KAPA HyperCap DS NHL Panel

A research solution for highly sensitive detection and longitudinal analysis of ctDNA in NHL samples

The KAPA HyperCap Design Share (DS) non-Hodgkin lymphoma (NHL) Panel is a research solution that covers single nucleotide variants (SNVs) in coding and/or untranslated regions of 383 genes, plus additional intergenic regions for a total capture size of 341 Kb. These genomic regions are enriched in genomic alterations associated with NHL. This panel can be used in combination with the KAPA HyperCap workflow[1] and open-source KAPA bioinformatics analysis for longitudinal detection of circulating tumor DNA (ctDNA).[2]

Insights from ctDNA-based NHL research now within reach

Pioneer advances in NHL research by using a unique panel developed for ctDNA monitoring

- Develop new NHL clinical research applications
- Study samples across various NHL subtypes
- Have access to all research data with an in-house sequencing workflow

Unique panel design proven in a large pivotal study

Leverage a panel that is based on years of rigorous research and panel design that was used with research samples from the POLARIX study to validate ctDNA as a prognostic biomarker[3]

- Take advantage of over a decade of R&D by Roche scientists and academic researchers[1]
- Advance your research with panel content that has been used to analyze over 1000 samples[3]
- Unlock insights from a panel with strong proof of principle data[1,2]

Simplified and reliable NHL research workflows

Utilize robust and streamlined KAPA workflows with the new KAPA HyperCap DS NHL panel and customizable open-source bioinformatics analysis

- Leverage the robust KAPA HyperCap workflows[1]
- Easily customize an open-source analysis pipeline to better meet your needs[2]
- Scale up by using an automation-friendly workflow

High sequencing quality

- Highly sensitive ctDNA detection by providing deep median unique coverage, greater than 5000X
- Highly specific sequencing with median percent selected bases greater than 74%
- Confident result enabled by minimizing sequencing errors by the use of UMI (unique molecular identifiers)

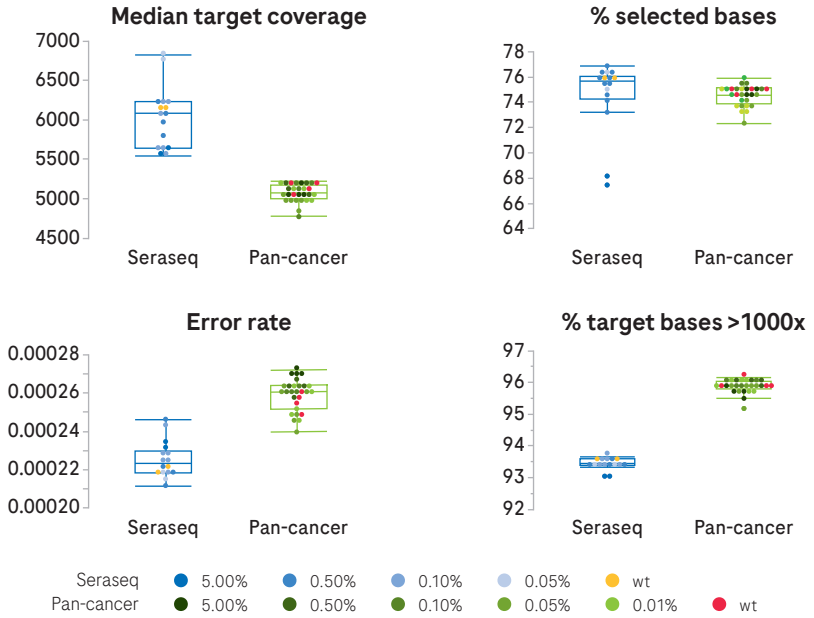

Figure 1. KAPA HyperCap DS NHL panel met key sequencing performance criteria and exhibited high sequencing efficiency as exhibited by the deduped samples.*

*In this study, commercial reference samples were used to prepare "contrived" samples—with known variants at allele frequencies (AFs) ranging from 0% to 5%—that mimic NHL samples. Next-generation sequencing (NGS) libraries were prepared using the KAPA HyperPrep Kit and KAPA HyperCap cfDNA Workflow v1.1 or the KAPA HyperPlus Kit and KAPA Hypercap Workflow v3.4. Libraries were enriched by hybridization to the KAPA HyperCap Design Share NHL Panel. Sequencing was performed on an Illumina® NextSeq™ 500/550 instrument using standard protocols. Data analysis was performed using open source bioinformatic tools.[2] The KAPA HyperCap DS NHL panel sequencing metrics met expectations for a 8-sample run on Illumina® NextSeq™ 500/550 flow-cell with an average number of reads >88 M per sample. After unique molecular identifier (UMI) deduplication, the median number of reads returned for Complete Mutation Mix (Seraseq®) libraries was 44 M, compared to the median of 33 M reads for Pan-cancer Reference Standard libraries. This translated to a median coverage depth of 6100X and 5000X, respectively.

