twist Bioscience. Human Core Exome Kit. Available at: https://www.twistbioscience.com/resources/product-sheet/human-core-exome-kit. AccessedFeb2022.
5. Miroculus. New Class of Technology. Available at: https://www.integra-biosciences.com/global/en/ngs-automation/miro-canvas.
6. Miroculus, Inc. Miro Universal Library Prep (Twist) Protocol. Available at: https://www.integra-biosciences.com/global/en/ngs-automation/miro-canvas. Accessed: Feb 2022.
7. Miroculus, Inc. Miro Human Exome Hyb Capture (Twist) Protocol. Available at: https://www.integra-biosciences.com/global/en/ngs-automation/miro-canvas. Accessed: Feb 2022.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	M-01-0001-001-01	MIRO CANVAS NGS prep system	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-02-0001-001-03	MIRO Cartridge	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-03-0001-001-01	MIRO Dropgloss	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
Twist Biosciences	102026	Twist Human Core Exome Kit, 12 Reactions	https://www.twistbioscience.com/products/ngs/fixed-panels/human-core-exome?tab=overview
Coriell Institute for Medical Research	NA12878	Genomic DNA from LCL	https://www.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA12878&Product=DNA

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