A novel AI-driven spatial genomics platform enables tumor enrichment and analysis of heterogeneity

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Abstract

Spatial genomics and digital pathology continue to advance at a rapid pace, generating large amounts of data. There is a critical unmet need for advanced tools to expedite spatial data generation, visualization, and analysis and to facilitate an improved understanding of health and disease, especially with the advancement of personalized cancer treatments. In this study we present a novel method for the extraction and analysis of genomic information from specific regions of interest (ROI) within a series of formalin-fixed paraffin embedded (FFPE) breast cancer adenocarcinoma tissue sections. An artificial intelligence-based tumor profiling algorithm first identified tumor, stroma, inflammatory, and necrotic regions within the tissue, and segmentation results were confirmed by a Board-Certified Pathologist. A crude lysate was extracted from the selected ROIs using an ink-jet based masking technology and novel ink chemistry. The lysis process was performed directly on the tissue slide and the crude lysates generated were transferred to a standard collection tube for subsequent NGS. DNA was extracted from the selected areas before being purified and analyzed using a targeted multiplex amplicon NGS panel. Library QC showed optimal coverage at an average of >95% uniformity. Sequencing results showed an average coverage of 95% at 250x across all amplicons of the panel. The workflow combined Reveal Biosciences' imageDx™ tumor profiling digital assay,, Quantumcyte's OncoMask technology and Pillar Biosciences' OncoReveal Multi-Cancer Panel powered by SLIMamp® (See figure 1). Variant calling was successfully performed using the PiVAT data analysis platform available from Pillar Biosciences. Sequencing data is presented to demonstrate the ability to extract quality DNA as well as the resulting tumor heterogeneity. This novel technology provides an efficient solution for the enrichment of tumor content with high sequencing coverage from FFPE tissue sections. Novel spatial data visualization and analysis techniques allowed a comprehensive assessment of tumor heterogeneity.

Workflow

Figure 1. The study combined Reveal Biosciences imageDx™ AI based digital assay with Quantumcyte's OncoMask technology that is designed to incorporate Reveal's digital annotations for Qcyte's proprietary masking technology. DNA isolated using OncoMask was prepared and analyzed using Pillar Biosciences ONCO/Reveal gene panel. NGS was performed on an Illumina MiSeq. Data was analyzed at Quantumcyte.

Study Design

The goal of this study was to demonstrate technical feasibility of a novel workflow for performing molecular pathology studies. The developed workflow begins by pairing a standard tumor biopsy pathology staining protocol (H&E) a machine learning-based digital pathology algorithm to segment tumor regions, novel system for stratifying relevant ROI and masking around specific tumor cell regions of interest then increases cellular level accuracy that is ultimately reflected in off the shelf DNA extraction and variant analysis library preparation chemistry, NGS, and secondary analysis. Figure 2 summarizes the study design.

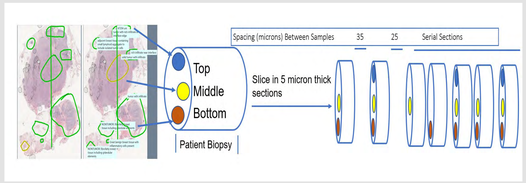

Figure 2. Three tumor regions in a single biopsy were identified using the imageDx™ tumor profiling digital assay. A Board-Certified pathologist verified the annotations. Each region was designated as "Top", "Middle" and "Bottom as shown above. 7, 5 micron thick serial sections were produced from the block and mounted to Superfrost slides. DNA was isolated from each region using Quantumcyte's OncoMask workflow. The DNA was extracted directly off of the slide using a Protein Kinase based buffer and processed using the Pillar Biosciences SLIMamp® Protocol through the Onco/Reveal Multi-cancer panel. A crude lysate was extracted from the three regions on the same slide and placed into an Eppendorf tube for subsequent nucleic acid prep. Regions studied are shown above.

Materials

Biopsy: A HER2+, invasive ductal carcinoma, grade III poorly differentiated breast cancer FFPE biopsy collected in 1999 from a 50 year old human female was purchased from Discovery Life Sciences, Los Osos California as 5 micron sections mounted to superfrost slides. All slides were deparaffinized prior to masking and lysis.

Reagents and consumables: All slides were deparaffinized using a standard procedure that included heat, Xylene and ethanol treatments. H&E staining followed a standard protocol. Lysis was performed directly off the slide using a proteinase K based buffer. Lysis buffer (50 ul) was placed on the slide and a coverslip placed on top of the buffer and slide. DNA purification was done using the QIAamp DNA FFPE Tissue kit from Qiagen. Purified DNA was prepared using the Pillar Biosciences ONCO/Reveal Multi-Cancer Panel workflow. The panel is a robust NGS assay that interrogates 56 genes of interest across multiple solid tumor cancer types. The assay uses proprietary Stem-Loop Inhibition-Mediated amplification (SLIMamp®) technology, a tiled amplicon-based library prep chemistry for efficient single-tube target enrichment. The prepped libraries were run on a MiSeq using the MiSeq reagent kit V3 150 cycle.

