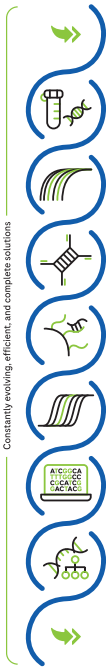
Enrich ACCURACY

KAPA Adapters enrich accuracy and throughput

Sample barcoding increases the efficiency of Next Generation Sequencing by allowing pooling of hundreds of samples in the same sequencing lane while Unique Dual-Indexed (UDI) adapters provide essential mitigation of index misassignment (index hopping). Molecular barcoding with Unique Molecular Identifiers (UMI) within each sample, allows proper molecule counting that further increases accuracy in low-frequency variant detection.

Roche Sequencing Sample Prep Solutions offers a comprehensive portfolio of KAPA Adapters compatible with Illumina® instruments, featuring:



Full length adapters

KAPA UDI Adapters, a set of 96 **full-length** UDI adapters ideal for PCR-free sample preparation

Truncated adapters

KAPA HyperPlex Adapters which is a **complete set** of adapters:

- **KAPA Universal Adapter**: Truncated universal adapter
- **KAPA Universal UMI Adapter**: Truncated universal adapter with UMI
- **KAPA UDI Primer Mixes 1 – 384**: UDI Primer Mixes validated with both KAPA Universal Adapters (with or without UMI) for KAPA Target Enrichment applications

Benefits of KAPA Adapters

Unique Dual-Indexing	with non-redundant dual-sequencing barcode combinations that mitigate index misassignment
Functionally tested	to confirm high library construction efficiency and minimal levels of adapter-dimer formation
Sequencing-based QC testing	for barcode cross-contamination
Automation friendly	plated format for the KAPA UDI Adapters and the KAPA UDI Primer Mixes

Unique Dual-Indexed Adapters for multiplexed sequencing

- High-quality, unique dual-indexed adapter system for ligation-based library construction in Illumina® sequencing workflows
- KAPA Unique Dual-Indexed Adapters comprise 96 adapters with non-redundant index combinations to mitigate the impact of index misassignment
- KAPA Universal Adapter offered with or without UMI is a truncated adapter which together with the KAPA UDI Primer Mixes allow up to 384 UDI combinations
- Low (≥2-plex) to high (up to 384-plex) levels of sequencing multiplexing are supported on one-, two-, and four-channel Illumina instruments, on patterned and non-patterned flow cells

KAPA Adapter selection guide*

		KAPA HyperPlex Adapters with KAPA UDI Primer Mixes	
	KAPA UDI Adapters	KAPA Universal Adapter	KAPA Universal UMI Adapter
Number of UDIs (unique dual-indexes)	96	384	
Number of 8-nt non-redundant P5 + P7 barcodes	96 + 96	384 + 384	
Barcodes identical to those used in adapters supplied by Illumina	No[1]	No[1]	
Recommended for all Illumina instruments with patterned or non-patterned flow cells	Yes	Yes	
PCR-free library preparation	Yes	No	
Molecular barcoded	No	No	Yes
Suitable for:			
KAPA EvoPrep and EvoPlus V2 Kits, KAPA HyperPrep and HyperPlus Kits	Validated	Compatible	Compatible
KAPA RNA HyperPrep Kit	Validated	Validated	Compatible
KAPA HyperCap and KAPA HyperPETE Workflows	Compatible[2]	Validated	Validated
Compatible with KAPA Universal Enhancing Oligos	Yes	Yes	
Adapter formulation	Full-length, ready-to-use, 15 µM 4 reactions/adapter[3]	Truncated, ready-to-use, 15 µM 1 reaction/adapter[3]	Truncated, ready-to-use, 33 µM 1 reaction/adapter[3]
Kit configuration	Hard-shell 96-well plate with automation-friendly labeling, overfills and replacement seals[4]	Adapter tube and hard-shell 96-well primer mixes plates with automation-friendly labeling, overage and replacement seals[4]	
KAPA Adapter Dilution Buffer	Yes (25 mL per kit)	No	

[1]The sets of 192 and 768 barcode sequences used in KAPA UDI Adapters and KAPA UDI Primer Mixes (respectively) are exclusive to Roche and different between the two sets.

