EuroClonality-NDC Assay

Quick Reference Guide

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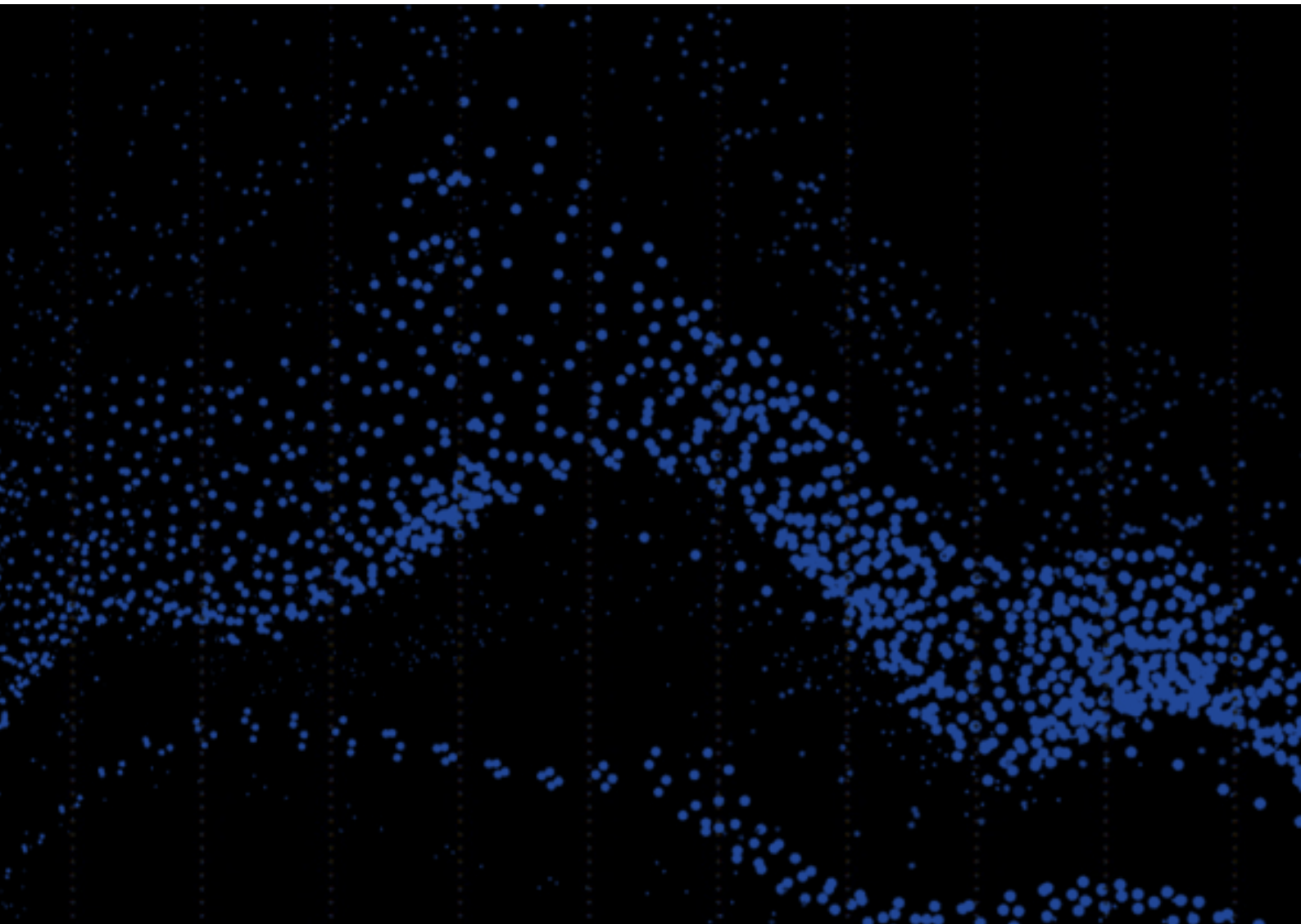
For **Research** Use Only. Not for use in diagnostic procedures.



EuroClonality-NDC Assay

This quick reference document is intended to guide the users through the instructions of use for the EuroClonality-NDC assay and associated laboratory procedures. Although every effort has been made to keep the information correct and up to date, we cannot guarantee that there are no unwilling errors or omissions. We encourage our customers to provide feedback which may lead to modifications and improvements in future versions of this document.

Warranties

Univ8 Genomics warrants that the EuroClonality-NDC assay as sold has passed the required internal quality controls and meets the performance characteristics specified in this document until the expiry date. This is provided the user stores the reagents according to the specifications and precisely follows all the instructions provided in this document and any additional instructions provided on the website.

No other warranties are provided, explicitly or implied and Univ8 Genomics does not accept any liability for any consequences, direct or indirect arising from the use of the EuroClonality-NDC assay or any of its associated third-party reagents, equipment or analytical tools.

