KAPA Adapters for NGS

Enhanced accuracy, greater throughput

The efficiency of **next-generation sequencing (NGS)** can be increased greatly by using indexed adapters to barcode samples, making it possible to run many pooled samples on a single sequencing run (multiplexing); the reads are then sorted bioinformatically.

However, some high-throughput sequencers are prone to index mis-assignment (index hopping), which can reduce data quality; unique dual-indexing (UDI) strategies help to mitigate the impact of these events.

Data accuracy—especially for quantitative data—can be further increased by the use of molecular barcoding with unique molecular identifiers (UMIs). UMIs assign each individual input molecule with a short tag that enables sequencing reads in the final data to be traced back to individual input molecules. This enables the identification of PCR duplicates and sequencing artifacts, and the removal of these reads from the final data.

- **Deliver high library conversion efficiency** for targeted sequencing, PCR-free whole genome sequencing (WGS), with-PCR library preparation workflows, and somatic oncology research with cell-free DNA (cfDNA)
- **Mitigate index mis-assignment** with KAPA Unique Dual Indexing (UDI) Adapters, which provide dual, non-redundant sample barcode combinations. KAPA UDI Adapters undergo sequencing-based QC testing to reduce the potential for index misassignment resulting from barcode cross-contamination
- **Save valuable time and resources** with automation-friendly, single-use plate formats for UDI primer mixes

Full-length Adapters *for PCR-free Sample Prep with up to 96 index combinations*	**Truncated Adapters** *for up to 384 unique index combinations, for greater number of sample barcodes and with optional UMIs*
KAPA UDI Adapters, set of 96	KAPA Universal Adapter KAPA Universal UMI Adapter KAPA UDI Primer Mixes 1-384, validated for KAPA Universal Adapters (UMI or non-UMI)

Deliver high library conversion efficiency

KAPA Universal Adapter and KAPA UDI Primer Mixes work together to provide high library conversion efficiency and improve data quality and analysis. Together, they have been validated for DNA library preparation (with KAPA HyperPrep and KAPA HyperPlus Kits), hybridization-based target enrichment (with the KAPA HyperCap Workflow v3), and RNA library preparation (with KAPA RNA HyperPrep Kits).