[2]Theoretically possible but not fully tested.

[3]This is sufficient for four or one library preps (KAPA UDI Adapter plate or KAPA UDI Primer Mixes plate respectively) with the KAPA HyperPrep or KAPA HyperPlus Kit if no dilution is required. Generous overfill supports use on automated liquid handling systems.

[4]KAPA UDI Primer Mixes and KAPA UDI Adapter plates are shipped with peelable seals. Replacement seals (three per plate) are peelable and pierceable.

*KAPA HyperPlex Adapters and KAPA UDI Adapters are not compatible with methyl-seq applications.

Fewer misassigned reads improve confidence in results

- Index misassignment during multiplexed sequencing may be the result of index hopping, barcode or sample cross-contamination, template switching during PCR amplification of pooled samples, and/or sequencing/analysis errors; some of which may be mitigated by adapter design and quality
- Unique dual-index combinations in KAPA UDI Adapters allow reads with unexpected barcode combinations to be filtered out prior to data analysis
- KAPA Adapters are manufactured using stringent procedures and undergo sequencing-based QC testing to reduce the potential for index misassignment resulting from barcode cross-contamination

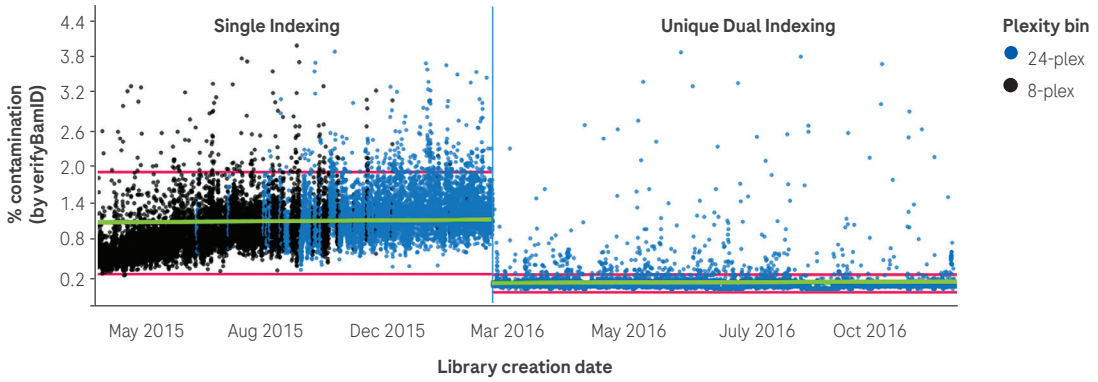


Figure 1. Unique dual indexing mitigates the impact of index misassignment during multiplexed sequencing on patterned flow cells. Each dot in this dataset represents a PCR-free human whole-genome library, sequenced in pools of 8 (black) or 24 (blue) on Illumina® HiSeq X instruments. Contamination rates, calculated with VerifyBamID, reflect potential sample cross-contamination based on genotype analysis. Index hopping contributes to observed contamination rates, and was significantly reduced after implementing the non-redundant dual indexing strategy and barcodes utilized in KAPA UDI Adapters (instead of the original single indexing workflow). Green lines represent the mean and red lines the upper and lower statistical control limits of the data, analyzed with JMP. Data courtesy of the Broad Institute (Cambridge, MA, USA).

Figure 2. Sequencing-based QC testing of KAPA Adapters for barcode cross-contamination improves confidence in results. In this in-house developed QC assay, each KAPA UDI Adapter is ligated to a unique, synthetic linear insert. The 96 libraries are pooled and sequenced on an Illumina NextSeq™ 500 instrument. Data are subsampled to 500,000 reads per library, before adapter trimming and alignment to the synthetic reference sequences. Aligned bam files are downsampled to the lowest aligned read count for final calculations. The heat map shown here is a representative barcode cross-contamination test result for KAPA UDI Adapters, and shows the percentage of reads associated with each insert (columns) and barcode (rows) combination. Dark green blocks across the diagonal correspond to correct UDI-insert combinations. Every other block corresponds to the percentage of reads for a particular insert associated with one of the other expected index combinations in the set of 96, and is colored according to the scale given on the right. The test confirms that adapters are plated in the correct wells, and that index misassignment attributable to cross-contamination with UDI combinations that can't be filtered out is extremely low compared to misassignment from other potential sources, such as index hopping.[1] A similar in-house developed QC assay is followed for the KAPA UDI Primer Mixes.

