# FUSION*Plex*™-HT and VARIANT*Plex*™-HT: Automation Ready Solutions for Anchored Multiplex PCR

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## Introduction

Anchored Multiplex PCR (AMP™) assays, VARIANT*Plex* and FUSION*Plex* are amplicon-based, next generation sequencing (NGS) workflows that identify genomic variations present in DNA or RNA input, respectively. AMP reagents are available in a lyophilized format which are user-friendly and convenient to store. However, lyophilized reagents are not suitable for automated liquid handling (ALH) or high-throughput (-HT) environments. Thus, we developed AMP reagents in liquid format to satisfy the need for a platform-agnostic method of high-throughput AMP library preparation. Using an ALH platform we developed, tested and optimized workflows for VARIANT*Plex*-HT and FUSION*Plex*-HT liquid reagents. Taken together, we provide a solution for increasing throughput for AMP-based NGS customers while reducing hands-on time.

## Methods

The performance of our liquid reagent based VARIANT*Plex*-HT and FUSION*Plex*-HT workflows were evaluated relative to legacy lyophilized reagents using several inputs of varying quantity, quality and type (RNA & DNA). We examined liquid reagent performance across multiple AMP panels, which target solid tumor and blood cancers variants. We also demonstrate the relative performance of the automated FUSION*Plex*-HT and VARIANT*Plex*-HT workflows to manual library preparation using SeraSeq® FFPE and Myeloid input materials with AMP-based panels. Samples were sequenced on the Illumina Nextseq® or MiSeq® system and analyzed using Archer™ Analysis v7.0 software. Two-sided, unpaired Student's t-Test was used to indicate a statistically significant difference between means. For multi-group comparisons against a single control condition, the Dunnett test was performed. *P<0.05; **P<0.0; ***P<0.001; n.s., not significant.

## New! Archer™ Liquid Reagents


- Available in 24- and 96- reaction kits
- Reagents are color matched to protocol steps for ease of use
- Each reagent supplied with 20% overage
- Compatible with all VARIANT*Plex* and FUSION*Plex* panels
- Features new liquid adapters
  - 96-well plate format
  - 192 unique P5 MBC + P7 adapters for a total of 36,864 unique combinations!
  - Reduced PhiX Control requirement to 1-2%

## Results - FUSION*Plex*-HT

| Input | Input Mass | Panel Name | Panel Size | Depth |
|---|---|---|---|---|
| Seraseq Fusion RNA Mix v4 | 20ng | FUSION*Plex* Lung v2 | 17 genes | 1M |
| Seraseq Fusion RNA Mix v4 | 50ng | FUSION*Plex* Lung v2 | 17 genes | 1M |
| Seraseq Fusion RNA FFPE v4 | 50ng | FUSION*Plex* Pan ST v2 | 137 genes | 3.5M |

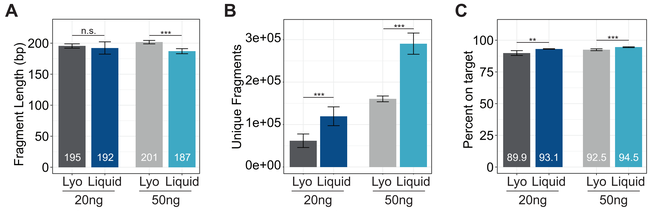
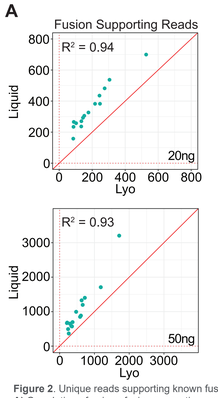
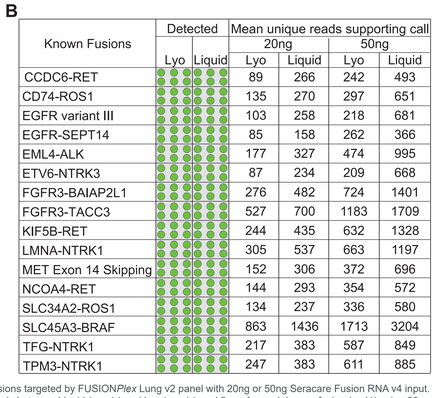



