POSTER NOTE
As presented at AGBT 2021

ROCHE PRIMALSeq:

An Adaption of the ARTIC PrimalSeq Workflow using Roche's KAPA Library Preparation Kits

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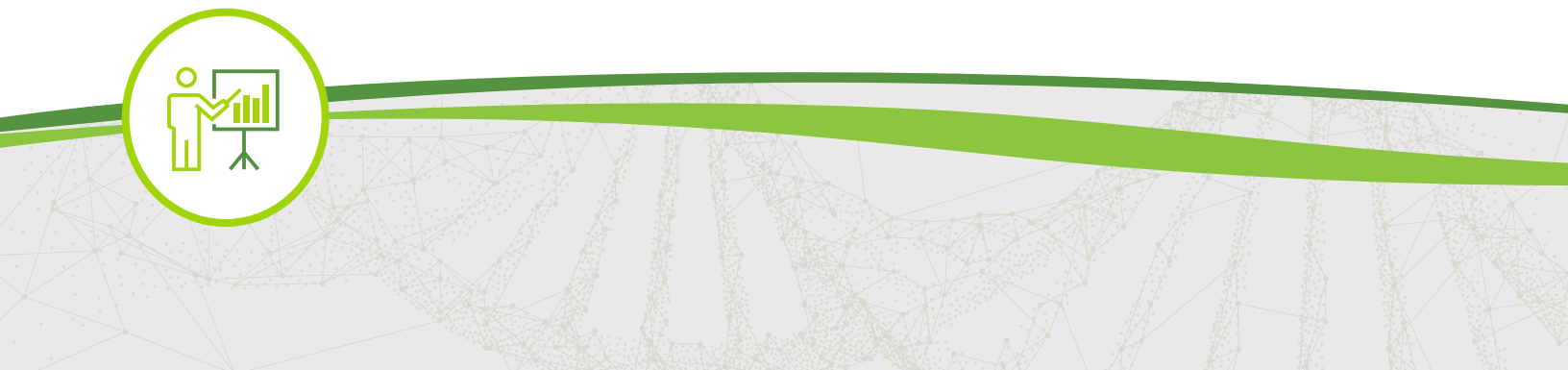

INTRODUCTION

Scientists around the globe have pivoted their research to focus on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the rampant and fatal COVID-19 pandemic. Amplicon sequencing is a critical tool that uses ultra-deep sequencing of PCR products (amplicons) which allows efficient variant identification and characterization in specific genomic regions. Valuable information such as this is used to monitor the evolution and transmission of the virus. Better understanding of the virus's genetic composition could ultimately save lives by shaping strategies for public health and clinical care, as well as facilitating the production of therapies and treatments to combat the virus.

The increasing number and accessibility of SARS-CoV-2 sequencing protocols that are compatible across different products gives researchers the flexibility to use resources that are trusted and convenient for them, which is critical as the virus continues to quickly spread. The ARTIC network has responded to the pressing need to understand the human coronavirus by making a set of materials widely available to help researchers with PrimalSeq amplicon sequencing for SARS-CoV-2.

The Roche PrimalSeq workflow is an adaptation of the previously published PrimalSeq-Nextera XT workflow and demonstrates the benefits of using PCR and NGS reagents from a single vendor, including trusted KAPA polymerases and library prep kits; these reagents are designed to work together seamlessly, simplifying NGS workflows. This protocol integrates reverse transcription of RNA, multiplexed PCR, DNA library preparation, deep sequencing and data analysis, enabling accurate reproducible sequencing readouts. Here, the Roche PrimalSeq workflow is compared to the PrimalSeq protocol as written using Nextera XT products and performance metrics are reported.

EXPERIMENTAL DESIGN

The overall approach of the PrimalSeq workflow is to (1) reverse-transcribe RNA samples into cDNA, (2) use virus-specific primers to amplify the SARS-CoV-2 sequences in small ~400 base pair overlapping fragments, and (3) convert the fragments to sequencing libraries for Illumina sequencers.

