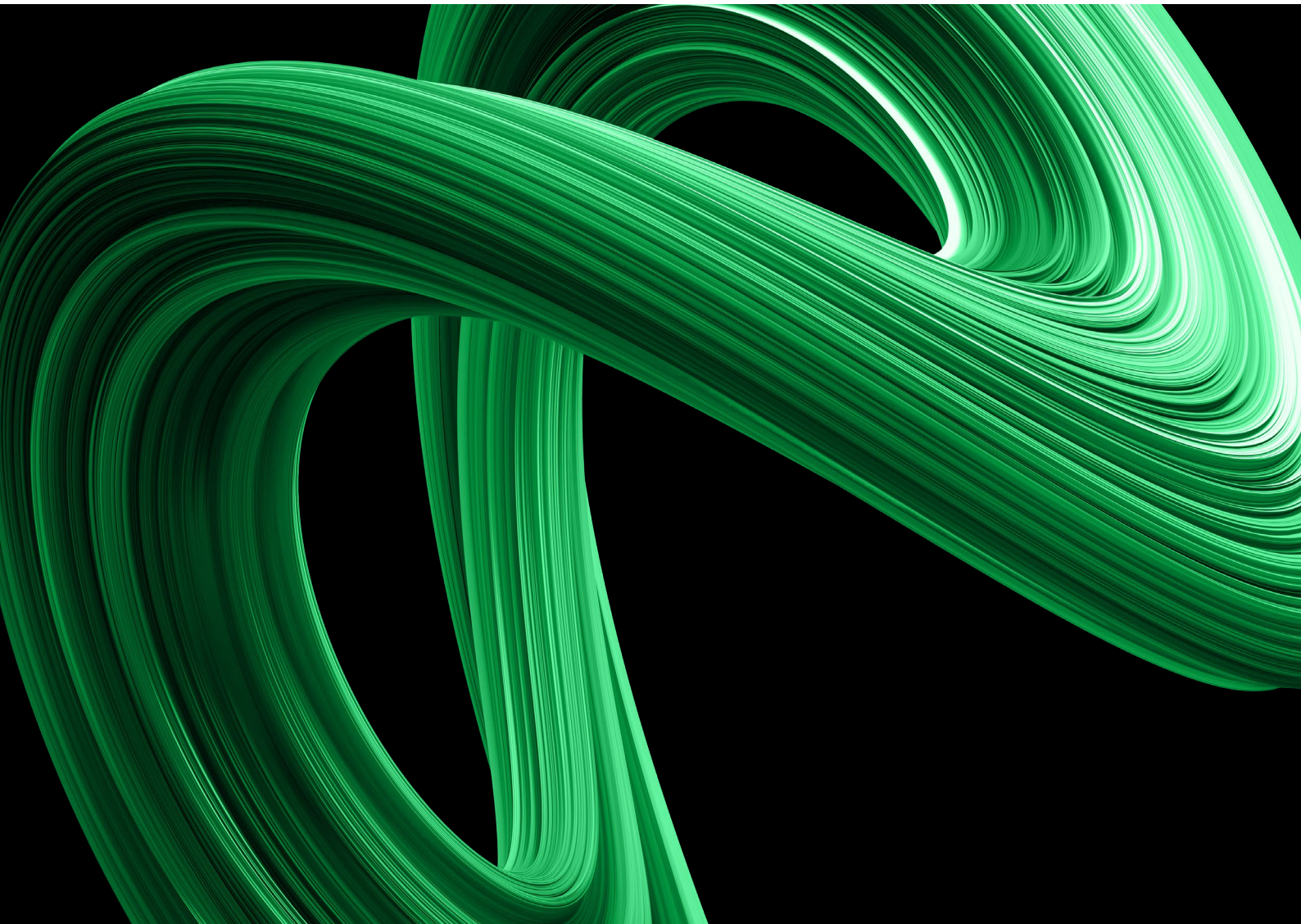
univ8® GENOMICS | MYELOID NDC

Myeloid-NDC Assay

Quick Reference Guide

Release: February 2024

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



Myeloid-NDC Assay

This quick reference document is intended to guide the users through the instructions of use for the Myeloid-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 Myeloid-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 Myeloid-NDC assay or any of its associated third-party reagents, equipment or analytical tools.

Trademarks

All trademarks are the property of their respective owners.