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Introduction

Intended Use

The EuroClonality-NDC is a capture-based targeted next generation sequencing (NGS) assay with customised bioinformatics pipeline for research use only (RUO). It is intended for the integrated and qualitative detection of clonal immunoglobulin (IG) and T cell receptor (TR) rearrangements, translocations, copy number variants (CNV) and sequence variants from suspected cases of lymphoproliferative disorders or lymphoid populations. EuroClonality-NDC has been validated for use with genomic DNA (gDNA) isolated from peripheral blood, bone marrow, fresh-frozen tissue and formalin fixed paraffin embedded (FFPE) tissue.

The EuroClonality-NDC has undergone a large multi-centre validation, performed across seven European laboratories, in a wide range of samples from more than 15 categories of lymphoid malignancies and including high molecular weight (HMW) and FFPE gDNA. These results highlight the robustness of the assay as performed in academic or clinical laboratories with a varying degree of experience (from very experienced to first timers) with DNA capture hybridization protocols[1].

Test Principle

Figure 1 provides an overview of the KAPA HyperCap Workflow v3.0 [HyperPlus] (Roche Sequencing Solutions) used for the EuroClonality-NDC assay. Briefly, whole genome shotgun library preparation is performed from 100 ng of HMW or FFPE gDNA using the KAPA HyperPlus kit (Roche Sequencing Solutions, Pleasanton, CA, US). Hybridisation of the libraries to the EuroClonality-NDC enrichment probes is performed using the KAPA HyperCapture Reagent Kit and KAPA HyperCapture Bead Kit (Roche Sequencing Solutions). The enriched and amplified libraries are subsequently sequenced on the NextSeq 500/550 (Illumina, Cambridge, UK) using a 75 bp paired-end strategy performed on a 150-cycle NextSeq 500/550 Mid Output Kit (Illumina). Following completion of the sequencing run, the binary base call (BCL) files undergo conversion and demultiplexing to sample specific FASTQ files. The FASTQ file is the required input file for the bioinformatics analyses.

Limitations

- The EuroClonality-NDC assay is designed for research use only.
- Refer to the performance characteristics and technical details of the assay, which describes the limitations in the detection of the different types of genomic alterations tested.
- Performing a proficiency assessment using cell lines detailed in the "Supporting Information" section is recommended for laboratories as part of initial verification of the assay and for those with less experience in this technology.
- False negatives are more likely to occur in samples with a tumour infiltration close to the limits of detection for rearrangements, translocation, sequence variants or CNV being analysed.
- The EuroClonality-NDC assay is a qualitative assay and has not been validated for accurate quantification of genomic alterations.
- Lower quantity or quality of gDNA than recommended can lead to poor performance of the assay.
- Copy number analysis is only validated for HMW-DNA and it should be optimised in each laboratory setting, based on quality of DNA and number of samples per sequencing run.

Library Preparation	Target Enrichment	Sequencing
DNA (Enzymatic) Fragmentation	Sample Pooling	Denaturation and Dilution of Libraries
End Repair & A-Tailing	Sample Hybridisation with EuroClonality-NDC	Sequencing of Libraries
Universal Adapter Ligation	Post-Hybridisation Washes	
Pre-Capture PCR Amplification	Post-Capture PCR Amplification	**Analysis**
Quality Control of Libraries	Quality Control of Libraries	EuroClonality-NDC App

Figure 1 EuroClonality-NDC assay overview. *Library preparation can be completed on Day 1 followed by overnight hybridisation enabling the pooled enriched library to be ready for sequencing at the end of Day 2.*

EUROCLONALITY-NDC ASSAY

Kit Components

Prior to setting up the hybridisation make sure you have the reagent listed below. Please note each vial in the box contains sufficient material to perform four hybridisations. It is recommended that, on first use, the reactions are split into single-use (4 µL) aliquots to minimise freeze/thaw cycles.

Note: there is excess volume in the vial to allow 4 x 4 µL aliquots.

Catalogue #	Reactions per tube
U8001	4 (4 µL per rxn)

Kit Stability and Storage Conditions

The EuroClonality-NDC assay is shipped on dry ice. Upon receipt the kit should be stored at -15°C to -25°C. To minimise freeze-thaw cycles the EuroClonality-NDC panel can be aliquoted into single-use aliquots (we recommend doing this on first use/thaw).

Before You Begin

Before commencing library preparation check that all the required equipment, compatible labware and sufficient consumables are available. The user should ensure they are familiar with the protocol including the time required to perform the steps specified for each day of the workflow. Consideration should be given to the inclusion of control samples on each run e.g. high-quality reference gDNA with known alterations and a non-template control. It is recommended that laboratories introduce a methods-based proficiency run to ensure competency in performing the EuroClonality-NDC assay. A table of commercially available B and T cell lines previously used for a proficiency run in the multi-site validation of the EuroClonality-NDC assay is included in the Supporting Information along with tables listing expected rearrangements, translocations and copy number alterations[1].