Confident variant calling

- High performance demonstrated even at 0.5% variant AF
- Confident variant calling enabled by high specificity‡ and high sensitivity
- Reproducible variant calling exhibited in replicates across samples

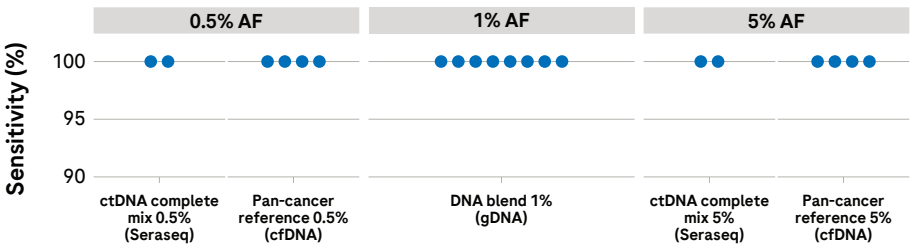

Figure 2. High sensitivity was exhibited across samples with 0.5%, 1%, and 5% AF.†

†Each blue dot represents a replicate. SNV calling sensitivity was demonstrated by using a blended sample of gDNA NA24631 (98%) and NA24149 (2%), targeting 1% AF, with characterized variants covered by the KAPA NHL Panel to assess variant calling performance for the germline workflow (DNA blend 1%: 10 true positive (TP); 8 replicates) and pre-fragmented reference cfDNA samples with known variants at specific AF covered by the KAPA HyperCap DS NHL Panel to assess somatic variant calling performance for the plasma cfDNA workflow (Seraseq® ctDNA complete mix AF 0.5% and AF 5% from SeraCare: 3 SNVs, 2 replicates each; cfDNA Pan-cancer Reference Standard AF 0.5% and 5% from Twist: 12 SNVs, 4 replicates each). For the germline workflow, 10 out of 10 (100%) TP were observed in all gDNA replicates. For the plasma cfDNA workflow, all expected SNVs were found in all cfDNA replicates that were investigated.

‡High SNV calling specificity was demonstrated by 0.06 errors every 10 Kb of the panel (as calculated by a set of 23 healthy donor samples). In the gDNA NA24149/NA24631 blend, 53 out of 53 true negative (TN) were observed in all eight replicates.

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

Highly sensitive minimal residual disease (MRD) analysis

- Background correction pipeline increases result confidence
- Confident longitudinal mutation positivity down to 0.05% AF
- Good reproducibility of reporter variant detection at AF of 0.01%

Table 1. Summary of longitudinal mutation analysis results for contrived cfDNA Pan-cancer reference standard samples*

Sample	n_mutations	total alt reads	p-value	decision
AF 0.5%	9	199	0.0001	100% positive
AF 0.1%	9	43	0.0001	100% positive
AF 0.05%	9	25	0.0001	100% positive
AF 0.01%	9	11	0.0001 – 0.2858	83% positive
WT (AF 0%)	9	8	0.0031 – 0.1217	100% negative

*Data generated from samples were subsequently processed using the three-stage KAPA bioinformatics analysis for longitudinal detection of ctDNA to demonstrate the use of the KAPA HyperCap DS NHL Panel for longitudinal analysis of NHL-associated variants in circulating tumor DNA. Reporter variants were successfully detected in all contrived T_N (subsequent samples after baseline) Pan-cancer samples. The Monte Carlo p-value[4,5] threshold for ctDNA positivity in simulated longitudinal samples was set at 0.003 since this was the lowest value observed in the wild type sample. The number of reporter variants with non-zero supporting reads, as well as the total number of supporting alt reads, drops as the expected AF % decreases from 0.5% to 0.01%. Mutation positivity was accurately called in all replicates of the AF 0.5%, AF 0.1%, and AF 0.05% samples (Monte Carlo p-values <0.003). For the AF 0.01% sample, mutation positivity was accurately called in five out of six replicates. All replicates of the wild type sample were called negative.

Gene list

The KAPA HyperCap DS NHL Panel targets 383 genes with complete or partial coverage, plus additional intergenic regions for a total capture size of 341 Kb.

- Cover multiple NHL subtypes with a single panel of genes and regions selected by experts
- Explore various research use cases such as ctDNA monitoring, MRD analysis, and cell-of-origin determination
- Increase ctDNA detection power by targeting content that was used in Phase 3 POLARIX study[3] to validate ctDNA as a prognostic biomarker

