Evaluation of the Singular Genomics' G4™ Sequencing Platform with Resolution ctDx Assays on cell-free DNA

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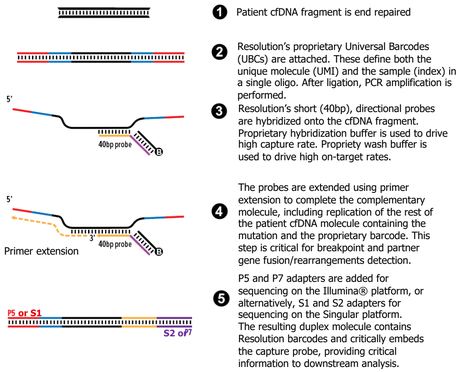
INTRODUCTION

Isolating cfDNA from blood to assess cancer variants is minimally invasive and can inform clinical decisions with significant benefit to patients. Resolution ctDx assays are targeted-hybrid capture, next generation sequencing (NGS) liquid biopsy assays which use circulating cell-free DNA (cfDNA) isolated from plasma. They are designed to combine efficient targeted sequencing with custom-built bioinformatics to allow sensitive detection of substitutions, insertions, deletions, fusions and copy number variations.

Cell-free tumor DNA (ctDNA) comprises only a small fraction of cfDNA, which in itself is present in plasma at low concentrations. Ultrasensitive detection and accuracy is therefore required to reliably identify potential cancer variants; sensitivity and quality are therefore important in the sequencing outputs. The G4 sequencing platform from Singular Genomics is a benchtop sequencer with novel, high-performance chemistry and advanced engineering, optimized to deliver faster results, greater output per hour, and the versatility of 4 independent flow cells. We therefore sought to evaluate the feasibility of the Resolution ctDx assays on the Singular Genomics' G4 pre-production sequencing platform, comparing it to the Illumina® NextSeq™ 500/550 currently used in clinical studies.

STUDY DESIGN

Libraries were prepared from cfDNA from three WT commercially-available plasma samples collected by plasmapheresis and preserved in citrate, from sheared genomic DNA (NA12878), or from SeraSeq ctDNA Mutation Mix with variants at minor allele frequency (MAF) of 0.25% or 1%. Libraries were captured with Resolution ctDx panels. Final libraries were prepared using compatible adaptor sequences for the respective sequencing platforms.



1. Patient cfDNA fragment is end repaired
2. Resolution's proprietary Universal Barcodes (UBCs) are attached. These define both the unique molecule (UMI) and the sample (index) in a single oligo. After ligation, PCR amplification is performed.
3. Resolution's short (40bp), directional probes are hybridized onto the cfDNA fragment. Proprietary hybridization buffer is used to drive high capture rate. Propriety wash buffer is used to drive high on-target rates.
4. The probes are extended using primer extension to complete the complementary molecule, including replication of the rest of the patient cfDNA molecule containing the mutation and the proprietary barcode. This step is critical for breakpoint and partner gene fusion/rearrangements detection.
5. P5 and P7 adapters are added for sequencing on the Illumina® platform, or alternatively, S1 and S2 adapters for sequencing on the Singular platform. The resulting duplex molecule contains Resolution barcodes and critically embeds the capture probe, providing critical information to downstream analysis.

G4 sequencer from Singular Genomics

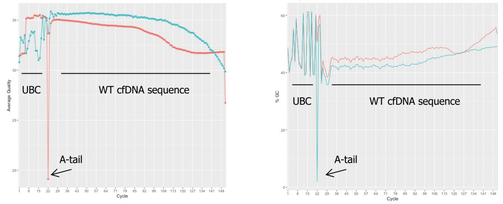
Resulting captured libraries were sequenced on either the Illumina® NextSeq™ 500/550 instrument or the Singular Genomics G4 pre-production instrument. Sequencing data was analyzed with Resolution Bioscience's proprietary analysis pipelines.

RUN QUALITY METRICS

Average Quality

The Rqc package from Bioconductor was used to evaluate read quality of output from either the Illumina® NextSeq™ 550 or Singular G4 sequencing platforms.