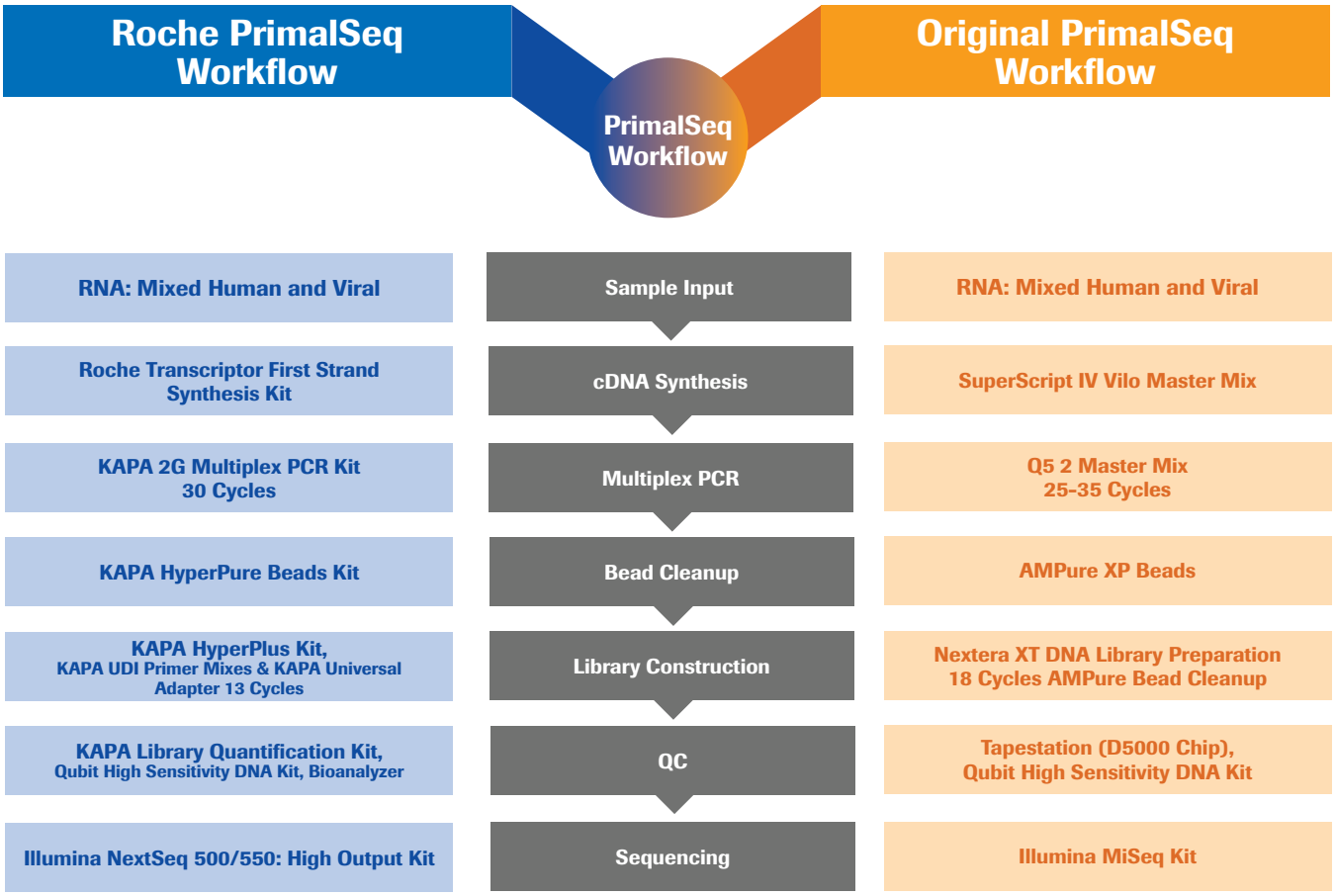

Figure 2: Comparison of the Roche PrimalSeq workflow and the original PrimalSeq workflow. The original workflow incorporates Nextera XT DNA Library Preparation Kit, which circumvents the 250/300 base pair read length requirement for downstream sequencing. In contrast, the the Roche workflow uses the KAPA HyperPlus Kit to enzymatically shear amplicons to the appropriate length for downstream sequencing. In this study, the RNA input for both workflows was a mixture of 50 ng of Universal Human RNA (UHR) and 10,000 copies of the SARS-CoV-2 genome (comprising 6 variants mixed in equal parts). For both methods, n=3.