Recommended DNA Sample Requirements

The EuroClonality-NDC assay has been validated and optimised for the DNA quality and quantity recommended in the table below, however the assay's robustness allows users to use lower than the recommended amounts of genomic HMW DNA or high-quality FFPE, without significantly compromising performance. Results for poor-quality samples (e.g. <500 bp median fragment size) can be significantly compromised and therefore higher DNA amounts are recommended where possible.

Recommended DNA integrity and quantity for optimal performance of the EuroClonality-NDC

DNA Type	DNA Integrity[a]	DNA Quantity Recommended[b]
High Molecular Weight	> 4,000 bp	100 ng
Formalin Fixed Paraffin Embedded	> 1,000 bp	100 ng
Formalin Fixed Paraffin Embedded	< 1,000 bp	200 ng

a DNA quality can be assessed by DNA fragment size and distribution.

b gDNA should be quantified using a method specific for dsDNA.

Interferences

For gDNA samples, a knowledge of the buffer composition the gDNA has been eluted in is required to ensure optimal fragmentation. Enzymatic fragmentation is sensitive to the presence of EDTA. If existing DNA samples have been eluted in an EDTA-containing buffer (e.g. Tris-EDTA buffer) then purification of the DNA (e.g. with magnetic beads or silica-membrane spin columns) and elution in ultra-pure H_2O is recommended. Alternatively, the addition of Conditioning Solution can mitigate the effect of the EDTA (refer to KAPA HyperPlus Kit Technical Data Sheet (Roche Sequencing Solutions) for details on preparing the working concentration of Conditioning Solution based on the EDTA concentration of the elution buffer). For prospective samples it is recommended to elute gDNA in ultra-pure H_2O to increase performance of the EuroClonality-NDC assay. If the gDNA has not been eluted in ultra-pure H_2O, the performance of the EuroClonality-NDC assay may be compromised.

Sample Quantity Assessment

The EuroClonality-NDC assay underwent a multi-site validation using both HMW and FFPE-derived gDNA material. The gDNA was extracted in multiple laboratories employing different DNA extraction systems.

Note: It is important not to use DNA extraction methods that produce significant amount of DNA degradation or single-stranded DNA molecules, as these will significantly hamper the efficiency of the library preparation.

Assessment of DNA quality for FFPE samples is recommended to determine the size distribution of the extracted gDNA. The gDNA quantity should be measured using a suitable fluorescent dye-based method with specificity for double stranded DNA. The quality and quantity of double stranded gDNA used for the library preparation can affect yield of the enriched library and impact downstream sequencing metrics.

PROTOCOL

The EuroClonality-NDC assay has been optimised and validated using the KAPA HyperCap (HyperPlus) Workflow v3.0 (Roche Sequencing Solutions) which can be accessed by contacting your Roche representative or service.sequencing@roche.com.

The following sections provide information regarding sample preparation and any modifications or specifications required for the EuroClonality-NDC assay using the Roche HyperCap Workflow v3.0.

Workflow Part 1: Library Preparation

For library preparation, batches of up to 24 libraries can be prepared per hybridisation reaction, which could be comprised of up to 22 samples along with a positive and a negative control.

Workflow Description	Chapter	Step	EuroClonality-NDC Specifications
Fragmentation	3	4	Fragmentation time: 22 min
End Repair & A-Tailing (ERAT)	3	6(a)	Use HyperPlus ERAT Enzyme Mix
Universal Adapter Ligation	3	7	No modifications
Post-Ligation Cleanup	3	8(c)	Incubation time: 15 min
Pre-Capture PCR Amplification	4	2.7	Perform a total of 6 cycles
Post-PCR Cleanup	4	3.3	Incubation time: 15 min
Amplified Library QC	4	4	No modifications

Workflow Part 2: Target Enrichment

For the Target Enrichment, all samples are pooled for the hybridisation by mixing equal amounts of each amplified library to achieve a total of 1.5 µg DNA for hybridisation (e.g., approximately 65 ng per sample if using 23 samples + NTC). If the number of samples to hybridise is low, you should use at least the recommended amount of gDNA in the library preparation to ensure sufficient library is obtained to reach the combined 1.5 µg required.

