Identification of relevant genetic variants in hereditary cancer research samples

Genexus Integrated Sequencer for a rapid, automated NGS workflow

Introduction

Ion AmpliSeq™ On-Demand Panels allow you to build custom panels from over 5,000 pretested genes that are most relevant in research on inherited diseases, including hereditary cancer, primary immunodeficiency, hearing loss, muscular dystrophy, and many more. Available in practical pack sizes, these panels allow you to iterate panel design for your research with efficiency and convenience. With the Ion Torrent™ Genexus™ System and Ion AmpliSeq On-Demand Panels, you can analyze genetic variants of interest in a highly automated workflow that allows you to go from specimen to variant report in as little as a single day.*

Benefits of Ion AmpliSeq On-Demand Panels on the Genexus System for inherited disease research:

- **Unprecedented automation and ease of use, from specimen to variant report**—just 10 minutes of hands-on time and two user touchpoints*
- **Single-day turnaround time**—from biological specimen to variant report
- **Easy panel design iteration**—based on the leading Ion AmpliSeq targeted sequencing technology, Ion AmpliSeq On-Demand Panels are customizable targeted next-generation sequencing (NGS) panels with pretested genes for research on germline diseases such as hereditary cancer

Genexus System workflow for inherited disease research

- Whole blood
- Plasma

Automated nucleic acid extraction, purification, and quantification*

Automated library prep, template prep, sequencing, and variant analysis

* Specimen-to-report workflow will be available after the Genexus Purification System and integrated reporting capabilities are added in 2020.

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Analyzing hereditary cancer research samples using Ion AmpliSeq On-Demand Panels on the Genexus Integrated Sequencer

In this application note, we demonstrate the NGS workflow—from nucleic acid to variant report—on the Ion Torrent™ Genexus™ Integrated Sequencer using two Ion AmpliSeq On-Demand Panels for hereditary cancer research samples. Results were generated from uncharacterized cell line samples and clinical research samples using both the Genexus Integrated Sequencer and Ion GeneStudio™ S5 System. In the Genexus Integrated Sequencer workflow, library prep, template prep, and sequencing were all automated on the integrated sequencer using the Ion Torrent™ GX5™ Chip. In the Ion GeneStudio S5 System workflow, library prep was performed manually, template prep was automated on the Ion Chef™ System, and sequencing was done using the Ion 530™ Chip. Concordance in results was observed between the two systems.

Key findings

- Collaboration with two genetic service providers resulted in identification of single-nucleotide variants from two different hereditary cancers using the Ion AmpliSeq On-Demand Panels run on the Ion GeneStudio S5 System and the Genexus Integrated Sequencer, which showed highly concordant (>95%) data.
- The Genexus Integrated Sequencer provides a fast, highly automated, and easy workflow to go from nucleic acid to variant report, making the technology accessible to clinical research or testing labs regardless of the level of NGS expertise.
- Ion AmpliSeq™ technology enables identification of known variants and discovery of novel variants through accurate and automated variant calling that helps eliminate bias when characterizing molecular markers.

Methods

- DNA inputs (20 ng for the Ion GeneStudio S5 System and 28 ng for the Genexus Integrated Sequencer) from cell lines NA12878, NA19240, NA24385, NA24631 (Coriell Institute) were analyzed using Hereditary Cancer Research Panel 1 (33 genes, 982 amplicons) and Hereditary Cancer Research Panel 2 (49 genes, 1,127 amplicons).
- For the Genexus Integrated Sequencer workflow, library prep, template prep, and sequencing were fully automated with a single touchpoint on the instrument using the Ion Torrent GX5 Chip (Cat. No. A40269). Libraries were prepared with the Ion Torrent™ Dual Barcode Kit (Cat. No. A39360).
- For the Ion GeneStudio S5 System workflow, libraries were constructed manually in duplicate, template prep was automated on the Ion Chef System, and sequencing was done on the Ion GeneStudio S5 System using the Ion 530 Chip (Cat. No. A27764). Libraries were prepared with Ion Torrent™ IonCode™ Barcode Adapters (Cat. No. A29751).