**Figure 1**. FUSION*Plex* Lung v2 panel performance with 20 or 50ng Seracare Fusion RNA v4 input. **A)** Mean fragment length. **B)** Mean number of unique reads passing read cleaning. **C)** Mean percent of unique reads that mapped on target. Data shown is aggregated between 2 users (n=3 technical replicates per condition per user). All samples were subsampled to 1M reads.



| Known Fusions | Detected | | Mean unique reads supporting call | | | |
|---|---|---|---|---|---|---|
| | | | 20ng | | 50ng | |
| | Lyo | Liquid | Lyo | Liquid | Lyo | Liquid |
| CCDC6-RET | | | 89 | 266 | 242 | 493 |
| CD74-ROS1 | | | 135 | 270 | 297 | 651 |
| EGFR variant III | | | 103 | 258 | 218 | 681 |
| EGFR-SEPT14 | | | 85 | 158 | 262 | 366 |
| EML4-ALK | | | 177 | 327 | 474 | 995 |
| ETV6-NTRK3 | | | 87 | 234 | 209 | 668 |
| FGFR3-BAIAP2L1 | | | 276 | 482 | 724 | 1401 |
| FGFR3-TACC3 | | | 527 | 700 | 1183 | 1709 |
| KIF5B-RET | | | 244 | 435 | 632 | 1328 |
| LMNA-NTRK1 | | | 305 | 537 | 663 | 1197 |
| MET Exon 14 Skipping | | | 152 | 306 | 372 | 696 |
| NCOA4-RET | | | 144 | 293 | 354 | 572 |
| SLC34A2-ROS1 | | | 134 | 237 | 336 | 580 |
| SLC45A3-BRAF | | | 863 | 1436 | 1713 | 3204 |
| TFG-NTRK1 | | | 217 | 383 | 587 | 849 |
| TPM3-NTRK1 | | | 247 | 383 | 611 | 885 |

**Figure 2**. Unique reads supporting known fusions targeted by FUSION*Plex* Lung v2 panel with 20ng or 50ng Seracare Fusion RNA v4 input. **A)** Correlation of unique fusion supporting reads between Liquid (*y-axis*) and Lyo (*x-axis*) workflows for each known fusion (n=11) using 20ng (*Top*) or 50ng (*Bottom*) **B)** Mean unique fusion supporting reads supporting call of expected fusions. Each green dot indicates the fusion was detected in each replicate (n=3 technical replicates per condition for each user). All samples were subsampled to 1M reads.

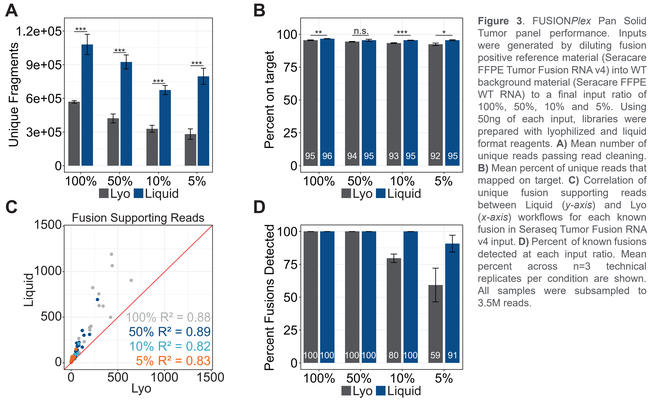



**Figure 3**. FUSION*Plex* Pan Solid Tumor panel performance. Inputs were generated by diluting fusion positive reference material (Seracare FFPE Tumor Fusion RNA v4) into WT background material (Seracare FFPE WT RNA) to a final input ratio of 100%, 50%, 10% and 5%. Using 50ng of each input, libraries were prepared with lyophilized and liquid format reagents. **A)** Mean number of unique reads passing read cleaning. **B)** Mean percent of unique reads that mapped on target. **C)** Correlation of unique fusion supporting reads between Liquid (*y-axis*) and Lyo (*x-axis*) workflows for each known fusion in Seraseq Tumor Fusion RNA v4 input. **D)** Percent of known fusions detected at each input ratio. Mean percent across n=3 technical replicates per condition are shown. All samples were subsampled to 3.5M reads.

