Highly Sensitive Longitudinal ctDNA Analysis in NHL Using the KAPA HyperCap Evolved Workflow

Now supported by the KAPA EvoPrep and KAPA EvoPlus V2 Kits and pre-capture multiplexing, the KAPA HyperCap Design Share NHL Panel enables highly sensitive and efficient longitudinal analysis of non-Hodgkin lymphoma (NHL) circulating tumor DNA (ctDNA) in a streamlined KAPA HyperCap Evolved Workflow. A user friendly, cloud-based software analysis pipeline powered by DNAnexus® completes a robust and integrated research solution.

Introduction

Non-Hodgkin lymphoma (NHL) is one of the most common hematological malignancies,[1] with an estimated 544,000 new cases globally leading to nearly 260,000 deaths in 2020.[2] Tumors are genetically diverse, with associated disease ranging from indolent to aggressive, and curable to refractory.[3] Global research efforts are not only focused on the introduction of new therapies (including stem cell transplants, chemotherapy, immunotherapy, and targeted therapies), but also on the development of genetic testing methods to improve disease detection and management.

Current diagnostic methods for lymphoid neoplasms include histopathology, flow cytometry, cytogenetics, immunohistochemistry, and molecular techniques. Next-generation sequencing (NGS)-based genomic profiling and gene expression analysis are playing an increasingly important role in accurate tumor classification, as this drives therapy selection.[3]

Molecular monitoring during and after treatment is also critical, as the radiological and nuclear imaging methods typically used to assess treatment response are unable to provide information on clonal evolution and minimal residual disease (MRD)—both of which impact final outcomes.[4] Cell-free circulating tumor DNA (cfDNA/ctDNA), typically assessed using targeted deep sequencing, has emerged as an important, non-invasive and highly sensitive biomarker in the monitoring of patient status.[5,6]

While next generation sequencing holds promise for patient management in the future, research efforts are currently underway to demonstrate future applicability in patient management. One such research tool available for scientists is the KAPA HyperCap Design Share NHL Panel.

We have previously described an integrated RUO workflow for the longitudinal detection of

Authors

Parul Agarwal, *Scientist II, Reagent and Assay Development;* **Andrea Ramos**, *Senior Manager, Reagent and Assay Development;* **Renee Stokowski**, *Director, Reagent and Assay Development;* **Bowdoin Su**, *Medical Affairs Leader, Medical Affairs;* **Richard Chien**, *Principal Bioinformatics Scientist II, Assay Informatics;* **Tina Thompson**, *Senior International Product Manager*
Roche Diagnostics Solutions, Pleasanton & Santa Clara, CA, USA

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variants associated with NHL, using the KAPA HyperCap Design Share (DS)* NHL Panel in combination with the KAPA HyperCap Workflow and open-source bioinformatic tools.[7,8] The NHL panel was released in 2023 and is suitable for the detection of both somatic and germline variants in blood and tissue samples.[9]

In this white paper, we demonstrate further improvements to the efficiency of the workflow, through the incorporation of (i) KAPA EvoPrep and KAPA EvoPlus V2 library preparation kits (which employ the potent, engineered KAPA EvoT4 DNA ligase in a streamlined workflow using pre-master-mixed reagents),[10,11] and (ii) pre-capture multiplexing during the target enrichment process. In addition, we have simplified access to the previously demonstrated analysis pipeline, which is now offered on DNAnexus® (https://www.dnanexus.com; user account required for access).

Detection of single nucleotide variants (SNVs) in contrived cfDNA samples was investigated with allele frequencies (AF) in the range of 1 – 5%. The use of this integrated solution for the longitudinal detection of SNVs is also described.

Materials and methods

1. Experimental Design

This study was designed to demonstrate the performance of the KAPA HyperCap Design Share NHL Panel for the longitudinal detection of ctDNA with (i) library preparation kits that offer higher unique molecular recovery rates, (ii) pre-capture multiplexing of two or four libraries, as compared to the standard single-plex enrichment protocol, and (iii) a user-friendly, on-cloud version of the previously available bioinformatic pipeline.

The KAPA HyperCap Design Share NHL Panel (IRN: 1000028225) covers single nucleotide variants (SNVs) located in the coding and/or untranslated regions of 383 genes (see Table A.1, *Appendix*) previously identified in NHL patients, particularly those diagnosed with diffuse large B-cell lymphoma (DLBCL).[5,9] The panel, which also contains genes associated with other B-cell lymphomas, enables longitudinal detection of variants associated with NHL.[7]

*The Roche Design Share platform offers NGS target enrichment panels designed and developed by Roche in collaboration with leading researchers from around the world.