Workflow Description	Chapter	Step	EuroClonality-NDC Specifications
Library Enrichment	5	2.2	Mix equal amount of each amplified library to achieve a total of 1.5 µg DNA for hybridisation.
Library Enrichment	5	3	Prepare Hybridization Master Mix using volumes for Target Enrichment designs < 40 Mbp
Prepare Capture Beads	6	2	Follow directions for Target Enrichment designs < 40 Mbp
Bind hybridised DNA to Capture Beads	6	3	No modifications
Wash Capture Beads Plus Bead-Bound DNA	6	4	No modifications
Post-Capture PCR Amplification	7	3	Perform a total of 11 cycles
Post-PCR Clean-up	7	4.4	Incubation time: 15 min
Amplified Library QC	7	5	No modifications

Workflow Part 3: Sequencing

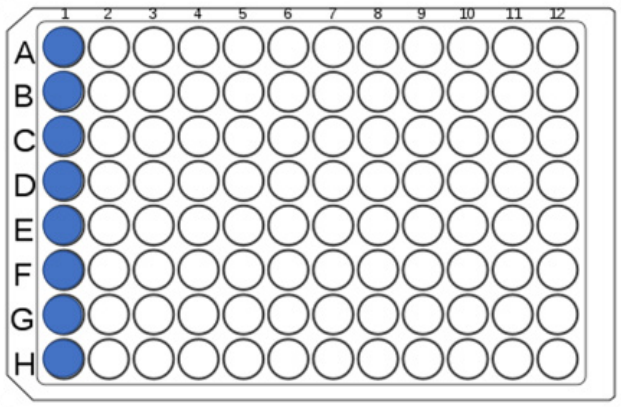
The EuroClonality-NDC assay was validated for use with the NextSeq 500/550 system (Illumina Inc) using a 75 bp paired-end strategy performed on a 150-cycle NextSeq 500/550 Mid Output Kit (Illumina Inc). If using other sequencing instruments/ cartridges, the users must keep the 75 bp paired-end sequencing protocol to avoid additional artefacts and reduce the impact on copy number analysis.

Prepare the amplified and enriched pooled library for sequencing on the NextSeq 500/550 by adhering to the Illumina NextSeq Denature and Dilute Guidelines using the following parameters:

- Protocol A (Standard Normalization Method)
- Final dilution of library is to 1.5 pM for Mid Output kits
- Final PhiX (sequencing control) spike-in percentage is 1% of the final library.

The sequencing recommendations detailed below are for the NextSeq 500/550 using the 'Run Monitoring and Storage' configuration option. The 'Run Monitoring and Storage' option requires the uploading of a sample sheet during setting up of a sequencing run. The 'Run Monitoring Only' option can be run if bcl2fastq conversion is to be performed in a local server.

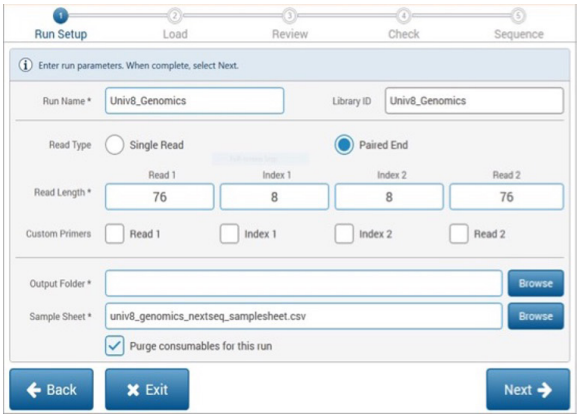
An editable sample sheet for use with the NextSeq analyser, is available from the Resources section on the website. The sample sheet is complete with all 96 available indexes in the KAPA UDI Primer Mix kit with the layout reflecting samples added to a 96 well plate in a column-based pattern (see figure). Delete all rows containing UDI primers not utilised on the current sequencing run. Additionally, investigator name, experiment name and date can also be modified. Further details on sample sheets, such as permitted characters for sample names (note that illegal characters will result in errors), can be found in the Illumina Sequencing Sample Sheet Format Specifications.



Sample_Well	I7_Index_ID	index	I5_Index_ID	index2	Sample_Project	Description
A01	i7_UDIP01	TGGATCGA	i5_UDIP01	AGCTAGGT	EuroClonality-NDC	SampleSheetTemplateforNextSeq
B01	i7_UDIP02	CAAGCTAG	i5_UDIP02	GATCGAAC	EuroClonality-NDC	SampleSheetTemplateforNextSeq
C01	i7_UDIP03	GTACCAAG	i5_UDIP03	GAACCATG	EuroClonality-NDC	SampleSheetTemplateforNextSeq
D01	i7_UDIP04	ACGTTGGA	i5_UDIP04	AGGTTGCA	EuroClonality-NDC	SampleSheetTemplateforNextSeq
E01	i7_UDIP05	TCGTGGTA	i5_UDIP05	ATGGTGCT	EuroClonality-NDC	SampleSheetTemplateforNextSeq
F01	i7_UDIP06	CTACAACG	i5_UDIP06	GCAACATC	EuroClonality-NDC	SampleSheetTemplateforNextSeq
G01	i7_UDIP07	GTTCAAGG	i5_UDIP07	GGAACTTG	EuroClonality-NDC	SampleSheetTemplateforNextSeq
H01	i7_UDIP08	ACCTGGAA	i5_UDIP08	AAGGTCCA	EuroClonality-NDC	SampleSheetTemplateforNextSeq
A02	i7_UDIP09	TCTTAGCG	i5_UDIP09	GCGATTCT	EuroClonality-NDC	SampleSheetTemplateforNextSeq
B02	i7_UDIP10	CTCCGATA	i5_UDIP10	ATAGCCTC	EuroClonality-NDC	SampleSheetTemplateforNextSeq
C02	i7_UDIP11	GTCCTACT	i5_UDIP11	TCATCCTG	EuroClonality-NDC	SampleSheetTemplateforNextSeq

