ion torrent

Automation meets reliability: Use of Oncomine™ Precision Assay on the Genexus™ System for identification of cancer biomarkers in FFPE and liquid biopsy samples

Jeff Schageman, Nicholas Siepert, Brian Hradecky, Emilia Ostrowska, Iris Casuga*, Kris Lea, Priyanka Kshatriya, Jian Gu, Ru Cao, Angie Cheng, Kelli Bramlett, Thilanka Jayaweera. Thermo Fisher Scientific, * 180 Oyster Point Blvd., South San Francisco, CA 94080; 2130 Woodward St, Austin, TX

INTRODUCTION

Accurate and early detection of oncogenic markers may one day be the key to fighting cancer. However, with complex workflows, long turnaround times, and numerous user touch points of most sequencing platforms do not make the process attainable. In contrast, the fully automated Genexus™ system provides a specimen-to-report workflow for cancer research with minimal user touchpoints and single day turnaround time. FFPE (formalin-fixed paraffin embedded) tissues and liquid biopsy are two of the main sample types used in oncology research. Here we report the use of the Oncomine™ Precision Assay (OPA) with the Genexus™ System, which provides a comprehensive genetic profile across 50 key genes using DNA and RNA from FFPE tissues or cfTNA (cell free total nucleic acid) from liquid biopsy samples.

The Ion Torrent Genexus™ Integrated Sequencer is part of the Genexus™ System, the first turnkey next-generation sequencing (NGS) solution. Comprised of two instruments, the Genexus™ Purification System and the Genexus Integrated Sequencer, the Genexus™ System enables a workflow from biological specimen all the way to the final report. The Ion Torrent Genexus™ Purification System automates nucleic acid extraction, purification, and quantitation on a single platform to provide a consistent and efficient workflow solution for next-generation sequencing (NGS) sample preparation. The Genexus™ Integrated Sequencer automates NGS library preparation, templating and sequencing. Genexus™ software links the two instruments, tracks the sample information and provides a report.

One of many oncology research assays that can be used on Genexus™ system is Oncomine™ Precision Assay (OPA). OPA analyzes 78 variants, including mutations (45), CNVs (14), and fusion variants (19), across 50 key genes. Included are tumor suppressor genes such as TP53, cancer drivers, and resistance mutations. Content has been carefully curated to include relevant targets and also targets of emerging importance in precision oncology clinical research. OPA is compatible with formalin-fixed, paraffin-embedded (FFPE) tissue as well as liquid biopsy samples.

The OPA assay only requires 10ng of DNA and RNA from FFPE samples and 20ng of cfTNA from liquid biopsy samples. Genexus™ purification instrument onboard quantitation data showed successful extraction of NA exceeding the required yields for library preparation. Excess NA was automatically aliquoted into an archive plate and stored for future use. Sequencing results for four samples of FFPE or liquid biopsy were reported within 24 hours. Both Control and clinical research samples showed expected assay metrices including read coverage, molecular coverage, and uniformity. The results reported all expected variants at correct allele frequencies, including BRAF V600E, KRAS G12C, PIK3CA N345K, AKT1, etc.

Ion Torrent™ Genexus™ System

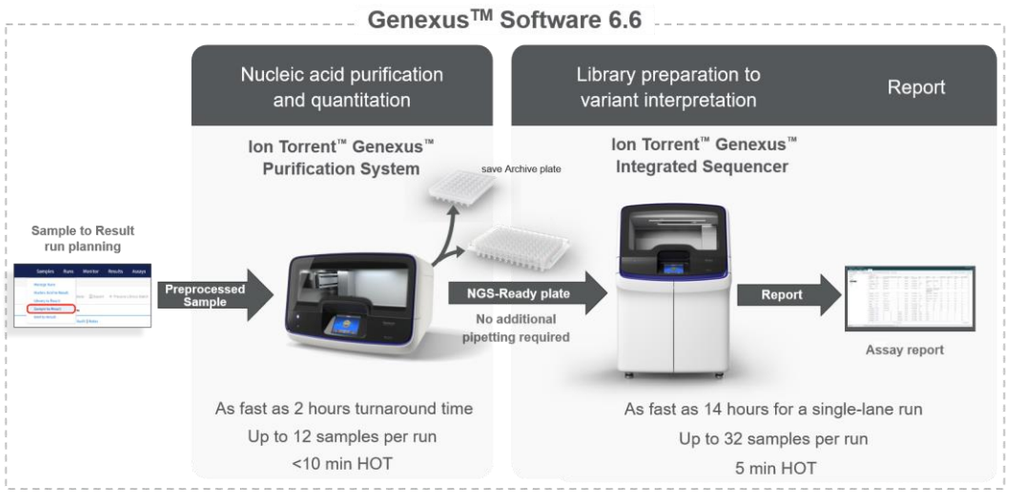


Figure 1. Ion Torrent™ Genexus™ System and workflow.