OPTIMIZATION OF THE ROCHE PRIMALSeq WORKFLOW

MULTIPLEX PCR PARAMETERS WERE MODIFIED TO INCREASE SPECIFICITY

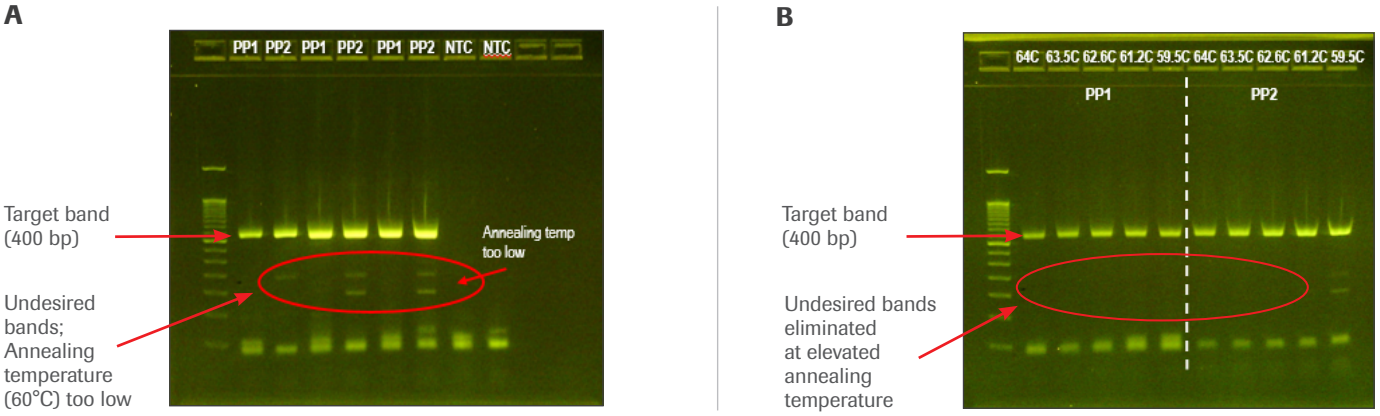

Figure 3: Optimal PCR conditions include increased annealing temperature and fewer PCR cycles. The KAPA2G Fast Multiplex PCR Kit protocol recommends an annealing temperature of 60°C. However, when used with the PrimalSeq primers, these cycling conditions resulted in nonspecific ~200 bp bands in addition to the target 400 bp amplicons **(A)**; this suggested that the annealing temperature was too low. To increase the specificity, PCR was performed with an annealing temperature gradient **(B)** and the number of PCR cycles was decreased. Optimal cycling conditions were determined to include an annealing time of 90 seconds at 62.6°C, for 30 cycles.

