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

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ABSTRACT

Next-Generation Sequencing technology has enhanced oncology research by enabling the detection of relevant variants for clinical 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 workflow from sample to result in 30hrs. This study demonstrates ≥ 90% accuracy for detecting SNV/Indel/CNV and Fusion variants.

The Ion Torrent™ Genexus™ System 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 first to extract and quantify 6 FFPE sections from human colon and lung tissue for matching DNA & RNA pairs. The nucleic acid output plate from the purification instrument is directly transferred to the sequencer for library preparation and sequencing using the Oncomine™ Comprehensive Assay v3. Variant calling analysis of 6 DNA & RNA pairs are completed the following day. Results show that the variants were detected with ≥ 90% Sensitivity when compared to the Ion GeneStudio™ S5 System as an orthogonal method. All variants report p-values ≤ 10⁻⁵ signifying more confidence that the variant call is correct. The Ion Torrent™ Genexus™ System is a seamless solution for sample to variant calling results.

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 FFPE lysates into DNA and RNA. The nucleic acid output plate is 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 variant calls of FFPE samples using the Oncomine™ Comprehensive Assay v3 (OCAv3), an oncology library panel used in translational and clinical research. The Ion GeneStudio™ S5 System is used as an orthogonal method to compare variant calls using the same sample source. 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. 6 unique FFPE tumor sections from human colon and lung tissue were used. Each FFPE lysate was prepared from two, 10micron 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.
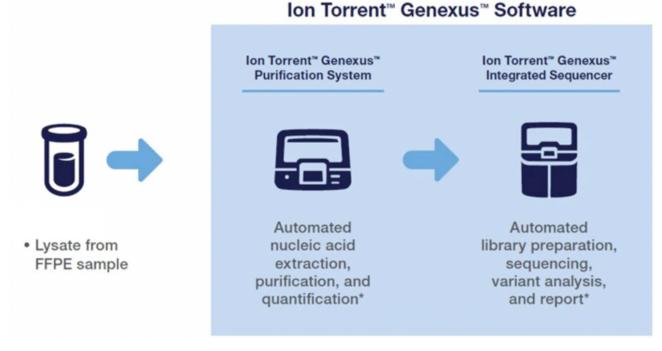
$$Sensitivity = (True\ Positives\ /\ (True\ Positives + False\ Negatives))$$
$$Positive\ Predictive\ Value = True\ Positive\ /\ (True\ Positive + False\ Positive)$$

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

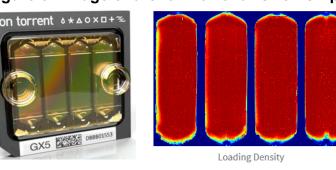Left: 6 DNA + 6 RNA FFPE samples can be sequenced on a single GX5™ chip across 4 lanes

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

RESULTS

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 1. Analysis of 6 unique FFPE samples with OCAv3

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

Sample Name	Sample Origin	Variant Type	Gene	Copy Number	Genexus System	Ion GeneStudio Rep 1	Ion GeneStudio 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 as an orthogonal method to detect variant calls using 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 2. 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.

CONCLUSIONS

This poster 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 CN variant. Rare fusion variants were not detected among the 6 samples tested.

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.

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