Copyright

Table of Contents

Introduction

Intended Use

The Myeloid-NDC assay is a capture-based targeted next generation sequencing (NGS) laboratory assay combined with a customised bioinformatics analysis application for research use only (RUO). It is intended for the integrated and qualitative detection of chromosomal translocations, copy number variants (CNV) and sequence variants from suspected or confirmed cases of myeloid malignancies. The Myeloid-NDC assay has been validated for use with high molecular weight (HMW) genomic DNA (gDNA).

The Myeloid-NDC assay has been designed as an integrated tool covering most genomic alterations recommended for the analysis of acute myeloid leukaemias (AML), myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN)[1-4]. The Myeloid-NDC assay provides a simple solution to allow laboratories to produce integrated reports according to the newest guidelines and recommendations, using just a single DNA sample. Detection of translocations (fusion genes) is included in the assay, with no need for an RNA sample.

The Myeloid-NDC assay utilises the same technology as the EuroClonality-NDC assay, which had undergone a large multi-centre validation across seven European laboratories[5]. This means that laboratories can now process all their suspected haematological malignancy samples at the same time using a universal protocol, reducing labour costs, increasing economy of scales, and speeding turnaround time to produce fully integrated genomic reports.

Test Principle

Figure 1 provides an overview of the KAPA HyperCap Workflow v3.0 [HyperPlus] (Roche Sequencing Solutions) used for the Myeloid-NDC assay. Briefly, whole genome shotgun library preparation is performed from 100 ng of HMW gDNA using the KAPA HyperPlus kit (Roche Sequencing Solutions, Pleasanton, CA, US). Hybridisation of the libraries to the Myeloid-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

- Refer to the performance characteristics and technical details of the assay, describing 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 type of technology.
- False negatives are more likely to occur in samples with a tumour infiltration close to the limit of detection.
- The Myeloid-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 may need to 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 Myeloid-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	Myeloid-NDC App

Figure 1 Myeloid-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.*

MYELOID-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
U8002	4 (4 µL per rxn)

Kit Stability and Storage Conditions

The Myeloid-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 Myeloid-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 Myeloid-NDC assay. A table of commercially available cell lines and reference material used for validation of the Myeloid-NDC assay is included in the "Supporting Information" section, along with tables listing expected translocations, sequence variants and copy number variants.

Recommended DNA Sample Requirements

The Myeloid-NDC assay has been validated and optimised for use with 100 ng of high molecular weight (HMW) gDNA eluted in ultra-pure double-distilled H_2O. Enzymatic fragmentation is sensitive to the presence of EDTA. If existing gDNA 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 Myeloid-NDC assay. If the gDNA has not been eluted in ultra-pure H_2O, copy number analysis using the Myeloid-NDC assay may be compromised.

PROTOCOL

The Myeloid-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 Myeloid-NDC assay using the Roche HyperCap Workflow v3.0.

Workflow Part 1: Library Preparation

For library preparation, batches of 8 to 24 libraries can be prepared per hybridisation reaction, with the recommended multi-plexing being 16 samples along with a positive and a negative control.

Workflow Description	Chapter	Step	Myeloid-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.

Workflow Description	Chapter	Step	Myeloid-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

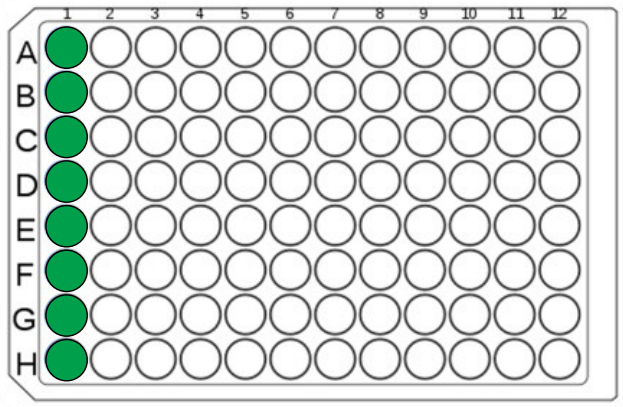
The Myeloid-NDC assay was validated for use with the NextSeq 500/550 system (Illumina Inc) using a 75 bp paired-end strategy performed on a 150-cycle NextSeq 500/550 Mid Output Kit (Illumina Inc). If using other sequencing instruments/cartridges, the users must keep the 75 bp paired-end sequencing protocol 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.