On the NextSeq Control Software:

1. Click the 'Sequence' icon
2. On the next window (Select run, monitoring and storage mode) check the box beside the 'Use BaseSpace Sequencing Hub'
3. Select 'Run Monitoring and Storage' and click Next
4. You will be prompted to log in to your BaseSpace account
5. Following successful logging on, select the respective workgroup (if applicable) and click Next
6. On the next window (shown below) select Setup Runs Using 'Manual' and enter Run Name and Library ID.



7. For read type select 'Paired End'
8. For read length input the following parameters for each of the fields:
   a. Read 1: 76 cycles; Index 1: 8 cycles; Index 2: 8 cycles Read 2: 76 cycles;
9. Select Browse and choose the output folder where the data is to be stored for your facility
10. Select Browse and navigate to the sample sheet which has been edited for the specific run to be sequenced
11. Check the box to 'Purge consumables for this run'.
12. Proceed with the remainder of the NextSeq Control Software prompts for preparing the instrument for sequencing.

For a detailed step-by-step protocol with easy to use instructions and tips please visit https://link.springer.com/protocol/10.1007/978-1-0716-2115-8_9.[2]

PERFORMANCE SPECIFICATIONS

The validation and performance characterisation of the EuroClonality-NDC was carried out on 280 samples comprising a range of different B and T cell malignancies using a different version of library preparation and hybridisation methodologies[1]. The EuroClonality-NDC assay and analysis workflow have been updated since to improve performance and target coverage.

Overall performance of the EuroClonality-NDC assay was assessed by identifying underperforming regions using DNA extracted from a panel of 91 normal tissue samples. Regions were classed as underperforming if coverage was >2 standard deviations (SD) below the mean in ≥50% samples. Only 10 underperforming coding regions have been identified that could affect mutation detection in the genes and exons shown in the table below.

Gene Name	Chromosome	Exon
ARID1A	chr1	6
CARD11	chr7	12
JAK2	chr9	15
TRAF2	chr9	1
PAX5	chr9	10
STAT5B	chr17	18
TCF3	chr19	7
BTK	chrX	18
PHF6	chrX	6
PHF6	chrX	3

Expected Metrics:

The table below shows the approximate expected metrics of the EuroClonality-NDC assay based on 22 samples per hybridisation reaction (plus controls) and run on a NextSeq500/550 (Mid-Output 150 cycles cartridge) at 2 x 75bp. These can vary depending on DNA quality and quantity, technical factors in each laboratory, differences in equipment, etc.

Parameter	Expected HMW-DNA	Expected FFPE-DNA
Library yield (ng)	3,000 ng (range: 500-6,000)	
Library average fragment size (bp)	330 bp (range: 275 – 450 bp)	290 bp (range: 245 – 330 bp)
Average on/near target bases (%)	80% (range: 70 - 85%)	
Duplicate reads (%)	40% (range: 30 – 50%)	
Mean unique target coverage (reads)	1,000x (range: 500 – 1,500x)	

This improved version of the EuroClonality-NDC assay and workflow underwent a verification bridging study with gDNA extracted from 22 FFPE and 44 HMW samples which were included in the original validation study, and which comprised of a broad range of B and T cell malignancies. The following table represents the observed analytical performance:

	Sensitivity	Specificity	Limit of Detection
IG/TR Rearrangements	>97%	>97%	5% VAF
Translocation	>95%	>99%	5% VAF
SNV	>99%	>99%	4% VAF
CNV*	>95%	>97%	40%**

*SNV: single nucleotide variant; CNV: copy number variation. *CNV have been validated in HMW-DNA for trisomy 12 and deletions of 11q, 13q and 17p. No performance data available for FFPE or other genomic regions.**Deletions can be detected if present in ≥ 40% of the cells. The threshold baseline fold-change for deletions detected by EuroClonality-NDC is 0.8 and 1.3 for losses and gains, and 0.7 and 2.0 for deletions and amplifications, respectively.*

