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

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ABSTRACT

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.

To evaluate the nucleic acid (NA) extraction and sequencing performance of the Genexus™ system, contrived control samples with known variants and clinical research samples were used in the OPA FFPE and liquid biopsy workflows (n = 30). NA was quantified using the Genexus™ purification instrument's onboard Qubit™ quantitation feature after purification. Output plates with extracted NA were transferred to the Genexus™ Integrated Sequencer for library preparation and sequencing using the OPA assay. Data were analyzed using the Ion torrent Genexus™ software to evaluate assay performance and variant calling.

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.

Overall, this study demonstrates that the Genexus™ system provides a user-friendly workflow with automated NA purification, quantitation, sample dilution, library preparation, sequencing, and data analysis with minimal hands-on time that can be performed with limited expertise to obtain results within 24 hours. Our study demonstrated that the Genexus™ System and OPA assay successfully identified the presence of pre-specified variants from both control and clinical research samples from both FFPE and liquid biopsy sample types, supporting the use of the assay and system in clinical oncology research.

INTRODUCTION

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.

MATERIALS AND METHODS

The Genexus Purification System was used to isolate DNA and RNA using the Genexus™ FFPE DNA and RNA Purification Kit, and Genexus™ total cell free nucleic acid purification kit to isolate cfTNA from liquid biopsy samples. Nucleic acid was quantified using the onboard quantitation assay and sequenced on the Genexus™ Integrated Sequencer using Oncomine™ Precision Assay.

18 DNA and RNA samples including commercially available analytical control, Horizon® HD789 and Seracare® fusion control were sequenced from FFPE samples. 12 cfTNA samples in duplicate including internally generated control were sequenced from liquid biopsy samples. Run reports were generated by the Genexus™ Software (V6.6).

Ion Torrent™ Genexus™ System

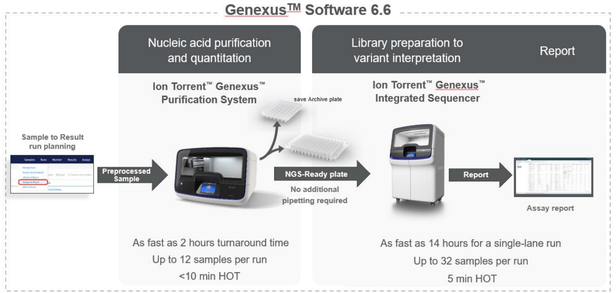


Figure 1. Ion Torrent™ Genexus™ System and workflow.

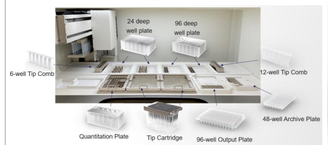


Figure 2. Genexus™ Purification System interior deck components and stations

Figure 3. Genexus™ FFPE DNA and RNA purification kit, and Genexus™ quantitation kit.

Genexus™ system is comprised of the Purification System and Genexus™ Integrated Sequencer. Genexus™ Software allows the integration of two instruments to perform end-to-end NGS workflows starting from run planning to generating a report. The Genexus™ purification system uses Genexus™ FFPE DNA and RNA purification kit, or Genexus™ total cell free nucleic acid purification kit along with Genexus™ quantitation kit and consumables which contain pre-filled reagents. All consumables are loaded on to instrument guided locations on the deck and are tracked by the automated barcode scanning.

Figure 4. Reagents and consumables used in Genexus™ Integrated sequencer. Reagents, Chips and Oncomine™ Precision Assay are configured to provide flexibility in sample run configurations and lane usage. 4 FFPE (4 DNA and 4 RNA samples) or one liquid biopsy cfTNA sample can be sequenced on one lane. Red color lanes in the right-hand side picture indicates successful loading of 3 out of 4 lanes in the Chip according to the Genexus™ Software where it can be reused to run the remaining lane.

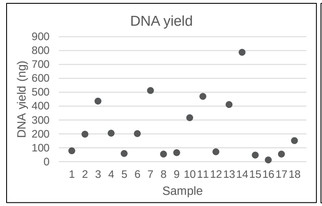
Oncomine™ Precision Assay (OPA)

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 5. 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



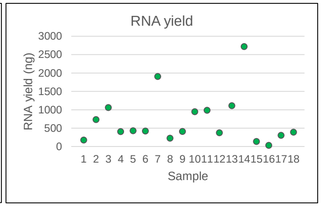
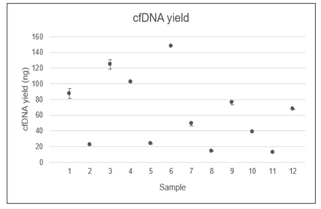


Figure 6 - 7. Sequential DNA and RNA extraction from FFPE samples. One 10µm curl per sample was used for the extraction. All samples yielded at least 10ng of DNA (Min = 12.43ng, Max = 787.8ng, Avg = 229.5 ng) and RNA (Min = 31.39ng, Max = 2718.34ng, Avg = 709.63 ng) recommended for the assay.



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 9. 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 8. 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 10. 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 11. 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 12. 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 13. 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 14. 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

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- Oncomine™ Precision Assay - https://www.thermofisher.com/us/en/home/clinical/preclinical-companion-diagnostic-development/oncomine-oncology/oncomine-precision-assay.html

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DISCLAIMER