Imaging, Regions ID & Data Analysis

Digital images were analyzed at Reveal Biosciences using their imageDx™ tumor profiling digital assay to segment tumor regions and identify tumor cells. Using the masked image, three regions were selected for the analysis. A Board-Certified pathologist confirmed the classification analysis Coordinates for the regions were converted into print files that were inserted into Quantumcyte's ink-jet based masking workflow. The workflow uses the print file to direct the application of a proprietary ink using ink-jet technology directly on to the slide such that only the areas surrounding the three regions were exposed to the ink. Figure 3 summarizes this workflow. Primary sequence data was analyzed using Pillar Biosciences PiVAT data analysis workflows. Final analysis was performed by Hugo Lam at hypahub.com using hierarchical clustering.

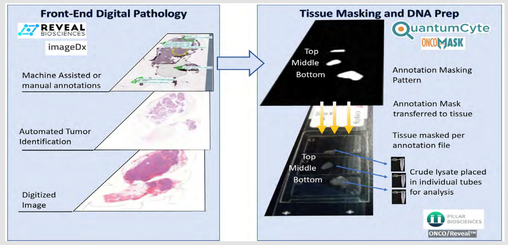

Figure 3. Tissue processing from digital imaging to nucleic acid prep for NGS. Slides taken at intervals through the serial sections were H&E stained and assessed with Reveal Biosciences' imageDx™ digital assay to segment tumor regions to identify tumor cells. Three different tumor regions (Top, Middle and Bottom) were identified and verified by a Board-Certified pathologist. The annotations helped generate a print file which was used to direct Quantumcyte's proprietary ink chemistry application using ink-jet technology directly on to the tissue sections. The ink covered the areas outside of the three tumor regions and is impermeable to the lysis reaction. A coverslip was placed over the regions and a proteinase K based lysis buffer was added to the three regions. After a 90' incubation at 56C, a crude lysate was collected, placed in to three different Eppendorf tubes, and DNA purified individually before library preparation using Pillar Biosciences ONCO/Reveal Multi-Cancer panel workflow and sequenced on the Illumina MiSeq.

Results

The ONCO/Reveal multicancer panel detects >7200 loci (SNVs and Indels) in a single tube from a purified sample of DNA. The panel was chosen to determine if the workflow could detect differences in variant calls between samples within and across a FFPE tumor block. Figure 4 shows that data from each sample as a heat map. Based on the data, we do see variant profile differences across and within each sample. As an example, TP53, a known oncogenic target, has been highlighted (see Figure 4).

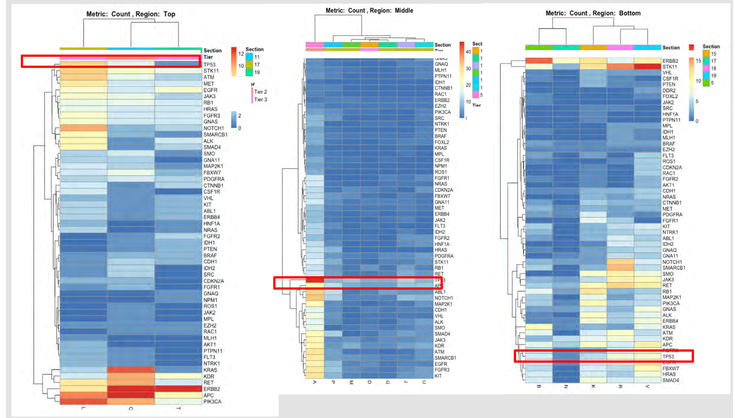

Figure 4. Heat map analysis. The data indicates that there are variant profile differences between the various samples. TP53 is an example of a known oncogene that exhibits these differences.

Conclusions

The goal of this study was to demonstrate a workflow that integrates AI driven automated pathology with a unique platform that combines high spatial resolution with a proven multicancer NGS workflow for use in genomic analysis in DNA variant calling. The data indicates successful completion of this workflow. I addition, variant profile differences were seen throughout the individual samples indicating that the system is capable of detecting heterogeneity in breast cancer. Further insights into tumor heterogeneity through the increased spatial resolution offered by this workflow may lead to a more accurate representation of cancer biology.

Next Steps

Additional studies are currently being designed to evaluate this workflow to precision and personalized medicine applications. We are extending the platform to specific applications including NASH, cardiac disease, and various cancers including prostate, colorectal and head and neck to name a few. We are doing this in collaboration with academic hospitals, pathology labs, therapeutics and pharma companies. Our Silicon Valley localized lab is working with our collaborators to develop clinical applications.

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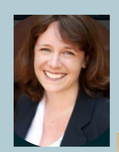

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