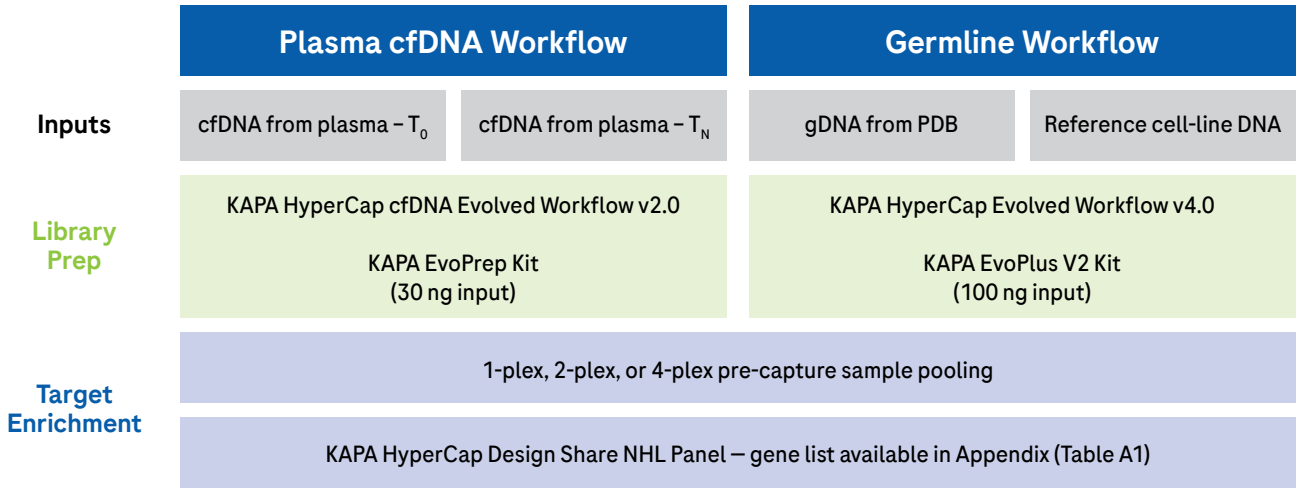

Figure 1. Experimental design for this study. Sample preparation, library construction, target enrichment, sequencing, and data analysis were performed as described in *Materials and methods*.

As outlined in Figure 1, libraries were prepared from plasma-derived cell-free DNA (cfDNA) using the KAPA EvoPrep Kit (KAPA HyperCap cfDNA Evolved Workflow v2.0[12] or "plasma cfDNA workflow"), and from genomic DNA purified from plasma depleted blood (PDB gDNA) or reference cell line gDNA using the KAPA EvoPlus V2 Kit (KAPA HyperCap Evolved Workflow v4.0[13] or "germline workflow"). Target enrichment was performed with the KAPA HyperCap DS NHL Panel. Sequencing was performed on an Illumina® NextSeq™ 550 instrument using standard protocols. Data analysis was performed with the previously described Roche KAPA Longitudinal Mutation Analysis RUO pipeline[8] on DNAnexus®. Key sequencing metrics and the results of longitudinal analysis are compared for single-plex vs. 2- and 4-plex captures.

2. Samples, DNA Extraction, and DNA QC

Plasma cfDNA workflow: Contrived samples were created by blending plasma-derived cfDNA from healthy donors. Cell-free DNA was extracted from plasma using the cobas® cfDNA Sample Preparation Kit (Roche PN: 07247737190). Extracted cfDNA was quantified using a fluorescence microplate reader and the Quant-iT™ dsDNA High-Sensitivity Assay Kit (Thermo Fisher Scientific). Cell-free DNA quality was assessed by qPCR as described in Saelee et al., 2022[14] and the original white paper.[7]

To mimic T_0 baseline samples, cfDNA from four unique, healthy donors were combined in specific ratios to create allele frequencies AF of known variants in the range of 1 – 5%. To mimic T_N follow-up samples, each baseline sample was further diluted with the corresponding major background donor in a specific ratio to achieve AFs of known variants in the range of 0.1 – 1%. Two such sets of samples were created from healthy donor cfDNA (Sample1 and Sample2; see Table 1, line 1).

Germline workflow: Two samples were derived from plasma-depleted blood of healthy donors (Table 1, line 2) and two from cell lines obtained from a commercial supplier (Table 1, lines 3 and 4). PDB gDNA was extracted using the KAPA NGS DNA Extraction Kit (Roche PN: 09189823001 or 09190023001). DNA was quantified using a fluorescence microplate reader and the Quant-iT™ dsDNA High-Sensitivity Assay Kit (Thermo Fisher Scientific).

