Rapid and Accurate Variant Calling of FFPE Samples with the Genexus System

Iris Casuga, Frances Chan, Milton Huynh, Gregory R. Govoni, Kayla Zochowski, Thilanka Jayaweera, Janice Au-Young
Thermo Fisher Scientific, 180 Oyster Point, South San Francisco, CA, USA, 94080

ABSTRACT

Next-Generation Sequencing technology has enhanced oncology research by enabling the detection of all cancer related variants into one assay for research and drug discovery programs. The Oncomine™ Comprehensive Assay v3, a pan-cancer panel, used with the Ion Torrent™ Genexus™ System allows for formalin-fixed paraffin embedded (FFPE) samples to be examined across 161 unique genes in an automated sample-to-result workflow in 30hrs. This study demonstrates ≥ 95% Sensitivity and PPV for detecting SNV, Indel, and Copy Number variants of clinical FFPE samples along with fusions. When tested using analytical controls, the Genexus System achieves ≥ 98% Sensitivity for hotspot variants, ≥ 95% for de novo variants, and 100% Sensitivity for fusion variants.

The Ion Torrent™ Genexus™ System is fully automated and consists of two software linked instruments, the Ion Torrent™ Genexus™ Purification System and the Ion Torrent™ Genexus™ Integrated Sequencer. For this study, the purification system was used to sequentially extract and quantify DNA & RNA samples from human colon and lung FFPE tumor tissue. The purification instrument provides minimal hands-on-time, ease of use and compatibility with the integrated sequencer. It extracts and quantifies the nucleic acids, records the quantitation values and transfers the nucleic acids to an output plate. The plate is transferred from the purification instrument directly to the sequencer for sample dilution, library preparation, and sequencing using the Oncomine™ Comprehensive Assay v3 panel.

Variant calling analysis of the DNA & RNA pairs is completed immediately following sequencing. Results show that the variants are concordant with ≥ 95% Sensitivity and PPV when compared to the Ion GeneStudio™ S5 System as an orthogonal method. All variants in the report were correct with p-values ≤ 10^{-5} . Additionally, a subset of the extracted samples was evaluated using Sanger Sequencing as an orthogonal method to verify hotspot mutations found in samples sequenced on both the Genexus™ System and GeneStudio™ S5 System. Results show that all expected hotspot variants were detected. In summary, the Ion Torrent™ Genexus™ System is a reliable and fast turnaround solution for sample-to-variant calling results. When used with the Oncomine™ Comprehensive Assay v3 panel, the system provides accurate identification of tumor markers for oncology research.

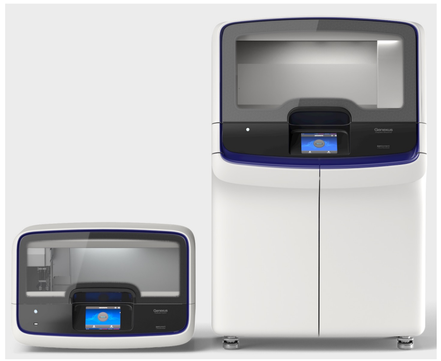
INTRODUCTION

The Ion Torrent™ Genexus™ System consists of two software linked instruments, the Ion Torrent™ Genexus™ Purification System and Ion Torrent™ Genexus™ Integrated Sequencer. The purification instrument extracts and quantifies DNA and RNA from FFPE lysates. The nucleic acid output plate from the purification instrument along with nucleic acid quantitation values are transferred directly to the sequencer instrument for library preparation, templating, and sequencing. The process is automated, has little hands-on time, and allows for flexible experiment planning. This poster demonstrates the use of the Ion Torrent™ Genexus™ System to determine variants of FFPE samples using the Oncomine™ Comprehensive Assay v3 (OCAv3), a solid tumor library panel used in translational and clinical research. The Ion GeneStudio™ S5 System is used as an orthogonal method to compare variant calls of the same samples. This study reports overall 100% Sensitivity and 100% Specificity.

