Scalable and Flexible Library Preparation for High-Sensitivity Applications

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Introduction

Next-generation sequencing (NGS) has become a foundational tool for biological research and in-vitro diagnostics, though there remains a need for DNA sequencing platforms that combine high accuracy, speed, and flexible throughput for research and clinical applications. Here, we apply the novel Singular Genomics G4™ Sequencing Platform to perform metagenomic and human CNV analysis via the Watchmaker Genomics DNA Library Prep Kit, a leading solution for high sensitivity applications. We demonstrate excellent performance as determined by sequencing of reference materials and comparison to the leading NGS platform, indicating seamless integration of the G4 Platform with Watchmaker library preparation solutions.

Watchmaker Library Prep with the G4™

Library Preparation. Metagenomic libraries were created from a mock microbial community (MSA-1003) using an input titration of 0.1, 1, and 10 ng of gDNA. The fragmentation reactions were carried out at 37°C for 20 minutes. Human gDNA libraries were constructed using DNA from the NA12878, HT-29, DU-145, and MDA-MB-231 cell lines, with 50 ng of input DNA, and fragmentation reactions were done at 30°C for 3.5 minutes. All libraries were prepared using the Singular U loop adapters, followed by cleavage and PCR amplification using Singular Indexed primers. The libraries were characterized using the TapeStation (Agilent) instrument and qPCR. Sequencing was performed using the Singular Genomics G4 Platform and the F2 sequencing kit, with 2 x 150 bp reads (plus 12 bp dual indices). The human CNV libraries were also sequenced in parallel on the Illumina MiSeq platform, using the same read configuration.

Power	Speed
400 Gb	Daily Sequencing
Flexibility	**Accuracy**
1 – 4 flow cells, 16 lanes	80 – 90% bases ≥Q30

Figure 1. The Singular Genomics G4™ Sequencing Platform.

Consistent Fragmentation Delivers Uniform Inserts

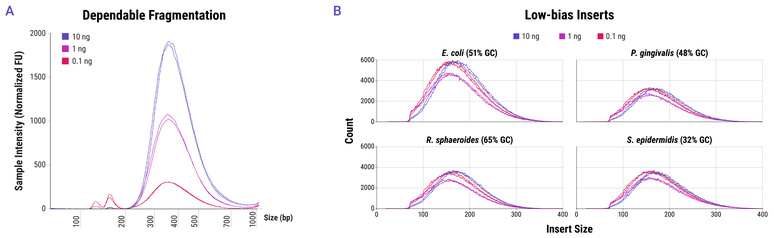

Figure 2. Consistent fragmentation across a broad input titration. Libraries were prepared in duplicate using uniform fragmentation conditions (37°C for 20 minutes) across an input titration ranging from from 0.1 to 10 ng of mock microbial community gDNA (MSA-1003). **(A)** The final library distributions were assessed using a D1000 assay on a TapeStation (Agilent). **(B)** The combined workflow of Watchmaker library prep and sequencing on the Singular Genomics G4 Platform produced consistent insert sizes across a wide range of DNA input amounts and diverse microbial genomes.

Reliable Coverage of Metagenomics Samples

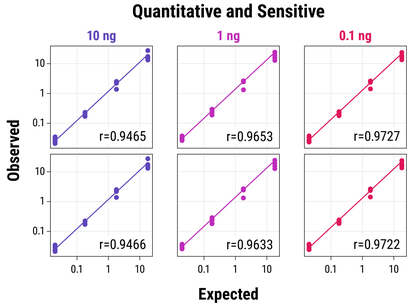

Figure 3. Sensitive and accurate quantitative coverage of microbial populations. High correlation between observed and expected abundance of 20 microbial species represented in the microbial community standard MSA-1003. Notably, a high correlation is observed even with low input (0.1 ng) samples.

