A FULLY AUTOMATED ONCOREVEAL® PAN-CANCER SOLID TUMOR ASSAY FOR THE CHARACTERIZATION OF *EGFR* AND *KRAS* GENES IN FORMALIN-FIXED, PARAFFIN-EMBEDDED (FFPE) TISSUES

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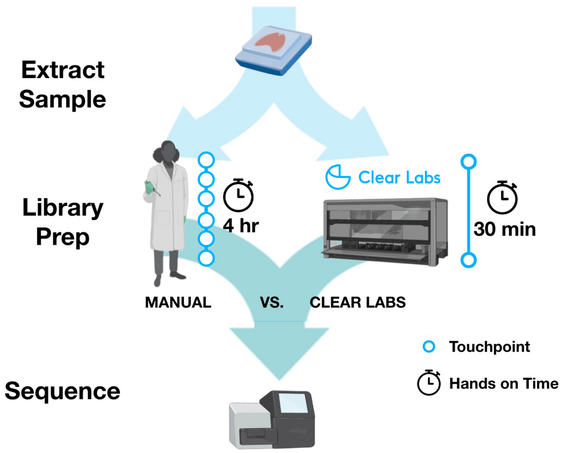
INTRODUCTION

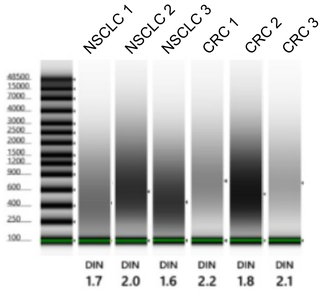
The oncoReveal® pan-cancer solid tumor assay is a robust multi-cancer test for key mutations present in DNA from solid tumors, enabling localized and distributed NGS testing to reduce time to results and testing cost. This assay uses amplicon-based target enrichment technology for the detection of SNVs, insertions and deletions in 22 genes using DNA isolated from FFPE tumor tissue samples and sequenced on the Illumina MiSeq instrument. However, the assay workflow requires significant hands-on time and has complex manual steps. Here we developed and evaluated an automated solution for the oncoReveal™ solid tumor assay using the Clear Dx™ platform with the aim of further reducing the turnaround time for results, ease of performing a complex assay by less skilled technicians, as well as reducing the chances of human error.

The automated oncoReveal® application has fewer touchpoints than the manual protocol and less hands-on time required by laboratory technicians



METHODS

Six archival FFPE research samples from colorectal cancer (CRC, *n*=3) and non-small cell lung cancer (NSCLC, *n*=3) were extracted manually using the QIAamp DNA FFPE Tissue Kit (Qiagen). Eluate was then loaded onto an oncoReveal™ solid tumor assay, comprising targeted gene amplification (*EGRF* and *KRAS*), library generation, sequencing and data analysis. These same samples were processed manually as per Pillar Biosciences' oncoReveal™ solid tumor assay user guide for comparison.

