

CTC-SEQ: ACCURATE MUTATION DETECTION FROM SINGLE CANCER CELLS USING THE CELLSEARCH® SYSTEM AND QUANTIDEX® TARGETED NGS PANELS

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SUMMARY

- Circulating tumor cells (CTCs) harbor genetic signatures that may inform the clinical management of patients with targeted therapies.
- Advances in single-cell isolation, genome amplification and low input mutation detection (1- 20 cells) are needed to enable genetic profiling of liquid biopsy samples.
- Here we describe initial evaluation of a comprehensive solution for targeted next-generation sequencing (NGS) of CTCs, or CTC-Seq, using cancer cell lines spiked into blood as a model.
- Accurate mutation detection can be achieved from as little as a single cell, with more robust results when using 5 or more cells.

INTRODUCTION

Liquid biopsies can forecast outcomes in cancer patients and report shifts in tumor progression in real-time. Enumeration of CTCs in peripheral blood has been clinically validated to predict overall survival of patients with metastatic breast, colorectal and prostate cancers (CELLSEARCH® CTC Test). Yet CTCs also harbor genetic signatures that may inform the clinical management of patients with targeted therapies. NGS is a compelling option to profile “druggable” mutations in CTCs, but advances are needed to accommodate low CTC counts. Here we describe initial evaluation of a comprehensive solution for targeted NGS of CTCs, or CTC-Seq. This approach combines sample qualification, targeted enrichment of clinically-actionable cancer genes, and a customized bioinformatics pipeline tuned for high positive predictive value (PPV).

METHODS

Four cancer cell lines were spiked into 7.5 mL of normal human blood, enriched using the CELLSEARCH® System (Janssen Diagnostics, JDX) and further purified by individual cell picking. Control cells were directly purified from culture by individual cell picking. The cell lines represent 6 “driver” mutations in 4 oncogenes (BRAF, EGFR, KRAS and PIK3CA). Phi29-based whole genome amplification (WGA) (REPLI-g SC, Qiagen) was used to amplify genetic material from low input cell samples (1, 3, 5, 10 or 20 picked cells) and high input control gDNA (10 ng). Amplifiable DNA inputs into NGS were qualified with the QuantideX® qPCR DNA QC Assay (Asuragen, Asu). PCR-based target enrichment, library preparation, and variant analysis were performed using the QuantideX® NGS Pan Cancer Kit* and QuantideX® NGS Reporter software (Asu) and sequenced on the MiSeq® System (Illumina).

RESULTS

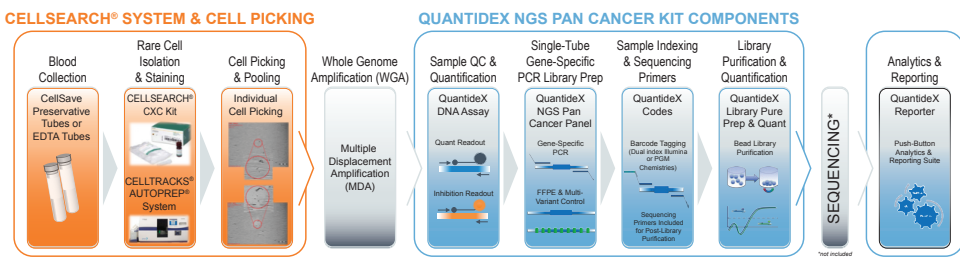


Figure 2. Integrated CTC-Seq workflow. Cancer cell lines were enriched by the CELLSEARCH® System and further purified by cell picking (JDX). WGA from as little as a single cell was conducted by two independent sites (JDX and JDX). QuantideX NGS Pan Cancer Kit and NGS Reporter software was used for sample QC, NGS library prep and data reporting (Asu). Sequencing was performed on the MiSeq System (Illumina).