[1]Costello M, et al. BMC Genomics. 2018;19:332.

KAPA UDI Adapters deliver high library conversion efficiency for PCR-free Whole-Genome Sequencing

- KAPA Adapters undergo functional testing in an NGS library construction workflow to confirm high levels of library construction efficiency, minimal levels of adapter-dimer formation and consistent performance across all 96 index combinations
- PCR-free workflows have become the gold standard for human whole-genome sequencing (WGS)
- High-quality, full-length KAPA UDI Adapters used in combination with KAPA EvoPrep Kits and KAPA HyperPrep Kits support flexible and highly efficient library construction protocols needed for high-throughput, PCR-free WGS

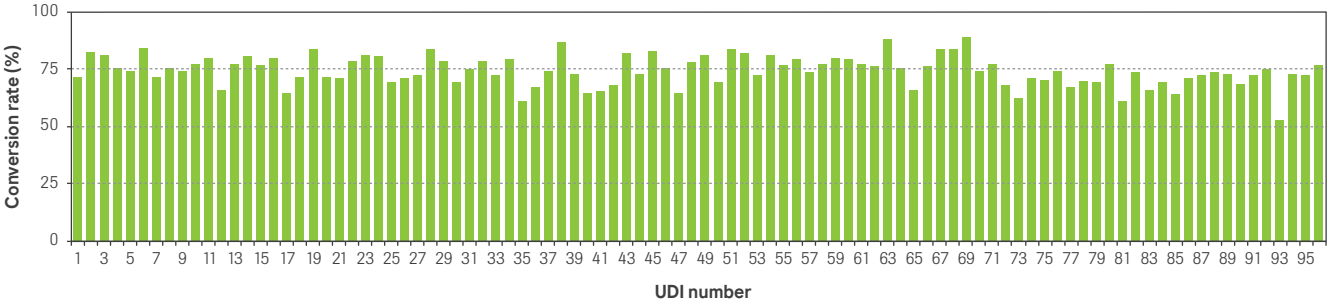


Figure 3. KAPA UDI Adapters support high and consistent library construction efficiency across all 96 index combinations. PCR-free shotgun libraries were prepared from 10 ng inputs of bulk Covaris®-sheared *E. coli* genomic DNA, using the KAPA HyperPrep Kit on a Tecan Freedom EVO® liquid handling system. A different KAPA UDI Adapter was used for each library. Libraries were quantified on a Roche LightCycler® 480 qPCR System using the KAPA Library Quantification Kit. Standard protocols were followed, and the conversion rate (% input DNA converted to sequencing-ready library) was calculated for each adapter. The average conversion rate of 74.5% ±6.5% across the 96 index combinations is representative of data generated across several independent experiments.

KAPA HyperPlex Adapters deliver high library yields in applications that require library amplification

- When input material is limited, robust library amplification delivers the maximum yield with the minimum number of cycles
- The KAPA HyperPlex Adapters combine high library conversion rate with robust amplification efficiency
- High complexity libraries deliver the required yield with less amplification cycles and reduced PCR duplication