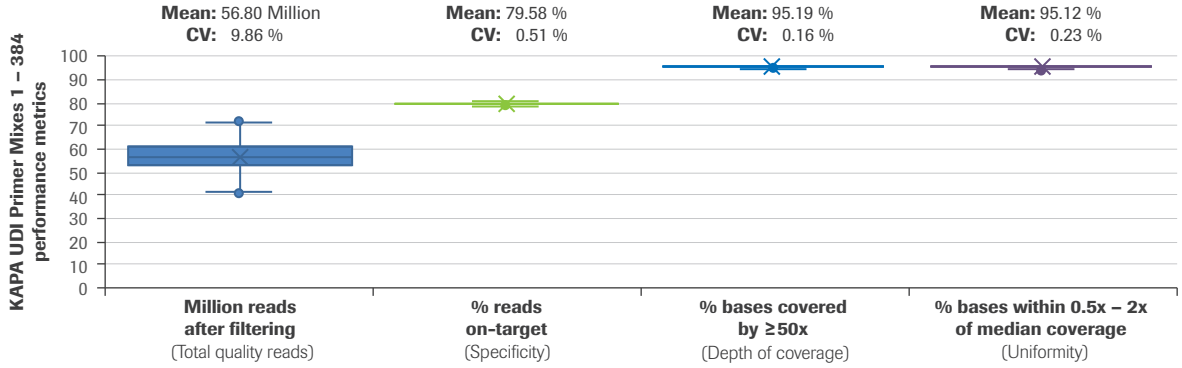

Figure 1. KAPA Universal Adapter with all 384 KAPA UDI Primer Mixes perform consistently in the KAPA HyperCap Workflow v3. High reproducibility was demonstrated across all UDI pairs following the sequencing of duplicate libraries containing all 384 UDI pairs in a single run. Filtered reads delivered high specificity (% reads on-target), deep target coverage (% bases covered by > 50X), and high uniformity (% bases within 0.5X – 2X of median coverage). Total duplicate rate was 3.2 % + 0.2 % and fold-80 base penalty was 1.32 + 0.01. **Experimental design:** Duplicate libraries were prepared with the KAPA HyperCap Workflow v3 using the KAPA HyperCap Heredity Panel (10 Mb capture target) and the KAPA HyperPlus Kit; input = 100 ng of human genomic DNA (NA12878; Coriell Institute). Libraries were multiplexed prior to capture, a total of 12 x 32-plex captures (384 enriched libraries total). All enriched libraries were then pooled and sequenced on a NovaSeq™ 6000 System lane at 2 x 100 bp, resulting in a mean of ~56.8 Million reads per sample after quality filtering. After down-sampling at 20 Million reads per sample, analysis followed the technical note "How To Evaluate KAPA Target Enrichment Data" (March 2020)[1]. Total duplicate rate was 3.2 % + 0.2 % and fold-80 base penalty was 1.32 + 0.01.

Somatic oncology research using cell-free DNA (cfDNA)

Accurate molecule counting is essential for somatic oncology applications, especially from low inputs of cfDNA where every molecule counts. This accuracy is enhanced by the use of Unique Molecular Identifiers (UMIs) incorporated into NGS adapters, providing barcodes for individual molecules.

- Prevent errors in the identification of UMIs with the proprietary design of KAPA Universal UMI Adapters
- Increase accuracy of molecule counting and achieve higher duplex recovery and lower error rates with KAPA UMI Adapters (compared to UMI adapters from another supplier)

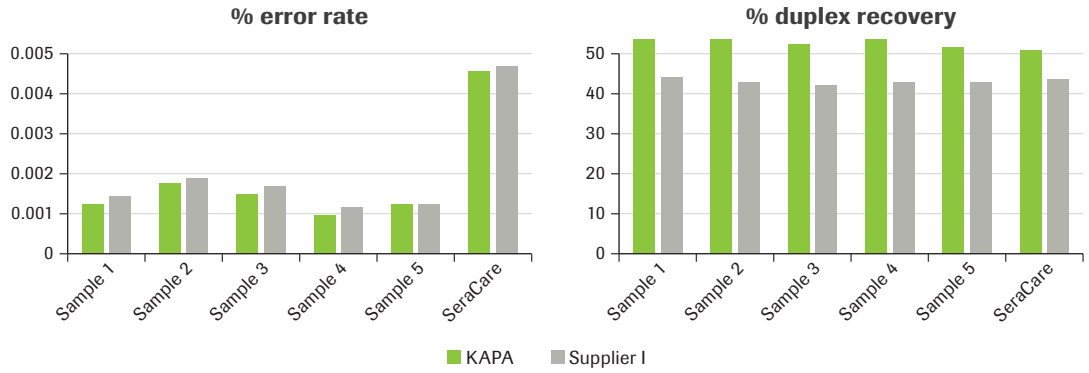

Figure 2. KAPA UMI Adapter supports highly accurate molecule counting and high recovery of duplex molecules from 10 ng cfDNA. Five healthy donors' cell-free DNA samples and the SeraSeq® ctDNA Complete™ Reference Material AF0.5% from SeraCare were tested in duplicate for library preparation and target 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.

1 Meyer, J, et al. Roche Application Note SEQ100183. 2018.

Mitigate index mis-assignment with UDI strategies

The use of unique dual-indexing strategies can improve data quality by enabling the removal of reads containing unexpected barcode combinations, such as those caused by index hopping or index mis-assignment, prior to data analysis **(Figure 3)**.

Such index mis-assignment can occur during several steps of multiplexed sequencing, including library preparation (cross-contamination of barcodes or samples), amplification of pooled samples (template switching during PCR), and/or errors during sequencing and analysis—especially when patterned flow cells are used for sequencing.

