

Validation of an Automated Method for Library Preparation for a Next-Generation Sequencing-Based Assay for Oncology

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BACKGROUND

Targeted Next Generation Sequencing (NGS) technology is rapidly being adopted to assess the mutational status of multiple genes on formalin-fixed, paraffin-embedded (FFPE) tumor specimens in clinical settings

Library preparation is a critical, hands-on and time-consuming step in the NGS workflow. During library preparation, each library is prepared in an independent well of a 96-well plate, encompassing several washes and magnetic bead-binding steps.

This format increases the number of technical hours as more samples/libraries are prepared, while increasing the risk of human-introduced error. Automation and scalability of library preparation is much needed to not only reduce these issues, but to allow for the laboratory to increase the sample throughput.

Here, we present the validation and implementation of an open liquid handling platform, the VERSA™ 1100 GENE (Aurora Biomed, Vancouver, BC) for medium to high-throughput library preparation for routine utilization with the Ion AmpliSeq™ Cancer Hotspot Panel v2 (CHP2) assay on FFPE clinical specimens, including FFPE Quality Control (QC) materials (1).

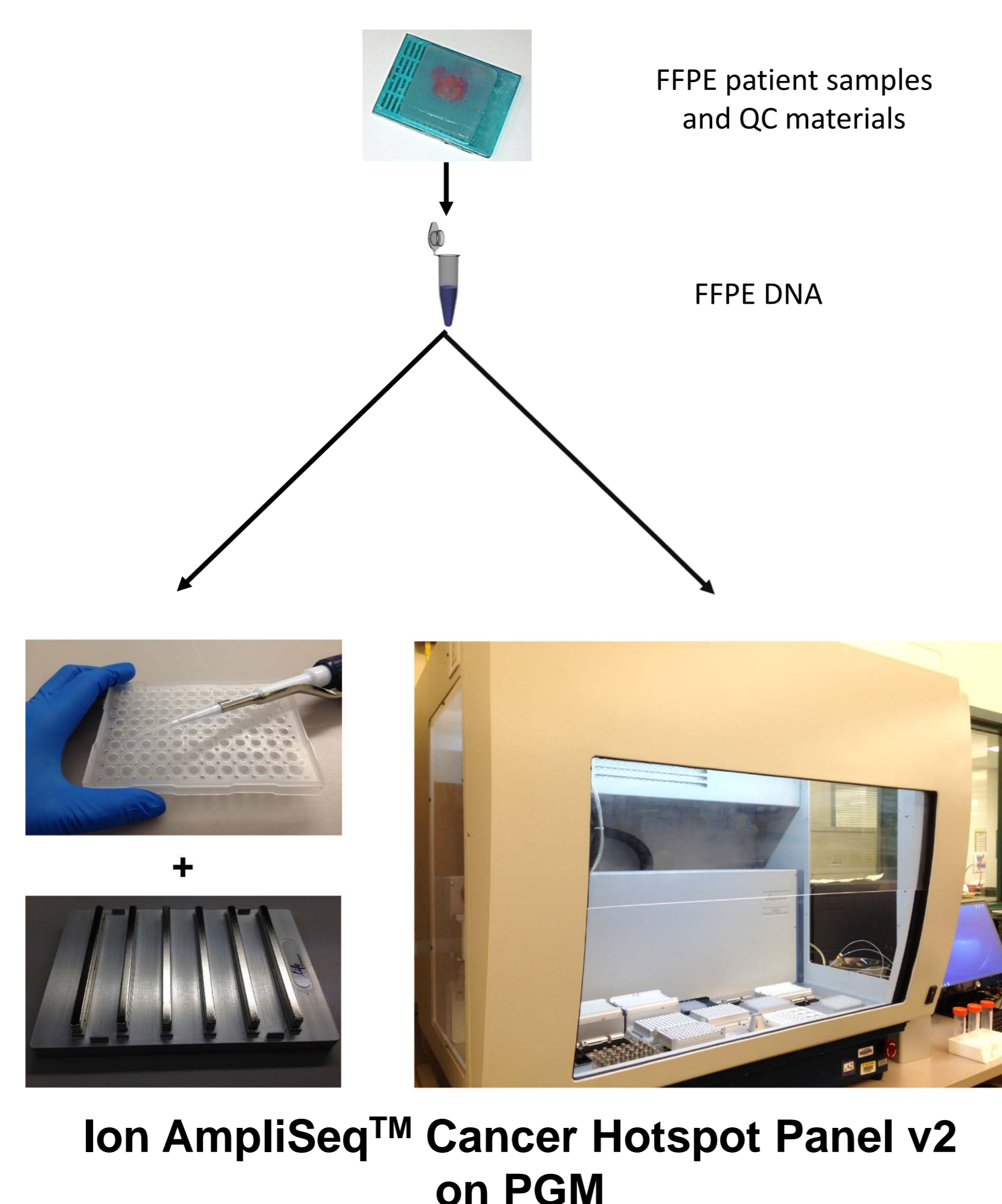
MATERIALS AND METHODS

Figure 1. Experimental Design

A – Cross-contamination (Checkerboard Experiments)

B – Reproducibility

C – Accuracy



RESULTS

Figure 2. Cross-contamination (Checkerboard Experiments)