THE ROCHE PRIMALSeq WORKFLOW YIELDS CONSISTENTLY SIZED LIBRARIES

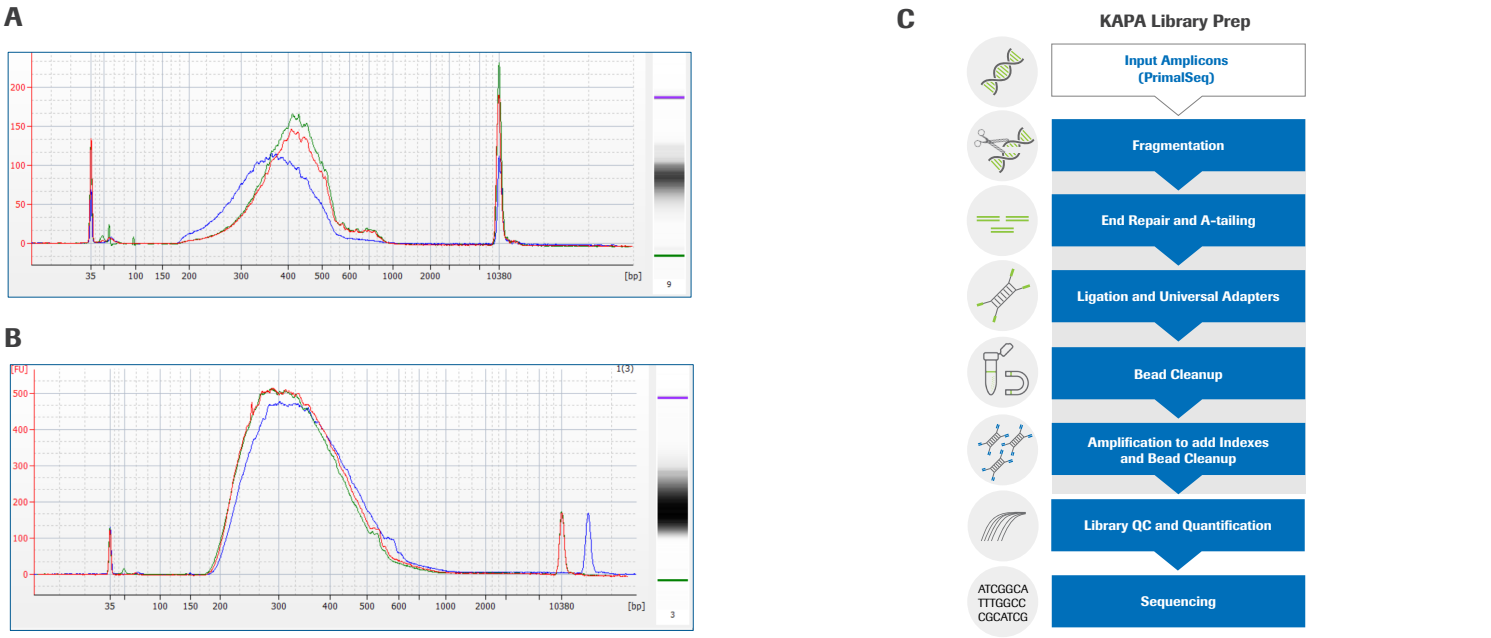

Figure 4: Optimal fragmentation time of the input amplicons was determined to be 40 minutes for the Roche PrimalSeq workflow. The libraries created using the original protocol with Nextera XT **(A)** fall within the acceptable size range for sequencing; however there is detectable adapter dimer present. Libraries created using the Roche PrimalSeq workflow **(B)** following fragmentation of input amplicons for 40 minutes at 37ºC (the recommended temperature for the KAPA HyperPlus workflow, shown in **(C)**, were successful at consistently shearing the DNA to ~150 base pairs in length (prior to adapter ligation) for optimal downstream sequencing, and generating libraries with no adapter dimer present.

THE ROCHE PRIMALSeq WORKFLOW YIELDS EQUAL-OR-BETTER TARGET COVERAGE AND UNIFORMITY COMPARED TO THE ORIGINAL WORKFLOW

The effectiveness of both methods at covering the SARS-CoV-2 genome was compared by assessing the sequencing results of all samples. Sequencing metrics show that the Roche PrimalSeq protocol with the KAPA HyperPlus Kit performs at least as well as the previously published PrimalSeq protocol using Nextera XT library construction.

