Improving PCR amplification of different NGS methods using **repliQa HiFi ToughMix**

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Abstract

Next-Generation Sequencing (NGS) technologies have transformed our understanding of the genome and transcriptome by providing enormous amounts of information within a short period of time. Most library preparation methods involve PCR amplification at some stage. Amplicon-seq or targeted NGS sequencing methods utilize primer sets to amplify target region(s) followed by barcode incorporation. On the other hand, whole genome, whole transcriptome and panel-based sequencing methods often require amplification of the entire genome or transcriptome using a single universal primer sequence previously added to the dsDNA fragments either via ligation or tagmentation. In both cases unbiased amplification of a wide range of GC containing targets and improved yield are the key factors for choosing a PCR reagent.

Here we present repliQa HiFi ToughMix, a 2X concentrated PCR master mix to fulfill a wide range of PCR amplification needs for different NGS applications.

Amplicon-seq NGS applications like 16S metagenomic sequencing on both Illumina and ONT platforms were benefited by amplifying targets using repliQa HiFi ToughMix. Libraries were prepared faster and received improved results from crude DNA extracts without compromising the accuracy of microbial species detection. RNA virus sequencing utilizing long cDNA synthesis followed by long range PCR using repliQa HiFi ToughMix improved the speed of the library preparation while also providing flexibility to utilize the resulting long PCR products on both short read and long read sequencing platforms. When repliQa HiFi ToughMix was used to amplify whole genome libraries with a wide range of GC content and composition, it produced higher yield, uniform genome coverage over a wide range of GC content and low duplication rates.

Overall, repliQa HiFi ToughMix provides great advantage over other PCR reagents for a wide range of NGS applications that require PCR amplification within the library preparation process.

repliQa HiFi ToughMix

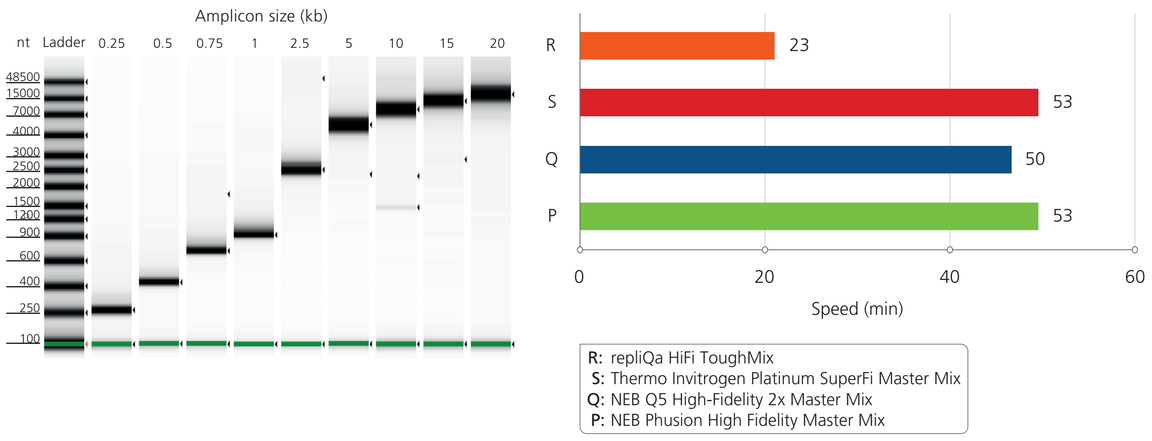


Figure 1 **The repliQa HiFi ToughMix is a high-fidelity PCR master mix.** (**A**) Using *E. coli* gDNA as a template, targets of different lengths were amplified using repliQa HiFi ToughMix (**B**) A 2 kb fragment was amplified according to the recommended protocol. The repliQa HiFi ToughMix (R) took only 23 min, >50% time saving compared to the competitor products. The competitor products used here include Thermo Platinum SuperFi (S), NEB Q5 (Q), and NEB Phusion (P).