## Results - VARIANT*Plex*-HT

| Input | Input Mass | Panel Name | Panel Size | Depth |
|---|---|---|---|---|
| Seraseq Myeloid DNA | 50ng | VARIANT*Plex* Core Myeloid | 17 genes | 3M |
| Seraseq Compromised FFPE DNA | 50ng | VARIANT*Plex* Complete ST | 137 genes | 50M |
| Horizon Moderate FFPE DNA | 10ng | VARIANT*Plex* Core ST | 60 genes | 4.5M |
| Horizon Moderate FFPE DNA | 50ng | VARIANT*Plex* Core ST | 60 genes | 4.5M |

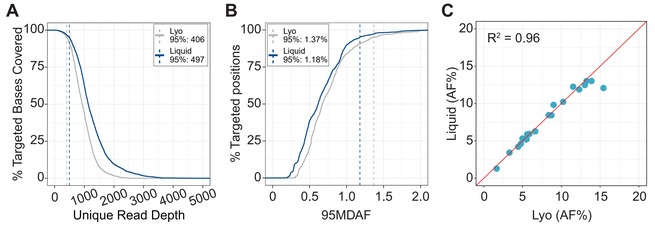



**Figure 4**. VARIANT*Plex* Core Myeloid panel performance with 50ng Seracare Myeloid DNA mix input. **A)** Mean unique read depth at bases targeted by the panel. *Upper right;* mean unique read depth 95 percentile. **B)** Minimum detectable allele frequency (MDAF) at α = 0.05 across bases targeted by the panel. Upper left; 95MDAF 95 percentile. **C)** Correlation of observed allele frequency (AF). 151/154 (97%) and 154/154 (100%) of expected variants were observed across Lyo and Liquid workflows, respectively. All data shown is aggregated between 2 users with at least n=3 technical replicates per condition per user. All samples were subsampled to 3M reads.

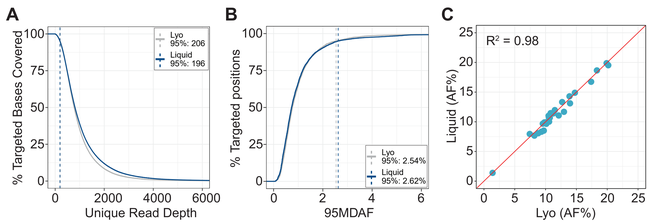



**Figure 5**. VARIANT*Plex* Complete Solid Tumor panel performance with 50ng Seracare compromised FFPE DNA input. **A)** Mean unique read depth at bases targeted by the panel. *Upper right;* mean unique read depth 95 percentile. **B)** Minimum detectable allele frequence (MDAF) at α = 0.05 across bases targeted by the panel. Lower right; 95MDAF 95 percentile. **C)** Correlation of observed allele frequency (AF). 84/84 (100%) of expected variants were observed across both Lyo and Liquid workflows. All samples were subsampled to 50M reads (n=3 technical replicates per condition).

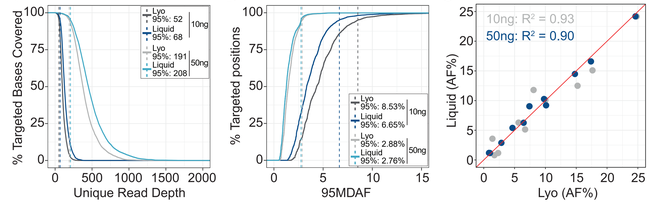



**Figure 6**. VARIANT*Plex* Core Solid Tumor panel performance with 10 or 50ng Seracare Hozizon Moderate FFPE DNA input. **A)** Mean unique read depth at bases targeted by the panel. *Upper right;* mean unique read depth 95 percentile. **B)** Minimum detectable allele frequence (MDAF) at α = 0.05 across bases targeted by the panel. Lower right; 95MDAF 95 percentile. **C)** Correlation of observed allele frequency (AF). 152/154 (98%) and 163/165 (98%) of expected variants were observed across both Lyo and Liquid workflows. All data shown is aggregated between 2 users with at least n=3 technical replicates per condition per user. All samples were subsampled to 4.5M reads.