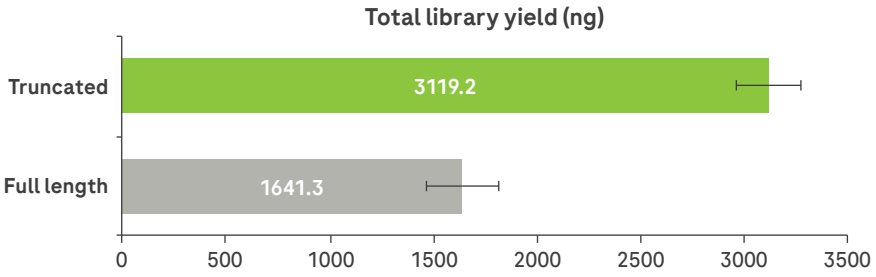


Figure 4. Truncated adapters can deliver as much as almost twice the library yield generated by conventional full length adapters. Libraries were prepared from 50 ng of human genomic DNA (NA12878; Coriell Institute) using the KAPA HyperPlus Kit and two different adapters. Twelve (*n*=12) DNA replicates were ligated to a standard Illumina®-compatible full length Y-adapter and amplified for 7 cycles with standard Illumina-compatible library amplification primers. Another twelve (*n*=12) DNA replicates were ligated to an Illumina-compatible truncated Y-adapter and amplified for 7 cycles with UDI Primer Mixes. All other conditions such as DNA fragmentation time, final adapter, and primer concentration, as well as bead cleanup ratios were kept identical between the two sample sets. Libraries were quantified with a Qubit Fluorometer (Thermo Fisher Scientific). The libraries prepared with a full length adapter had a mean yield of 1641.3 ng ±178 ng compared to a mean yield of 3119.2 ng ±157 ng for the libraries prepared with the truncated adapter.

High performance – KAPA HyperCap Target Enrichment

- Consistent high performance target enrichment workflows require extensive testing and optimization with complete reagent and kit offering to achieve reproducible quality
- KAPA Universal Adapter and KAPA UDI Primer Mixes have been validated with the KAPA HyperCap Workflow v3 and are compatible with KAPA EvoPrep, KAPA EvoPlus V2, KAPA HyperPrep, KAPA HyperPlus, and the KAPA RNA HyperPrep Library Preparation Kit

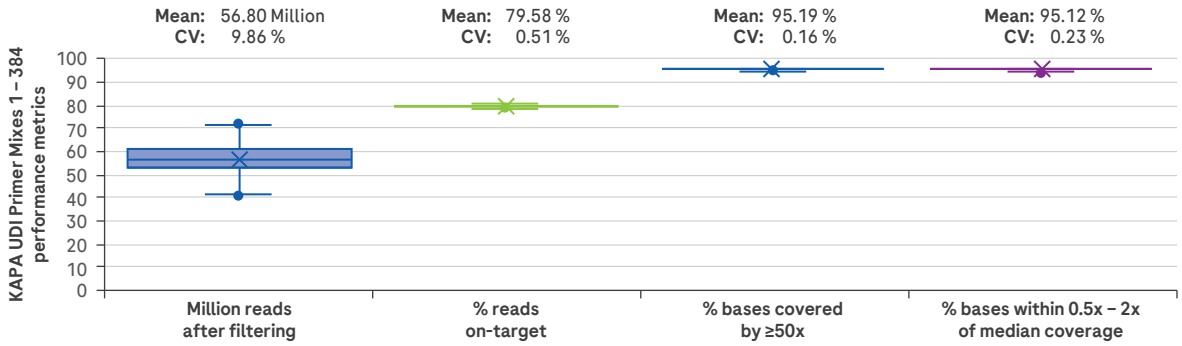


Figure 5. KAPA Universal Adapter with all 384 KAPA UDI Primer Mixes perform consistently in the KAPA HyperCap Workflow v3. High reproducibility was demonstrated when replicate libraries prepared with all 384 UDIs were pooled in the same sequencing run, delivering high specificity (% reads on-target), high uniformity (% bases within 0.5x – 2x of median coverage) and deep target coverage (% bases covered by >50x). The KAPA HyperCap Heredity Panel (10 Mb capture target) was used to enrich libraries which were prepared from 100 ng replicate inputs of human genomic DNA (NA12878; Coriell Institute) with the KAPA HyperPlus kit in the KAPA HyperCap Workflow v3. Pre-capture libraries were quantified with a Qubit Fluorometer (Thermo Fisher Scientific). An average pre-capture yield of 3.9 ±0.4 µg was obtained across the 384 libraries that were multiplexed by 12 in 32 hybridizations. All 384 final enriched libraries (32 captures) were sequenced on a NovaSeq™ 6000 System lane at 2 x 100 bp, resulting in a mean of ~28.4 Million clusters (56.8 M reads) per sample after quality filtering. After down-sampling to 10 Million clusters per sample, analysis followed the technical note "How To Evaluate KAPA Target Enrichment Data" (March 2020). Total duplicate rate was 3.2 % ±0.2% and fold-80 base penalty was 1.32 ±0.01.