SUPPORTING INFORMATION

EuroClonality-NDC Assay Composition

List of genes with full coding regions mutation assessment on EuroClonality-NDC

ARID1A	CDKN2B	H1-2	MAPK1	RHOA	TNFAIP3
ATM	CDKN2C	H1-3	MYC	RUNX1	TP53
B2M	CKS1B	H1-4	PAX5	SAMHD1	TRAF2
BCL2	EP300	ID3	PHF6	SOCS1	TRAF3
BTG1	ERG	KLF2	POT1	TCF3	WT1
BTK	FAT1	KMT2D	PTEN	TENT5C	
CDKN2A	H1-5	MAP3K14	PTPRD	TET2	

List of genes with select regions for mutation assessment on EuroClonality-NDC

Gene Name	Region Covered	Gene Name	Region Covered
ABL1	Exons 4-9	JAK1	Exons 12-15
ASXL1	Hotspot in last exon	JAK2	Exons 12-15
BIRC3	Exons 8 and 9	JAK3	Exons 11-24
BRAF	Exons 11 and 15	KIT	Exons 9, 11, 13, 17 and 18
CARD11	Exons 2-16	KRAS	Exons 2, 3 & 4
CCND1	Exon 1	MAP2K1	Exons 1-8
CCND3	Exon 5	MEF2B	Exons 2-4
CD79A	Exons 4 & 5	MYD88	Exon 3-5
CD79B	Exons 4-6	NFKBIE	Exon 1
CREBBP	Exons 2-30	NOTCH1	Exons 26, 27 & 34
CXCR4	Hotspot in exon 2	NOTCH2	Exon 34
DIS3	Exons 1-3 and 9-18	NRAS	Exons 2-4
DNMT3A	Exons 8-23	NT5C2	Exons 9-15
EGR2	Hotspot in exon 2	PIK3CA	Exons 2, 5 10 & 21
EZH2	Exons 15-17	PLCG1	Exons 1, 11, 15-17, 19 and 29
FBXW7	Exons 1-12	PLCG2	Exons 19, 20 & 24
FOXO1	Exon 1	SF3B1	Exons 12-17
IDH1	Exon 4	STAT3	Exons 10-16 and 20-21
IDH2	Exons 4 and 7	STAT5B	Exons 13-19
IKZF1	Exons 2-7	STAT6	Exons 5, 9, 11, 12 and 14
IL7R	Exon 6	XPO1	Exons 15-16
IRF4	Exons 2-3		

List of additional regions assessed for translocations on EuroClonality-NDC

Chromosome	Gene Name	Chromosome	Gene Name
chr1	STIL	chr11	CCND1
chr2	ALK	chr11	BIRC3
chr3	BCL6	chr11	KMT2A
chr6	DUSP22	chr18	BCL2
chr8	MYC	chr19	TCF3
chr9	CD274	chrX	CRLF2
chr9	PDCD1LG2		

List of additional chromosome regions assessed for copy number variation on EuroClonality-NDC

9pTEL chr9	9pCEN chr9	11qCEN chr11	11qTEL chr11	RB1 chr13
13qMDR[a] chr13	13qTEL chr13	17pTEL chr17	17pCEN chr17	

[a] *Probes span a minimally deleted region (MDR) comprising the DLEU1 and DLEU2 genes*

Materials required but not provided

The protocol has been optimised and validated using the KAPA HyperCap (HyperPlus) Workflow v3.0 (Roche Sequencing Solutions) for library preparation and target enrichment followed by the sequencing reagents (Illumina Inc) listed below.

Kit Description	Company	Catalogue No.	Kit Size
KAPA HyperPlus Kit	Roche Sequencing Solutions, Inc.	07962428001	96 rxn*
KAPA Universal Adapter	Roche Sequencing Solutions, Inc.	09063781001	96 rxn
KAPA UDI Primer Mixes	Roche Sequencing Solutions, Inc.	09134336001	96 rxn
KAPA HyperPure Beads	Roche Sequencing Solutions, Inc.	08963851001	60 mL*
KAPA HyperCapture Bead Kit	Roche Sequencing Solutions, Inc.	09075780001	24 rxn*
KAPA Hypercapture Reagent Kit	Roche Sequencing Solutions, Inc.	09075810001	24 rxn*
NextSeq 500/550 Mid Output Kit v2.5 (150 Cycles)	Illumina, Inc.	20024904	NA
PhiX Sequencing Control V3	Illumina, Inc.	FC-110-3001	NA

** Different kit sizes are available for these products*

Proficiency Samples

Recommended Cell Lines for Proficiency Run

Cell Line	B/T Cell Line	Disease	ATCC[a]	DMSZ[b]
CA-46	B	BL	CRL-1648	ACC 73
H929	B	PCM	CRL-9068	ACC 163
HAL-01	B	B-ALL	-	ACC 610
Namalwa	B	BL	CRL-1432	ACC 24
REH	B	B-ALL	CRL-8286	ACC 22
RS4;11	B	B-ALL	CRL-1873	ACC 508
TOM-1	B	B-ALL	-	ACC 578
CML-T1	T	T-ALL	-	ACC 7
Loucy	T	T-ALL	CRL-2629	ACC 394
P12-Ichikawa	T	T-ALL	-	ACC 34

