A novel dual-strand whole-genome sequencing (WGS) method for ultra-sensitive molecular residual disease detection (MRD)

Jeff Tsai[1], Dhamayanthi Pugazhendi[2], Jacob Gibson[1], Milana Gataric[2], Jessica Moore[2], Esther Musgrave-Brown[2], Trevor Ho[2], Alex Calderwood[2], Christopher Edlund[1], Seong Won Cha[1], Sven Bilke[1], Dale Weekes[2], Huihong You[1], Chris Truong[1],Khai Luong[1], Li Liu[1], Yunjiao Zhu[1], Jeff Fisher[1], Traci Pawlowski[1], James Han[1], Fiona Kaper[1], Cande Rogert[1], Andrew Slatter*[2]

[1]Illumina, Inc., San Diego, CA, USA
[2]Illumina, Inc., Cambridge, Cambridgeshire, UK

INTRODUCTION

Whole genome sequencing (WGS) of circulating cell-free DNA (cfDNA) enables ultra-sensitive circulating tumor DNA (ctDNA) detection by leveraging genome-wide mutations. However, the detection sensitivity remains limited by errors. Here we describe the performance of a new, high-Q WGS library prep method, which enables >Q65 data quality, and demonstrate its application in molecular residual disease (MRD) detection.

METHODS

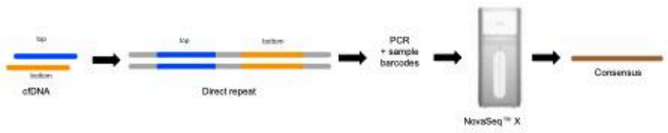


Figure 1: Dual strand WGS method

- **Novel dual-strand WGS library prep** generates direct repeat fragments of top and bottom strands from double stranded cfDNA. Direct repeats allow for information from original top and bottom strands to be contained in a single strand with the same polarity, simplifying amplification and sequencing. This enables duplex sequencing for the whole genome without the high depth requirement of unique molecular identifiers (UMIs).
- **Integrated assay tools for high accuracy data** starting from library prep, sequencing on a standard NovaSeq™ X with custom run configuration, and bioinformatics enables a streamlined workflow that results in high quality data output. Duplex sequencing allows for the correction of errors originating from DNA damage, the library preparation process, or sequencing. Residual errors on either strand are informatically detected and masked, creating duplex consensus reads. A novel q-score prediction algorithm is employed to predict the probability a reported base is an error.
- **Low noise and ultra-high sensitivity assay performance** enables single-digit parts per million (ppm) ctDNA detection through a tumor-informed WGS MRD approach. The assay is compatible with as little as 5ng cfDNA input, supporting over 90% of samples from a typical 4ml plasma yield in a 10ml blood draw tube (based on internal cfDNA extraction yield from 4mls of plasma).

RESULTS

High Q data quality

Libraries prepared using the direct repeat or standard library prep from nucleosomal-prepared DNA (npDNA) or cfDNA were evaluated for Q-score across the read position up to 200bp length. The predicted Q-score in the dual strand WGS method showed 80% of bases with ≥Q67 for npDNA and ≥Q65 for cfDNA, with up to 20% bases with ≥Q71 for npDNA, compared to majority of bases at Q40 for standard WGS prep. This demonstrates the dual strand WGS technology capability of down to 10^{-7} error rate.

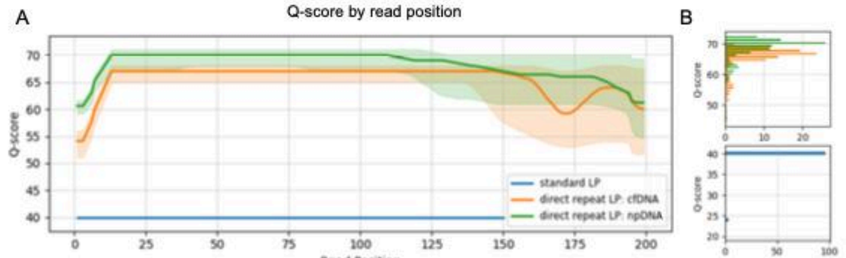


Figure 2: Data quality by WGS library prep methods. Libraries from the dual strand WGS method and standard WGS prep were sequenced up to 200bp on a NovaSeq™ X. (A) The predicted Q-score was reported across all read positions and plotted by median Q-score from npDNA (solid green line) or cfDNA (solid orange line) or standard WGS prep (solid blue line) with 20-80th percentile (shade). (B) Histogram distribution of the percent (%) bases across Q-score.