Average Quality (left) and % GC (right) by Cycle. Red lines represent Read 1 from Illumina sequencing of WT cfDNA while blue lines represent Read 1 from Singular sequencing of the same libraries.



- Overall sequencing quality is comparable between the two sequencing platforms
- Low diversity at the A-tailing site results in a drop in quality on the Illumina® sequencing platform, which is not seen with Singular G4 sequencing.
- Singular G4 sequencing consistently detects the A/T at the low-diversity location.

Sequencing Accuracy

Because the Resolution Bioscience analysis pipelines require 100% match to the known Universal Barcode sequence, the percentage of unknown barcodes can be an indication of potential sequencing errors.

Percent Unknown Barcodes in Singular G4 pre-production and Illumina® NextSeq™ sequencing runs

Assay	Singular G4	Illumina® NextSeq™ 550
Resolution ctDx Panel 1	5.97%	5.67%
Resolution ctDx Panel 2	6.51%	5.69%

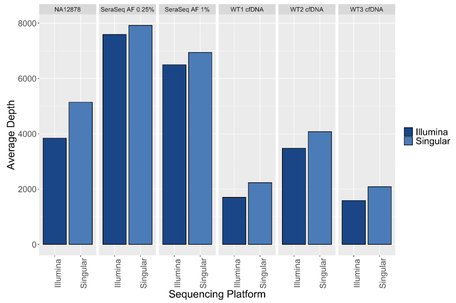
- The percentage unknown barcodes is similar between sequencing platforms, suggesting that the rate of potential sequencing errors is comparable.

DEPTHS AND READS

Average Unique Read Depth

Average depth was determined by the Resolution Bioscience analysis pipelines and is an indication of the number of unique reads observed across the panel in each sample.

Average Unique Read Depth Across Two Sequencing Platforms

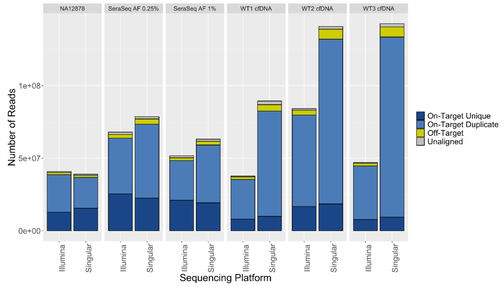


- Average depths were similar between the Singular G4 and Illumina® NextSeq™ 550 platforms, for both contrived and sheared gDNA, and for cfDNA.

Assignment of Unique/ Duplicate/ Off-Target Reads

Total reads varied based on the number of samples sequenced per flow cells. With one sample / flow cell, the three WT cfDNA samples were over-sequenced on the Singular platform, which resulted in an increase in duplicated reads. However, the number of unique reads showed consistency between sequencing platforms.

Number of Reads for 6 Samples Across Two Sequencing Platforms



- The number of unique reads are consistent across sequencing platforms
- Comparable off-target and unaligned reads suggests sequence quality is comparable between platforms

SENSITIVITY

Variant Detection

SeraSeq ctDNA Mutation Mix was diluted into sheared NA12878 gDNA to 0.25% or to 1% AF. Libraries were hybridized with the Resolution ctDx assay and variants detected with the Resolution Bioscience analysis pipeline, which assigns a confidence score based on the number of reads containing the variant sequence, as well as the quality of the sequencing at the variant site.

Variant Detection in SeraSeq ctDNA Mutation Mix at 0.25% and 1% AF

- Expected variants were detected similarly in the SeraSeq ctDNA Mutation Mix at both 0.25% AF and 1% AF when analyzed with the Resolution ctDx assay and sequenced with either Illumina® NextSeq™ 550 or with the Singular Genomics G4 instruments.

SUMMARY

The G4 pre-production sequencer from Singular Genomics was evaluated and compared with Illumina®'s NextSeq™ 500/550 .

- **Read quality from the Singular Genomics G4 sequencing is consistently high**
- **Average Depth and Number Unique Reads are concordant with those seen on the Illumina® NextSeq™ 550**
- **Analysis indicates comparable sensitivity in variant detection with the Resolution ctDx assay**