Somatic oncology research from cell-free DNA

- Accurate molecule counting facilitated by molecular barcoded adapters is essential in somatic oncology applications, especially from low inputs of cfDNA where every molecule counts
- The KAPA Universal UMI Adapter has a proprietary design that prevents single errors in the UMI sequence to result in false counting of spurious molecules
- The KAPA Universal UMI Adapter shows improved performance in molecule counting, showing higher duplex recovery and lower error rate than Supplier I's UMI adapter

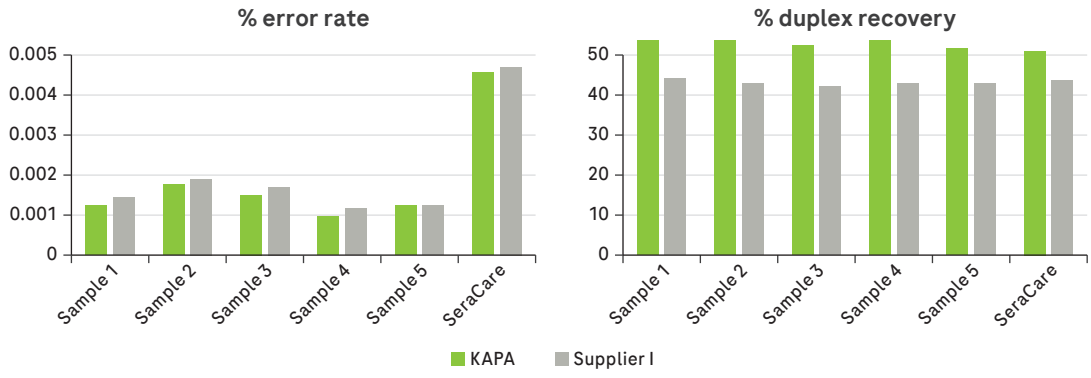


Figure 6. KAPA UMI Adapter supports highly accurate molecule counting and high recovery of duplex molecules from 10 ng cfDNA. Five healthy donors' cell-free DNA samples and the SeraSeq® ctDNA Complete™ Reference Material AF0.5% from SeraCare were tested in duplicate for library preparation and target enrichment with the KAPA HyperCap Oncology Panel (214 Kb capture target). Libraries from 10 ng cfDNA were prepared with the KAPA HyperPrep Kit and captured according to the KAPA HyperCap Workflow v1 for cfDNA in single hybridizations per sample. Sequencing clusters of 2 x 150 bp from a NextSeq™ 500 System were downsampled to 50 Million quality filtered clusters per sample prior to analysis.

Data on file.
For Research Use Only. Not for use in diagnostic procedures.

Process more samples successfully, get more information from every sample, and optimize your sequencing resources with solutions that are **Proven**, **Simple**, and **Complete**.

Ordering information for KAPA Adapters

KAPA UDI Adapters – Full Length

Roche cat. no.	KAPA code	Description	Pack size
08861919702	KK8727	KAPA Unique Dual-Indexed Adapter Kit (15 µM)	96 x 20 µL
08278539001	KK8721	KAPA Adapter Dilution Buffer	25 mL

KAPA HyperPlex Adapters - Truncated

Roche cat. no.	KAPA code	Description	Pack size
09063781001	N/A	KAPA Universal Adapter, 15 µM 960 µL	960 µL
09063790001	N/A	KAPA Universal Adapter, 15 µM 4 x 960 µL	4 x 960 µL
09329862001	N/A	KAPA Universal UMI Adapter, 960 µL	960 µL
09134336001	N/A	KAPA UDI Primer Mixes, 1 – 96, 96 rxn	96-well plate
09329838001	N/A	KAPA UDI Primer Mixes, 97 – 192, 96 rxn	96-well plate
09329846001	N/A	KAPA UDI Primer Mixes, 193 – 288, 96 rxn	96-well plate
09329854001	N/A	KAPA UDI Primer Mixes, 289 – 384, 96 rxn	96-well plate

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