Amplicon-Seq: 16S Metagenomic Sequencing Workflows

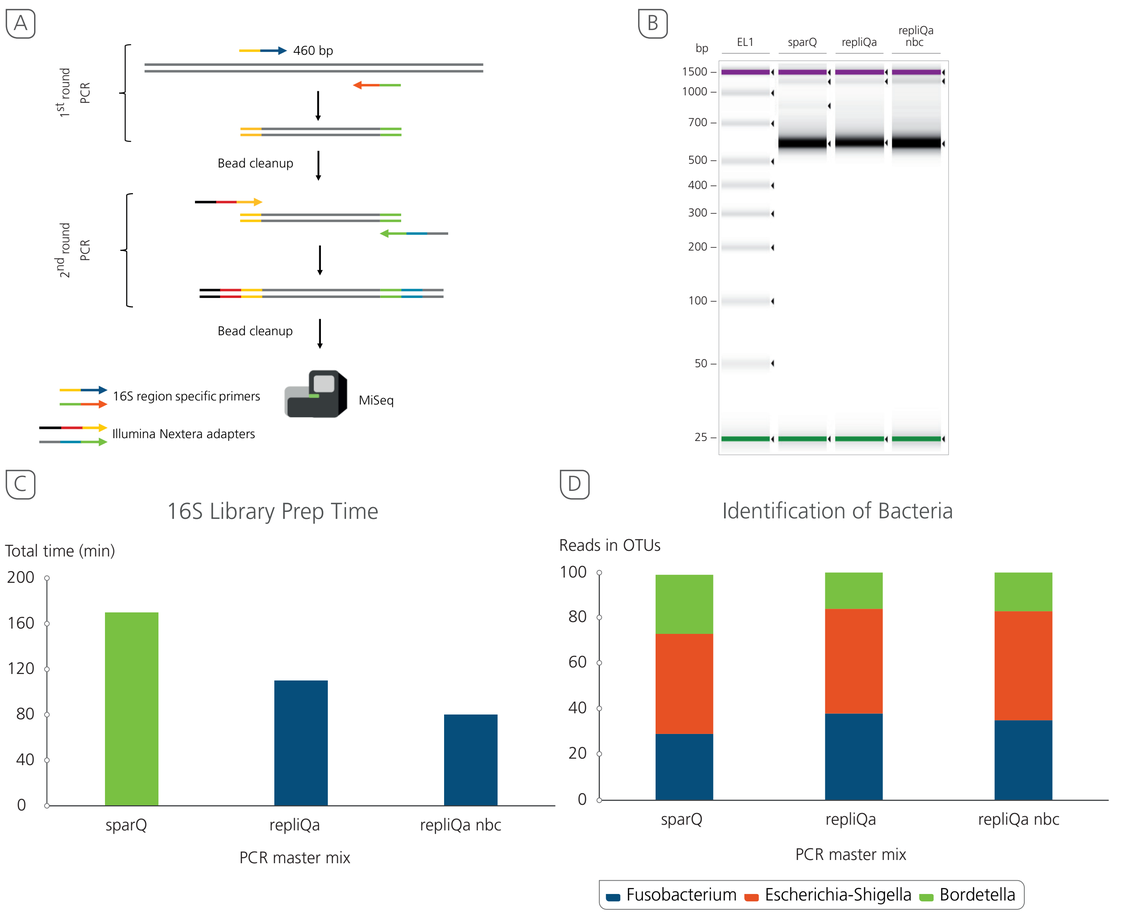
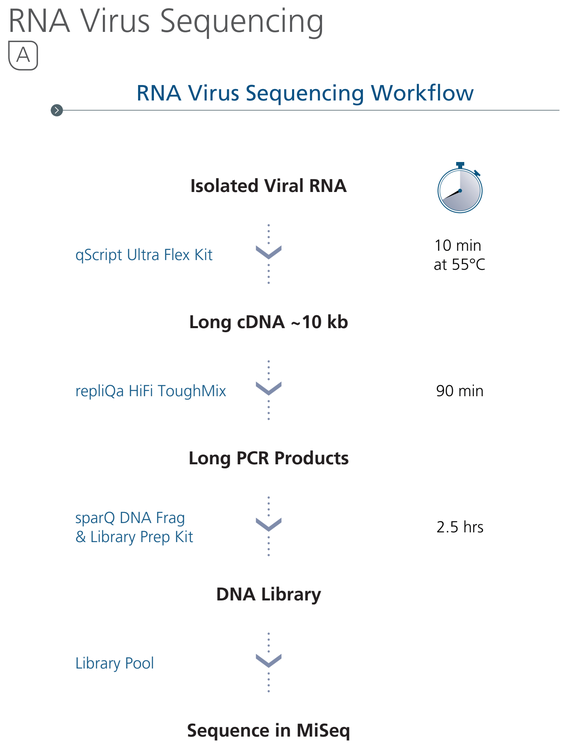
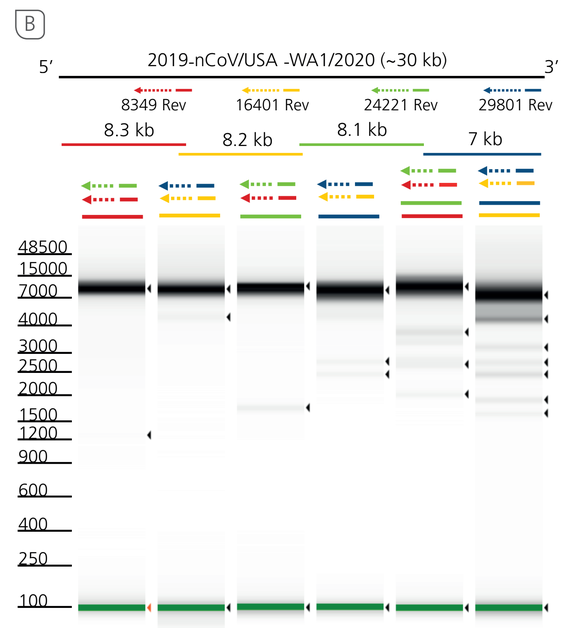
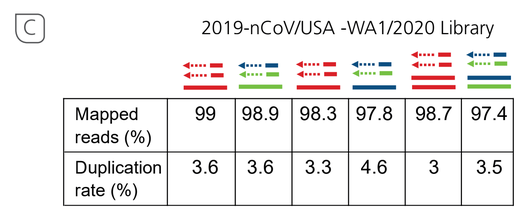