3. Library Preparation and Target Enrichment

Library preparation for plasma cfDNA workflow: Triplicate libraries were prepared from 30 ng inputs of each of the two plasma cfDNA T_0 (baseline) and each of the two T_N (follow-up) samples, for a total of 12 libraries (Table 2). Library construction was performed as outlined in the KAPA HyperCap cfDNA Evolved Workflow v2.0 Instructions for Use,[12] using the KAPA EvoPrep Kit (Roche PN: 10154039001 or 10096039001), KAPA Universal UMI Adapter (Roche PN: 09329862001 or 09329889001), KAPA UDI Primer Mixes 1 – 384 (Roche PN:9134336001, 9329838001, 9329846001, or 9329854001), and KAPA HyperPure Beads (Roche PN: 08963835001, 08963843001, 08963851001, or 08963878001).

Library preparation for germline workflow: Triplicate libraries were prepared from 100 ng inputs of each of the two PDB gDNA and each of the two reference cell line gDNA samples, for a total of 12 libraries (Table 3). Library construction was performed as outlined in the KAPA HyperCap Evolved Workflow v4.0 Instructions for Use,[13] using the KAPA EvoPlus V2 Kit (Roche PN: 0942003001, 09420053001, 09420339001, or 9420428001), KAPA Universal Adapter (15 µM; Roche PN: 09063781001), KAPA UDI Primer Mixes 1 – 384 (Roche PN:9134336001, 9329838001,

Table 1. Samples used in this study

	Sample name/ part number	Sample description	Genotype	Workflow	Sample type	Precision INDEL
1	Sample1, T_0 and T_N Sample2, T_0 and T_N	Two sets of contrived samples, derived from plasma of healthy donors	WT	Plasma cfDNA	Plasma	cfDNA blends, representing AFs of known variants in the range of 1 – 5% (T_0) or 0.1 – 1% (T_N)
2	PDB1 gDNA PDB2 gDNA	gDNA samples from healthy donors	WT	Germline	PDB gDNA	N/A
3	NA24631	GIAB NA24631	WT	Germline	Reference (cell line)	N/A
4	NA24149	GIAB NA24149	WT	Germline	Reference (cell line)	N/A

GIAB: Genome in a bottle (see: https://www.coriell.org/1/NIGMS/Collections/NIST-Reference-Materials).

9329846001, or 9329854001), and KAPA HyperPure Beads (Roche PN: 08963835001, 08963843001, 08963851001, 08963878001, or 08963860001).

Pre-capture Library QC: Quantification of amplified libraries is critical for pre-capture multiplexing. Amplified libraries were diluted 1/10 for analysis of fragment size distribution using an Agilent 4200 TapeStation System and DNA High Sensitivity D1000 ScreenTape Assay (Agilent Technologies). The same diluted material was used for library quantification using a fluorescence microplate reader and the Quant-iT™ dsDNA High-Sensitivity Assay Kit (Thermo Fisher Scientific). The concentration of each undiluted, amplified sample library was calculated and these values were used to determine input masses for single-plex and multiplex target enrichment.

Pre-capture multiplexing for plasma cfDNA workflow: For single-plex captures, 30 µL of a uniquely indexed, amplified library were processed as described in the KAPA HyperCap cfDNA Evolved Workflow v2.0 Instructions for Use.[12] For multiplex captures, equal amounts (in ng) of each uniquely dual-indexed, amplified DNA library were mixed together to obtain a combined DNA mass of 1500 ng (Table 4). PCR-grade water was added to achieve a final volume of 30 µL for each multiplex sample library pool.

Pre-capture multiplexing for germline workflow: For single-plex captures, 1000 ng of every uniquely dual-indexed, amplified library were diluted in PCR-grade water to achieve a final volume of 45 µL. For multiplex captures, equal amounts (in ng) of each uniquely indexed, amplified library were mixed together to obtain a combined DNA mass of 1500 ng (Table 5). PCR-grade water was added to achieve a final volume of 45 µL in each multiplex sample library pool.

Target enrichment: Probe-based hybridization capture was performed as described in the KAPA HyperCap cfDNA Evolved Workflow v2.0 Instructions for Use[12] or KAPA HyperCap

Table 2. Breakdown of libraries prepared with the KAPA EvoPrep Kit for the plasma cfDNA workflow

Capture strategy	Number of libraries prepared from sample:		Number of libraries	Number of captures
	Plasma cfDNA T_0 (30 ng input)	Plasma cfDNA T_N (30 ng input)		
1-plex	2	2	4	4
2-plex	2	2	4	2
4-plex	2	2	4	1
TOTAL	**6**	**6**	**12**	**7**

The two libraries prepared from each plasma sample represent biological replicates, as one library was derived from Sample set 1 and one from Sample set 2 (ref. Table 1).

Table 3. Breakdown of libraries prepared with the KAPA EvoPlus V2 Kit for the germline workflow

Capture strategy	Number of libraries prepared from sample:		Number of libraries	Number of captures
	PDB gDNA (100 ng input)	Reference (cell line) gDNA (100 ng input)		
1-plex	2	2	4	4
2-plex	2	2	4	2
4-plex	2	2	4	1
TOTAL	**6**	**6**	**12**	**7**

The two libraries prepared from each sample represent biological replicates, as PDB gDNA was derived from two different donors, and two different reference cell lines were used (ref. Table 1).