MATERIALS AND METHODS

The Ion Torrent™ Genexus™ System, Ion Chef™ Instrument, Ion GeneStudio™ S5 instrument and consumables used in this work were manufactured by Thermo Fisher Scientific. The OCAv3 assay targets SNVs, InDels, CNVs, and fusions from 161 cancer-related genes. Libraries were made using OCAv3 GX panel for the Ion Torrent™ Genexus™ System and OCAv3M panel for the Ion GeneStudio™ S5 System. Six unique FFPE tumor sections from human colon and lung tissue were used. Each FFPE lysate was prepared from two, 10 micron sections and processed in Autolys tubes for protease digestion. The FFPE lysates were then processed on the Ion Torrent™ Genexus™ Purification Instrument using the Genexus™ FFPE DNA and RNA Purification kit into 6 DNA & 6 RNA matched pairs. Results were collected directly from the Torrent Suite analysis reports. Sensitivity is calculated by confirming the variants detected using the Ion Torrent™ Genexus™ System are also detected using Ion Chef™ Instrument and Ion GeneStudio™ S5 System. Specificity is the positive predictive value (PPV). A true positive is defined as a variant identified by prior testing on the Ion GeneStudio™ S5 platform. A false negative is defined as a variant previously reported by Catalogue of Somatic Mutations in Cancer (COSMIC) database but not detected by the Ion Torrent™ Genexus™ System. Qualifying variants have p-values ≤ 10^{-5} and Quality scores ≥ 60 signifying more confidence that the variant call is correct.

Figure 1. The Ion Torrent™ Genexus™ System



Left: The Ion Torrent™ Genexus™ Purification Instrument.
Right: The Ion Torrent™ Genexus™ Integrated Sequencer.

Figure 2. The Ion Torrent™ Genexus™ System Workflow

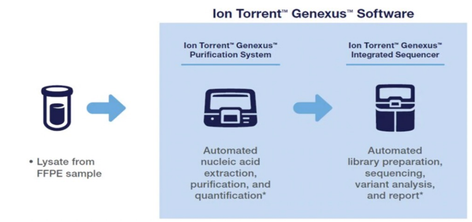
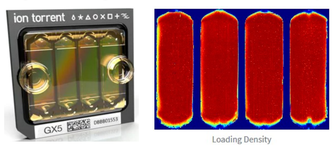


Figure 3. Image of the Ion Torrent™ GX5™ Chip & Chip Loading Density



Left: 6 DNA + 6 RNA samples from FFPE can be sequenced on a single GX5™ chip across 4 lanes

Right: Chip loading image from Torrent Suite analysis report of a 4 lane run with OCAv3 panel.

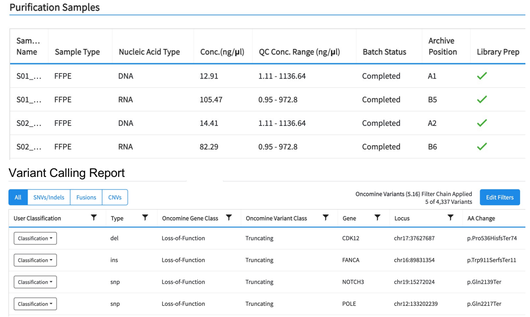
RESULTS

Table 1. Run Time for OCAv3 DNA & RNA Purification to Sequencing Run

Number of Samples	Genexus Purification Run Time	Genexus Integrated Sequencer Run Time
6 DNA + 6 RNA	3hrs 15min	25hrs 45min

The table reports the instrument run time. Time does not include processing FFPE curls into lysates. Hands on time handling of the instrument consumables is minimal.

Figure 4. Example of an Ion Torrent™ Genexus™ Analysis Report



Results from the Ion Torrent™ Genexus™ System are generated automatically into a single report.
Top: Screen capture of quantitation results of FFPE samples extracted using the Ion Torrent™ Genexus™ Purification Instrument showing sample yield values meeting concentration range to proceed to library preparation.
Bottom: Screen capture of variant calling results from the analysis report of an FFPE sample analyzed with the OCAv3 Panel.