Ion AmpliSeq On-Demand Panels for hereditary cancer research

Two Ion AmpliSeq On-Demand Panels that include genes associated with hereditary cancer research were designed using the Ion AmpliSeq™ Designer (Figure 1).

Ion AmpliSeq Designer version 7.4
Amplicon designs target 125–275 bp length

Hereditary Cancer Research Panel 1	Hereditary Cancer Research Panel 2
• Genes: 33	• Genes: 49
• Amplicons: 982	• Amplicons: 1,127
• Panel size: 185.7 kb	• Panel size: 213.6 kb
• *In silico* coverage: 99.0%	• *In silico* coverage: 100.0%

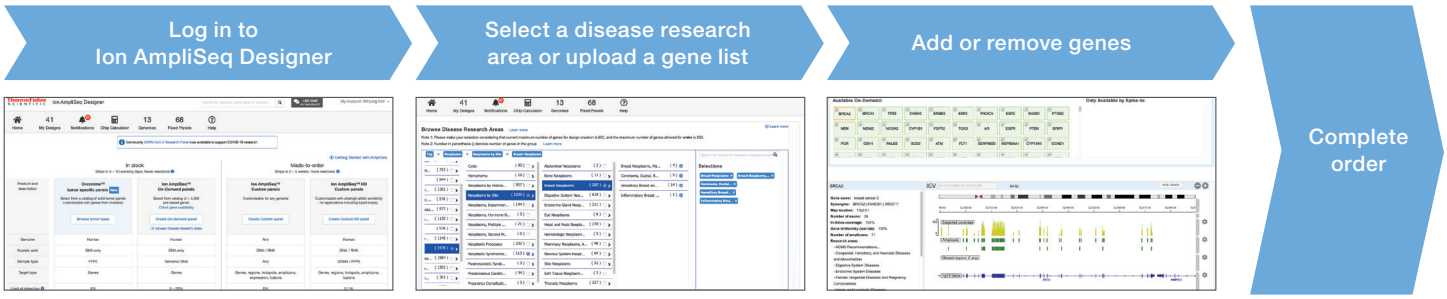

Figure 1. The Ion AmpliSeq On-Demand Panel design workflow. Start your panel design in Ion AmpliSeq Designer (**ampliseq.com**) by either selecting a disease research area or uploading a list containing the genes of your choice. Easily configure the panel design by adding or removing genes. After the design is finalized, consult with a sales or field support representative for assistance with ordering.

Ion AmpliSeq On-Demand Panels contain full coding sequence (CDS) designs and have been optimized for unbiased target design to cover multiple variants, including single-nucleotide polymorphisms (SNPs), short indels, and copy number variants (CNVs). All gene designs in the catalog have been pretested to assure that *in silico* designs translate to high coverage and uniformity in your lab. Ion AmpliSeq On-Demand Panels are made using a 2-pool DNA and 125–275 bp amplicon design, based on the hg19 reference genome.

Workflow

The demonstrated data in this application note were generated using the following nucleic acid–to–variant report workflow on the Genexus Integrated Sequencer.

Select targets	Set up NGS run	Automate library prep, template prep, sequencing and analysis	
• Use the intuitive Ion AmpliSeq Designer	• Load primers to the Ion Torrent™ Genexus™ Primer Pool Tubes • Complete Genexus Integrated Sequencer setup in as little as 5 minutes	• Genexus Integrated Sequencer • GX5 Chip	Genexus Software 6.0 for variant calling and annotation Plug-ins: • Torrent Variant Caller • Coverage analysis

Results

Consistent performance of Ion AmpliSeq On-Demand Panels between the Genexus Integrated Sequencer and Ion GeneStudio S5 System was observed with detection of concordant variants (Figures 2–4, Table 1).