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Evolved Workflow v4.0 Instructions for Use,[13] using the following kits: KAPA HyperCapture Reagent Kit (Roche PN: 09075810001 or 09075828001), KAPA HyperCapture Bead Kit (Roche PN: 09075780001 or 09075798001) and the KAPA HyperCap Design Share NHL Panel, described in Table 6.

Post-enrichment Library QC: Post-capture (enriched) libraries were diluted 1/10 for analysis of fragment size distribution using an Agilent 4200 TapeStation System and DNA High Sensitivity D1000 ScreenTape Assay (Agilent Technologies). The same diluted material was used for the quantification of sequencing ready libraries using a fluorescence microplate reader and the Quant-iT™ dsDNA High-Sensitivity Assay Kit (Thermo Fisher Scientific).

3. Sequencing and Data Analysis

Sequencing: Libraries were pooled for multiplexed, paired-end (2 x 151 bp) sequencing using an Illumina® NextSeq™ 550 System. Pools representing eight samples each (i.e., 8 x 1-plex libraries, 2 x 4-plex, or 4 x 2-plex libraries) were configured to be run on a single flowcell.

Data Analysis: Data analysis was performed with the Roche KAPA Longitudinal Mutation Analysis RUO Pipeline on DNAnexus®. This analysis pipeline utilizes the methods described in a previously published White Paper,[8] and consists of three main workflows: (i) single sample processing, then (ii) blocklist generation and reporter selection, and (iii) longitudinal mutation analysis. Data were downsampled to 72 million FASTQ reads (36 million read pairs) per sample (before deduplication). Graphs were generated with JMP® 16.

Table 4. Multiplexing strategy for the plasma cfDNA workflow

Capture strategy	Number of captures	Sample input for multiplexing	Total input for target enrichment
1-plex	4	30 µL per sample	30 µL per sample
2-plex	2	(750 ng per sample) x 2	1500 ng
4-plex	1	(375 ng per sample) x 4	1500 ng

Table 5. Multiplexing strategy for the germline workflow

Capture strategy	Number of captures	Sample input for multiplexing	Total input for target enrichment
1-plex	4	1000 ng per sample	1000 ng
2-plex	2	(750 ng per sample) x 2	1500 ng
4-plex	1	(375 ng per sample) x 4	1500 ng

Table 6. Details of the target enrichment panel used in this study

Workflow	Panel name	Key features
Plasma cfDNA	KAPA HyperCap Design Share NHL Panel	• Covers SNVs in coding and/or untranslated regions of 383 genes associated with NHL and other B-cell lymphomas[9] • Enables longitudinal detection of variants associated with NHL[7]
Germline		

The panel is ordered as KAPA HyperChoice MAX 3Mb T1 (Roche PN: 09052631001, IRN: 1000028225).

Results and discussion

1. Assessment of pre-capture multiplexing performance

The performance of the KAPA HyperCap DS NHL Panel with pre-capture multiplexing of libraries prepared using the KAPA EvoPrep (for cfDNA) or KAPA EvoPlus V2 kits (for PDB/cell line gDNA) was evaluated by assessing key sequencing QC metrics for each workflow. Longitudinal mutation analysis was subsequently performed.

Library QC metrics: All pre-capture libraries met the yield and size distribution criteria for single-plex and multiplex target enrichment. All post-capture (enriched) libraries and library pools also met the yield and size distribution criteria for sequencing[12,13] (data not shown).

Sequencing performance metrics: Sample and analysis QC metrics for each sequencing run were generated using the Roche KAPA Longitudinal Mutation Analysis RUO Pipeline[8] on DNAnexus®. A subset of these metrics are described in Table 7.

Results obtained for plasma cfDNA baseline (T_0) and follow-up (T_N) libraries prepared with the KAPA EvoPrep Kit and three capture strategies are shown in Figure 2, with median values reported in Table 8. Values for all sequencing metrics were highly similar, with no correlation with the level of pre-capture plexing. The median for the uniformity metric, fold 80 base penalty (which indicates the amount of additional sequencing required to ensure that the mean coverage is achieved for 80% of target bases), was 1.6 for 4-plex samples compared to 1.7 for both the 1-plex and 2-plex captures. Penalty values of approximately 1.6 indicated good coverage uniformity across all libraries. Results obtained for germline (PDB and cell line gDNA) libraries prepared with the KAPA EvoPlus V2 Kit and three capture strategies are shown in Figure 3, with median values reported in Table 9. Results were again highly similar and within expected ranges across all levels of pre-capture multiplexing.