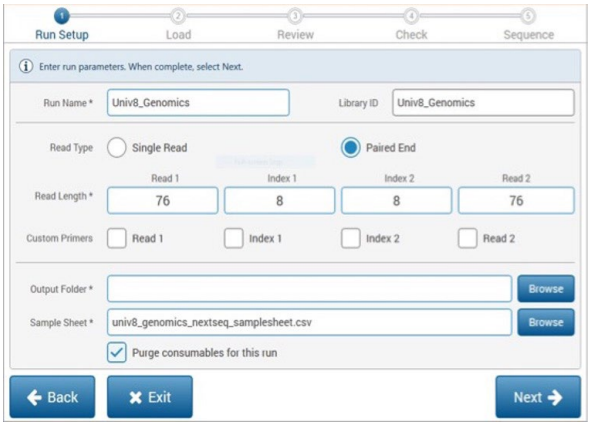
An editable sample sheet for use with the NextSeq analyser, is available from the Resources section on the website. The sample sheet is complete with all 96 available indexes in the KAPA UDI Primer Mix kit with the layout reflecting samples added to a 96 well plate in a column-based pattern (see figure). 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_Wel	I7_Index_ID	index	I5_Index_ID	index2	Sample_Project	Description
A01	i7_UDIP01	TGGATCGA	i5_UDIP01	AGCTAGGT	Myeloid-NDC	SampleSheetTemplateforNextSeq
B01	i7_UDIP02	CAAGCTAG	i5_UDIP02	GATCGAAC	Myeloid-NDC	SampleSheetTemplateforNextSeq
C01	i7_UDIP03	GTACCAAG	i5_UDIP03	GAACCATG	Myeloid-NDC	SampleSheetTemplateforNextSeq
D01	i7_UDIP04	ACGTTGGA	i5_UDIP04	AGGTTGCA	Myeloid-NDC	SampleSheetTemplateforNextSeq
E01	i7_UDIP05	TCGTGGTA	i5_UDIP05	ATGGTGCT	Myeloid-NDC	SampleSheetTemplateforNextSeq
F01	i7_UDIP06	CTACAACG	i5_UDIP06	GCAACATC	Myeloid-NDC	SampleSheetTemplateforNextSeq
G01	i7_UDIP07	GTTCAAGG	i5_UDIP07	GGAACTTG	Myeloid-NDC	SampleSheetTemplateforNextSeq
H01	i7_UDIP08	ACCTGGAA	i5_UDIP08	AAGGTCCA	Myeloid-NDC	SampleSheetTemplateforNextSeq
A02	i7_UDIP09	TCTTAGCG	i5_UDIP09	GCGATTCT	Myeloid-NDC	SampleSheetTemplateforNextSeq
B02	i7_UDIP10	CTCCGATA	i5_UDIP10	ATAGCCTC	Myeloid-NDC	SampleSheetTemplateforNextSeq
C02	i7_UDIP11	GTCCTACT	i5_UDIP11	TCATCCTG	Myeloid-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 onto your BaseSpace account
5. Following successfully logging, 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.

A detailed step-by-step protocol with easy to use instructions and tips for the EuroClonality-NDC assay -which is fully compatible with the Myeloid-NDC assay- is available here: https://link.springer.com/protocol/10.1007/978-1-0716-2115-8_9.[6]

PERFORMANCE SPECIFICATIONS

Expected Metrics:

The table below shows the approximate expected metrics of the Myeloid-NDC assay based on 16 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, performance of libraries, etc.

Parameter	Expected value
Library yield (ng)	3,000 (range: 1,000-6,000)
Library average fragment size (bp)	330 (range: 275-450)
Average on/near target bases (%)	80% (range: 70%-85%)
Duplicate reads (%)	25% (range: 15%-35%)
Mean target unique coverage (reads)	1,000x (range: 500x-1,500x)

The following table represents the observed analytical performance for the Myeloid-NDC assay:

	Sensitivity	Specificity	Limit of Detection
SV	>95%	>99%	5%
VAR	>99%	>99%	4%
CNV*	>95%	>97%	40% (tumour cells)

SV: structural variants, VAR: sequence variants, CNV: copy number variants.
**The threshold baseline fold-change for deletions detected by Myeloid-NDC is 0.8 and 1.3 for losses and gains, and 0.7 and 2.0 for deletions and amplifications, respectively.*