Oncomine™ Precision Assay (OPA)

DNA hotspots					CNVs		Inter-genetic fusions		Intra-genetic fusions
AKT1	*CHEK2*	*FGFR3*	*KIT*	*NTRK3*	*ALK*	*FGFR1*	*ALK*	*NTRK1*	*AR*
AKT2	*CTNNB1*	*FGFR4*	*KRAS*	*PDGFRA*	*AR*	*FGFR2*	*BRAF*	*NTRK2*	*EGFR*
AKT3	*EGFR*	*FLT3*	*MAP2K1*	*PIK3CA*	*CD274*	*FGFR3*	*ESR1*	*NTRK3*	*MET*
ALK	*ERBB2*	*GNA11*	*MAP2K2*	*PTEN*	*CDKN2A*	*KRAS*	*FGFR1*	*NUTM1*	
AR	*ERBB3*	*GNAQ*	*MET*	*RAF1*	*EGFR*	*MET*	*FGFR2*	*RET*	
ARAF	*ERBB4*	*GNAS*	*MTOR*	*RET*	*ERBB2*	*PIK3CA*	*FGFR3*	*ROS1*	
BRAF	*ESR1*	*HRAS*	*NRAS*	*ROS1*	*ERBB3*	*PTEN*	*MET*	*RSPO2*	
CDK4	*FGFR1*	*IDH1*	*NTRK1*	*SMO*			*NRG1*	*RSPO3*	
CDKN2A	*FGFR2*	*IDH2*	*NTRK2*	*TP53*					

Figure 2. Oncomine™ Precision Assay (OPA) analyzes 78 variants, including mutations (45), CNVs (14), and fusion variants (19), across 50 key genes.

RESULTS

Sample purification on Genexus™ Purification System

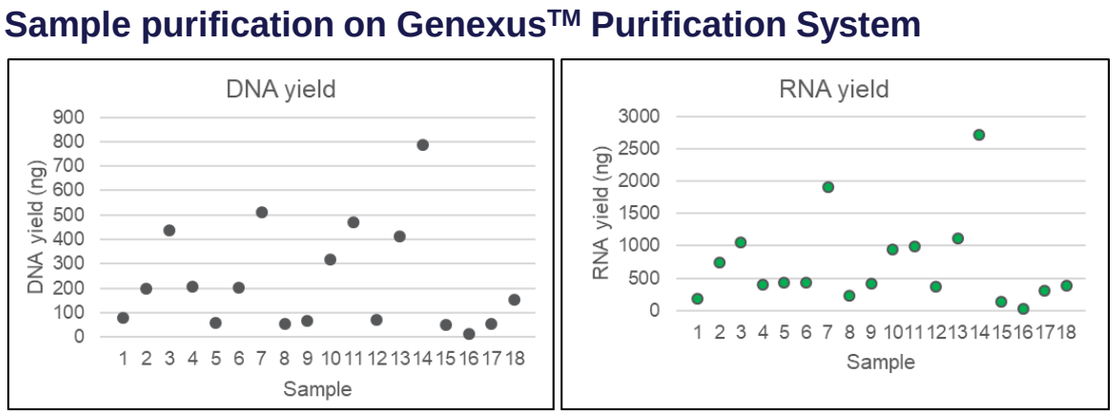
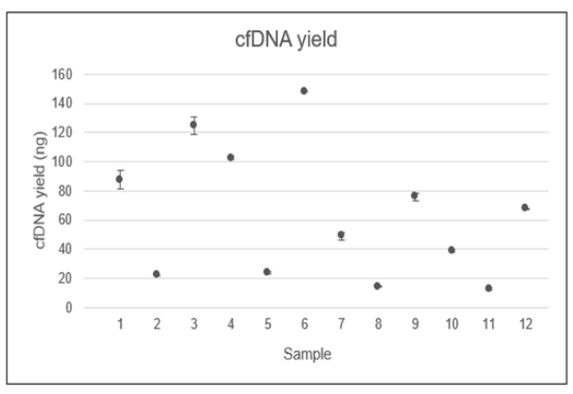


Figure 3 - 4. Sequential DNA and RNA extraction from FFPE samples. One 10µm curl per sample was used for the extraction. All samples yielded at least 10ng of DNA (Min = 12.43ng, Max = 787.8ng, Avg = 229.5 ng) and RNA (Min = 31.39ng, Max = 2718.34ng, Avg = 709.63 ng) recommended for the assay.