## Results - Liquid Automation

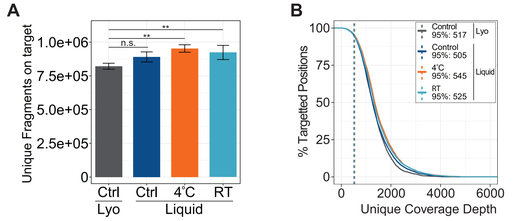



**Figure 7**. VARIANT*Plex* Core Myeloid panel performance with 50ng male Genome in a Bottle input. Liquid mastermixes were prepared at the step where required (Liquid Ctrl; Control) or all mastermixes were prepared at the start of the library prep and stored at the designated temperature (4°C or RT; room-temperature) until required at the designated step of the workflow. **A)** Mean unique reads mapped on target. B) Mean unique read depth at bases covered by the panel. All samples were subsampled to 3M reads (n=3-4 technical replicates per condition).

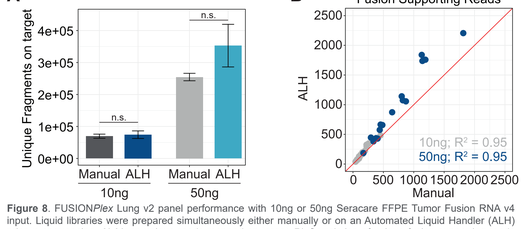



**Figure 8**. FUSION*Plex* Lung v2 panel performance with 10ng or 50ng Seracare FFPE Tumor Fusion RNA v4 input. Liquid libraries were prepared simultaneously either manually or on an Automated Liquid Handler (ALH) prior to sequencing. **A)** Mean unique reads mapped on target. **B)** Correlation of unique fusion supporting reads between Manual and ALH-prepared samples. 48/48 (100%) of expected fusions were detected by both workflows. All samples were subsampled to 1M reads (n=3 technical replicates per condition). Error bars are mean ± SEM.

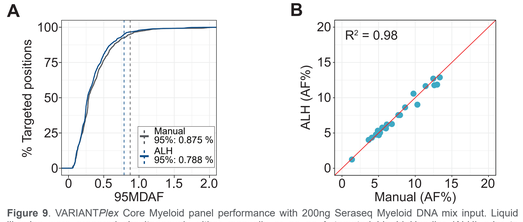



**Figure 9**. VARIANT*Plex* Core Myeloid panel performance with 200ng Seraseq Myeloid DNA mix input. Liquid libraries were prepared simultaneously either manually or on an Automated Liquid Handler (ALH) prior to sequencing. **A)** Minimum detectable allele frequency (MDAF) at α = 0.05 across bases targeted by the panel. Lower right; 95MDAF 95 percentile. **B)** Correlation of observed allele frequency (AF). 66/66 (100%) of expected variants were observed across both Manual and ALH-prepped samples. All samples were subsampled to 3M reads. (n=3 technical replicates per condition).

## Conclusions

Our liquid reagent-based VARIANT*Plex*-HT workflow yielded libraries with parallel performance to our lyophilized products across a variety of input types, masses and panel sizes, including our most comprehensive panel to date, VARIANT*Plex* Complete Solid Tumor. The FUSIONPlex-HT workflow displayed greater numbers of unique reads supporting fusion calls across multiple input types, panels and at various dilutions of Fusion-positive material relative to lyophilized reagents. Automation of FUSION*Plex*-HT and VARIANT*Plex*-HT workflows resulted in libraries with equivalent numbers of unique molecules and power to call variants relative to manually prepped samples. The FUSIONPlex-HT automated workflow detected all known fusions associated with FFPE input. Similarly, the VARIANT*Plex*-HT automated workflow detected all known variants associated with Myeloid input.

In conclusion, we have formulated and optimized automation-friendly liquid reagents that perform equivalently to legacy lyophilized products, providing a solution for mid- to high-throughput AMP-based NGS applications.

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