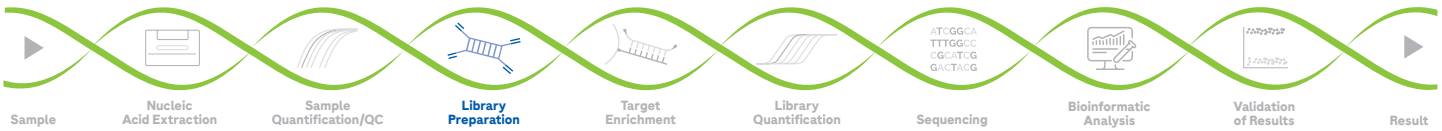
KAPA EvoPlus V2 Kit

Perform convenient enzymatic prep without sacrificing performance

Achieve higher confidence & increased sequencing efficiency in a streamlined, automation-friendly workflow with **KAPA EvoPlus V2 Kits**. This enhanced enzymatic DNA library preparation solution features improved fragmentation performance, insensitivity to inhibitors, increased conversion efficiency & fewer sequencing artifacts.

KAPA EvoPlus V2 Kits are designed to rival the performance of mechanical-shearing methods while delivering unparalleled convenience for whole-genome sequencing (WGS), whole-exome sequencing (WES) & targeted sequencing applications.

- **Increase efficiency & convenience** with all-in-one enzyme & buffer ReadyMixes—available in either automation-friendly reagent plates or in tubes
- **Begin with as little as 0.1 ng (100 pg) input DNA** & increase library yields with KAPA EvoT4 DNA Ligase
- **Ensure consistent fragment sizes across a range of DNA inputs & elution buffers** while retaining highly tunable fragmentation
- **Improve library prep performance & minimize wasteful artifacts**
- **Achieve comparable or greater sequencing success compared to mechanical fragmentation methods**, even with challenging samples like FFPET



Increase efficiency & convenience

- Simplify library prep & reduce the risk of human error with a simplified workflow, including one-step fragmentation & A-tailing
- Decrease hands-on-time & the number of reagents with ReadyMix formulations
- Produce sequencing-ready or target-capture-ready libraries in 1.5 hr (PCR-free) to 2.5 hr (with-PCR)

