Automated Nucleic Acid Purification on Ion Torrent™ Genexus™ to Support Variant Detection in Research of Myeloid Malignancies

Sarah Brozio, Jennifer Burke, Nitisha Thapa, Brendan Deal, Jiajie Huang, Collyn Seeger, Crystal Harris, Caemanii Rowe, Emilia Ostrowska, Tasha Delacour, Thilanka Jayaweera, Sophie Rozenzhak, Seth Sadis and Marina Sedova

INTRODUCTION

- Myeloid malignancies are associated with a diverse set of genomic alterations.
- Accurate detection of these variants and rapid turnaround time are essential to providing informative results to researchers.
- Sufficient yield of high-quality nucleic acid is critical to sequencing outcomes; however, isolation of nucleic acids is highly dependent on sample condition.
- The Genexus™ Purification System provides a robust automated purification of nucleic acids from fresh and frozen, blood, buffy coat and bone marrow samples to be used with the Oncomine™ Myeloid GX v2 Assay (OMAv2) on the Ion Torrent™ Genexus Sequencer (Figure 1).
- Oncomine Myeloid GX v2 assay can detect DNA variants in 45 target genes, and >800 fusion isoforms in 35 driver genes (Table 1).

Figure 1.

Table 1. Oncomine Myeloid v2 Gene Targets

Hotspot genes (28)		Full genes (17)		Fusion Driver Genes (30)		Expression genes (5)	Expression control genes (5)
ABL1	*KRAS*	*ASXL1*	*PRPF8*	*ABL1*	*MET*	*BAALC*	*EIF2B1*
ANKRD 26	*WT1*	*BCOR*	*RB1*	*ALK*	*MLLT10*	*MECOM*	*FBXW2*
BRAF	*MPL*	*CALR*	*RUNX1*	*BCL2*	*MLLT3*	*MYC*	*PSMB2*
CBL	*MYD88*	*CEBPA*	*SH2B3*	*BRAF*	*MYBL1*	*SMC1A*	*PUM1*
CSF3R	*NPM1*	*ETV6*	*STAG2*	*CCND1*	*MYH11*	*WT1*	*TRIM27*
DDX41	*NRAS*	*EZH2*	*TET2*	*CREBBP*	*NTRK3*		
DNMT3A	PPM1D	*IKZF1*	*TP53*	*EGFR*	*NUP214*		
FLT3	*PTPN11*	*NF1*	*ZRSR2*	*ETV6*	*NUP98*		
GATA2	*SETBP1*	*PHF6*		*FGFR1*	*PDGFRA*		
HRAS	*SF3B1*			*FGFR2*	*PDGFRB*		
IDH1	*SMC1A*			*FUS*	*RARA*		
IDH2	*SMC3*			*HMGA2*	*RBM15*		
JAK2	*SRSF2*			*JAK2*	*RUNX1*		
KIT	*U2AF1*			*KMT2A (MLL-PTD)*	*TCF3*		
				MECOM	*TFE3*		

MATERIALS AND METHODS

Fresh and frozen replicates of 35 unique samples from healthy donors and patients diagnosed with Acute Myeloid Leukemia (AML) were analyzed. All samples were purified using the Genexus purification instrument. RNA extraction samples were thawed/mixed with PK buffer (supplied in kit) before being purified using the Genexus Total RNA extraction kit. DNA extraction samples were mixed with DNA homogenization buffer (supplied in kit) before being purified using the Genexus Mulitsample DNA extraction kit. The on-instrument Qubit™ Fluorometer measured nucleic acid concentration.

A Bioanalyzer™ (Agilent Technologies) instrument was used to assess RNA quality using the RNA 6000 Pico Kit. The Genexus integrated Sequencer was used to dilute samples to optimal concentration and to sequence 209 DNA and RNA replicates using the OMAv2. The optimized Genexus software analysis pipeline was used to produce variant calling results. Data were analyzed using JMP software.

ION TORRENT GENEXUS™ INSTRUMENTS PROVIDE AUTOMATED NUCLEIC ACID PURIFICATION AND OMAv2 SEQUENCING OF MULTIPLE SAMPLE TYPES

RNA %DV200 PREDICTS SEQUENCING OUTCOMES

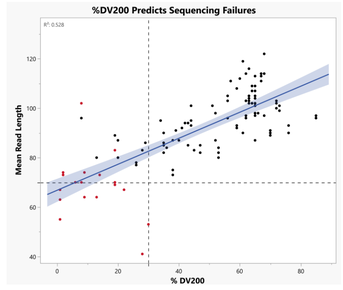


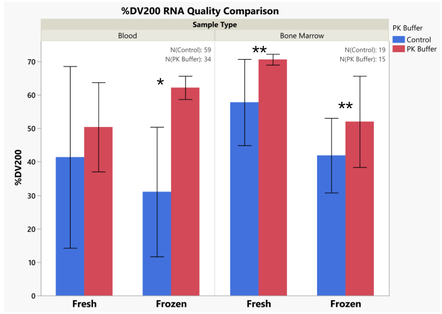
Figure 2.

- %DV200 is the percentage of RNA fragments > 200 nucleotides long.
- RNA that is highly fragmented will have low %DV200 scores.
- Samples with %DV200 < 30 are at risk of failing sequencing QC .
- Failing samples shown as red markers (Figure 2).
- 23/32 samples with %DV200 <30% failed sequencing, while 100% of samples >30% passed.

HIGH QUALITY RNA PURIFIED FROM FROZEN SAMPLES

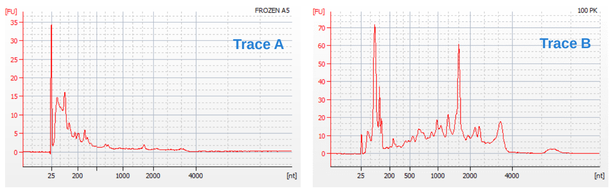
Figure 3.

