Title

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A novel single-tube multimodal NGS assay using total nucleic acid for comprehensive genomic profiling of hematologic malignancies.

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Introduction

A cost effective and comprehensive genomic profiling (CGP) approach for diagnosis, risk stratification and therapy would be useful for the evaluation of oncologic specimens. Available approaches involving additive testing for DNA and RNA abnormalities through traditional methods (e.g. Sanger, FISH, cytogenetics, qRT-PCR) are not comprehensive, require multiple different workflows and are sample consuming, often resulting in incomplete testing. While there are next generation sequencing (NGS) assays designed for detecting DNA and RNA abnormalities, they have separate workflows that require twice the amount of sample and effort. To address this, we developed a novel total nucleic acid (TNA) extraction method and single tube workflow utilizing TNA and a custom multimodal chemistry designed for hematologic malignancies. This consolidated workflow enables an efficient discovery based approach for both DNA/RNA abnormalities including single nucleotide variants (SNVs), InDels, copy number variants (CNVs), large structural changes from DNA and gene fusions and gene expression levels from RNA. This method maximizes data derived from valuable samples while delivering a comprehensive profile of the patient's tumor which can help guide therapeutic and clinical decisions.

Methods

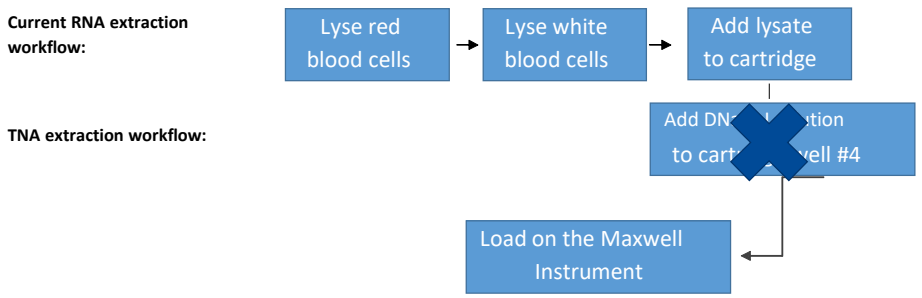
Total nucleic acid (TNA) was extracted from bone marrow and peripheral blood of the patients (CML, CMML, CLL, AML and myeloid disorders). 297 genes that have DNA mutations specific to hematological cancers were targeted, along with 213 genes that were targeted for clinically significant RNA abnormalities. The QIAGEN's QIAseq Multimodal Targeted DNA/RNA chemistry workflow was used for the library preparation. The enriched genomic and transcriptomic regions of interest from the patients were successfully sequenced with unique dual indices on an Illumina NovaSeq 6000. DNA variant detection as well as fusion detection from RNA were compared to traditional orthogonal NGS assays that use DNA input or compared to qRT-PCR and Sanger sequencing assays that use RNA as input.