Figure 2 **Rapid 16S metagenomic sequencing.** (**A**) 16S metagenomic library prep workflow, a common amplicon-based sequencing application. (**B**) Libraries prepared with sparQ HiFi PCR MasterMix (sparQ), repliQa HiFi ToughMix (repliQa), or repliQa HiFi ToughMix without the first bead cleanup step (repliQa nbc) (**C**) Total library preparation time using different PCR master mixes (**D**) Libraries prepared with different master mixes were sequenced on an Illumina MiSeq instrument and the reads in operational taxonomic units (OTUs) identified. All three species in the test sample were identified regardless of the library preparation method.

RNA Virus Sequencing







Mapped reads (%)	99	98.9	98.3	97.8	98.7	97.4
Duplication rate (%)	3.6	3.6	3.3	4.6	3	3.5

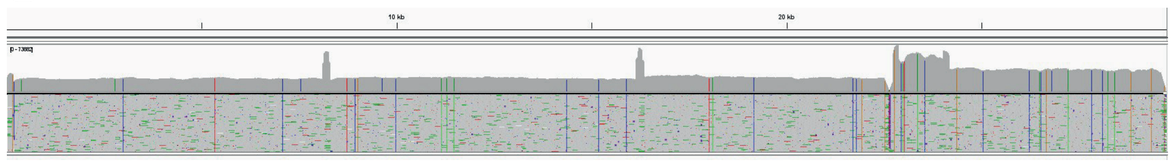


Figure 3 **RNA virus sequencing.** (**A**) A representative workflow for RNA virus sequencing. (**B**) Long-range amplicons were generated using SARS-CoV-2 RNA as a template either by singleplex PCR or multiplex PCR. (**C**) Sequencing QC matrices showed a high percentage of mapped reads and low duplication rates after mapping. (**D**) A representative IGV view of the BAM files generated with SARS-CoV-2 RNA sequencing reads.