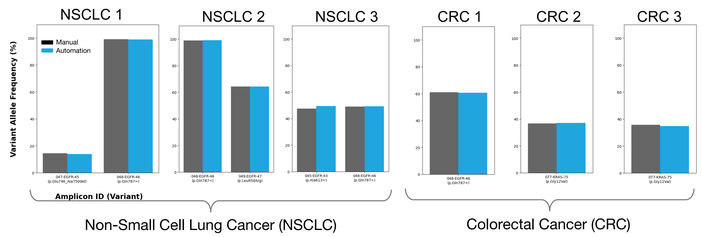


Genomic DNA manually extracted from 6 different FFPE samples

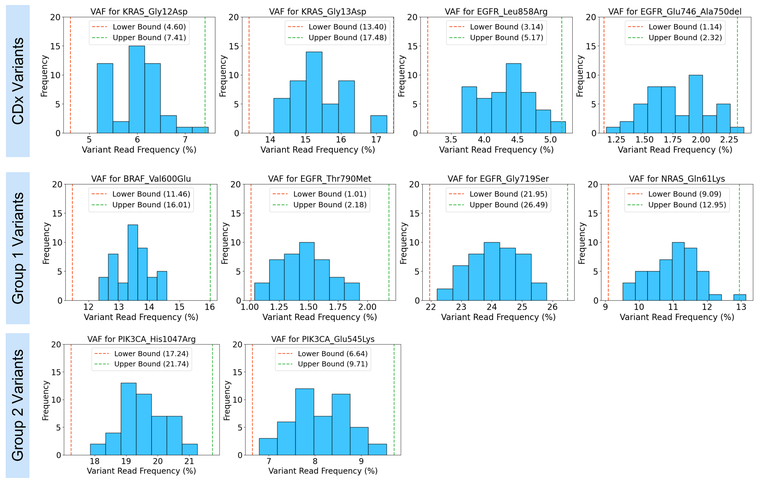
RESULTS

There was high level overall concordance of results generated between the manual and automated workflows. The number of touchpoints and hands on time were significantly lower using the fully automated workflow as compared to the manual workflow. Data generated by the fully automated workflow was also found to be highly reproducible.

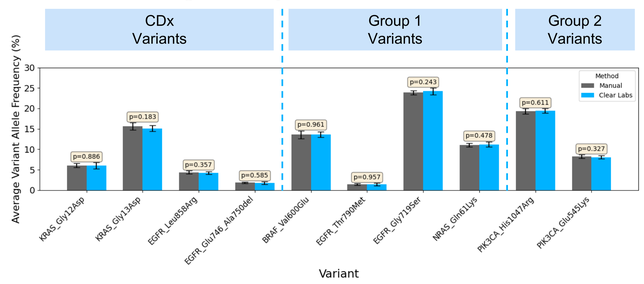
Variant allele frequency (VAF) of* EGFR *and* KRAS *in each FFPE sample are highly correlated between manual and automated workflows



Automation-generated VAFs from positive control sample (HD799) fall within tolerance intervals generated by manual baseline data

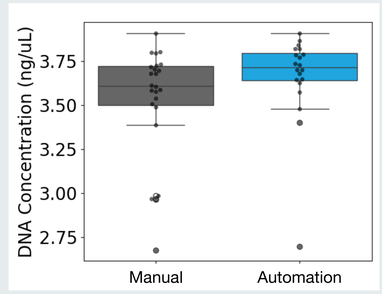


Automation-generated VAFs of positive control samples (HD799) are not significantly different than manual

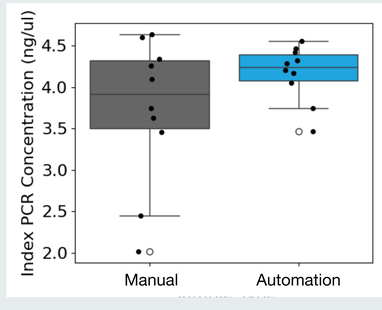


AUTOMATION OF INTERMEDIATE STEPS

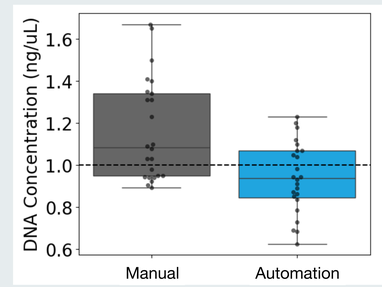
Automation of the gene-specific PCR workflow generated equivalent product yield when compared to the manual process



Automation of the workflow from sample input through to library preparation (Index PCR) generated more precise library yields when compared to the manual process



During quantification and library pooling, dilutions of 2 ng/µl stock to 1 ng/µl performed using automation were more accurate than manual



CONCLUSIONS

- Automating Pillar Biosciences' oncoReveal® pan-cancer solid tumor assay on Clear Labs' Clear Dx™ platform is a promising approach for accurate and unbiased testing for *EGFR* and *KRAS* mutations from archival NSCLC and CRC FFPE research samples, respectively.
- The results generated using automation are highly reproducible and comparable to the manual workflow and are for research evaluation only
- Currently, ongoing experiments using more archival FFPE research samples are underway to increase the power of this study.

The Clear Dx™ platform has the versatility to potentially automate any oncology NGS assay.

Come chat with us at **Booth #811**!

For more information regarding Clear Dx™ products, please contact inquiries@clearlabs.com
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