Table 2. Analysis of 6 unique FFPE samples with OCAv3

Sample Name	Sample Origin	Variant Type	Gene	AA Change	Genexus System	Ion GeneStudio	
						Rep 1	Rep 2
FFPE_1	NSCLC Tumor	SNV	BRAF	p.Gly469Ala	✓	✓	✓
		SNV	CHEK2	p.Arg523Cys	✓	✓	✓
		MNV	NF1	p.[Leu90=;Glu91Ter]	✓	✓	✓
		SNV	SETD2	p.Gln109Ter	✓	✓	✓
		SNV	SMAD4	p.Glu390Ter	✓	✓	✓
FFPE_2	NSCLC Tumor	SNV	NF1	p.Gln543Ter	✓	✓	✓
		SNV	TP53	p.Gly245Cys	✓	✓	✓
FFPE_3	NSCLC Tumor	SNV	TERT	p.[Val197=;Glu198Ter]	✓	✓	✓
		SNV	CDKN2A	p.Asp84Tyr	✓	✓	✓
		SNV	TP53	p.?	✓	✓	✓
FFPE_4	Colon Tumor	SNV	BRCA1	p.Lys339ArgfsTer2	✓	✓	✓
		SNV	FANCA	p.Trp911SerfsTer11	✓	✓	✓
		SNV	BRAF	p.Val600Glu	✓	✓	✓
FFPE_5	Colon Tumor	INDEL	CDKN1B	p.Ser160PhefsTer44	✓	✓	✓
		SNV	SMAD4	p.Arg361His	✓	✓	✓
FFPE_6	Colon Tumor	SNV	BRAF	p.Val600Glu	✓	✓	✓
		SNV	CDK12	p.Arg1356Ter	✓	✓	✓

Sample Name	Sample Origin	Variant Type	Gene	Copy Number	Genexus System	Ion GeneStudio	
						Rep 1	Rep 2
FFPE_3	NSCLC Tumor	CNV	EGFR	Gain of function	12.36	12.13	12.29

Total Variants	18	18
False Negatives	0	0
False Positives	0	0
Sensitivity	100%	100%
PPV	100%	100%

The Ion GeneStudio™ S5 System was used to show concordance for the variant calls with the same FFPE sample source. Libraries were created and templated on the Ion Chef™ instrument using the Ion Ampliseq™ DL8 Kit for library preparation and Ion 540™ Kit for templating. Two Ion 540™ chips were sequenced using the Ion GeneStudio™ S5 System and the sequencing data was transferred to the Ion Reporter™ software for variant calling analysis. Results show that the Ion Torrent™ Genexus™ System and Ion GeneStudio™ S5 System variant calls are in agreement with each other with 100% Sensitivity and 100% PPV. Qualifying variants report p-values ≤ 10^{-5} and Quality scores ≥ 60 signifying more confidence that the variant call is correct. No fusion variants were detected in these samples; Only SNV/InDel and CN variants were found. Copy Number values are similar across platforms.

Table 3. Analysis of analytical control samples with OCAv3

Control Type	Analytical Control	Number of Replicates	Average Sensitivity	Average PPV
DNA	Acrometrix™ Oncology Hotspot Control	9	98.7%	98.9%
	Seraseq® Trilevel Tumor Mutation Mix v2	8		
	Seraseq® Breast CNV Mix +6 copies	3		
RNA	Seraseq® Fusion RNA Mix v4	15	100%	100%

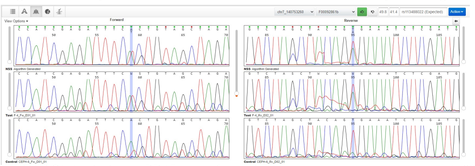
Analytical control samples were used with the OCAv3 GX panel on the Genexus System to calculate Sensitivity and Specificity of variant detection of SNV/InDel, CNV, and Fusion variants. Analysis of the Acrometrix™ Oncology Hotspot Control done by Kayla Zochowski PhD.