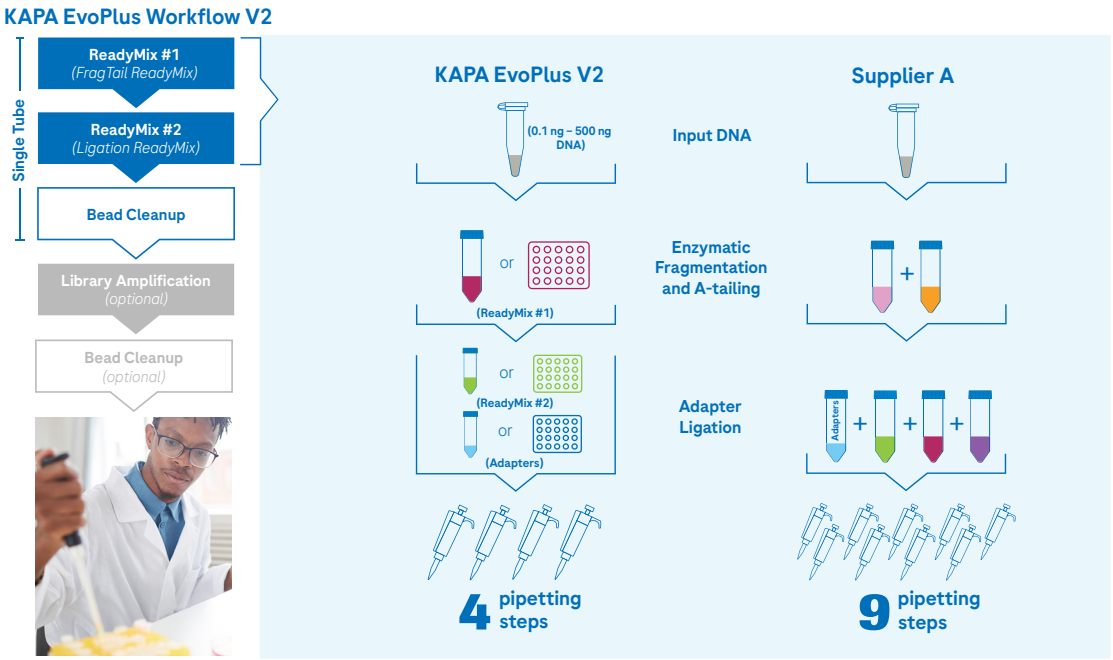


Figure 1: Comparison of the core KAPA EvoPlus V2 workflow to another enzymatic fragmentation library preparation kit. Both workflows are carried out in a single tube up to the library amplification step, which is optional when full-length indexed adapters are used. KAPA EvoPlus V2 ReadyMix reagents are ready-to-use; no master mix preparation is required. With KAPA EvoPlus V2, only two reagents (ReadyMix #1 & ReadyMix #2) are added to input DNA vs. the Supplier A workflow which requires additional reagents & mixing steps, thus increasing the quantity of consumables used & the number of pipetting steps. The core reaction (all non-optional steps) for KAPA EvoPlus V2 can be completed in only four pipetting steps when using full-length adapters, vs. Supplier A which requires nine. NOTES: KAPA full-length UDI adapters are only available in 96-well plates. KAPA Universal Adapters, which are used in combination with KAPA UDI Primer Mixes and require amplification, are available in plates or tubes. KAPA HyperPure Beads, UDI Adapter Kits & KAPA Library Amplification Primer Mix (10X) are sold separately.

Begin with as little as 0.1 ng (100 pg) input DNA

- Increase conversion efficiency with new KAPA EvoT4 DNA Ligase, for higher library yields
- Create high-quality libraries for somatic & germline applications from a range of input DNA amounts (from 0.1 to 500 ng) & sample types
- Achieve successful library construction with clinically relevant samples & PCR-free workflows (from as little as 50 ng)

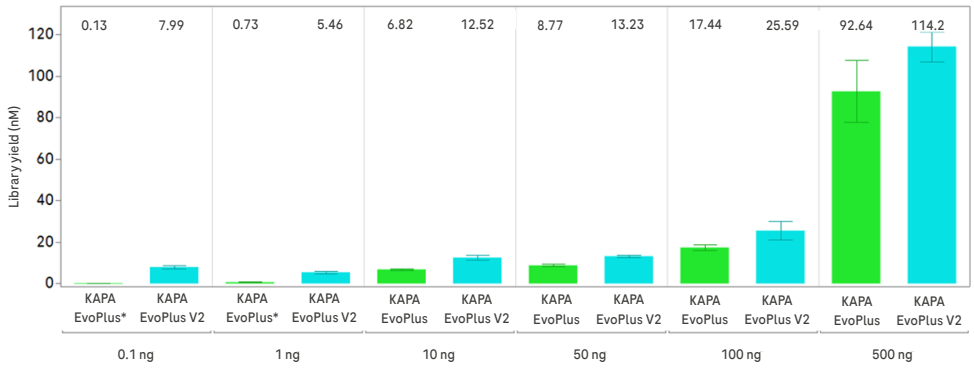


Figure 2: KAPA EvoPlus V2 chemistry enables high library conversion across a range of input DNA 0.1 ng - 500 ng of high-quality human genomic DNA was fragmented for15 minutes & used to prepare libraries with KAPA Universal Adapters with KAPA UDI Primer Mixes at the recommended adapter: insert molar ratio following the KAPA EvoPlus Kit & KAPA EvoPlus V2 Kit Instructions for Use. *Data bars represent the mean, and error bars represent standard deviation (n=4). Non-validated input (outside of the input range) of KAPA EvoPlus Kit - optimized cycle number for KAPA EvoPlus V2 Kit inputs used.

Ensure consistent fragment sizes across a range of DNA inputs & elution buffers

- Adjust library insert sizes by varying fragmentation time
- Obtain reproducible insert sizes across a range of DNA input amounts
- Eliminate impact of inhibitors on fragmentation; KAPA EvoPlus V2 is insensitive to EDTA (≤2 mM) as well as numerous other inhibitors