2. Longitudinal mutation analysis

Longitudinal mutation analysis was performed with the Roche KAPA Longitudinal Mutation Analysis RUO Pipeline on DNAnexus®, with the objective of determining the presence or absence of reporter variants in T_N (follow-up) samples. For each T_N sample, the FASTQs for the corresponding T_0 baseline and the major background donor (the germline sample for subtraction) were used as inputs for the analysis. Mutation positivity test results are given in Table 10. Mutation positivity was called when the Monte Carlo empirical p-value was less than 0.005.[7,15] As shown in Table 10, all T_N samples were called as positive, irrespective of the capture strategy.

Table 7. Key sequencing QC metrics reported in this study

Metric	Description
Total reads	Number of reads prior to fastp processing for quality and adapter trimming, including all PF and non-PF reads. When CATEGORY equals PAIR this value will be 2X the number of clusters.
UMI barcode deduplicated reads	Reads after UMI barcode (plasma cfDNA workflow) and position (plasma cfDNA and germline workflows) based removal of duplicate reads
Median target coverage	Median depth of coverage over the capture target
Percent target bases 2500X	The fraction of all target bases for which 2500X or greater coverage was achieved
Fold 80 base penalty	The fold additional sequencing necessary to raise 80% of bases in “non-zero-cvg” targets to the mean coverage level in those targets
Median insert size	The **median** insert size of all paired-end reads of which both ends mapped to the same chromosome
Error rate	Mismatch rate calculation from ctDNAtools package get_background_rate.R. Defined as (the sum of mismatches) ÷ (sum of read depths) for all bases in the target.