SUPPORTING INFORMATION

Myeloid-NDC Assay Composition

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

Gene Name	Region Covered
ABL1	All coding
ANKRD26	Exon 1
ARAF	Exons 4, 7, 10, 15
ASXL1	All coding
ASXL2	Exons 9-13
ATG2B	Exon 1
ATRX	All coding
BCOR	All coding
BCORL1	All coding
BRAF	Exons 11, 12, 15
CALR	Exons 8, 9
CBL	Exons 4-9
CBLB	Exons 8-17
CCND2	All coding
CCND3	Exon 5
CDKN1B	All coding
CDKN2A	All coding
CDKN2B	All coding
CDY1B	Exon 1 (partial)
CEBPA	All coding
CHEK2	All coding
CREBBP	All coding
CSF3R	Exons 14-17
CSNK1A1	All coding
CTCF	Exons 3 (partial), 4-8
CTNNA1	Exons 6-17
CUX1	All coding
DDX41	All coding
DHX15	Exons 2-5
DIS3	All coding
DNMT3A	Exons 4-23
EP300	All coding
ERBB3	Exons 3-8, 15, 22-24
ETNK1	Exon 3
ETV6	All coding
EZH2	All coding
FBXW7	All coding
FLT3	Exons 11, 13-15, 16, 20
GATA1	All coding
GATA2	All coding
GATA3	Exon 6
GNAS	All coding
GNB1	Exons 5-6
HRAS	Exons 2-4

Gene Name	Region Covered
IDH1	Exon 4
IDH2	Exon 4
IKZF1	All coding
JAK2	Exons 8-19
JAK3	Exons 11-22
KDM6A	All coding
KDR	Exons 11, 12, 23
KIT	Exons 8-11, 13, 17-18
KMT2A	Exons 6-27
KMT2C	All coding
KMT2E	All coding
KRAS	Exons 2-4
MAP2K1	Exons 2-3
MAP3K1	Exons 14-20
MAPK1	Exons 4, 7
MBD4	All coding
MGA	Exons 3, 9, 18
MPL	Exons 10-12
MST1	Exons 7-14
MYC	Exon 2
NF1	All coding
NFE2	All coding
SRP72	Exons 6-10
SRSF2	Exon 1
STAG2	All coding
STAT5B	Exons 14-16
TERC	Exon 1
TERT	All coding and promoter
TET2	All coding
NPM1	Exons 10-11
NRAS	Exons 2-4
NSD1	All coding
NUP98	All coding
PHF6	All coding
PIK3CA	Exons 10, 21
PIK3CD	Exons 9-11
PML	Exon 6
PPM1D	Exon 6
PTPN11	Exons 3, 8, 12-13
RAD21	All coding
RB1	All coding
RIT1	Exon 5
RUNX1	Exons 4-9

Gene Name	Region Covered
RUNX1	Exons 4-9
RUNX1T1	All coding
SAMD9	Exon 3
SAMD9L	Exon 5
SETBP1	Exon 4
SF3B1	Exons 13-18
SH2B3	Exons 2 (partial), 3-8
SMC1A	All coding
SMC3	All coding
TP53	Exons 2-10
U2AF1	Exon 2
UBA1	Exon 3
WT1	All coding
ZBTB7A	Exon 2
ZRSR2	All coding

List of regions assessed for translocations on Myeloid-NDC

ABL1	FLT3	MNX1	PDGFRB
BCR	FUS	MYH11	PML
BRAF	GATA2	NPM1	RARA
CBF	GLIS2	NTRK1	RBM15
CBFA2T3	JAK2	NUP214	RUNX1
DEK	KMT2A-PTD	NUP98	RUNX1T1
FGFR1	KAT6A	PDGFRA	

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

5q	7q22	8q	17q
5q31.2	7q36	12p	X
7p	8p	17p13.1	Y

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[a]	Disease	ATCC[b]	DMSZ[c]
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] These B and T cell lines were selected as they have available data and performance metrics for the EuroClonality-NDC assay. For additional data on myeloid cell lines check our Resources section on the website or email: contact@univ8genomics.com