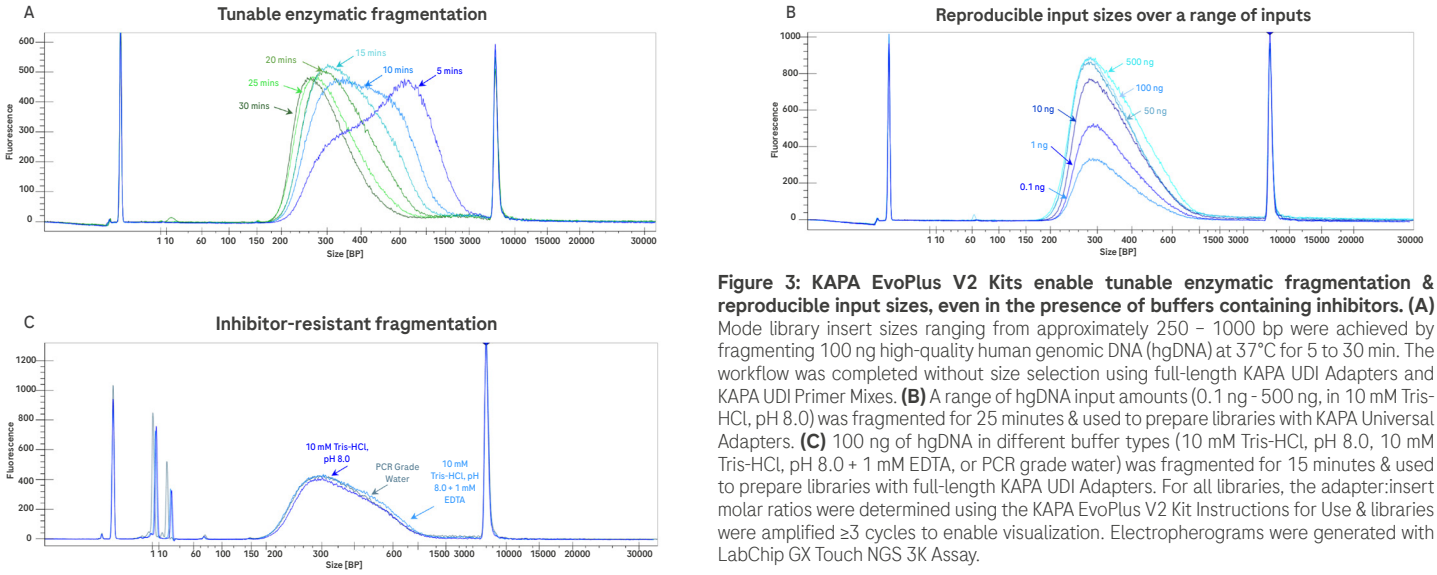


Figure 3: KAPA EvoPlus V2 Kits enable tunable enzymatic fragmentation & reproducible input sizes, even in the presence of buffers containing inhibitors. (A) Mode library insert sizes ranging from approximately 250 – 1000 bp were achieved by fragmenting 100 ng high-quality human genomic DNA (hgDNA) at 37°C for 5 to 30 min. The workflow was completed without size selection using full-length KAPA UDI Adapters and KAPA UDI Primer Mixes. **(B)** A range of hgDNA input amounts (0.1 ng - 500 ng, in 10 mM Tris-HCl, pH 8.0) was fragmented for 25 minutes & used to prepare libraries with KAPA Universal Adapters. **(C)** 100 ng of hgDNA in different buffer types (10 mM Tris-HCl, pH 8.0, 10 mM Tris-HCl, pH 8.0 + 1 mM EDTA, or PCR grade water) was fragmented for 15 minutes & used to prepare libraries with full-length KAPA UDI Adapters. For all libraries, the adapter:insert molar ratios were determined using the KAPA EvoPlus V2 Kit Instructions for Use & libraries were amplified ≥3 cycles to enable visualization. Electropherograms were generated with LabChip GX Touch NGS 3K Assay.

Improve library prep performance & minimize wasteful artifacts

- Achieve high-performing fragmentation without the drawbacks common to enzymatic fragmentation workflows
- Increase confidence in data with improved sequencing metrics & measurably reduced sequencing artifacts