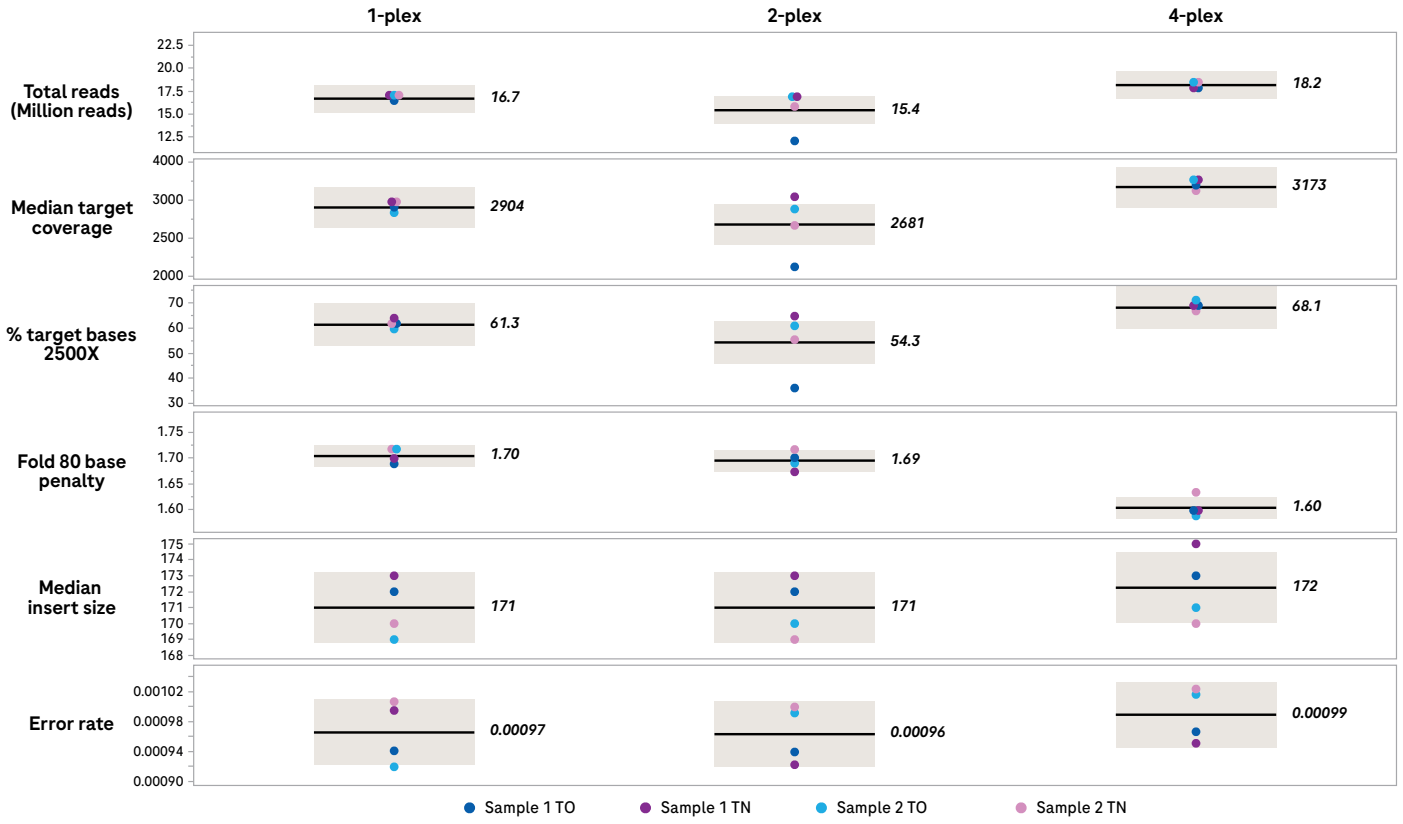

Figure 2. Key sequencing metrics for the plasma cfDNA workflow. Results shown are after UMI barcode deduplication. Libraries were prepared from 30 ng inputs of healthy donor cfDNA blends; enriched, and sequenced as outlined in *Materials and methods*. Data were analyzed with the Roche KAPA Longitudinal Mutation Analysis RUO pipeline on DNAnexus®. Refer to Table 8 for median values for each of the three plexing conditions.

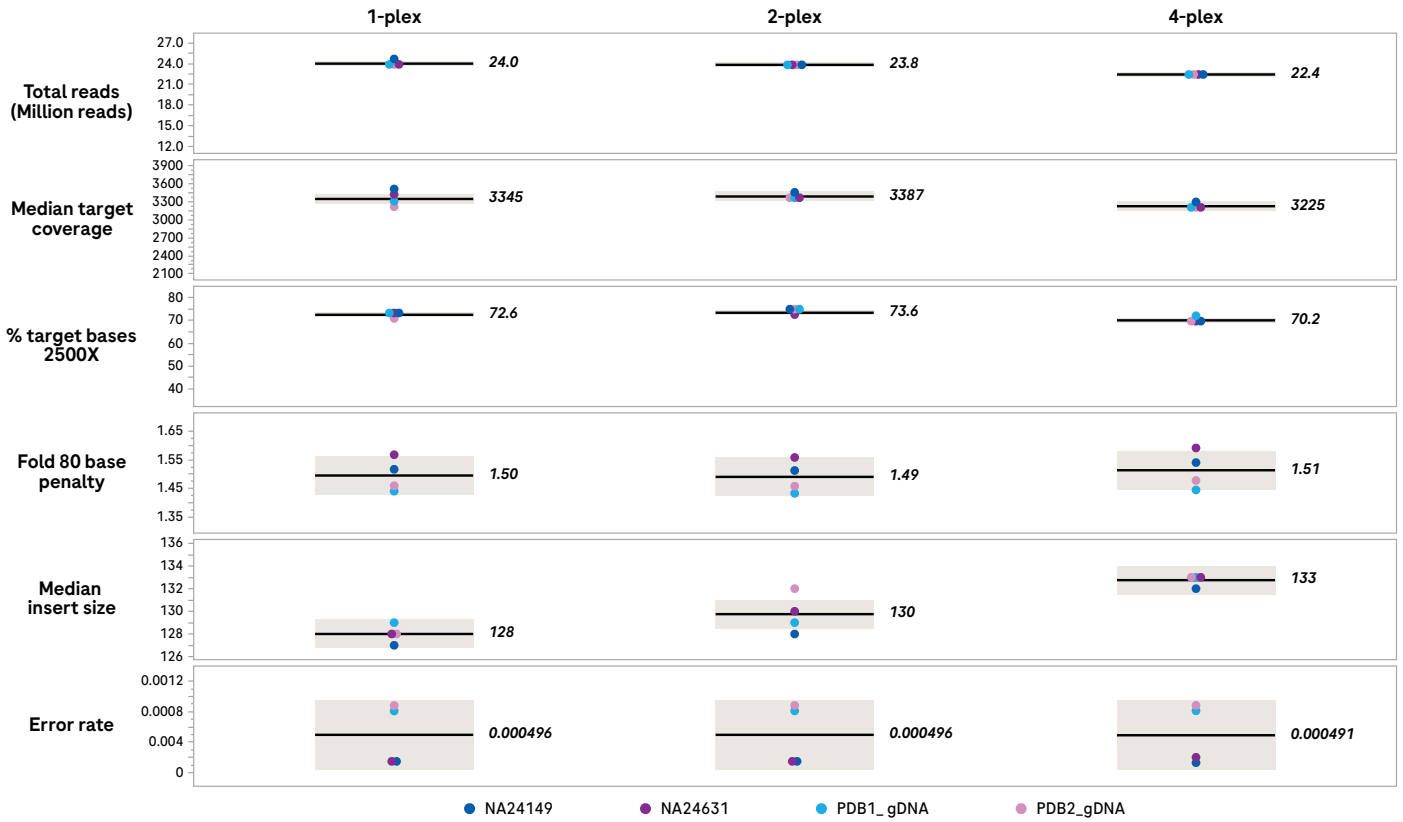

Figure 3. Key sequencing metrics for the germline workflow. Results shown are after position deduplication. Libraries were prepared from 100 ng inputs of healthy donor PDB gDNA and gDNA from two reference cell lines; enriched, and sequenced as outlined in *Materials and methods*. Data were analyzed with the Roche KAPA Longitudinal Mutation Analysis RUO pipeline on DNAnexus®. Refer to Table 9 for median values for each of the three plexing conditions.