[b] https://www.lgcstandards-atcc.org

[c] https://www.dsmz.de

Proficiency Samples : Expected results using Myeloid-NDC

Cell Line	Variant type	Gene	Variant
HD829*	VAR	BCOR	c.3621dup p.Gln1208ThrfsTer8
	VAR	KRAS	c.38G>A p.Gly13Asp
	VAR	RUNX1	c.801G>A p.Met267Ile
	VAR	GATA2	c.599del p.Gly200ValfsTer18
	VAR	ASXL1	c.1926_1927insG p.Gly646TrpfsTer12
	VAR	GATA1	c.355C>T p.Gln119Ter
	VAR	NRAS	c.182A>T p.Gln61Leu
	VAR	TP53	c.722C>T p.Ser241Phe
	VAR	SF3B1	c.2219G>A p.Gly740Glu
	VAR	CBL	c.1208C>T p.Ser403Phe
	VAR	IDH2	c.515G>A p.Arg172Lys
	VAR	JAK2	c.1849G>T p.Val617Phe
	VAR	ASXL1	c.2388G>T p.Trp796Cys
	VAR	TET2	c.3782G>A p.Arg1261His
	VAR	DNMT3A	c.2644C>T p.Arg882Cys
	VAR	IDH1	c.394C>T p.Arg132Cys
	VAR	ABL1	c.944C>T p.Thr315Ile
	VAR	EZH2	c.1253G>A p.Arg418Gln
	VAR	JAK2	c.1611_1616del p.Phe537_Lys539delinsLeu
	VAR	FLT3	c.2503G>T p.Asp835Tyr
	VAR	NPM1	c.859_860insTCTG p.Trp288CysfsTer12
	SV	FLT3	FLT3-ITD dup (285bp)
CA46	VAR	TP53	c.743G>A p.Arg248Gln
	VAR	MYC	c.214C>T p.Pro72Ser
	VAR	MYC	c.224C>A p.Pro75His
	VAR	MYC	c.58G>A p.Val20Ile
	VAR	CSF3R	c.1919C>T p.Thr640Ile
	VAR	CSF3R	c.1748G>A p.Arg583His
	CNV	RIT1	CNV_gain
	CNV	NTRK1	CNV_gain
	CNV	CCND3	CNV_deletion
	CNV	7q22	CNV_gain
	CNV	7q36	CNV_gain
	CNV	SAMD9	CNV_amplification
	CNV	SAMD9L	CNV_amplification
	CNV	CUX1	CNV_gain
	CNV	KMT2E	CNV_gain
	CNV	BRAF	CNV_gain
	CNV	EZH2	CNV_gain
	CNV	KMT2C	CNV_gain
	CNV	MNX1	CNV_gain
	CNV	FLT3	CNV_gain
	CNV	RB1	CNV_gain
	CNV	DLEU2	CNV_gain
	CNV	DIS3	CNV_amplification
H929	VAR	NRAS	c.38G>A p.Gly13Asp
	CNV		Multiple alterations
HAL-01	VAR	EP300	c.5390A>G p.Gln1797Arg
	VAR	NRAS	c.34G>A p.Gly12Ser

Cell Line	Variant type	Gene	Variant
	VAR	PTPN11	c.1504T>C p.Ser502Pro
	VAR	CREBBP	c.1942-1G>T
	VAR	CEBPA	c.566_568delinsACC p.Pro189_Ser190delinsHisPro
	VAR	CEBPA	c.585_590dup p.Pro197_Pro198dup
	CNV	CDKN2A	CNV_deletion
	CNV	CDKN2B	CNV_deletion
Namalwa	VAR	TP53	c.743G>A p.Arg248Gln
	VAR	BCOR	c.728G>T p.Arg243Leu
	VAR	MYC	c.214C>T p.Pro72Ser
	VAR	MYC	c.59T>C p.Val20Ala
	VAR	MYC	c.790A>C p.Ser264Arg
	VAR	CDKN2A	c.316G>T p.Val106Leu
	VAR	TERC	n.490C>T -
	VAR	KMT2C	c.2722G>T p.Gly908Cys
	VAR	KMT2C	c.1179C>A p.Asn393Lys
	CNV		Multiple alterations
REH	VAR	BCOR	c.3857T>G p.Val1286Gly
	VAR	IKZF1	c.1079G>A p.Arg360His
	VAR	NSD1	c.4520C>T p.Thr1507Met
	VAR	DDX41	c.215G>A p.Gly72Glu
	VAR	TERT	c.785C>T p.Thr262Met
	VAR	RUNX1T1	c.1076G>A p.Arg359Gln
	VAR	RB1	c.929G>A p.Gly310Glu
	VAR	TERT	c.1321G>A p.Glu441Lys
	VAR	CEBPA	c.632C>T p.Ala211Val
	VAR	TP53	c.541C>T p.Arg181Cys
	VAR	TERT	c.2095G>A p.Ala699Thr
	VAR	NF1	c.3421C>G p.Leu1141Val
	VAR	CEBPA	c.214G>A p.Ala72Thr
	VAR	KMT2C	c.2431A>G p.Ile811Val
	SV	ETV6::CFAP54	t(12;12)
	CNV	MST1	CNV_deletion
	CNV	CCND3	CNV_gain
	CNV	CDKN2A	CNV_deletion
	CNV	CDKN2B	CNV_deletion
	CNV	12p	CNV_deletion
	CNV	ETV6	CNV_deletion
	CNV	CREBBP	CNV_gain
	CNV	GLIS2	CNV_gain
	CNV	MYH11	CNV_gain
	CNV	FUS	CNV_gain
	CNV	CBFB	CNV_gain
	CNV	CTCF	CNV_gain
	CNV	CBFA2T3	CNV_gain
	CNV	U2AF1	CNV_gain
RS4-11	VAR	KMT2A	c.4138C>T p.Leu1380Phe
	VAR	EP300	c.4452+2T>G
	VAR	FBXW7	c.452A>G p.Asp151Gly