Table 1- % bases at predicted Q-score by sample type

Sample	80% bases	50% bases	20% bases
npDNA	≥Q67	≥Q70	≥Q71
cfDNA	≥Q65	≥Q67	≥Q68

High duplex yield

The fraction of fragments that resulted in duplex reads from the direct repeat WGS prep was informatically calculated from the total reads. The results showed >80% of passed filter clusters deliver duplex reads for all tested sample types, with demonstrating up to 90% duplex yield for npDNA.

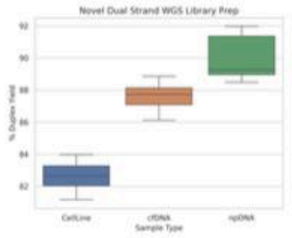


Figure 3: Direct Repeat WGS library prep data yield. Libraries prepared from 10ng of sample were assessed for duplex yield sequenced at >30x molecular coverage

RESULTS

Reduced noise level for MRD application

To demonstrate noise reduction for ctDNA detection in a tumor-informed MRD approach, libraries from 10 self-reported healthy donor cfDNA samples were prepared with the dual strand WGS method and standard WGS prep. Using the DRAGEN™ MRD pipeline, each sample was assessed for 9 unique tumor SNV signatures from NSCLC, CRC, bladder, melanoma, and breast cancer samples. Results show significant reduction in estimated VAF signal (up to 40-fold reduction) in the dual strand WGS approach, demonstrating improved noise reduction potential higher sensitivity in for MRD application

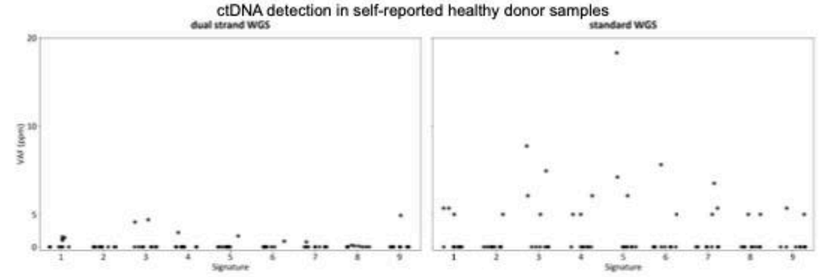


Figure 4: ctDNA detection in blank samples. Ten self-reported healthy donor samples were processed using the dual strand WGS method (left) or standard WGS prep (right). Plot represents detected tumor signal (estimated VAF) from 9 unique tumor SNV signatures for each sample (block dots).

High sensitivity ctDNA detection

To demonstrate the limit of detection (LoD) for the assay, Seraseq ctDNA MRD samples were diluted into normal background at targeted contrived tumor fractions (ppm). Using the DRAGEN™ MRD pipeline for ctDNA detection by a tumor-informed WGS MRD approach, results using showed 100% detection at 2.5 ppm. This demonstrates high sensitivity single-digit ppm capability for this technology.

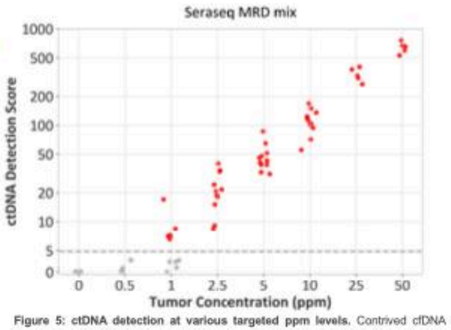


Figure 5: ctDNA detection at various targeted ppm levels. Contrived cfDNA at various targeted ppm levels (x-axis) were processed through the dual strand WGS method at >30x molecular coverage. A ctDNA score, statistical representation of ctDNA in DRAGEN™ MRD pipeline, at 4.8 (y-axis, dashed line) represents detection threshold for 99.8% specificity. Each dot represents a valid observation (red dots = ctDNA positive, grey dots = ctDNA negative).

CONCLUSIONS

- Dual strand WGS method through direct repeat library prep, sequencing, and informatics enables >Q65 data quality (down to 1 in $1x10^7$ error rate)
- Direct repeat library prep has high library conversion that results in >80% high quality duplex consensus reads
- This method enables low assay noise for cfDNA, enabling single-digit ppm ctDNA detection through a tumor informed WGS MRD approach

CAP# SP-00864

© 2026 Illumina, Inc. All rights reserved.

For Research Use Only. Not for use in diagnostic procedures.

illumina®