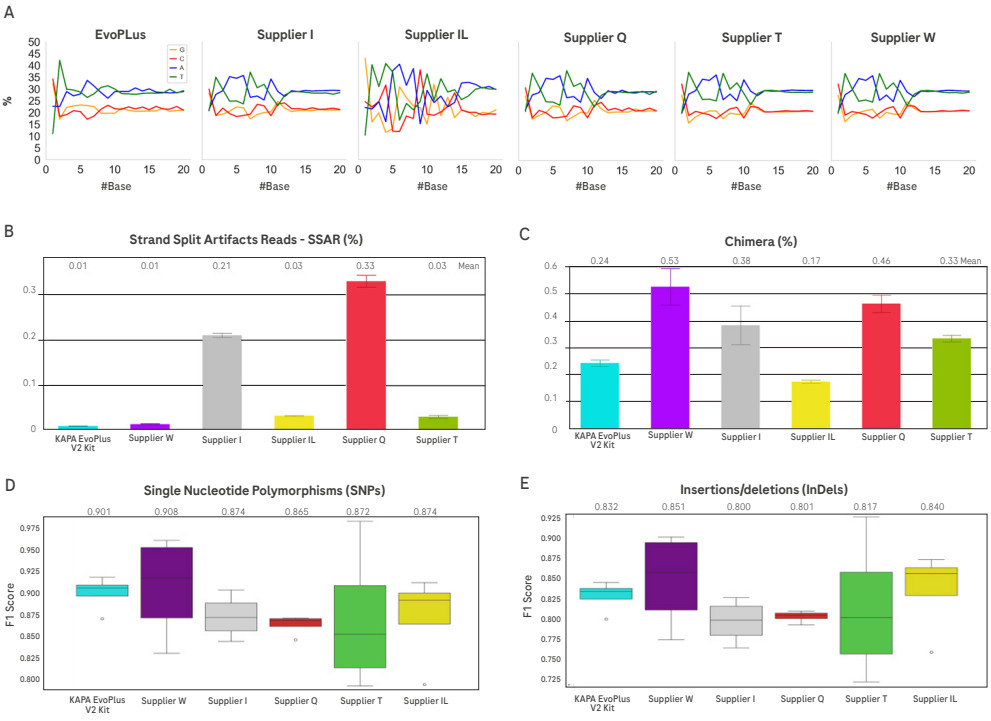


Figure 4: KAPA EvoPlus V2 Kit yields fewer sequencing artifacts in human WGS compared to enzymatic kits from other suppliers. PCR-free whole genome libraries were prepared using 100 ng of human genomic DNA (NA12878) with the KAPA EvoPlus V2 Kit, Supplier I, Supplier IL, Supplier Q, Supplier T & Supplier W, following each supplier's instructions for use. **(A)** The KAPA EvoPlus V2 Kit yielded the least start site bias compared to other Suppliers, resulting in higher data confidence[1], with the most start site bias associated with Supplier IL. **(B)** The KAPA EvoPlus V2 Kit yielded the lowest percentage of SSARs present compared to other Suppliers, with Supplier Q having the highest percentage of SSARs present. SSARs represent chimeric reads that appear to be derived from non-contiguous portions of the genome[2]. **(C)** The KAPA EvoPlus V2 Kit had a lower percentage of chimeras present, resulting in higher data confidence[3] compared to Supplier I, Supplier Q, Supplier T & Supplier W. **(D)** & **(E)** KAPA EvoPlus V2 Kit demonstrated consistent, high sensitivity and specificity of detecting known variants (SNPs & InDels) compared to other suppliers. All libraries were sequenced on an Illumina NovaSeq 6000 (2x150bp) and subsampled to 150 M reads.

Achieve comparable or greater sequencing success compared to mechanical fragmentation methods

- Achieve high on-target rates & deeper coverage in combination with target enrichment probes
- Effectively sequence even low-quality FFPET DNA
- Increase the recovery of unique reads and reduce sequencing artifacts