Cell Line	Variant type	Gene	Variant
	VAR	SMC3	c.121del p.Tyr43MetfsTer69
	VAR	DDX41	c.409A>G p.Thr137Ala
	VAR	KMT2E	c.4878del p.Pro1628LeufsTer25
	SV	AFF1::KMT2A	t(4;11)
	CNV	7p	CNV_deletion
	CNV	7q22	CNV_gain
	CNV	7q36	CNV_gain
	CNV	IKZF1	CNV_deletion
	CNV	SAMD9	CNV_gain
	CNV	SAMD9L	CNV_gain
	CNV	CUX1	CNV_gain
	CNV	KMT2E	CNV_gain
	CNV	BRAF	CNV_gain
	CNV	EZH2	CNV_gain
	CNV	KMT2C	CNV_gain
	CNV	MNX1	CNV_gain
	CNV	CDKN2A	CNV_deletion
	CNV	CDKN2B	CNV_deletion
TOM-1	VAR	BCOR	c.935_937del p.Gln312del
	VAR	KDM6A	c.3056_3057insC p.Thr1020TyrfsTer11
	VAR	BCR	c.3445G>A p.Ala1149Thr
	VAR	ASXL2	c.2636C>T p.Pro879Leu
	VAR	CHEK2	c.886G>T p.Asp296Tyr
	SV	BCR::ABL1	t(9;22)
	CNV	GNB1	CNV_deletion
	CNV	PIK3CD	CNV_deletion
	CNV	CCND3	CNV_gain
	CNV	7p	CNV_deletion
	CNV	IKZF1	CNV_deletion
	CNV	8p	CNV_gain
	CNV	FGFR1	CNV_gain
	CNV	KAT6A	CNV_gain
	CNV	RAD21	CNV_gain
	CNV	MYC	CNV_gain
	CNV	CDKN2A	CNV_deletion
CML-T1	VAR	FBXW7	c.1394G>A p.Arg465His
	VAR	STAG2	c.1109G>A p.Arg370Gln
	VAR	KMT2E	c.82G>A p.Val28Ile
	VAR	CREBBP	c.1813G>A p.Val605Met
	VAR	SMC3	c.121del p.Tyr43MetfsTer69
	VAR	TP53	c.868C>T p.Arg290Cys

