Combining High-Throughput, High-Resolution Targeted Spatial Microdissection with SLIMamp® NGS Chemistry for Sensitive and Accurate Molecular Characterization on Low-Quantity, Low-Quality FFPE Samples

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Background

- QuantumCyte has developed an automated microdissection platform, QCPRECISE!™, for sample enrichment of tissue biopsies including cancer.
- The system uses AI-based digital pathology to annotate slides and applies a printed mask to remove unwanted regions.
- The system can be easily incorporated into existing –omics and pathology workflows.
  - This enables an unprecedented level of precision and speed to the analysis of patient samples, especially those with low tumor content.
- The **objective** of this pilot study was to assess the ability of the QCPRECISE!™ platform (**Figure 1**) to extract DNA of sufficient quality and quantity from FFPE tissue microarrays for clinically significant NGS-based molecular characterization.

Figure 1: The QCPRECISE!™ System for high-resolution targeted spatial microdissection

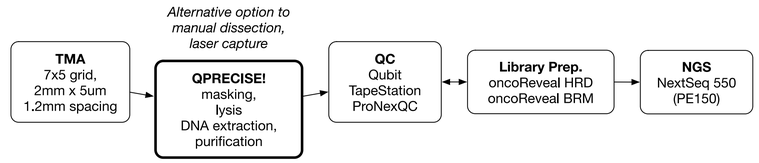
Methods and Materials

- Tissue microarrays (7x5 grid) were created using 2mm x 5um cores, with 1.2mm spacing, from 31 samples (23 FFPET and 8 cell line pellets) previously characterized with the Pillar Biosciences oncoReveal™ **HRD** and BRCA1 & RAD51C Methylation (**BRM**) panel NGS tests.
- DNA was extracted from selectively lysed tumor cells using the QuantumCyte methodology (**Figure 2**) and platform and was then purified.
- QCPRECISE! was incorporated into an existing next-generation sequencing workflow of tissue biopsies to provide spatially-targeted cellular analysis without sacrificing tissue quality.
- DNA concentration and quality were assessed with a TapeStation and the Qubit and ProNexQC assays (**Figure 3**).
- The resulting DNA was used for NGS amplicon library prep using the same panels and sequenced on a NextSeq 550 with PE150 reads.

Disclosures
This study was funded by AstraZeneca. JLF is a consultant for Pillar Biosciences; SZ, YC, YK, LR, & SM are full time employees of Pillar Biosciences. BC, KK, & JB are full time employees of QuantumCyte. SK, JA, PW, & JW are full time employees of AstraZeneca. PW is on the Scientific Advisory Board and is a stock holder of Pillar Biosciences. oncoReveal™ HRD and BRCA1 & RAD51C Methylation (BRM) are trademarks of Pillar Biosciences. QCPRECISE! Is trademark of QuantumCyte.

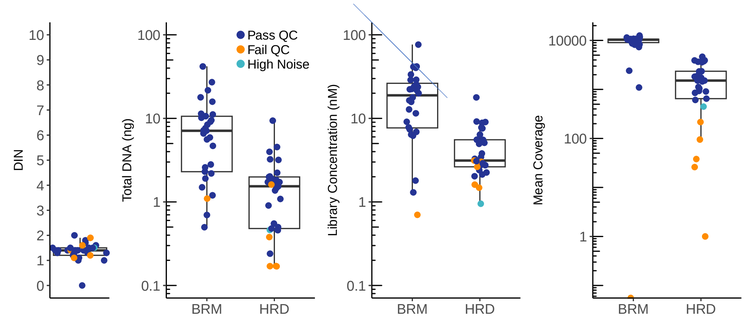
Materials and methods (continued)

Figure 2: QCPRECISE!™ was easily integrated into an existing workflow



- The resulting DNA was used for NGS amplicon library prep using the same panels and sequenced on a NextSeq 550 with PE150 reads.
- Bioinformatic analysis was performed with Pillar's PiVAT bioinformatics platform and custom analyses.

Figure 3: Confirmation of DNA quality and concentration extracted with QCPRECISE!™ and assessed on Pillar Biosciences oncoReveal™ TRD and BRM panels*



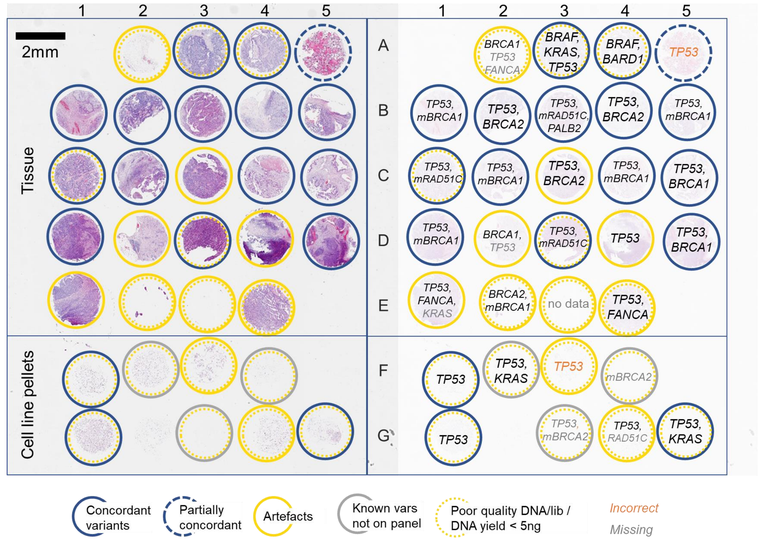
*Abbreviations: BRM, Pillar Biosciences BRCA1 *RAD51C Methylation Test; HRD, Pillar Biosciences oncoReveal HRD Test

Results

- Variants were called on all 31 samples (**Figure 4**)
  - 9 were clearly comprised of artefacts.
  - 3 samples (all cell line pellets) showed VAF peaks at allele. frequencies greater than 20% and, in most cases, greater than 40%, and contained variants that were not represented on the panels used here.
- Of the samples that could be compared, we could evaluate 29.
  - Of these, 76% of samples matched known variant VAFs with correlation of 0.93 using the HRD panel.
- VAFs ranged from 14–90%. For 24% of samples, known variants were not identified.
  - There were many artefactual variants between VAFs of 2.5–15%.
  - Many could be identified by applying a filter noting low confidence or C >T artefact.
- No cross-contamination was detected.

Results (continued)

Figure 4: Downstream molecular characterization of spatially-targeted cells collected with QCPRECISE!™



Conclusions

- We demonstrated that QuantumCyte's **QCPRECISE!™ platform can successfully extract DNA from very small, tightly packed tissue cores without cross-contamination.**
- We also showed that the resulting **DNA was sufficient for downstream molecular characterisation, if combined with a sensitive and robust NGS assay technology** and post-analysis filtering.
- The resulting DNA extracted by QuantumCyte's QCPRECISE! when combined with Pillar's robust and sensitive SLIMamp amplicon-based chemistry was **proven to provide sufficient downstream molecular characterization**.
- Leveraging the QCPRECISE! Platform in combination with a highly sensitive amplicon-based chemistry, such as Pillar's SLIMamp, will provide labs with the ability to **generate NGS results for samples that otherwise would have failed**.