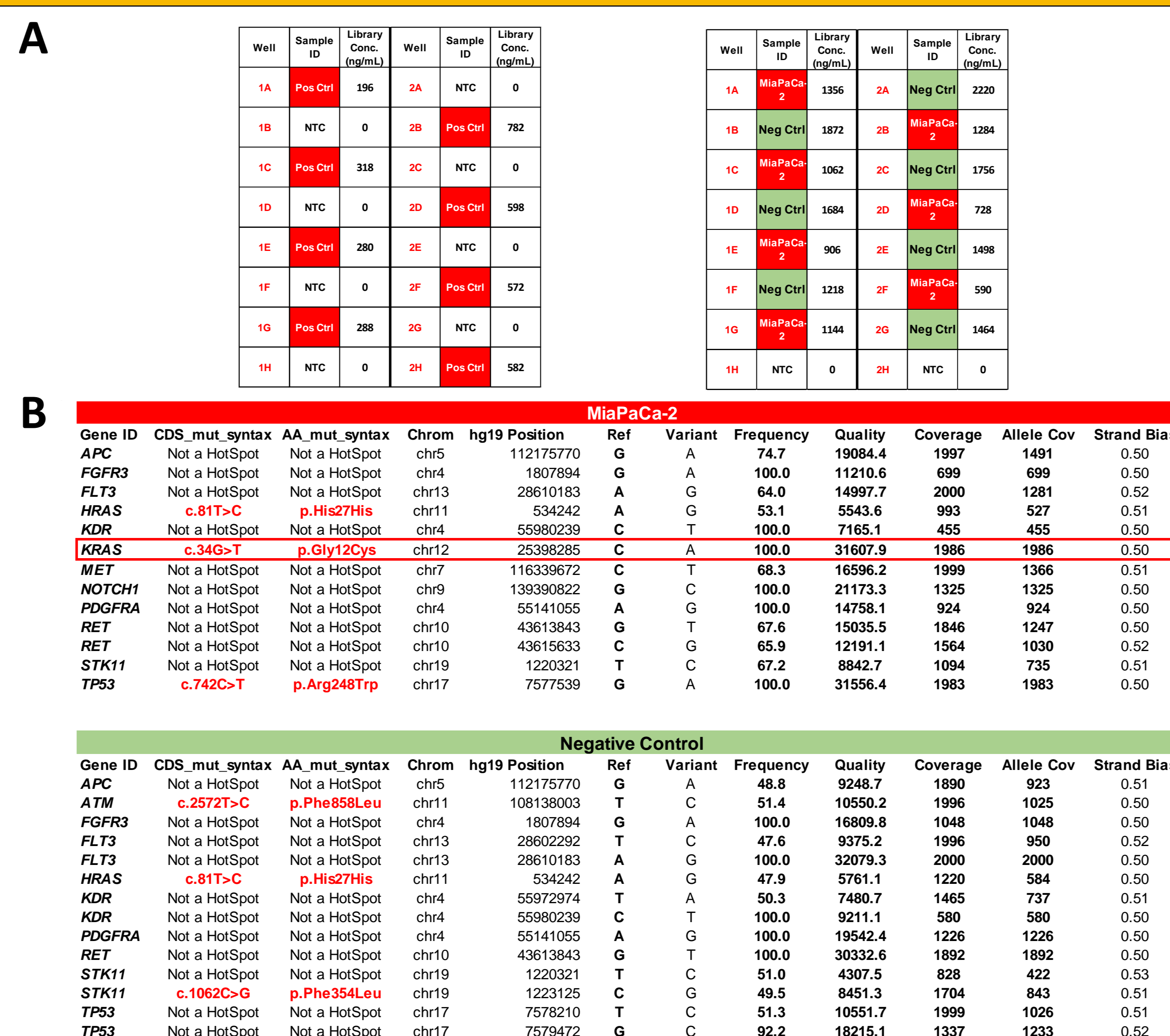


Fig. 2. Checkerboard library preparations. A- Library concentrations measured by the Qubit dsDNA HS Assay for the two checkerboard experiments. B- Representative variants called for the KRAS homozygous mutant pancreatic cancer-derived cell line, MiaPaCa-2, and the Negative Control libraries from the second checkerboard experiment. The expected p.Gly12Cys KRAS mutation in the **red box** was systematically detected in the MiaPaCa-2 libraries at 100% frequency, whereas it was not detected on any of the Negative Control libraries prepared by the VERSA™ 1100 GENE.

