



# Industry-leading **FIDELITY**

## Library Amplification Solutions

Roche Sequencing offers Library Amplification Kits that include KAPA HiFi DNA Polymerase, a novel enzyme engineered to provide industry-leading fidelity and robustness. This allows for multiple improvements to overall sequence quality including low amplification bias, more uniform coverage of difficult regions, and lower duplication rates.

KAPA HiFi Uracil+ DNA Polymerase—a uracil-tolerant variant—provides the same improvements to libraries constructed from bisulfite-converted DNA.

## Benefits of KAPA HiFi Kits

### Greater performance

- Higher success rates with different sample types/applications
- Higher and more uniform coverage with lower dropout of difficult regions

### Exceptional sequencing results

- Higher yields, low duplication rates and fewer wasted sequencing reads

### Convenient and reliable

- Convenience with the consistency of one core enzyme
- Trusted as shown through over 10 years in the industry as well as thousands of peer-reviewed publications

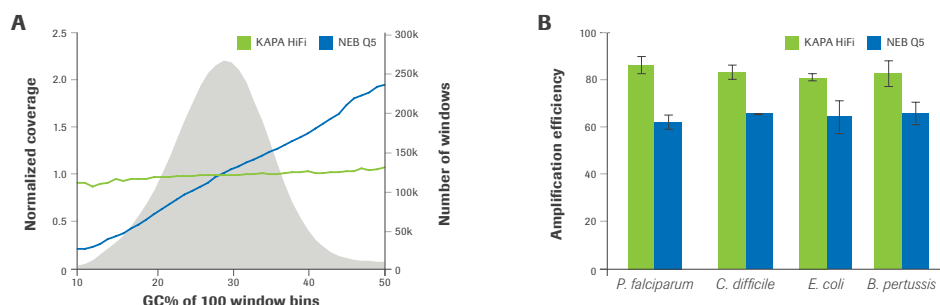


## KAPA Library Amplification Kits

High-fidelity, low-bias amplification for all NGS workflows that require PCR

### More efficient amplification of GC- and AT-rich fragments and genomes

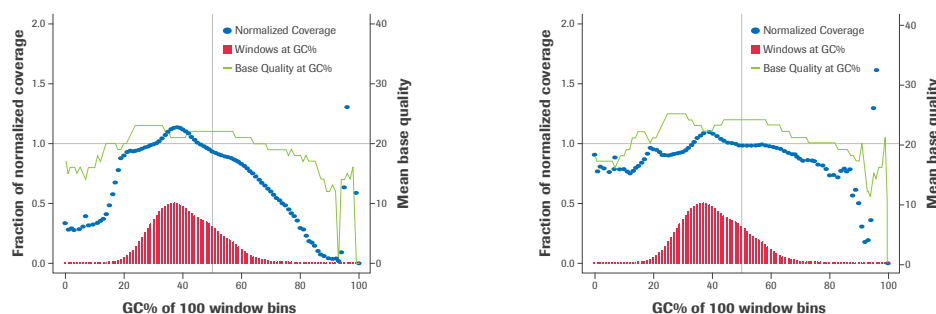
- Improved representation of all library fragments and sequence regions
- Due to higher amplification efficiency, fewer cycles are required to achieve equivalent yields



**Figure 1. KAPA HiFi has become the gold standard for NGS library amplification.** Libraries prepared from *P. falciparum* (19% GC), *C. difficile* (28%), *E. coli* (51% GC), and *B. pertussis* (68%) were amplified using KAPA HiFi HotStart ReadyMix or NEB Q5. **(A)** KAPA HiFi HotStart ReadyMix yielded significantly more even coverage of the *C. difficile* genome than NEB Q5. As a result, much less sequencing is needed to cover all regions to the requisite average coverage depth. **(B)** KAPA HiFi HotStart ReadyMix outperformed NEB Q5 with respect to amplification efficiency across all organisms. Less amplification has a positive impact on key sequencing metrics.

### Improved sequencing coverage

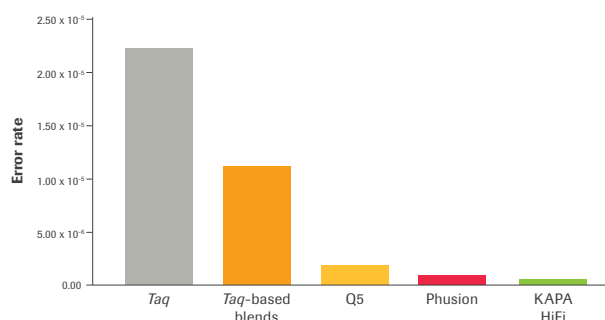
- Higher coverage uniformity across all GC windows



**Figure 2. KAPA HiFi HotStart ReadyMix reduces amplification bias and improves sequencing coverage.** Libraries were prepared for whole-genome shotgun sequencing, from 100 ng of Covaris-sheared human genomic DNA, using either the KAPA Library Preparation Kit (left) or a competitor kit (right). Lower amplification bias with KAPA HiFi HotStart ReadyMix resulted in more uniform coverage. Data courtesy of The Broad Institute. (Cambridge, MA, USA).