Table 8. Median values for key sequencing metrics reported for the plasma cfDNA workflow (from Figure 2)

Metric	1-plex (*n*=4)	2-plex (*n*=2)	4-plex (*n*=1)
UMI barcode deduplicated reads	16.6 M	16.3 M	18.1 M
Median target coverage	2909	2777	3179
Percent target bases 2500X	61%	58%	68%
Fold 80 base penalty	1.7	1.7	1.6
Median insert size	171	171	172
Error rate	0.001	0.001	0.001

Table 9. Median values for key sequencing metrics reported for the germline workflow (from Figure 3)

Metric	1-plex (*n*=4)	2-plex (*n*=2)	4-plex (*n*=1)
Position deduplicated reads	24.1 M	23.8 M	22.4 M
Median target coverage	3361	3377	3218
Percent target bases 2500X	73%	74%	70%
Fold 80 base penalty	1.5	1.5	1.5
Median insert size	128	130	133
Error rate	0.0005	0.0005	0.0005

Table 10. Summary of longitudinal mutation analysis results

Sample	Plex level	n_mutations	n_nonzero_alt	total_alt_reads	informative_reads	p-value	Decision
Sample1 T_N	1-plex	261	245	3008	653064	0.0001	Positive
	2-plex	233	226	2956	638700	0.0001	Positive
	4-plex	276	268	3396	718032	0.0001	Positive
Sample 2 T_N	1-plex	264	250	3138	633881	0.0001	Positive
	2-plex	264	245	2902	570349	0.0001	Positive
	4-plex	280	264	3284	681299	0.0001	Positive

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Conclusions

As previously demonstrated, the KAPA HyperCap Design Share NHL Panel and Roche KAPA Longitudinal Mutation Analysis RUO Pipeline[8] offer a robust, integrated workflow for highly accurate longitudinal mutation analysis from ctDNA samples. This study demonstrated the following improvements:

- More streamlined and effective library preparation from both ctDNA and gDNA samples, using the KAPA EvoPrep and KAPA EvoPlus V2 Kits, respectively. The engineered KAPA Evo T4 DNA ligase used in both kits ensures very high rates of unique molecule recovery, which is particularly important for challenging ctDNA samples.
- Further streamlining and a reduction in per-sample cost with the application of pre-capture multiplexing, without an impact on sequencing or functional performance.
- Improved, user-friendly and cloud-based access to the Roche KAPA Longitudinal Mutation Analysis RUO Pipeline on DNAnexus®.

This study lays the foundation for integrated workflows that seamlessly combine efficient library preparation, advanced capture strategies, expertly designed enrichment panels, and accessible analysis pipelines for diverse oncology, inherited, and complex disease research applications.

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Appendix – Supplemental Information

Table A1. KAPA HyperCap Design Share NHL Panel gene list[16]