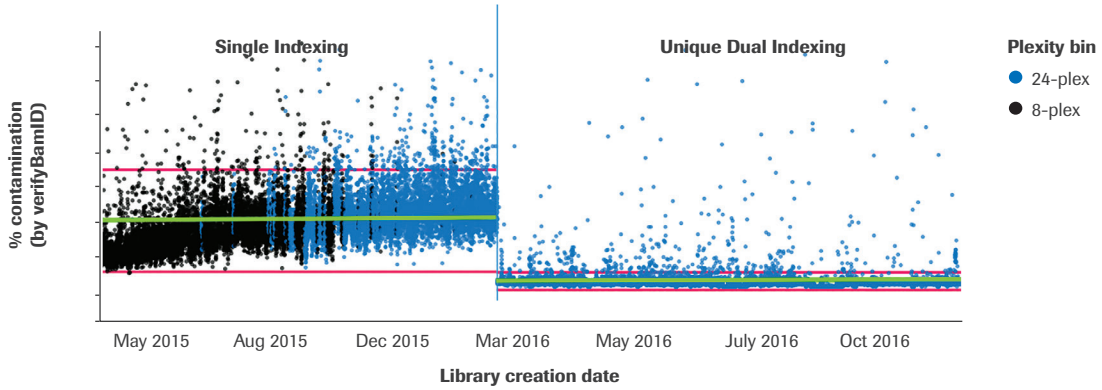

Figure 3. Unique dual indexing mitigates the impact of index mis-assignment during multiplexed sequencing on patterned flow cells. Each dot in this dataset represents a PCR-free human whole-genome library, sequenced in pools of 8 (black) or 24 (blue) on Illumina® HiSeq X instruments. Contamination rates, 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).

Reduce the potential for index mis-assignment resulting from barcode cross-contamination with KAPA Adapters, which are manufactured using stringent procedures and then undergo sequencing-based QC testing.

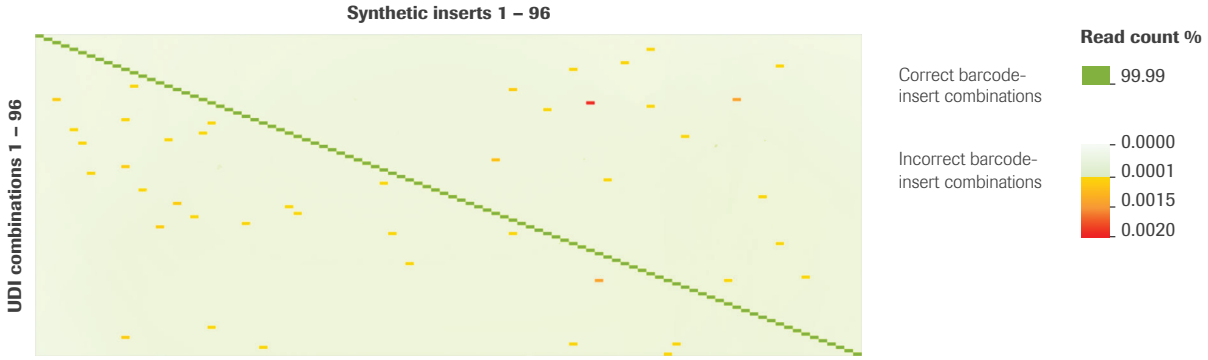

Figure 4. Sequencing-based QC testing of KAPA Adapters for barcode cross-contamination improves confidence in results. In this in-house developed QC assay, each KAPA UDI Adapter is ligated to a unique, synthetic linear insert. The 96 libraries are pooled and sequenced on an Illumina NextSeq™ 500 instrument. 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.

Save valuable time and resources

KAPA Adapters are a collection of high quality, unique dual-indexed adapters for ligation-based library construction. KAPA Adapters support many levels of sequencing multiplexing (2-plex up to 384-plex) on both two- and four-channel Illumina instruments that use either patterned or non-patterned flow cells.

- Mitigate the impact of index misassignment with full-length KAPA Unique Dual-Indexed (UDI) Adapters, offering 96 adapters containing non-redundant index combinations, or with KAPA Universal Adapters *plus* KAPA UDI primer mixes
- Create up to 384 uniquely-indexed libraries with truncated KAPA Universal Adapters (with or without UMIs) and KAPA UDI Primer Mixes

*KAPA Adapter selection guide**

		KAPA HyperPlex Adapters	
	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 HyperPrep and HyperPlus	Validated	Compatible	Compatible
KAPA RNA HyperPrep	Validated	Validated	Compatible
KAPA HyperCap	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 labelling, 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 adapter dilution is required. Generous overfill supports use on automated liquid handling systems.

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

*KAPA Universal Adapters, KAPA Universal UMI Adapters and KAPA UDI Adapters are not compatible with methyl-seq applications.

Ordering information for KAPA Adapters

Full-Length Adapters

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

Truncated Adapters

Roche cat. no.	KAPA code	Description	Pack size
09063781001	N/A	KAPA Universal Adapter, 15 uM 960 µL	960 µL
09063790001	N/A	KAPA Universal Adapter, 15 uM 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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915 Hague Road
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sequencing.roche.com

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