WorkFlow	Assay	Average GNXP:Manual ratio	SD	Percent Difference %
FFPE	DNA HS	0.98	0.16	2.37
FFPE	RNA BR	0.96	0.07	3.41
cfTNA	DNA HS	0.89	0.06	10.61

Figure 6. On-board quantitation accuracy of Genexus™ Purification system. On-board quantitation data were compared to manual qubit assay data and percent difference was calculated.

Figure 5. cfTNA extraction from liquid biopsy samples. 4 mL (samples 7-12) to 8mL (Samples 1-6) of plasma was used for the extraction. 83% of the samples yielded at least 20ng of cfTNA, recommended for the assay. All samples yielded at least 5ng of cfTNA, recommended minimum for the assay (Min = 12.03ng, Max = 148.21ng, Avg = 63.91ng).

Sequencing on Genexus™ integrated sequencer – FFPE samples

Sample	MRL	Mapped Reads	MAPD	Percent reads on target	Mean Depth	Target base coverage at 100x	uniformity of amplicon coverage	uniformity of base coverage
Clinical research samples	91	953564	0.235	90%	3319.5	100%	99%	99%
Control HD789	97	1081224	0.22	90%	4084	100%	99%	98%

Figure 7. FFPE DNA sequencing run matrices for clinical research samples and Horizon HD789 control. All samples met the run and sample QC specifications.

Sample	MRL	Total Reads	Mapped Reads	RNA Controls Detected
Clinical research samples	87	1130809	204880.5	7
Control SeraCare® FFPE Fusion	100	1322855	200147	7

Figure 8. FFPE RNA sequencing run matrices for clinical research samples and SeraCare ® FFPE Fusion control. All samples met the run and sample QC specifications.

	Horizon® HD789	SeraCare® Fusion
SNV sensitivity	99%	NA
SNV PPV	100%	NA
InDel Sensitivity	100%	NA
InDel PPV	100%	NA
SNV sensitivity	100%	NA
SNV PPV	100%	NA
Fusion Sensitivity	NA	100%
Fusion PPV	NA	100%

Figure 9. Performance of FFPE SNV, InDel ,CNV and fusion detection. Average sensitivity and PPV for SNV, InDel and CNV detection were calculated for the Horizon® structural multiplex reference standard HD789 control that includes 15 key cancer driver SNV/InDels and 1 CNV (MET). Fusion detection sensitivity and PPV were demonstrated using the SeraCare ® FFPE tumor fusion RNA v4 control that includes 15 RNA variants including 2 RNA exon variants, EGFR and MET.

Sequencing on Genexus™ integrated sequencer – Liquid Biopsy samples

Sample Name	DNA Mapped Reads	% of reads On Target	Uniformity	Median Mol Cov	MAPD
Clinical research samples	11898471	93.12%	99.27%	2754.071	0.193846
Control (0.1-0.5% AF)	12514320	93.65%	100%	3128	0.2

Figure 10. cfTNA sequencing run matrices for clinical research samples and control with 0.1- 0.5% allelic frequency. All samples met the run and sample QC specifications.

	0.1 - 0.5% AF	0.25 - 0.5% AF
Sensitivity	95.90%	98.10%
PPV	95.90%	

Figure 11. Performance of cfTNA SNV and InDel detection. Average sensitivity and PPV for SNVs and InDels were calculated using internally generated fragmented control mixtures that contain variants at 0.1%, 0.25% or 0.5% allelic frequencies.

CONCLUSIONS

- Genexus™ system provides a user-friendly workflow with automated nucleic acid purification, quantitation, sample dilution, library preparation, sequencing, and data analysis with minimal hands-on time that can be performed with limited expertise.
- Genexus™ system, consumables, reagents and Oncomine™ Precision Assay are configured to provide flexibility in sample run configurations and lane usage to increase workflow efficiency.
- Oncomine™ Precision Assay only requires minimum input amount of DNA and RNA from FFPE samples (10ng) and cfTNA from liquid biopsy samples (20ng) to examine 78 variants, including mutations (45), CNVs (14), and fusion variants (19), across 50 key genes.
- Comparable sample and run QC matrices for control and clinical research samples on Genexus™ system demonstrate the robustness of the system.
- High sensitivity and PPV in detecting control and clinical research sample variants using Oncomine™ Precision Assay on Genexus™ system demonstrates the reliability of the assay and workflow to be used in clinical research.

REFERENCES

- Ion Torrent™ Genexus™ System - https://www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-run-sequence/ion-torrent-genexus-system.html
- Oncomine™ Precision Assay - https://www.thermofisher.com/us/en/home/clinical/preclinical-companion-diagnostic-development/oncomine-oncology/oncomine-precision-assay.html

ACKNOWLEDGEMENTS

Thanks to all the individuals involved in the development of the Genexus™ system and Oncomine™ Precision Assay.

DISCLAIMER

ThermoFisher SCIENTIFIC