Whole Genome Library Amplification With 1 Second Extension Time

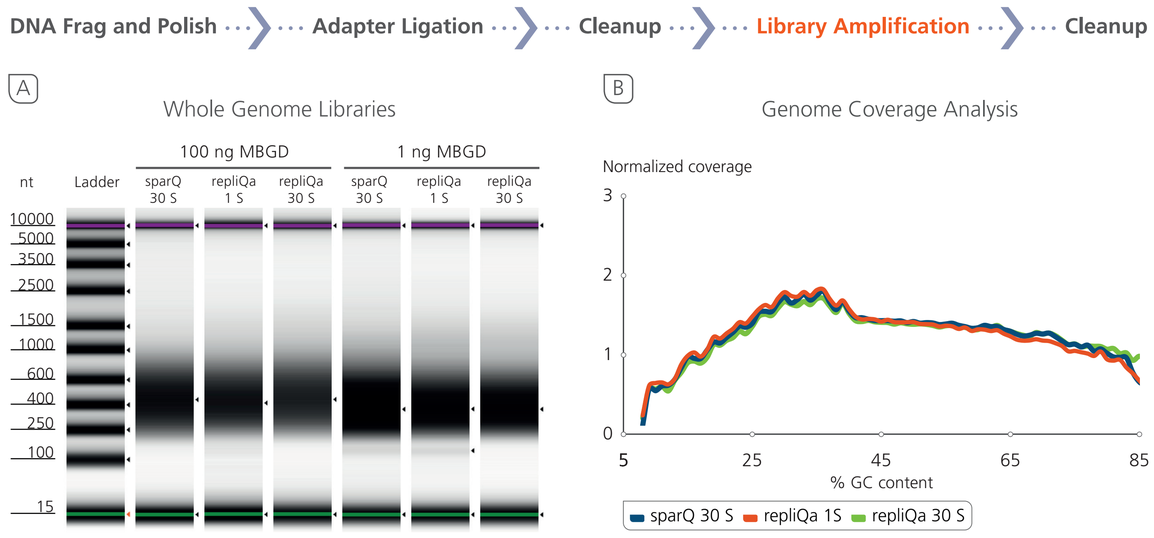


Figure 4 **Whole genome library amplification.** Whole genome libraries were prepared from mixed bacterial genomic DNA (MBGD) using sparQ DNA Frag & Library Prep Kit. Libraries were amplified with either sparQ HiFi PCR Master Mix with a standard 30 s extension time or repliQa HiFi ToughMix with either a 1 s or 30 s extension time. (**A**) TapeStation analysis of the prepared libraries. (**B**) Genomic coverage across 5 to 85% of GC content.

Input DNA (ng)	Amplification enzyme used	No. of PCR cycles	Extension time (sec)	Yield (ng)	Duplication rate (%)
100	sparQ HiFi PCR MasterMix	4	30	570	5.6
100	repliQa HiFi ToughMix	4	1	500	5.8
100	repliQa HiFi ToughMix	4	30	567	4.8
10	sparQ HiFi PCR MasterMix	9	30	1000	6.2
10	repliQa HiFi ToughMix	9	1	708	5.9
10	repliQa HiFi ToughMix	9	30	663	6.1

Table 1 **Summary of whole genome sequencing metrics**

Key Points

1. Compared to similar kits, repliQa HiFi ToughMix demonstrates extreme speed (reduction of run time by more than half), superior sensitivity (up to 3x the yield), and strong inhibitor resistance.
2. repliQa HiFi ToughMix is ideal for 16S or other amplicon-seq applications for rapid preparation of libraries from even difficult input samples.
3. Combination of long cDNA synthesis by qScript Ultra Flex Kit followed by long-range PCR using repliQa HiFi ToughMix is ideal for RNA virus sequencing. This method can detect multiple single nucleotide mutations and structural variations.
4. Whole genome library amplification with repliQa HiFi ToughMix saves time without affecting the yield, duplication rate, genomic coverage, and other sequencing matrices