### Amplify NGS libraries with industry-leading fidelity

- Enhanced proofreading (3' – 5' exonuclease) activity



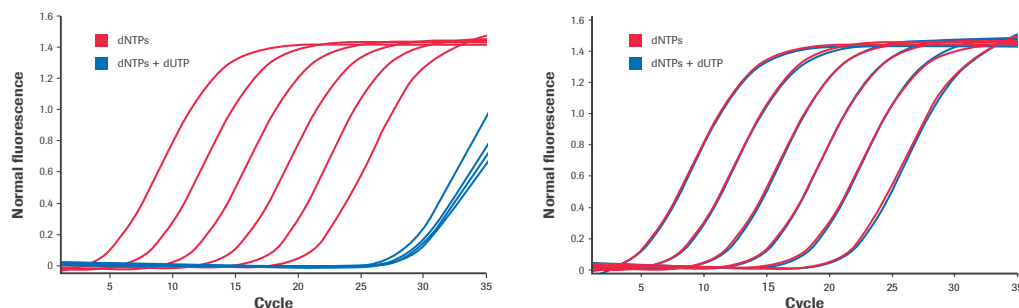
**Figure 3. Industry-leading fidelity confirmed by pyrosequencing.** Published error rates of *Taq*, *Taq*-based long-range PCR blends, and proofreading DNA polymerases (Q5, New England Biolabs and Phusion®, Thermo Scientific) are compared to the error rate of KAPA HiFi HotStart DNA Polymerase (1 error per 3.54 x 10<sup>-6</sup> nucleotides incorporated), which was confirmed by deep sequencing, and is 50 – 100 times lower than that of *Taq*.

## KAPA HiFi Uracil+ Kits

Accurate and efficient amplification of bisulfite-treated DNA in Methyl-Seq workflows

Amplify uracil-containing templates with high efficiency

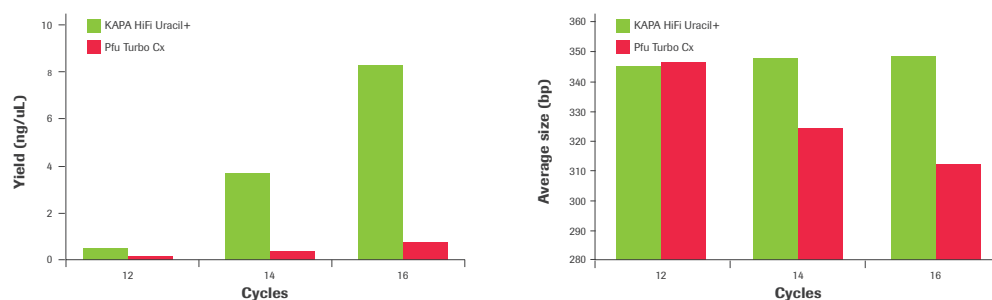
- Uracil-tolerant proofreading enzyme, derived from KAPA HiFi DNA Polymerase



**Figure 4. KAPA HiFi Uracil+ ReadyMix is not inhibited by dUTP.** SYBR® Green real-time PCR was used to monitor amplification of a 452 bp fragment from a ten-fold template dilution series (80 pM – 0.8 fM), in the presence or absence of 0.2 mM dUTP KAPA HiFi HotStart ReadyMix (left) shows typical inhibition by uracil, while KAPA HiFi Uracil+ ReadyMix (right) shows no amplification inhibition.