Panel Summary

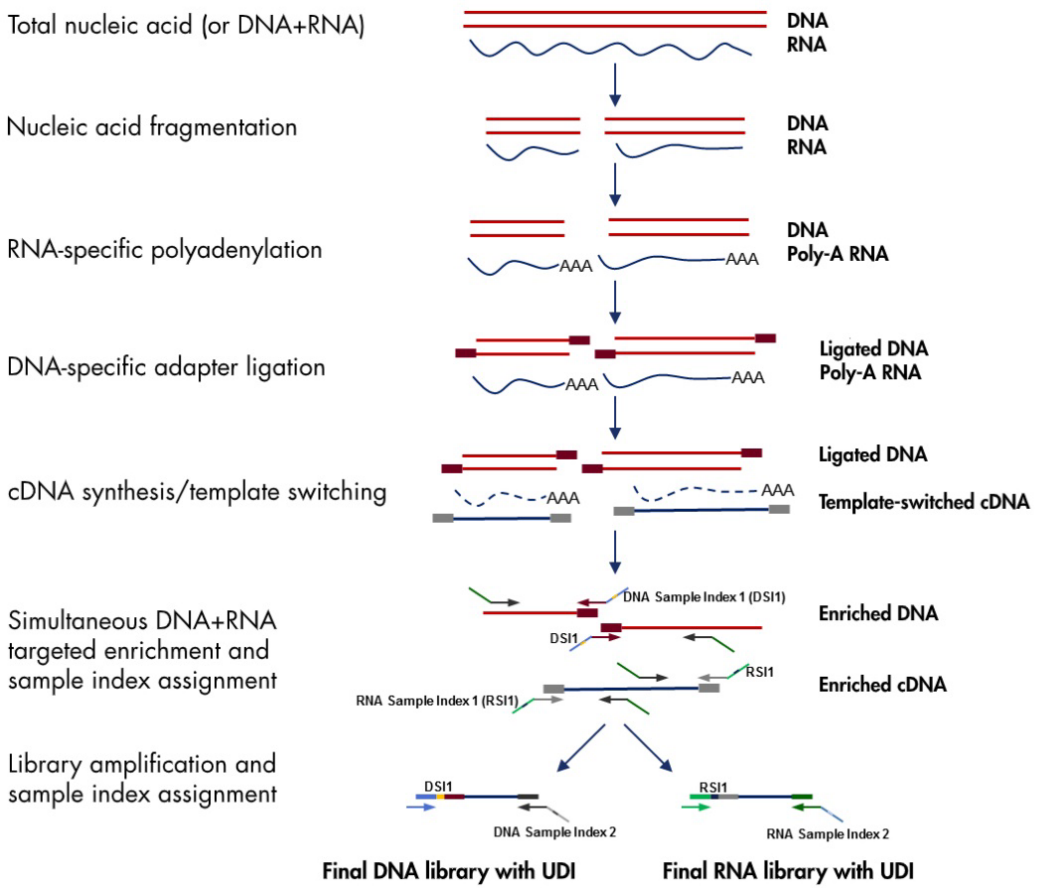
- Total targeted genes for DNA panel: 297 genes
- Targeted Gene fusions: 185 genes (Coverage based on guidelines, FISH test, NeoGenomics panel and NeoGenomics pathologists' clinical practice)
- Targeted Gene Expressions: 35 genes (7 genes also have fusion detection)

TNA extraction Workflow

- Maxwell® RSC simplyRNA Blood Kit by Promega
- Maxwell RSC 48 Instrument Operation SOP, RNA extraction
  Forgo the DNase I treatment step to achieve TNA in final elution

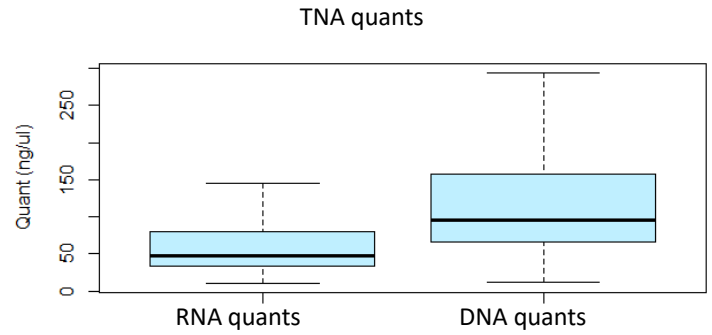


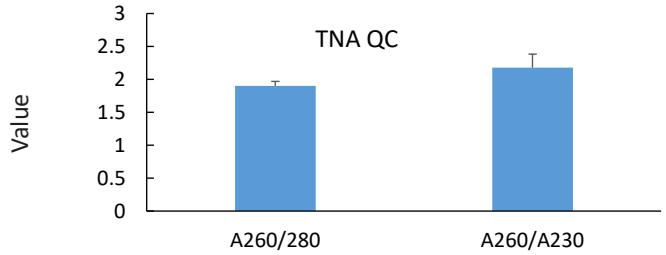
Multimodal NGS workflow



TNA Quality/Quantity Profiling - Yield and OD ratios

(48 MOL case samples and 48 NTP case samples)





