An automated tissue dissection platform improves NGS data quality of clinical lung and colon cancer biopsies.

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Abstract

Quantumcyte has developed an automated tissue dissection system capable of enriching regions of interest (ROIs) with 50um resolution. This system was used for enriching the neoplastic content in lung and colorectal cancer FFPE samples which were identified using whole slide imaging of H&E stained slides by a trained pathologist. Based on these annotations, automated and manual tissue dissection was performed. After tissue lysis and nucleic acid purification, AmpliSeq reagents were used for target amplification and library construction in order to interrogate a custom DNA panel (somatic solid tumour panel, hotspot regions of 27 genes) via NGS sequencing. Data analysis revealed a significantly higher neoplastic content in samples dissected with the Quantumcyte system when compared to manual dissection.

In Quantumcyte dissected samples, an average increase in allelic frequency of 69% (22%-130%) was detected for all three cases mutations. Similarly, Q scores increased 7-fold (2-10 fold). These data demonstrate the utility of the Quantumcyte platform to improve clinical grade sequencing metrics for NGS based workflows.

Introduction

Next generation sequencing workflows in a clinical setting are now routinely used to support treatment decisions. Enriching for neoplastic tissue in samples with a high degree of heterogeneity is crucial to gain actionable results from NGS data. Obtaining a sample with high enough purity, however, can be challenging. Currently, enriching neoplastic cells from slide-mounted FFPE tissue sections is primarily done through a manual process (termed manual macro-dissection) by physically scraping off tissue from a microscope slide with guidance from a pathologist. While this practice is adequate for the majority of cases, numerous cases (as many as 25%) fail to meet the critical purity specification to yield a clinically actionable result. Quantumcyte, Inc (Sunnyvale, CA) has developed a novel tissue dissection system that increases the neoplastic content from these difficult to dissect tumor tissues in order to meet the required specifications. The system can guide tissue dissection based on manual tissue annotation or automated, AI supported algorithms. Cell lysates containing DNA and RNA can be created from regions as small as 50 microns in diameter and lysates can be used directly for downstream nucleic acid purification. For this study, H&E stained slides from three tumor samples were whole slide imaged and annotated by a pathologist at Singapore General Hospital (SGH). Unstained slide mounted FFPE serial sections from each tumor were then shipped to Quantumcyte. The annotations were used to guide the Quantumcyte technology (system?) to extract a crude lysate only/exclusively from regions with high neoplastic content. Figure 2 outlines the workflow for this study.

Quantumcyte Tissue Dissection Workflow

Figure 1. Quantumcyte tissue dissection system. The system is capable of extracting a crude lysate from regions of interest on a FFPE tissue slide as small as 50 microns. DNA and RNA can be purified for use in NGS analysis. Annotations (manual or automated) from scanned tissue slides guide the system to target informative ROIs.

Workflow

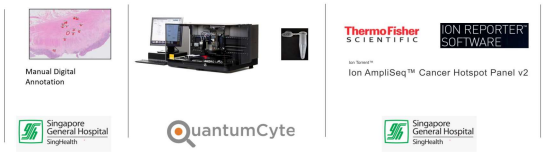

Figure 2. Experimental Workflow. H&E stained 5-micron thick tissue sections were scanned on a Phillips whole slide imaging system at SGH. 5 micron slide mounted serial sections from each tumor were shipped to Quantumcyte. Digital annotation files were used to guide the Quantumcyte tissue dissection system to target exclusively the tumor regions. The extracted a crude lysates were then shipped to SGH for sample prep and NGS analysis.

Results

H&E stained images of the three samples tested in this study are shown in Figure 3. The green highlighted areas for case 1 and 2 and the red highlighted areas for case 3 are the pathologists annotations identifying tumor tissue (see Figure 3A). The black and white images in Figure 3B are files generated from the annotations that were used to guide Quantumcyte's tissue dissection platform to extract the crude lysate from specified regions. Visual analysis of the images pre and post lysis confirmed digestion of cellular contents. For each case, the crude lysates generated from the manually macro-dissected tissue, and the Quantumcyte dissected tissue, were analyzed using Ampliseq reagents with a customized DNA panel (somatic solid tumour panel, hotspot regions of 27 genes) and sequencing on Ion Torrent PGM sequencer was performed. Data from each sample is presented in Figure 4. The sequencing quality was optimal with 100% coverage at 250x which is an accepted clinical grade metric. At high depth of 1000x, all three cases covered at least 97% of the panel target region. The mutations detected with the Quantumcyte workflow showed an average of 69% increase in allele frequency for all three cases, with a range of 22% to 130%. Quality scores showed an average increase of 7-fold, with a range of 2- to 10-fold.

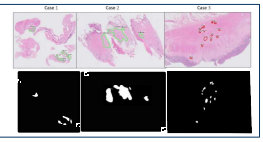

Figure 3. H&E stained images of the three cases tested in this study. Tumour-only regions were identified by a pathologist (Green in cases 1 and 2, Red in case 3). Figure 3A-H&E stained images from the 3 cases used in this study. Figure 3 B – Images used to guide the tissue dissection system for extract a crude lysate from the tumor only regions.

	Sequencing Quality (Panel Coverage)	Manual MD Quality Score (AF%)	QuantumCyte Quality Score (AF%)	Increase in Quality Score (AF%)
Case 1	100%(250X)	45.4 (3.9)	470 (9.0)	10 (130)
Case 2	100%(250X)	374 (9.2)	911 (12.3)	2 (2)
Case 3	100%(250X)	32 (4.6)	281 (7.2)	9 (56)

Figure 4. Results from crude lysates generated from manually macro-dissected (manual MD) tissue and crude lysates generated from the Quantumcyte tissue dissection workflow.

Materials and Methods

5-micron thick FFPE tissue sections from each case were mounted to standard microscope slides using a microtome. Serial sections were H&E stained, whole slide imaged using a Phillip scanner, and annotated using a touch screen by a certified pathologist. Unstained serial sections were manually macro-dissected by a certified histotechnologist or processed using the Quantumcyte tissue dissection system. Tissues were lysed using lysis buffer from Takara's Nucleospin total RNA FFPE XS Kit under the kit recommended conditions. Nucleic acid was purified using the Qiagen Ampliseq kit. Purified DNA was prepped and sequenced on an Ion Torrent PGM sequencing system. Sequencing metrics reported were generated using Ion Reported Software.

Conclusions and Discussion

The Quantumcyte tissue dissection system isolated tissue lysates from specific ROIs of clinical FFPE slides that yielded DNA meeting the clinical quality score. Interrogation of the DNA with an NGS panel produced cllinical grade sequencing results. Compared to manual tissue dissection, automated tissue dissection produced a significant increase in allelic mutation frequency, reflecting the higher neoplastic content. This study shows the tremendous potential of the Quantumcyte technology for use in clinical molecular diagnostics. We look forward to reporting the results of an extended study using this platform.

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