ABCB11	CAPZA3	DSEL	H1-5	IGHV1-58	IGKV2-24	IGLV3-25	LIPH	NTNG1	RIMS2	WDPCP
ACAD8	CARD11	DTX1	H2AC11	IGHV1-69	IGKV2-30	IGLV3-27	LIPM	P2RY8	RNF213	WRAP73
ACSS1	CCND3	DUSP2	H2AC16	IGHV2-26	IGKV2D-26	IGLV3-32	LIPN	PABPC1	RNLS	XBP1
ACTA2	CD274	DUSP3	H2AC17	IGHV2-5	IGKV2D-29	IGLV3-9	LRP10	PANK1	S1PR2	XPO1
ACTB	CD36	EBF3	H2AC6	IGHV3-11	IGKV2D-30	IGLV4-3	LRP1B	PAPSS2	SCML4	YTHDF2
ACTG1	CD53	EDIL3	H2AC8	IGHV3-13	IGKV3-20	IGLV4-60	LRP2	PCDH17	SERPINE3	ZEB2
ACVR2A	CD58	EFEMP1	H2BC11	IGHV3-15	IGKV3D-11	IGLV4-69	LRRN3	PCDHA6	SGK1	ZFP36L1
ADAMTS1	CD70	EGR1	H2BC12	IGHV3-16	IGKV3D-20	IGLV5-37	LRRTM4	PCLO	SI	ZFP42
ADAMTS16	CD79B	EHBP1	H2BC14	IGHV3-20	IGKV4-1	IGLV5-45	MAGEB16	PCSK5	SLC22A16	ZMYM6
ADAMTS9	CD83	ELAC1	H2BC17	IGHV3-21	IGKV5-2	IGLV5-48	MAGEC2	PCSK7	SLC9A4	ZNF577
AFF1	CDH12	ELAVL1	H2BC4	IGHV3-23	IGLJ2	IGLV5-52	MAP2K1	PDCD1	SLITRK1	ZNF608
AHCYL1	CDH19	ENSG00000281179	H2BC5	IGHV3-30	IGLJ3	IGLV6-57	MAP3K13	PDCD1LG2	SMAD4	ZNF649
ANKRD22	CDKN2A	ENSG00000282988	H2BC8	IGHV3-33	IGLJ4	IGLV7-43	MC5R	PIK3C2G	SOCS1	ZNF678
APC	CDKN2B	ENSG00000285938	H2BC9	IGHV3-35	IGLJ5	IGLV7-46	MDH1	PIK3CD	SORCS2	
ARHGAP5	CEP104	ENSG00000285947	H3C1	IGHV3-38	IGLJ7	IGLV8-61	MED12	PIK3CG	SRRM2	
ARID1A	CFAP276	EP300	H3C10	IGHV3-43	IGLL5	IGLV9-49	MEF2B	PIK3R1	STAT3	
ATP1B4	CFL1	EPHA7	H3C11	IGHV3-48	IGLV10-54	IRAG2	MEX3C	PIM1	STAT6	
ATP8B1	CLSTN2	EPS8	H3C2	IGHV3-49	IGLV11-55	IRF1	MFHAS1	PLCG2	STT3A	
B2M	CNTNAP2	ERICH1	H3C3	IGHV3-64	IGLV1-36	IRF4	MPDZ	PLCL1	TAF1	
BCHE	COL22A1	EZH2	H3C4	IGHV3-7	IGLV1-40	IRF8	MPEG1	POM121L2	TAS2R16	
BCL10	COL24A1	F2RL2	H3C7	IGHV3-72	IGLV1-44	ITPKB	MRO	POU2F2	TBC1D22A	
BCL11A	CREBBP	FAS	H4C12	IGHV3-73	IGLV1-47	IZUMO3	MSH6	POU2F3	TBL1XR1	
BCL2	CRISPLD1	FAT1	HAS2	IGHV3-74	IGLV1-50	JUNB	MTAP	PPP2R1B	TCL1A	
BCL6	CRYAB	FBXO11	HDAC7	IGHV4-28	IGLV1-51	KCND2	MYC	PPP4C	TET2	
BCR	CSMD1	FBXW7	HMCN1	IGHV4-31	IGLV2-11	KHDRBS3	MYD88	PRDM1	TGFBI	
BNC2	CSMD3	FGFR4	ID3	IGHV4-34	IGLV2-14	KIF2B	MYO15A	PSD3	THBD	
BORCS8	CTNNA2	FOCAD	IGHA1	IGHV4-39	IGLV2-18	KLF10	NARS1	PSRC1	THYN1	
BORCS8-MEF2B	CTNND2	FOXO1	IGHA2	IGHV4-59	IGLV2-23	KLHL14	NCAPD3	PTEN	TLR2	
BRAF	CXCR4	FXYD6	IGHG2	IGHV5-51	IGLV2-33	KLHL25	NCSTN	PWWP3A	TMEM30A	
BRCA2	CXCR5	FXYD6-FXYD2	IGHG4	IGHV6-1	IGLV2-8	KLHL4	NEDD4L	PXDN	TMSB4X	
BRINP3	DFFB	GK2	IGHM	IGHV7-81	IGLV3-1	KLHL6	NEXMIF	RASSF9	TNFAIP3	
BTG1	DHX33	GNA13	IGHV1-18	IGKV1-5	IGLV3-10	KLK11	NFATC1	RBFA	TNFRSF14	
BTG2	DKC1	GRM7	IGHV1-2	IGKV1-6	IGLV3-12	KLK13	NFIA	REL	TNFRSF1A	
BTG3	DLAT	GSDMC	IGHV1-24	IGKV1-8	IGLV3-16	KMT2D	NFKBIA	RER1	TP53	
BTK	DNAH5	H1-2	IGHV1-3	IGKV1D-17	IGLV3-19	KRAS	NFRKB	RFTN1	TRPS1	
CACNA1E	DPAGT1	H1-3	IGHV1-45	IGKV1D-43	IGLV3-21	LAMA1	NOTCH1	RHOA	USP34	
CACNA1S	DRG2	H1-4	IGHV1-46	IGKV1D-8	IGLV3-22	LINGO2	NOTCH2	RHOH	VPS8	

Data on File. KAPA HyperCap DS NHL Panel design files are available at sequencing.roche.com/designshare.
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