Results

In this study, we developed an efficient and high-quality TNA extraction method that can purify enough total nucleic acid from bone marrow, peripheral blood, cytogenetic pellets, flow suspension, and FFPE samples for the downstream NGS assay. The average OD 260/280 value was 1.9 and the OD 260/230 was 2.18. After sequencing, 256/262 (97.7% accuracy) SNV and Indel variants that were candidate pathogenic mutations were concordant from 38 patients. Meanwhile, 100% (7/7) of all BCR/ABL1 gene fusions which had an international scale (IS) value above 6.4% were concordant. In addition, 69 fusion positive samples containing 20 unique gene fusions which had been previously reported by an independent ArcherDX assay designed specifically for gene fusions were also evaluated with this chemistry. Analysis revealed a 92.5% (64/69) concordance. More importantly, the QIAseq multimodal TNA NGS assay detected both DNA and RNA abnormalities in a single tube. For example, in one myeloid leukemia patient, we not only identified pathogenic variants of ASXL1 and JAK2 which had been previously detected by a DNA NGS assay, but also detected a concurrent BCR-FGFR1 fusion which had been previously reported by a FISH assay. Moreover, we were able to provide more comprehensive genomic profiling by investigating many DNA and RNA abnormalities simultaneously. In our study, for 5 patients that previously been tested for BCR-ABL1 fusion only, we are able to assess BCR-ABL1 fusion status from RNA as well as identify pathogenic DNA variants at the same time, including JAK2 p.V617F, U2AF1 p.S34F, ASXL1 p.E635Rfs*15, BRCA p.S1982Rfs*22, and DNMT3A p.S708Vfs*71, which provides valuable information to assist diagnosis and treatment in a cost effective and efficient way.

Abnormalities of DNA and RNA are detected in one CML patient

Patient ID	DNA variants	Mutant Allele Frequency (%)	RNA fusions	FISH test
CML_P1	JAK2:K558N	49.7	BCR-FGFR1	FGFR1 GENE REARRANGEMENT;FGFR1 gene rearrangement in 87.5% of interphase nuclei (1R1G1F, normal <5.7%)
	ASXL1:S846Qfs*5	6.2		

Abnormalities of DNA and RNA can be assessed simultaneously in patients

Patient ID	BCR-ABL1 Standard p210, p190 Test result	BCR-ABL1 fusion by NGS	DNA variants	Mutant Allele Frequency (%)
P1	ND	ND	BRCA2: p.S1982Rfs*22	53.4
P2	ND	ND	JAK2: p.V617F	20.6
P3	ND	ND	ASXL1: p.E635Rfs*15	27.2
			JAK2: p.V617F	25
P4	ND	ND	DNMT3A: p.S708Vfs*71	49.7
P5	POS (IS 83.868%)	Detected	U2AF1: p.S34F	4.8

Extensive Orthogonal Validation of the Fusions

Sixty-nine samples with a list of 20 fusions, previously reported by ArcherDX, were tested by NGS resulting 92.5% (64/69) concordance.

Fusions	Expected	Detected	Fusions	Expected	Detected
BCR-ABL1	33	32	TCF3-PBX1	4	4
CRLF2-P2RY8	10	10	ZNF384-CREBBP	1	1
JAK2-PAX5	1	1	ZNF384-EP300	1	1
KMT2A-AFF1	3	3	ZNF384-SYNRG	1	1
KMT2A-MLLT3	1	1	ABL1-NUP214	1	1
KMT2A--UBE4A	1	1	ABL1-SRP9	1	1
MEF2D-HNRNPUL1	1	1	BCR-HBA2	1	0
MLLT10-PICALM	3	3	ETV6-PRKAR1A	1	0
PDGFRB-ATF7IP	2	2	RUNX1-ETS2	1	0
RUNX1-RUNX1T1	1	1	TAL1-SLC6A9	1	0

Conclusion

We developed a single tube TNA based workflow with a custom multimodal chemistry that simultaneously detects many DNA and RNA abnormalities in a cost effective and efficient way while reducing sample requirements. This unique TNA NGS assay provides comprehensive genomic profiling for hematologic malignancies and improves the diagnostic testing options for precise patient care.

Disclosure

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		Yes	Merck	Other: Speaker	
		Yes	Genentech	Other: Speaker	
		Yes	Bayer	Other: Speaker	
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