Table 4. Analysis of hotspot mutations using Sanger Sequencing

Sample Name	Sample Origin	Variant Class	Gene	AA Change	Mutation	Strand	Chromosome	Position (GRCh38.p12)	SNP	Amplicon Size	Mutation Detected
FFPE_1	NSCLC Tumor	Hotspot SNV	BRAF	p.Gly469Ala	G [GGA] > A [GCA]	Rev	7	140781602	rs121913355	388	Yes
		Hotspot SNV	CHEK2	p.Arg523Cys	R [CGT] > C [TGT]	Rev	22	28687962	rs149501505	395	Yes
FFPE_3	NSCLC Tumor	Hotspot SNV	TERT	p.[Val197=;Glu198Ter]	G > A	Fw	5	1295113	rs1242535815	586	Yes
		Hotspot SNV	CDKN2A	p.Asp84Tyr	D [GAC] > Y [TAC]	Rev	9	21971109	rs11552822	339	Yes
FFPE_5	Colon Tumor	Hotspot SNV	SMAD4	p.Arg361His	R [CGC] > H [CAC]	Fw	18	51065549	rs377767347	319	Yes
FFPE_6	Colon Tumor	Hotspot SNV	BRAF	p.Val600Glu	V [GTG] > E [GAG]	Rev	7	140753336	rs113488022	189	Yes

A subset of the FFPE samples were used to verify hotspot variants using Sanger Sequencing. Validated primer sets or newly designed sets were used to detect variants. PCR was done with 10ng of input per variant using Amplitaq™ 360 Gold MasterMix and Amplitaq™ 360 DNA Polymerase. Cycle sequencing and purification were done using BigDye™ Terminator v3.1 Cycling Kit and BigDye Xterminator™ Purification Kit. Primer design and Sanger Sequencing work completed by Gregory R. Govoni PhD.

Figure 5. Hotspot mutation detected in colon tumor sample using Sanger Sequencing



Hotspot SNV detected on BRAF gene of colon tumor sample, amino acid change p.Val600Glu.

CONCLUSIONS

This study demonstrates the use of the Ion Torrent™ Genexus™ System to determine variant calls of FFPE samples using the Oncomine™ Comprehensive Assay v3. Results show that the variants identified using samples purified from the Ion Torrent™ Genexus™ Purification Instrument and sequenced on the Ion Torrent™ Genexus™ Integrated Sequencer were also identified when the same samples were used on the Ion Chef™ Instrument and Ion GeneStudio™ S5 System with 100% Sensitivity. A total of 18 variants were detected across 6 DNA & 6 RNA FFPE matched pairs: 17 SNV/InDel variants and 1 copy number variant. Rare fusion variants are not expected and were not detected among the 6 samples tested with the Genexus or GeneStudio systems. In addition, a subset of FFPE samples were used to verify hotspot variants using Sanger Sequencing, with all expected hotspot variants detected. Analytical control samples were also used to demonstrate high variant detection capability with ≥ 98% Sensitivity for SNV, Indel, CNV, and Fusion variants.

The experiment reported here illustrates an automated workflow solution for sample purification, library preparation, templating and sequencing. Variant calling accuracy meets previously established standards for the Ion GeneStudio™ S5 System (2). The OCAv3 assays were used in this work to demonstrate the speed and ease-of-use of the system and equivalent performance to Ion GeneStudio™ S5 System.

REFERENCES

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Sample Vendors:
1. Biochain Institute Inc., https://www.biochain.com/
2. Disovery Life Sciences - Folio Conversant, https://www.dls.com/
3. SeraCare – www.seracare.com

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author: iris.casuga@thermofisher.com Thermo Fisher Scientific • 180 Oyster Point Boulevard • South San Francisco, CA 94080 • thermofisher.com