Robust Low-Input CNV Detection

Table 1. Sequencing performance data for the genome in a bottle (NA12878) control and human cancer cell lines (HT-29, DU-145, and MDA-MB-231) sequenced using the Singular Genomics G4

Cell Line	Mapping Rate	Chimera Fraction	Softclip Fraction
NA12878	0.998361	0.007632	0.015651
HT-29	0.999799	0.006520	0.015043
DU-145	0.999776	0.008248	0.017251
MDA-MB-231	0.999803	0.006537	0.015534

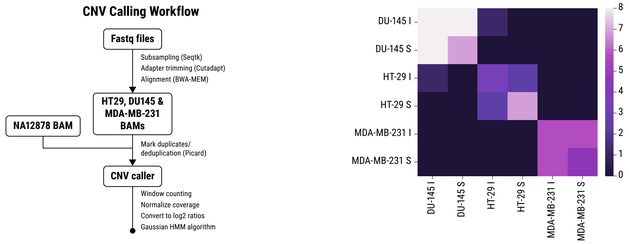

Figure 4. Hierarchical clustering indicates that Singular Genomics and Illumina sequencing platforms have similar CNV sensitivity and specificity. The heatmap shows that the samples cluster together based on their CNV profiles, regardless of the sequencing platform used.

Accurate CNV Detection

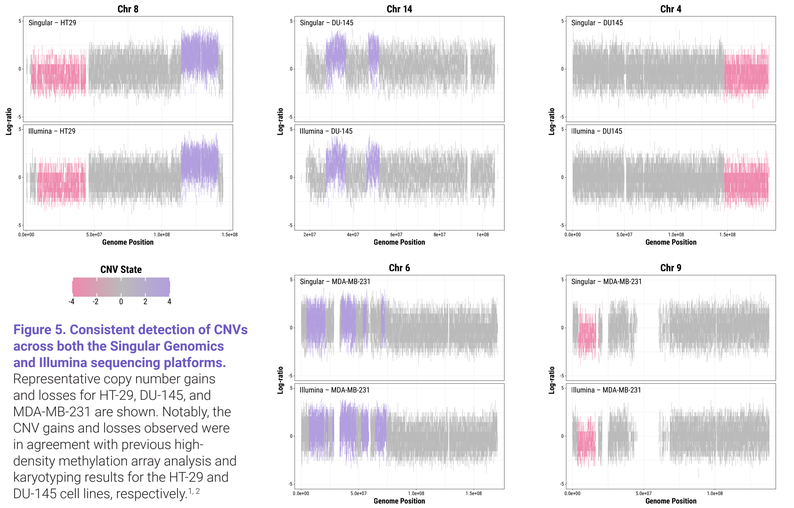

Figure 5. Consistent detection of CNVs across both the Singular Genomics and Illumina sequencing platforms. Representative copy number gains and losses for HT-29, DU-145, and MDA-MB-231 are shown. Notably, the CNV gains and losses observed were in agreement with previous high-density methylation array analysis and karyotyping results for the HT-29 and DU-145 cell lines, respectively.[1,2]

References

1. Gang Feng, Jennifer Hobbs, Xin Lu, Yue Yu, Pan Du, Warren A Kibbe, James Chandler, Lifang Hou and Simon M Lin (2013) A statistical method to estimate DNA copy number from Illumina high-density methylation arrays, *SystemsBiomedicine*, 1:2, 94 – 98, DOI: 10.4161/sysb.25896

2. Matsui S, LaDuca J, Rossi MR, Nowak NJ, Cowell JK. Molecular characterization of a consistent 4.5-megabase deletion at 4q28 in prostate cancer cells. *Cancer Genet Cytogenet*. 2005 May;159(1):18 – 26. DOI: 10.1016/j.cancergencyto.2004.09.010. PMID: 15860352.

Conclusions

- Watchmaker DNA Library Prep Kits with Fragmentation seamlessly integrate with the Singular Genomics G4 Sequencing Platform, providing accessible benchtop sequencing.
- Watchmaker DNA Library Prep Kit with Fragmentation delivers tunable insert sizes and excellent sequence accuracy with a highly scalable workflow.
- The Singular Genomics G4 Platform delivers fast, flexible and accurate sequencing for demanding applications such as microbial community and human CNV analysis.
- The G4 fastq file output is compatible with pre-existing bioinformatics tools and pipelines, yielding results that are highly correlated with those obtained from the leading NGS platform.