

Personal Automation for Whole Genome Sequencing: Evaluating Digital Microfluidics Across Two Different PCR-free Protocols

Genomics

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There is increasing demand for PCR-free WGS (e.g. rapid diagnosis in the NICU, Tumor/Normal somatic sequencing) enabling identification of more variants and without amplification-associated artifacts. Traditional automation requires commitment to a small set of protocols, necessitating high throughput and batching, in order to justify the investment of time, space, and capital.

Quality, consistent library preparation is essential for newer sequencers (e.g. NovaSeq) where low-quality libraries can negatively affect a costly flow cell, requiring more oversight or intervention on library preparation.

Fully automated library construction on Miro Canvas™ from Miroculus™ uses electromechanical forces for dispensing, moving reagents, mixing, thermocycling, magnetic bead clean-ups, and eluting. This occurs on a single-use electronics-free cartridge, preventing contamination.

PCR-free WGS protocols were compared to manual and high-throughput liquid handler library preparation: 1) Miro PCR-free WGS Library Prep Kit with mechanical fragmentation, 2) Illumina DNA PCR-free Prep Kit with tagmentation.

Miro PCR-free WGS libraries (n=178) were prepared using sheared gDNA inputs (75-500ng) from multiple sources (NA12878 and donor blood samples). Twenty-six Illumina DNA PCR-free libraries were generated using NA12878 inputs (50-500ng).

Miro Canvas libraries demonstrated equivalent or better sequencing metrics compared to both manual efforts and a high-throughput plate-based liquid handler using the same kit. Miro PCR-free WGS libraries consistently achieved more than 95% bases at 20X coverage and >98% HetSNP sensitivity with 109Gb sequenced across sheared DNA inputs (100-500ng). Libraries from the liquid handler system required 5Gb more to achieve the same metrics.

Shallow sequencing of the Illumina DNA PCR-free libraries demonstrated equivalent insert size, coverage and %excluded total metrics compared to manually-prepared libraries with an average insert size of 425bp and average yield of 10nM (300ng-500ng input).

“On-demand” personal automation for WGS PCR-free protocols using either mechanical fragmentation or tagmentation with Miro Canvas allows for more consistent, higher quality WGS libraries.