- Frozen samples were treated with PK buffer during thawing (1:1 ratio).
- PK buffer was mixed 1:1 ratio with fresh samples.
- Adding PK buffer to frozen samples significantly improved %DV200 outcomes (*p<0.0001, **p=0.03).
- In all sample conditions addition of PK buffer had no negative impact on RNA concentration.



Bioanalyzer traces demonstrated degradation prevention by addition of PK buffer.

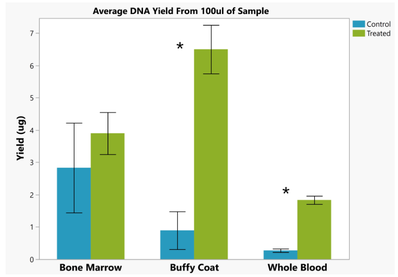
- **A**. RNA extracted from a frozen blood sample without pretreatment has almost no RNA fragments longer than 200nt.
- **B**. RNA integrity is improved by adding PK buffer before purification: >70% of RNA fragments >200nt long.



NEW SAMPLE TYPES ADDED WITH INCREASED DNA YIELD

Figure 4.

- DNA purification workflow uses up to 400ul sample volume.
- DNA homogenization buffer was added to lower volume samples to maximize sample input (example 100ul sample + 300ul buffer).
- Addition of DNA homogenization buffer to 100ul of sample significantly increases DNA yield (Figure 4). Buffy coat and Bone marrow had *p<0.0001.



RNA PRE-TREATMENT IMPROVES SEQUENCING OUTCOMES

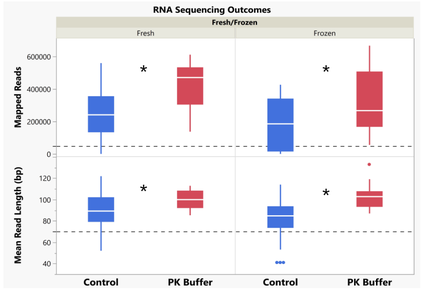


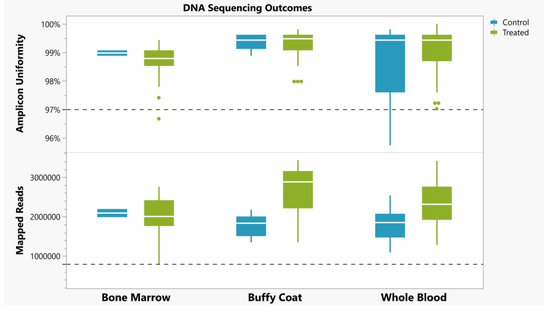
Figure 5.

- 20% of untreated RNA samples failed QC metrics (MRL >70bp, Mapped Reads >50,000)
- 100% of samples treated with buffer (fresh and frozen) passed QC metrics.
- Treated RNA performed significantly better, p<0.0001 across all conditions

HIGHER DNA YIELD DID NOT IMPACT SEQUENCING RESULTS

Figure 6. There was no significant difference in DNA sequencing outcomes between treated (n= 147) and control (n=62) samples.

- 100% of control and treated samples had >800,000 Mapped Reads .
- 97% of treated and 90% of control samples had Amplicon Uniformity >97%.
- Buffy coat is a new sample type that is now compatible with OMAv2.



RUNX1-ZFPM2 FUSION DETECTED IN AML BLOOD SAMPLE

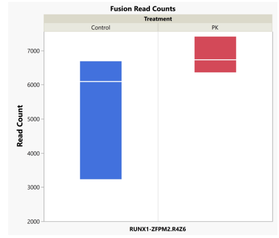


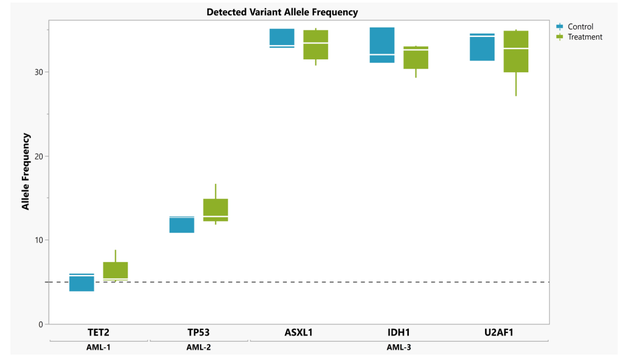
Figure 7.

- Fusion read counts in a frozen AML blood sample were compared using RNA purified with and without PK buffer addition.
- Six replicates were sequenced per condition.
- RUNX1 fusion read counts were higher and more consistent in RNA samples treated with PK Buffer (Figure 7).

ASXL1, IDH1 AND OTHER VARIANTS CALLED IN DNA SAMPLES

- DNA variant calling results were compared from 3 unique AML (1 fresh, 2 frozen) blood samples extracted using updated purification protocols.
- Samples were sequenced in 8 replicates per sample for each condition (Figure 8).
- Detected variants and their allele frequency were concordant between DNA purification methods.

Figure 8.



CONCLUSIONS

- Genexus instruments provide automated nucleic acid purification and sequencing of blood, bone marrow and buffy coat samples using the Oncomine Myeloid GX v2 assay.
- Pre-treatment of samples with PK buffer enabled the use of frozen specimen with Genexus Purification System for RNA isolation.
- DNA extraction on Genexus Purification System consistently provided high DNA yields with low volume input from multiple sample types, including buffy coat.
- DNA and RNA purified using Genexus Purification System had reliable sequencing outcomes, that consistently passed OMAv2 sample QC metrics.

ThermoFisher SCIENTIFIC