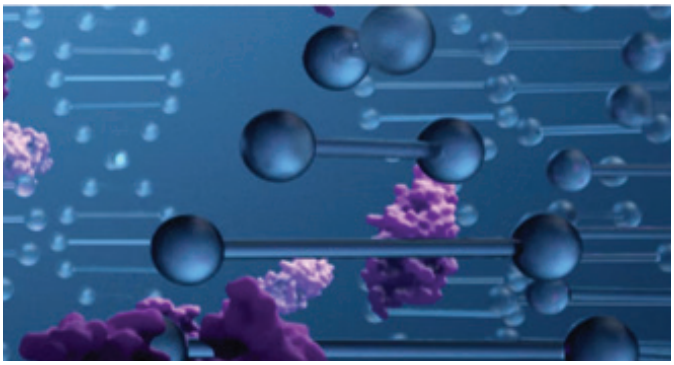
Tech Spotlight

High-performance WES

KAPA HyperExome Probes V2 and Element Biosciences™ Cloudbreak Freestyle™ Workflow

Streamlined, high-performance exome sequencing without conversion

Whole exome sequencing (WES) provides a well-established and cost-effective approach for enabling research into the genetic variants underlying well-characterized, rare, and complex genetic disorders.[1] Library pools for WES generated with the **KAPA EvoPlus V2** or **KAPA EvoPrep Kits** (using KAPA adapters and primers), **KAPA HyperExome V2 Probes**, and the **KAPA HyperCap Evolved Workflow** can be sequenced directly on **Element Biosciences AVITI™ sequencers** with the **Cloudbreak Freestyle workflow**. This obviates the need for reagent modifications and library conversion, and enables streamlined, high-performance WES.



[1] Rabbani, *et al*. The promise of whole-exome sequencing in medical genetics. 2014, *J of Human Genetics*, 59, 5 – 15

Direct compatibility with KAPA HyperExome V2 library pools enables streamlined, high-performance WES on AVITI™ sequencers