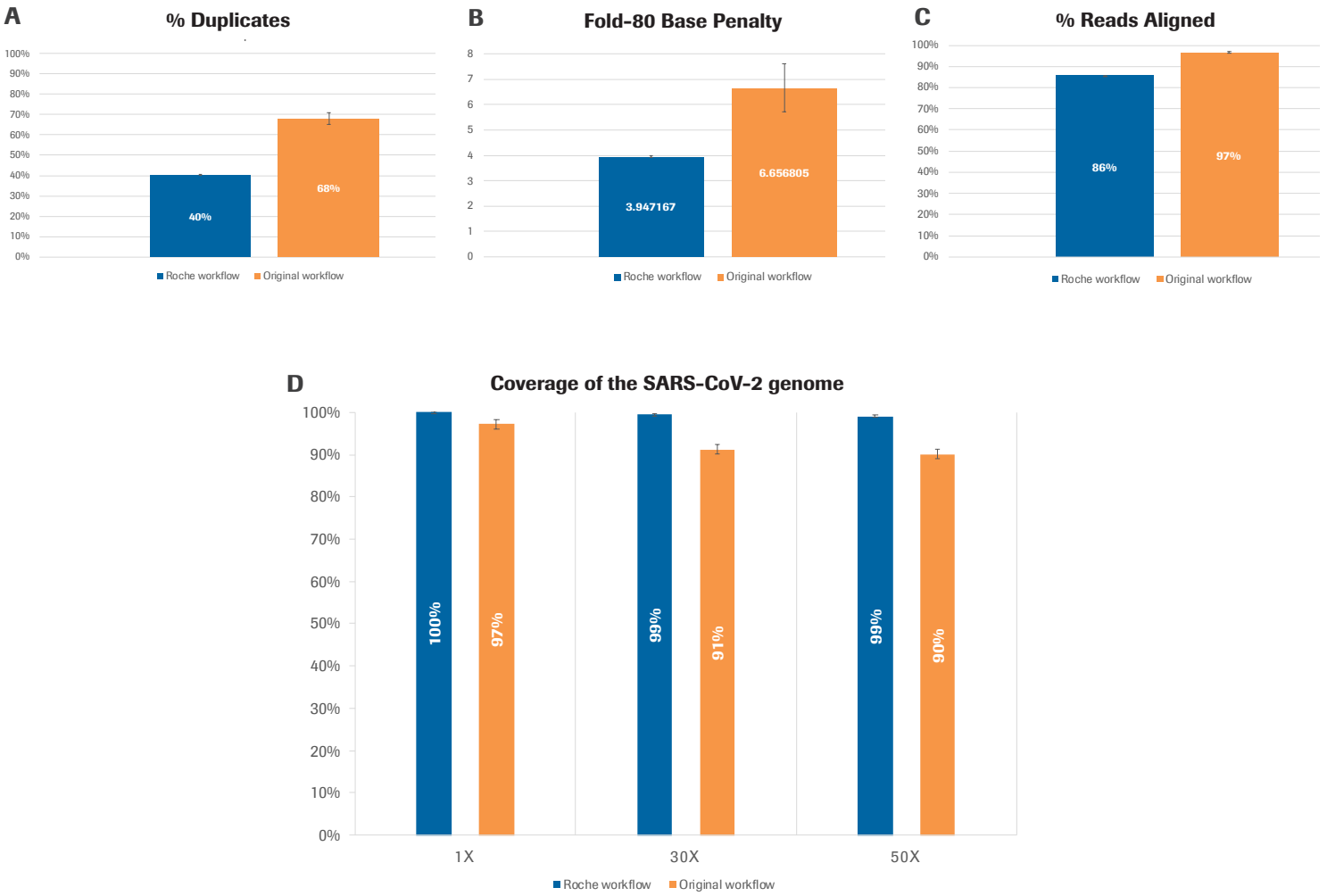

Figure 5: Coverage of the SARS-CoV-2 genome using the Roche PrimalSeq workflow is comparable or better than with the original PrimalSeq workflow. The Roche PrimalSeq workflow yields **(A)** lower duplication rates (duplication rates were lower for the majority of libraries prepared with the Roche PrimalSeq workflow; the average % duplicates for libraries (40%) represented a 28% decrease compared to the original protocol); **(B)** better coverage uniformity, shown as Fold-80 base penalty; **(C)** similar % of reads aligned to the reference genome; and **(D)** superior genome coverage. Sequencing was performed in two separate Illumina NextSeq 500/550 runs—the Roche PrimalSeq samples on one run and the original PrimalSeq workflow samples on the other.

CONCLUSIONS

The Roche PrimalSeq workflow achieves consistent and reproducible sequencing results when used used to detect and sequence the SARS-CoV-2 viral genome in a human RNA background, including:

- Similar-to-better fragmentation and library size consistency;
- Improved library complexity;
- Greater coverage uniformity;
- Reduced duplication rates;
- Single-vendor source for reagents and support.

Disclaimer: Although the results of this study are promising, this workflow is still in development and has not been fully validated by Roche.

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