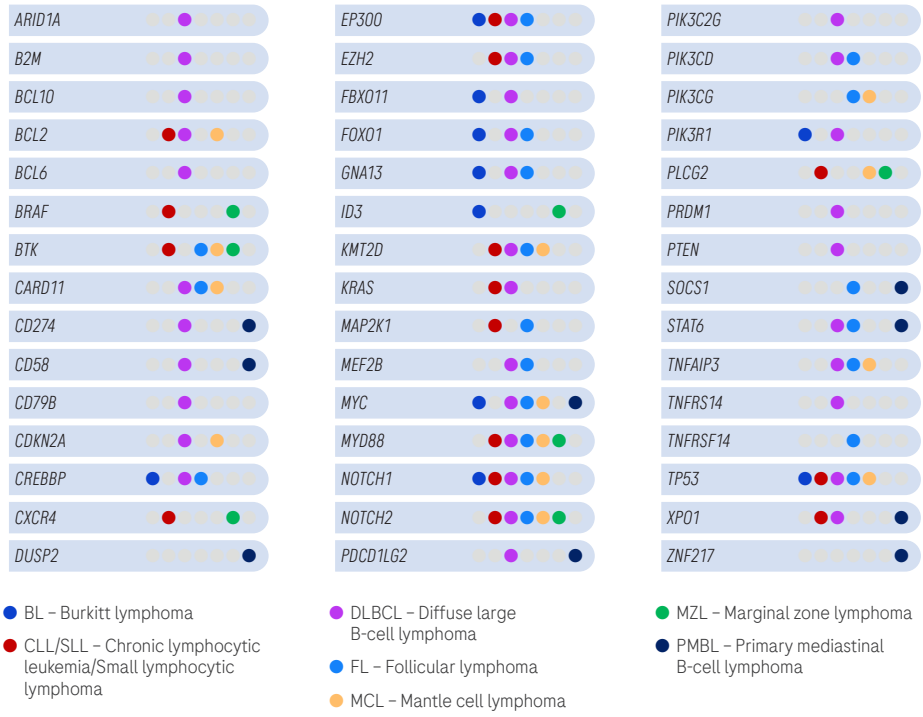

Figure 3. Genes known to be associated with NHL.†

†To access the full gene list, please visit https://sequencing.roche.com/us/en/products/group/design-share.html.

Ease of ordering

The KAPA HyperCap DS NHL panel is available in 24 and 96 sample sizes. For ease of ordering* there are 4 virtual kits (VKs) available that include the reagent kits that will be needed to build an in-house research workflow based on the *Longitudinal detection of non-Hodgkin lymphoma ctDNA white paper*.[1]

Please contact your local Roche sales representative to order these reagent kits to get started with NHL ctDNA monitoring research in your laboratory.

Material Number	Description	Samples	Workflow and Comments
10040084001	KAPA HyperCap DS NHL cfDNA 24 samples VK	24	Plasma cfDNA
10040076001	KAPA HyperCap DS NHL cfDNA 96 samples VK	96	Plasma cfDNA
10040068001	KAPA HyperCap DS NHL gDNA 24 samples VK	24	gDNA (plasma-depleted whole blood)
10040092001	KAPA HyperCap DS NHL gDNA 96 samples VK	96	gDNA (plasma-depleted whole blood)
09052593001	KAPA HyperChoice MAX 3Mb T1, 24 rxn	24	IRN: 1000028225
09052615001	KAPA HyperChoice MAX 3Mb T1, 96 rxn	96	IRN: 1000028225
Varies	KAPA HyperChoice MAX 3Mb T1	Varies	IRN: 1000028225, larger reaction pack sizes available—reach out to your local Roche sales representative

*To build an in-house NHL monitoring research workflow, one will have to order the VK per application along with the respective panel pack size (24 or 96 samples). The VKs include the required component kits such as cobas® cfDNA Sample Preparation Kit, KAPA NGS FFPE DNA QC Kit, KAPA HyperPrep Library Preparation Kit, KAPA HyperPure Beads, KAPA UDI Primer Mixes, 97 – 192, KAPA Universal UMI Adapter, KAPA HyperCapture Bead kit, KAPA HyperCapture Reagent kit, KAPA Probes resuspension buffer, KAPA NGS DNA Extraction Kit, and KAPA HyperPlus Library Preparation Kit.

References

1. Bermejo C, Agarwal P, Chien R et al. The KAPA HyperCap Design Share NHL Panel enables highly sensitive, longitudinal detection of non-Hodgkin lymphoma circulating tumor DNA. Roche white paper. MC--11981.
2. Chien, R. KAPA bioinformatics analysis for longitudinal detection of circulating tumor DNA. Roche white paper. MC--12095.
3. Herrera et al. Risk Profiling of Patients with Previously Untreated Diffuse Large B-Cell Lymphoma (DLBCL) By Measuring Circulating Tumor DNA (ctDNA): Results from the POLARIX Study. *Blood* 2022; 140 (supplement 1): 1297 – 1300. doi: https://doi.org/10.1182/blood-2022-157559.
4. Newman AM, Lovejoy AF, Klass DM, et al. Integrated digital error suppression for improved detection of circulating tumor DNA. *Nature Biotechnol* 2016;34(547 – 555). doi: 10.1038/nbt.3520.
5. Alkodsi A, Meriranta L, Pasenen A, Sirpa Leppä. ctDNAtools: An R package to work with sequencing data of circulating tumor DNA. *bioRxiv* 2020.01.27.912790. doi: 10.1101/2020.01.27.912790.

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