Unlock the true potential of bisulfite-converted libraries

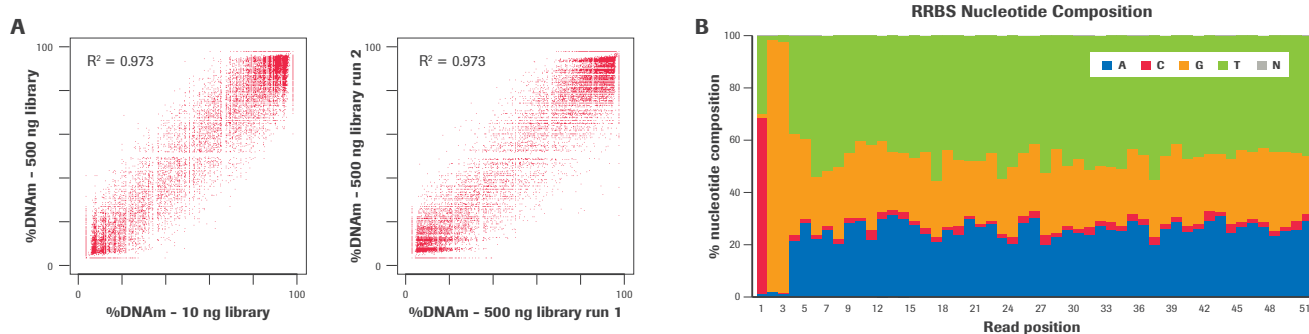
- Achieve higher library yields and employ fewer cycles of amplification to reduce PCR duplicates and bias
- High-efficiency amplification preserves longer library fragments



**Figure 5. KAPA HiFi Uracil+ ReadyMix enables higher yields and minimal size bias.** Human whole-genome bisulfite-treated libraries were amplified using standard protocols (12, 14, or 16 cycles), and analyzed using a Bioanalyzer® 2100 High Sensitivity DNA assay. When compared with Agilent® Pfu Turbo Cx HotStart DNA Polymerase, KAPA HiFi Uracil+ ReadyMix produced much higher yields (left) with very little size bias (right).

Integration into commonly used methyl-seq applications

- Efficient amplification of bisulfite-converted libraries
- Improved bisulfite-sequencing read quality



**Figure 6. KAPA HiFi Uracil+ ReadyMix enables successful library amplification of reduced-representation bisulfite-converted libraries.** Libraries were prepared using the KAPA HyperPrep Kit from 500 ng or 10 ng of mouse B-cell genomic DNA after digestion with *MspI*. KAPA HiFi Uracil+ ReadyMix was used for amplification. 10 cycles were performed for 500 ng libraries and 15 cycles for 10 ng libraries. **(A)** Methylation calls correlated well between 10 ng and 500 ng libraries (left) and duplicate 500 ng libraries (right). **(B)** Nucleotide composition plots for read position indicates that >95% of total reads starts with an *MspI* cut site (CCGG). Data courtesy of Emory University. (Atlanta, GA, USA)

## Ordering Information for KAPA Library Amplification Kits

Roche cat. no.	KAPA code	Description	Kit size
07958951001	KK2611	KAPA HiFi HotStart Library Amplification Kit	50 x 50 µL rxn
07958960001	KK2612	KAPA HiFi HotStart Library Amplification Kit	250 x 50 µL rxn
07958978001	KK2620	KAPA HiFi HotStart Library Amplification Kit with Primer Mix	50 x 50 µL rxn
07958986001	KK2621	KAPA HiFi HotStart Library Amplification Kit with Primer Mix	250 x 50 µL rxn
07958994001	KK2623	KAPA Library Amp Primer Mix	250 rxn
09420410001	N/A	KAPA Library Amp Primer Mix	384 rxn
09420479001	N/A	KAPA Library Amp Primer Mix 96-well plate	96 rxn
07959028001	KK2702	KAPA HiFi HotStart Real-Time Library Amplification Kit	250 x 50 µL rxn

## Ordering Information for KAPA HiFi Kits

Roche cat. no.	KAPA code	Description	Kit size
07958927001	KK2601	KAPA HiFi HotStart ReadyMix (1.25 mL)	50 rxn
07958935001	KK2602	KAPA HiFi HotStart ReadyMix (6.25 mL)	250 rxn
09420398001	N/A	KAPA HiFi HotStart ReadyMix (9.6 mL)	9.6 mL
07958889001	KK2501	KAPA HiFi HotStart PCR Kit with dNTPs	50 rxn
07958897001	KK2502	KAPA HiFi HotStart PCR Kit with dNTPs	125 rxn
07958838001	KK2101	KAPA HiFi PCR Kit with dNTPs	50 rxn
07958846001	KK2102	KAPA HiFi PCR Kit with dNTPs	125 rxn
07958960001	KK2801	KAPA HiFi HotStart Uracil+ ReadyMix (2X) Kit, 1.25 mL	50 x 50 µL rxn
07958951001	KK2802	KAPA HiFi HotStart Uracil+ ReadyMix (2X) Kit, 6.25 mL	250 x 50 µL rxn

**Note:** Library Amplification Primer Kit (07958994001//09420410001/09420479001) is recommended for the amplification of bisulfite-converted libraries constructed with Illumina® TruSeq® adapters.

Learn more at  
[sequencing.roche.com](https://sequencing.roche.com)

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