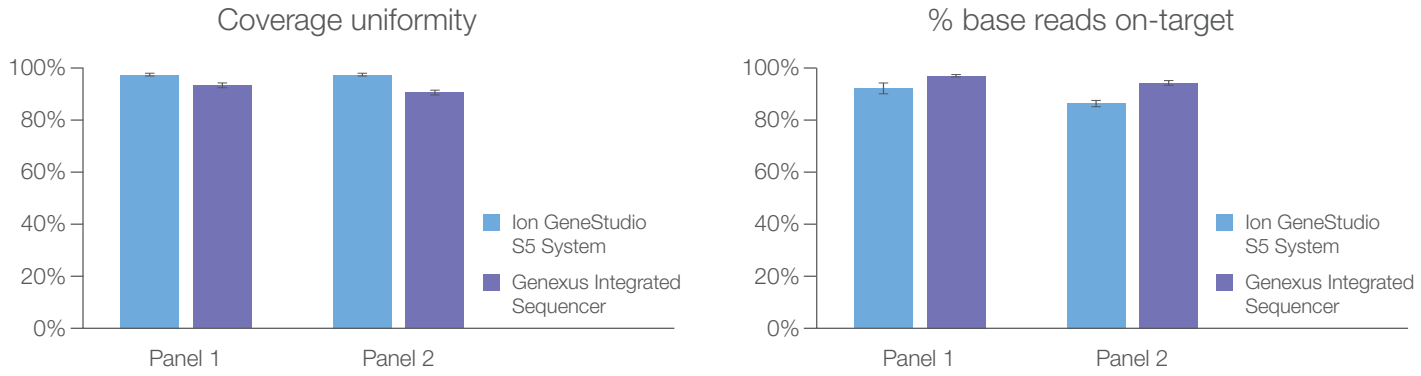

Figure 2. Coverage uniformity and percent base reads on-target for panel 1 and panel 2 on the Ion GeneStudio S5 System (n = 8) and Genexus Integrated Sequencer (n = 7).

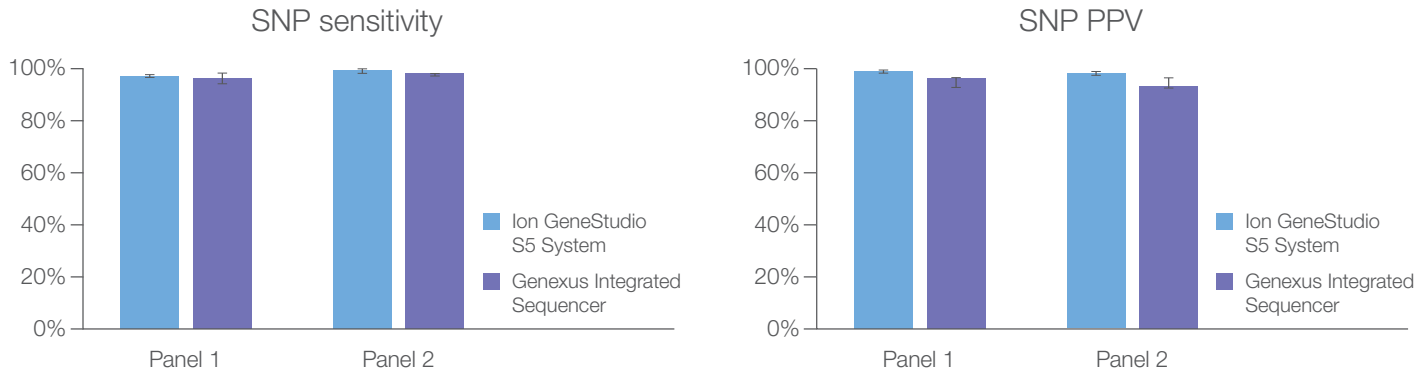

Figure 3. Sensitivity and PPV for SNPs, for panel 1 and panel 2 on the Ion GeneStudio S5 System (n = 6) and Genexus Integrated Sequencer (n = 5). For SNPs, sensitivity of >95% and PPV of >94% were observed for both the Ion GeneStudio S5 System and Genexus Integrated Sequencer.