Cell Line	Variant type	Gene	Variant
	VAR	MST1	c.1117C>T p.Arg373Trp
	VAR	CTCF	c.1129C>T p.Arg377Cys
	VAR	TP53	c.541C>T p.Arg181Cys
	VAR	PDGFRB	c.1436A>G p.Asn479Ser
	VAR	ZBTB7A	c.1150T>C p.Cys384Arg
	VAR	SAMD9	c.153del p.Glu52AsnfsTer16
	VAR	MST1	c.1382G>A p.Arg461His
	VAR	TP53	c.817C>T p.Arg273Cys
	SV	BCR::ABL1	t(9;22) complex 3-way translocation (including RXRA)
	CNV	CDKN2A	CNV_deletion
	CNV	CDKN2B	CNV_deletion
	CNV	KMT2A	CNV_deletion
	CNV	CBL	CNV_deletion
Karpas299	CNV	TP53	c.817C>T p.Arg273Cys
	SV	NPM1::ALK	t(2;5)
	CNV	RIT1	CNV_deletion
	CNV	NTRK1	CNV_amplification
	CNV	CCND3	CNV_gain
	CNV	GATA3	CNV_deletion
	CNV	SMC3	CNV_deletion
	CNV	KMT2A	CNV_deletion
	CNV	CBL	CNV_deletion
	CNV	CREBBP	CNV_amplification
	CNV	GLIS2	CNV_gain
	CNV	MYH11	CNV_gain
	CNV	17p	CNV_deletion
	CNV	TP53	CNV_deletion
Loucy	VAR	TP53	c.814G>A p.Val272Met
	VAR	CDKN1B	c.287_297del p.Cys99GlyfsTer22
	VAR	CBLB	c.1934A>G p.His645Arg
	VAR	KMT2C	c.2722G>T p.Gly908Cys
	CNV		Multiple alterations (including aneuploidy)
P12Ichikawa	VAR	FBXW7	c.1513C>T p.Arg505Cys
	VAR	TP53	c.743G>A p.Arg248Gln
	VAR	RUNX1	c.253C>A p.His85Asn
	VAR	TP53	c.743G>C p.Arg248Pro
	VAR	NRAS	c.35G>A p.Gly12Asp
	VAR	TP53	c.31G>C p.Glu11Gln
	CNV		Multiple alterations

** HD829 DNA reference standard and associated data are available at https://horizondiscovery.com*

Abbreviations

ALL	Acute Lymphoblastic Leukaemia
BCL	Binary base call file
BL	Burkitts' Lymphoma
CNV	Copy number variants
EDTA	Ethylenediaminetetraacetic acid
HMW	High-molecular weight
PCM	Plasma Cell Myeloma
QC	Quality control
SV	Structural variants
VAR	Sequence variants

References

1. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022 Sep 15;140(11):1200-1228. doi: 10.1182/blood.2022015850. PMID: 35767897; PMCID: PMC9479031.

2. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377. doi: 10.1182/blood.2022016867. PMID: 35797463.

3. Bernard E, Tuechler H, Greenberg PL, Hasserjian RP, Arango Ossa JE, Nannya Y, et al. Molecular International Prognostic Scoring System for myelodysplastic syndromes. NEJM Evid. 2022 doi: 10.1056/EVIDoa2200008.

4. Gerds AT, Gotlib J, Ali H, Bose P, Dunbar A, Elshoury A, et al. Myeloproliferative Neoplasms, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2022 Sep;20(9):1033-1062. doi: 10.6004/jnccn.2022.0046. PMID: 36075392.

5. Stewart JP, Gazdova J, Darzentas N, Wren D, Proszek P, Fazio G, et al; 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.

6. Stewart JP, Gazdova J, Srivastava S, Revolta J, Harewood L, Maurya M, Darzentas N, Gonzalez D. Immunoglobulin/T Cell Receptor Capture Strategy for Comprehensive Immunogenetics. Methods Mol Biol. 2022;2453:133-152. doi: 10.1007/978-1-0716-2115-8_9. PMID: 35622325; PMCID: PMC9761918.

FAQ

1. Can I run the Myeloid-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 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.

2. Can I use more or less than 16 samples (plus controls) per hybridisation run?
Yes, you can run any number of samples from 1 to 22. The percentage of target bases covered at 300x per sample (see summary QC files) should ideally be at least 90%, to ensure that most sequence and structural variants will be detected. Therefore, to achieve these figures, the ideal multiplexing value may be different for different laboratories, depending on the quality of library preparation, hybridisation, automation options, etc.

3. Can I run more than 16-22 samples per run?
If you want to run more samples, you can run multiple hybridisations of up to 16-22 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 HMW-derived DNA samples the final enriched library should ideally be between 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 preparation. If the values obtained are consistently below the above values, 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
2400013 r01	First released	February 2024

Technical Assistance

Univ8 Genomics Ltd
63 University Road
Belfast
Northern Ireland

Email: contact@univ8genomics.com
Web: univ8genomics.com