Figure 3. Reproducibility

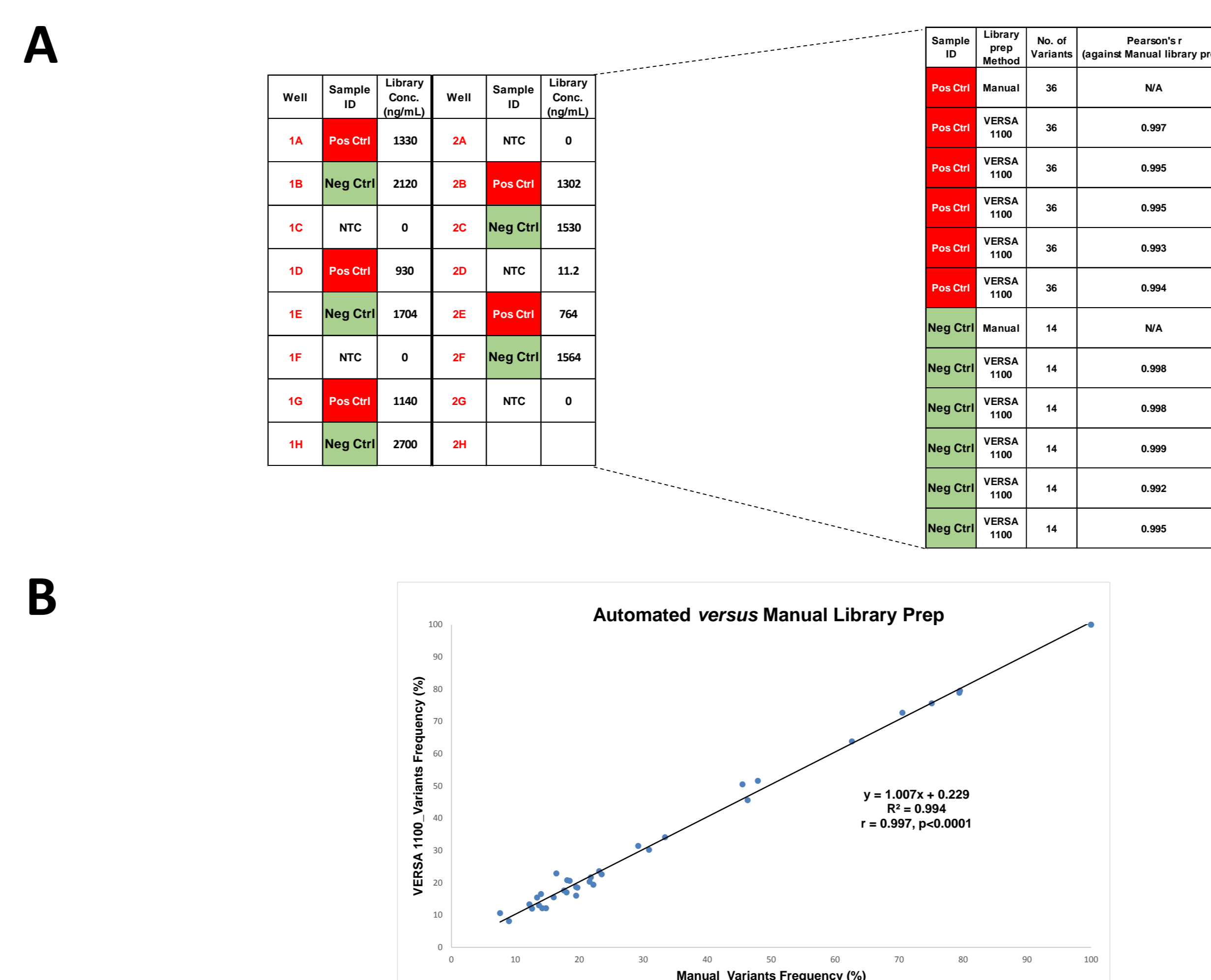


Fig. 3. Reproducibility of Control Samples. A- Library concentrations measured by the Qubit dsDNA HS Assay for five Positive and Negative control samples each (Left Panel) and number of variants and Pearson's correlations of variant frequencies with those obtained from manual library preparations (Right Panel). B- Representative curve showing Pearson correlation of the 36 variants frequency identified in the Positive Control sample by both library preparation methods.

Figure 4. Accuracy

| Sample ID | No. of PCR cycles | Library prep Method | No. of Variants | Pearson's r (against Manual library prep) |
|-----------|-------------------|---------------------|----------------------|---|
| Case_1 | 20 | Manual | N/A (library failed) | N/A |
| Case_1 | 23 | Manual | 19 | N/A |
| Case_1 | 20 | VERSA 1100 | 19 | 0.992 |
| Case_1 | 23 | VERSA 1100 | 19 | 0.992 |
| Case_2 | 20 | Manual | N/A (library failed) | N/A |
| Case_2 | 23 | Manual | 17 | N/A |
| Case_2 | 20 | VERSA 1100 | 17 | 0.996 |
| Case_2 | 23 | VERSA 1100 | 17 | 0.997 |
| Case_3 | 23 | Manual | 12 | N/A |
| Case_3 | 23 | VERSA 1100 | 12 | 0.995 |

Fig. 4. Accuracy in the variants called on FFPE patient samples. Difficult to amplify samples were chosen to compare the library yields and variants called from automatic versus manual library preparation protocols were used. Cases 1 and 2 failed to generate libraries using the manual protocol, so they were subjected to higher number of PCR cycles to generate libraries. For those samples, the VERSA™ 1100 GENE was used under both conditions, obtaining libraries even at fewer PCR cycles. The number and frequency of the variants found in every case were highly correlated.

CONCLUSIONS

➤ From the checkerboard experiments, we concluded that this automated liquid handling system shows no evidence of cross-contamination, by either no library on the no template control (NTC) wells, or no variants called on negative samples after sequencing using the CHP2 assay.

➤ Also, high reproducibility was observed in both, library yields and variants called across all technical replicates of the Quality Control materials.

➤ All patient DNA samples yield good quality libraries, including those difficult samples that had previously failed using the manual library preparation method, and variants were called with highly correlated (Pearson's $r > 0.990$) frequencies to those obtained with the manual method.

➤ Altogether, our results show that the performance of the VERSA™ 1100 Gene automated liquid handling workstation is very robust and might eliminate human-introduced errors, when compared to the manual library preparation method for the CHP2 assay.

REFERENCES

1- Dumur CI *et al.* Quality control material for the detection of somatic mutations in fixed clinical specimens by next-generation sequencing. *Diagn Pathol.* 2015;10(1):169. PMID: 26376646, PMCID: PMC4573924