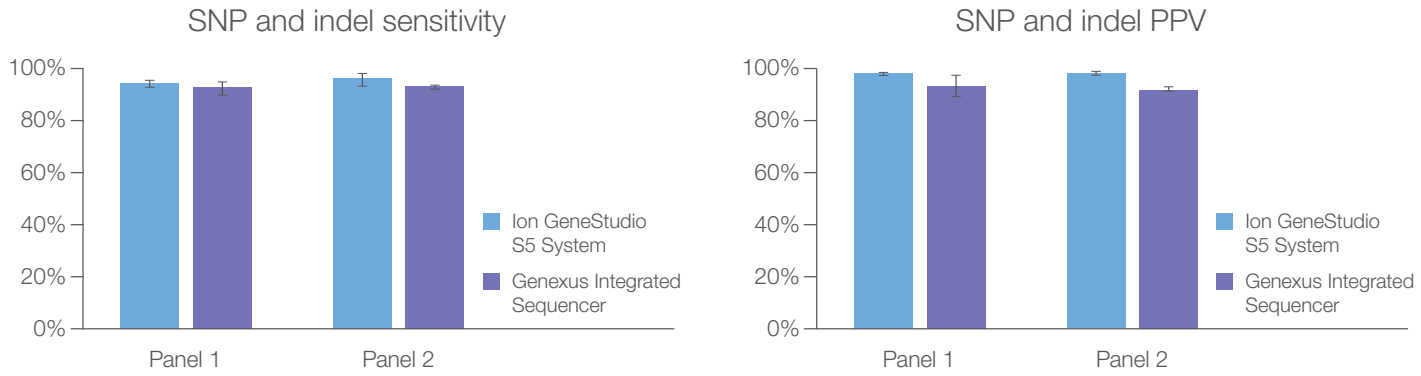

Figure 4. Sensitivity and PPV for SNPs and indels, for panel 1 and panel 2 on the Ion GeneStudio S5 System (n = 6) and Genexus Integrated Sequencer (n = 5). For SNPs and indels combined, sensitivity of >91% and PPV of >92% were observed for both the Ion GeneStudio S5 System and Genexus Integrated Sequencer.

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Table 1. Concordant results from hereditary cancer research samples analyzed using the Ion AmpliSeq On-Demand Panel. Clinical research samples (n = 5) obtained from a genetic service provider were analyzed in a blind study on both the Ion GeneStudio S5 System and the Genexus Integrated Sequencer using an Ion AmpliSeq On-Demand Panel for hereditary cancer research. Sample variant information for concordance assessment was obtained by the genetic service provider using the Ion GeneStudio S5 System.

Sample	n	Gene variant	Reference	Mutation	Concordance
Sample 1	3	*MSH6*	C	C>G	Yes
Sample 2	3	*BRCA1*	G	G>C	Yes
Sample 3	3	*ATM*	GA	DelGA	Yes
Sample 4	3	*BRCA1*	–	InsG	Yes
Sample 5	3	*BRCA1*	C	C>T	Yes

Conclusions

The Genexus System combined with Ion AmpliSeq On-Demand Panels will provide a highly automated NGS workflow from specimen to variant report in a single day,* enabling you to analyze variants at a speed never possible before. In the future, the hands-off, set-up-and-go workflow on the Genexus System will make NGS accessible even if your lab is new to the technology; and with less operational hands-on time compared to current technologies, this new solution can help improve your lab's productivity.

* Specimen-to-report workflow will be available after the Genexus Purification System and integrated reporting capabilities are added in 2020.

Find out more at **thermofisher.com/inherited-genexus**

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