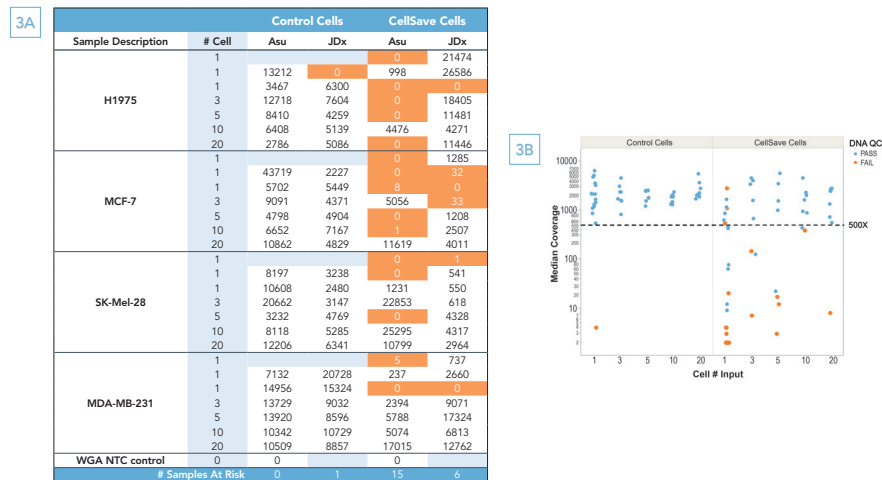


Figure 3. QuantideX qPCR DNA QC Assay predicts WGA sample performance on NGS. (A) WGA products from control cells and cells spiked into CellSave blood for < 24 hr prior to enrichment were QC'd. Samples with < 100 amplifiable copies/uL were flagged at risk for poor performance on NGS. 98% of the WGA samples from control cells (excluding a single 1 cell input) yielded sufficient amplifiable DNA. A 17% decrease in number of “usable” CellSave samples compared to controls (JDX site) was observed, especially below 5 cell input. Asu CellSave samples showed lower pass rate (46%) compared to JDX (78%), consistent with a batch handling effect. (B) Median amplicon coverage > 500X indicates successful performance on NGS; however high-sensitivity variant calling is possible down to ~200X.

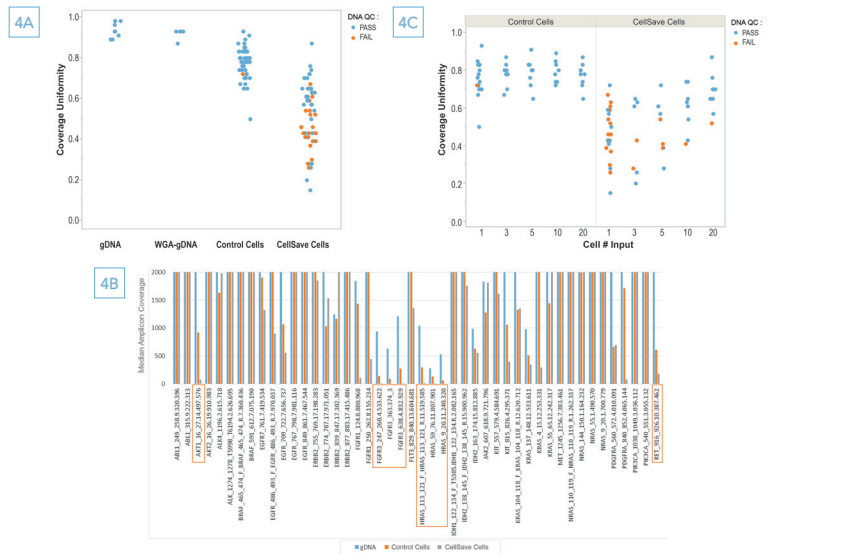


Figure 4. Coverage uniformity is a function of sample quality and input. The QuantideX NGS Pan Cancer Panel is optimized for low-quality FFPE DNA with > 90% coverage uniformity across all 46 amplicons. (A) Intact control gDNA and WGA-treated gDNA samples display similar uniformity results suggesting minimal WGA-induced bias; however, (B) combinatorial effects associated with low input WGA (1 - 20 cells) and DNA fixation in CellSave produced characteristic MDA-induced amplification bias resulting in allelic dropout in AKT1, FGFR3, HRAS, and RET regions (CellSave Cells). Of note, FGFR3 and HRAS loci are each GC-rich (60-73%) which may contribute to reduced amplification uniformity in the WGA step. (C) Coverage uniformity in CellSave improved as a function of cell number and amplifiable DNA inputs, where 80% of 20 cell inputs showed comparable uniformity to control cell samples.