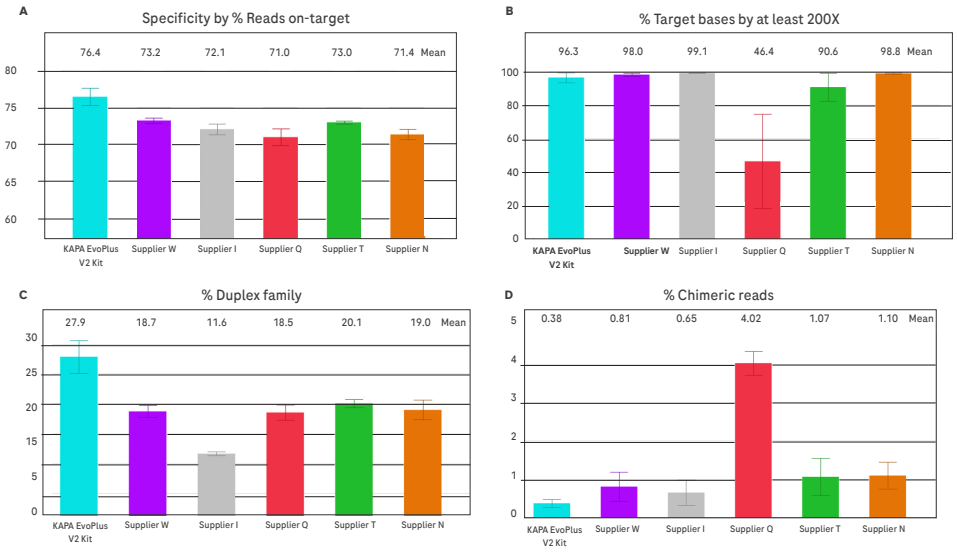


Figure 5: KAPA EvoPlus V2 Kit yields improved sequencing performance in the KAPA HyperCap FFPET target capture workflow, compared to other suppliers' mechanical fragmentation kits. **(A)** The KAPA EvoPlus V2 Kit yielded the highest percentage of reads on-target compared to the other library prep kits tested. **(B)** The KAPA EvoPlus V2 Kit yielded a very high percentage of target bases covered by at least 200X. **(C)** The KAPA EvoPlus V2 Kit yielded more unique reads (as determined by UMI sequences), indicating greater library complexity and a greater recovery of unique input molecules. **(D)** KAPA EvoPlus V2 Kit yielded the very low chimeric read artifacts, even when compared against mechanical fragmentation library preps. Methods: For all libraries, 50 ng of low-quality FFPET DNA was used to prepare triplicate libraries following the suppliers' instructions for use. All libraries were enriched with the KAPA HyperCap Oncology Panel (214 Kb) following the KAPA HyperCap FFPET Evolved workflow instructions. All libraries were sequenced on an Illumina NovaSeq 6000 (2x150bp) and subsampled to 150 M reads.

KAPA EvoPlus V2 Kits

Roche Cat. No.	Description
09420037001	KAPA EvoPlus V2 Kit (24rxn)
09420053001	KAPA EvoPlus V2 Kit (96rxn)
09420339001	KAPA EvoPlus V2 Kit (384rxn)
09420428001	KAPA EvoPlus V2 Kit, plated format (96rxn)
09420045001*	KAPA EvoPlus V2 Kit, PCR-free (24rxn)
09420304001*	KAPA EvoPlus V2 Kit, PCR-free (96rxn)
09420371001*	KAPA EvoPlus V2 Kit, PCR-free (384rxn)
09420436001*	KAPA EvoPlus V2 Kit, PCR-free, plated format (96rxn)

**KAPA Library Amplification Primer Mix (10X) not included.*

KAPA HiFi Hot Start Ready Mixes

Roche Cat. No.	Description
09420398001	KAPA HiFi HS RM (9.6ml)
09420444001	KAPA HiFi HS RM 96 well plate (96rxn)

KAPA EvoPlus V2 Kits + KAPA Library Amplification Mix

Roche Cat. No.	Description
10212284702**	KAPA EvoPlus V2 Kit + Lib Amp Primers (24rxn)
10212292702**	KAPA EvoPlus V2 Kit + Lib Amp Primers (96rxn)
10212306702**	KAPA EvoPlus V2 Kit + Lib Amp Primers (384rxn)
10212314702**	KAPA EvoPlus V2 Kit + Lib Amp Primers (96 rxn plate)

***Combined Virtual kits. Order one catalog number & receive both items.*

KAPA Library Amplification Mixes

Roche Cat. No.	Description
09420410001	KAPA Library Amp Primer Mix (384 rxn)
09420479001	KAPA Library Amp Primer Mix 96-well plate (96rxn)

1. McNulty, *et al.* (2020). Impact of reducing DNA input on next-generation sequencing library complexity & variant detection. The journal of Molecular Diagnostics, Volume 22, Issue 5, May 2020, Pages 720-727.
2. Haile, *et al.* (2019). Sources of erroneous sequences & artifact chimeric reads in next generation sequencing of genomic DNA from formalin-fixed paraffin-embedded samples. Nucleic Acids Research, 2019, 7,2. doi: 10.1093/nar/gky1142.
3. Chen, *et al.* (2024). Characterization & mitigation of artifacts derived from NGS library preparation due to structure-specific sequences in the human genome. BMC Genomics 25:227 https://doi.org/10.1186/s12864-024-10157-w.

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