[a] *https://www.lgcstandards-atcc.org*

[b] *https://www.dsmz.de*

Proficiency Samples : Expected results using EuroClonality-NDC (part I)

Cell Line	Locus	Rearrangement	Structural Variations (SV)	Copy number variants (CNV)
CA46	IGH	IGHD3-9>IGHJ4	t(8;14) MYC/IGHswc	Amp/Gain: CKS1B, 7q, 13q, DIS3 Del/Loss: CCND3, 13qTEL
	IGH	IGHV5-51>IGHJ4		
	IGK	IGKV2-28>IGKJ2		
H929	TRB	TRBD1>TRBJ2-2	t(4;14) NSD2(MMSET)/ IGHswc t(8;20) MYC/FAM242A	Amp/Gain: CKS1B, 11q, BCL2, TCF3, 20q Del/Loss: TENT5C, NOTCH2, BCL6, CARD11, chr9, ERG2, WT1, KRAS, 13q, PLCG2
	IGH	IGHD1-1>IGHJ3		
	IGH	IGHV3-9>IGHJ5		
	IGK	IGKV3-15>IGKJ1		
HAL-01	IGH	IGHV1-45>IGHJ6	t(17;19) HLF/TCF3 t(14;14)IGHswc/IGHD	Amp/Gain: None Del/Loss: None
	IGK	IGKV1-39>Kde		
	IGK	IGKV2-28>Kde		
	TRG	TRGV3>TRGJ1 / 2		
Namalwa	TRB	TRBD1>TRBJ2-5 / 2-6	None	Amp/Gain: CKS1B, PIK3CA Del/Loss: RHOA, IRF4, 17p/TP53
	IGH	IGHV1-69>IGHJ2		
	IGH	IGHV4 (4-4)>IGHJ4		
	IGK	IGKV4-1>IGKJ4		
	IGL	IGLV4-60>IGLJ2=IGLJ3		
	IGK	IGKV2-30>Kde		
	IGK	intron>Kde		
REH	TRB	TRBV27>TRBD2	t(1;2) HYDIN2/IGKV	Amp/Gain: chr16, ERG Del/Loss: RHOA, MYD88, CDKN2A/B (Hom)
	TRD	TRDV2>TRDD3		
	TRB	TRBV20-1>TRBJ2-7		
	TRA+D	TRDV2>TRAJ29		
	TRA+D	TRDV2>TRAJ61		
	TRG	TRGV4>TRGJ2		
	TRG	TRGV9>TRGJ2		
	IGH	IGHV3-15>IGHJ6		
	IGK	IGKV2-29>IGKJ4		
	IGL	IGLV2-8>IGLJ2=IGLJ3		
	IGL	IGLV3-21>IGLJ2=IGLJ3		
	IGK	IGKV3-20>Kde		
	IGK	intron>Kde		
RS4,11	TRA+D	TRDV2>TRAJ53	t(4,11) AFF1/KMT2A	Amp/Gain: 7q Del/Loss: 7p, CDKN2A/B (Hom)
	TRA+D	TRDV2>TRAJ29		
	IGH	IGHV3-20>IGHJ5		
	IGH	IGHV6-1>IGHJ4		
	IGK	IGKV4-1>IGKJ1		
	IGL	IGLV4-3>IGLJ2=IGLJ3		
	IGK	IGKV7-3>Kde		

Proficiency Samples : Expected results using EuroClonality-NDC (part I I)

Cell Line	Locus	Rearrangement	Structural Variations (SV)	Copy number variants (CNV)
TOM-1	TRD	TRDV2>TRDD3	IGHswc/IGHV	Amp/Gain: MYC Del/Loss: CARD11, CDKN2A, PAX5
	TRG	TRGV5>TRGJ2		
	IGH	~IGHV4 (4-55)>IGHJ6		
CML-T1	TRA	TRAV2>TRAJ3	t(6;7) AHI1/TRBJ	Amp/Gain: None Del/Loss: CDKN2A (Hom), CDKN2B(Het), 11q
	TRA	TRAV1-2>TRAJ3		
	TRB	TRBV19>TRBJ2-5		
	TRG	TRGV4/V2>TRGJ2		
	TRG	TRGV4/V2>TRGJ1		
Karpas299	TRB	TRBD1>TRBJ1-6	t(2;5) ALK/TLX3_NPM1	Amp/Gain: IRF4, 16p Del/Loss: CKS1B, 10q, 11q, 17p/TP53
	TRA	TRAV26-1>TRAJ10		
	TRA	TRAV1-1>TRAJ35		
	TRB	TRBV20-1>TRBJ2-7		
	TRG	TRGV2>TRGJP2		
	TRG	TRGV8>TRGJ2		
Loucy	TRD	TRDD2>TRDJ1	None	Amp/Gain: None Del/Loss: TNFAIP3, CDKN2A, CDKN2B, ABL1, PTEN, 16p
	TRA+D	TRAV23/DV6>TRDJ2		
	TRB	TRBV5-6>TRBJ2-1		
	TRB	TRBV20-1>TRBJ2-2		
	TRG	TRGV9>TRGJ2		
	TRG	TRGV2>TRGJ2		
P12_Ichikawa	TRB	TRBD1>TRBJ1-5	t(7;19) TRBJ-TRBV/LYL1	Amp/Gain: ID3, ARID1, chr6, CD79A, chr20 Del/Loss: 9p(Het), CDKN2A (Hom), KRAS, B2M
	TRB	TRBV6-5>TRBJ2-1		
	TRB	TRBV10-1>TRBJ2-3		
	TRD	TRDV1>TRDJ1		
	TRG	TRGV4>TRGJ2		
	TRG	TRGV9>TRGJ2		