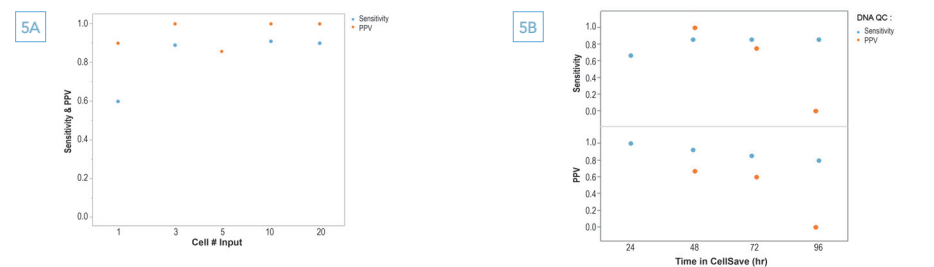


Figure 5. Mutation detection and time course stability in CellSave. (A) Accuracy (PPV) and sensitivity of mutation detection is summarized by site where WGA was conducted (JDX and Asu). Cellular inputs ranging from 1 to 20 cells (CellSave < 24 hrs) were tested in duplicate (triplicate for the single cell inputs). Expected mutations were identified from as few as a single cell in all 4 cell lines, with the most accurate results (86 - 100% PPV and analytical sensitivity) achieved with 5 or more cells. (B) Sample stability was evaluated using 3 and 5 cell inputs into CellSave blood for 24-96 hrs prior to enrichment and cell picking. Amplifiable copy number of the resulting WGA products were predictive of successful variant detection. PPV decreased with length of time in CellSave, while ~80% sensitivity was maintained across all points measured.

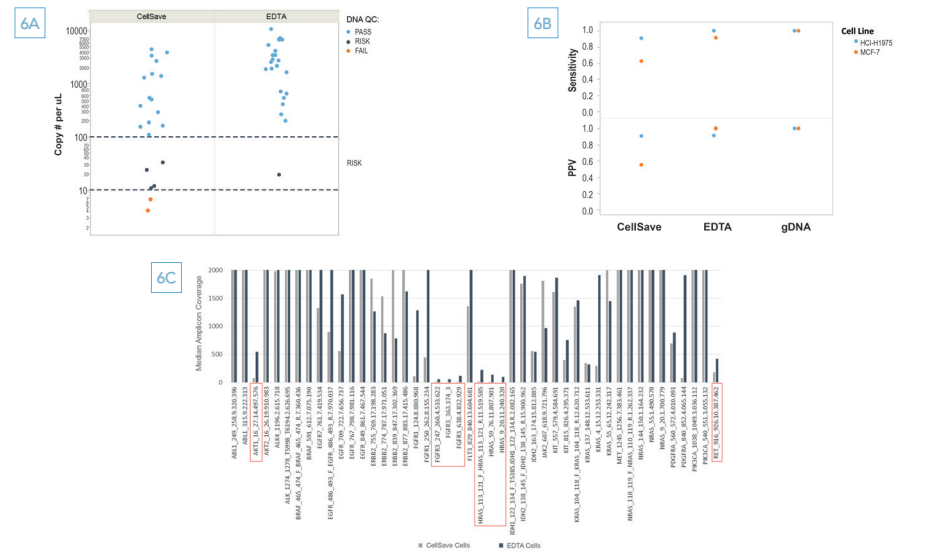


Figure 6. Sample collection comparison (CellSave vs. EDTA tubes) and implications on NGS performance. NCI-H1975 and MCF-7 cell lines at 3 and 5 cell inputs were spiked into CellSave blood (in duplicate) and EDTA blood (in triplicate) for up to 24 hrs. EDTA samples consistently outperformed CellSave samples in terms of usable samples (96% EDTA, 75% CellSave) (A), mutation detection (Sensitivity/PPV for NCI-H1975: 100%/92% EDTA, 91%/91% CellSave) (B) and coverage uniformity (up to 20% increase for EDTA) (C). Time course stability in EDTA suggests number of usable samples and mutation detection is comparable out to 96 hrs (data not shown). Successful attempts were made to recover samples in the “risk” DNA QC category (10 – 100 copies/uL); however samples with < 10 copies/uL consistently failed to yield usable sequence data.

CONCLUSIONS

- Our results demonstrate proof-of-concept for CTC-Seq mutation detection from 1-20 cancer cells captured by the CELLSEARCH® System and further purified by single cell picking.
- QuantideX qPCR DNA QC Assay was predictive of WGA sample performance on NGS.
- Adequate sequence coverage was reached for >90% of the QuantideX NGS Pan Cancer target genes enabling accurate mutation detection from as little as a single cell, with more robust results when using 5 or more cells.
- EDTA sample collection tubes support more robust NGS analysis and mutation detection than CellSave tubes.
- Further optimization of the CTC-Seq workflow and its application to clinical CTC specimens are in progress.

*Research Use Only – Not For Use In Diagnostic Procedures
Preliminary research data. The performance characteristics of this assay have not yet been established.
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