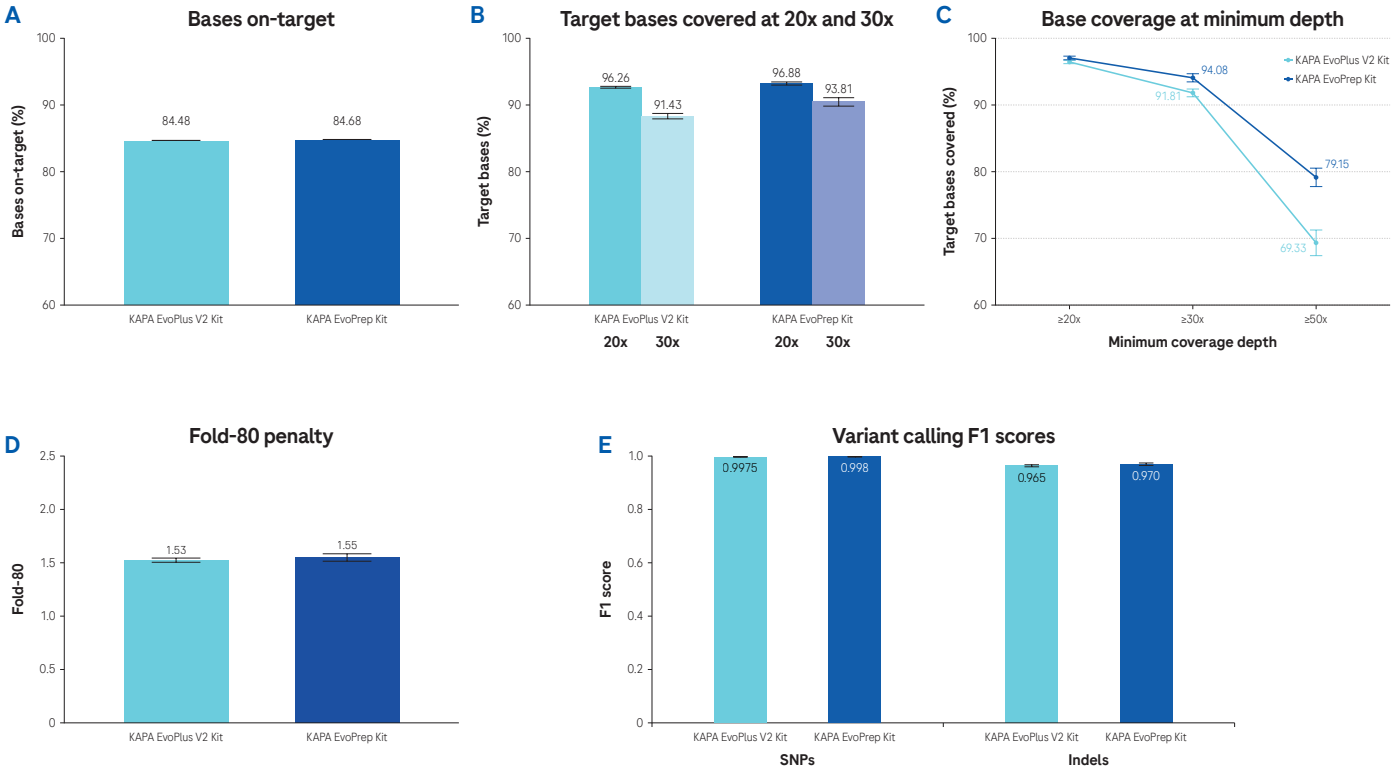


Figure 1. Highly efficient WES with the KAPA EvoPrep and KAPA EvoPlus V2 Kits, KAPA HyperExome V2 Probes, KAPA HyperCap Evolved Workflow, and the Element Biosciences Cloudbreak Freestyle Workflow. (A) Exceptional on-target rates, and **(D)** high coverage uniformity (low fold-80 penalty values) translate to high base coverage at different coverage depths **(B** and **C)**. These key metrics enable accurate, high-confidence variant calling **(E)**. Libraries (*n*=8) were prepared from 100 ng inputs of each of two reference samples (NA12878 and NA24385; Coriell Institute for Medical Research) using the KAPA EvoPlus V2 Kit (enzymatic fragmentation; light blue) or KAPA EvoPrep Kit (mechanical shearing, dark blue). Both kits contain the KAPA Evo T4 DNA Ligase, which offers exceptional ligation efficiency with only 5 min of ligation time. Libraries were prepared as described and 8-plex exome capture was performed as described in the KAPA HyperCap Evolved Workflow v5.0. The Cloudbreak Freestyle sequencing workflow was subsequently performed as described in the Element Biosciences Cloudbreak™ Sequencing User Guide (July 2025). The sequencing run (2 x 100 bp) was configured to yield at least 30 million read pairs per library. Data analysis was performed using bwa-mem2 (v2.2.1), Fastp (v0.23.2), GATK (v4.5.0.0), MultiQC (v1.24.1), DeepVariant (v.1.6.0), and hap.py (v0.3.15).

Data on file at Roche.

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WES workflow with KAPA library prep and target enrichment reagents and Cloudbreak Freestyle™ workflow on AVITI™ sequencers

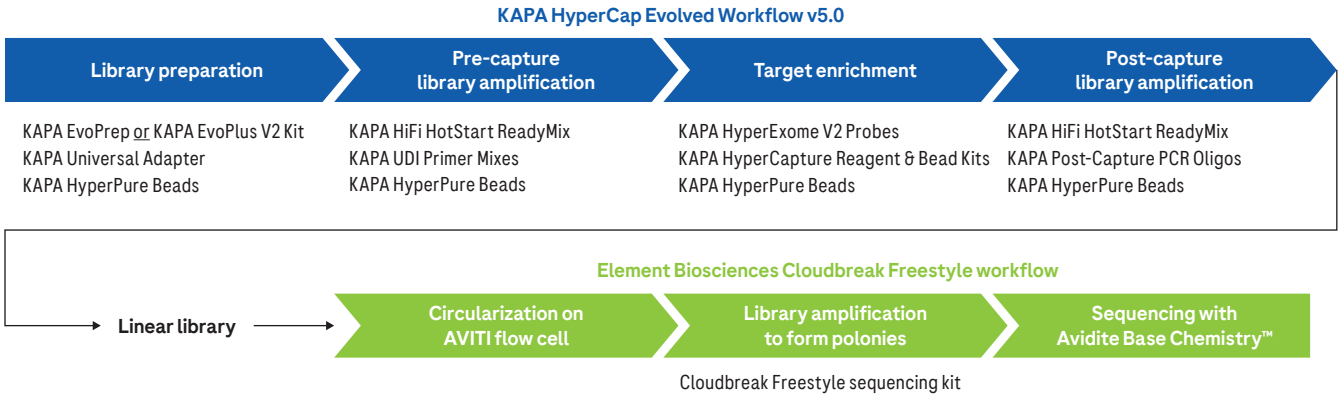

Figure 2. The Cloudbreak Freestyle workflow enables seamless sequencing of whole-exome libraries prepared with the KAPA HyperCap Evolved Workflow. Library preparation with the KAPA EvoPrep Kit or KAPA EvoPlus V2 Kit utilizes a streamlined workflow with reagent ready mixes, fewer pipetting steps, and less plasticware. The KAPA HyperCap target enrichment workflow has been optimized for automation with simplified hybridization and wash steps, all performed at 55°C. With the Cloudbreak Freestyle workflow, linear libraries generated with the KAPA workflow are directly loaded onto the AVITI flow cell for clonal amplification and sequencing.

Summary

KAPA EvoPrep and KAPA EvoPlus V2 Kits utilize an **evolved ligase**, **ready mixes**, and a **streamlined protocol** to enable rapid construction of highly complex sample libraries for exome sequencing. With the Cloudbreak Freestyle sequencing workflow, no special adapters, amplification primers, or conversion process is required for sequencing on AVITI systems.

Based on the most recent genomic databases and **empirically optimized using real-world samples**, KAPA HyperExome V2 Probes are designed to achieve coverage of the **most challenging exonic regions** in a **compact capture target** (43.2 Mb); with multiplexing and low sequencing requirements (30 million read pairs/library).

Element Biosciences' Avidite Base Chemistry™ (ABC) supports high-performance sequencing of KAPA whole-exome libraries. High on-target rates and high and even coverage translate to **reliable variant calling results** and **excellent sequencing economy**.

Explore more...

with **KAPA EvoPlus V2** or **KAPA EvoPrep Kits** and **KAPA HyperExome V2 Probes** using the Cloudbreak Freestyle workflow.

Click **here** or scan the code below:

Data on file at Roche.
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