Additional results for sequence variants and example outputs for cell lines are available under the "Resources" section on the website

Abbreviations

ALL	Acute Lymphoblastic Leukaemia
BCL	Binary base call file
BL	Burkitts' Lymphoma
CNV	Copy number variants
EDTA	Ethylenediaminetetraacetic acid
FFPE	Formalin fixed paraffin embedded
HMW	High-molecular weight
IG	Immunoglobulin
PCM	Plasma Cell Myeloma
QC	Quality control
SNV	Single nucleotide variants
TR	T cell receptor
WHO	World Health Organisation

References

1. Stewart JP, Gazdova J, Darzentas N, Wren D, Proszek P, Fazio G, Songia S, Alcoceba M, Sarasquete ME, Villarese P, van der Klift MY, Heezen KC, McCafferty N, Pal K, Catherwood M, Kim CS, Srivastava S, Kroeze LI, Hodges E, Stamatopoulos K, Klapper W, Genuardi E, Ferrero S, van den Brand M, Cazzaniga G, Davi F, Sutton LA, Garcia-Sanz R, Groenen PJTA, Macintyre EA, Brüggemann M, Pott C, Langerak AW, Gonzalez D; EuroClonality-NGS Working Group. Validation of the EuroClonality-NGS DNA capture panel as an integrated genomic tool for lymphoproliferative disorders. Blood Adv. 2021 Aug 24;5(16):3188-3198. doi: 10.1182/bloodadvances.2020004056. PMID: 34424321.

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FAQ

1. **Can I run the EuroClonality-NDC assay in a MiSeq or NovaSeq instrument, or with different read length?**
It is critical to maintain the 2 x 75bp read structure to avoid generating additional sequencing artefacts. It is possible, and might be more suitable for some laboratories with different projects and different throughputs, to run different sample numbers in different sequencers. We recommend scaling up or down according to the performance metrics expected for the sequencer/cartridge combination (e.g. maximum 5-7 samples for a MiSeq v3). However, the analysis App has been specifically validated using the NextSeq500/550 with a 150 cycles mid-output cartridge and 2x 75bp reads, and therefore some differences in performance may be expected. In particular, thresholds for clonality and translocation detection can significantly change, as well as performance of copy number analysis.

2. **Can I use less than 22 samples (plus controls) per hybridisation run?**
Yes, you can run any number of samples from 1 to 24 (if you don't use controls). In fact, for applications such as cell-free DNA (cfDNA) analysis, we recommend using not more than 8 samples per run (depending on starting cfDNA quantity).

3. **Can I use more than 22 samples (plus controls) per run?**
We don't recommend using more than 22 samples (plus controls) per hybridisation run, as this can affect the quality and performance of the assay. If you want to run > 24 samples, you can run multiple hybridisations of up to 24 each and then pool all enriched DNA libraries into one sequencing run, provided samples across all the pooled enriched libraries have different KAPA UDI Primer mixes (Please note this may require a run with a higher sequencing capacity, e.g., NovaSeq. See FAQ #1).

4. **What should be the final enriched library concentration prior to sequencing?**
For FFPE-derived DNA samples the final enriched library should ideally be between 2-4 ng/µL, while for HMW-derived DNA it should be higher, up to 5-8 ng/µL. These values are just for guidance and the actual results will depend on quality of DNA and performance of the library. If the values obtained are consistently well below the above, we recommend troubleshooting the library preparation and hybridisation processes.

5. **Can I use less than 4 µL of probe mix for hybridisation if I have low number of samples?**
No, the assay is designed to work with 4 µL of probe mix per hybridisation reaction, irrespective of the number of samples hybridise (from 1 to 24).

Revision History

Release	Summary of change	Date
2100011 r01	First released	September 2021
2100011 r02	Updated references, updated FAQs and minor corrections	February 2024

Technical Assistance

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