Enabling reproductive genomics and inherited disease research with the Ion S5 next-generation sequencing systems

Ion S5 systems and inherited disease research

- Predesigned sequencing panels support cost-effective detection of single-nucleotide variants (SNVs), indels, and copy number variants (CNVs) for inherited disease and reproductive genomics research
- Customize existing inherited disease research panel designs and deliver just the content needed using the simple-to-use Ion AmpliSeq™ Designer tool
- Rapid sequencing workflows with integrated data analysis
- Easy-to-use sequencing system with variable data outputs to match sample sizes and target multiplexing (Figure 1)

Introduction

Next-generation sequencing (NGS) has revolutionized the detection of genomic changes associated with genetic diseases. With the capability to multiplex thousands of targets and hundreds of samples in a single sequencing run, often at a lower cost per sample than conventional methods, NGS is enabling the replacement of multiple single-endpoint assays for human genetic disease research.

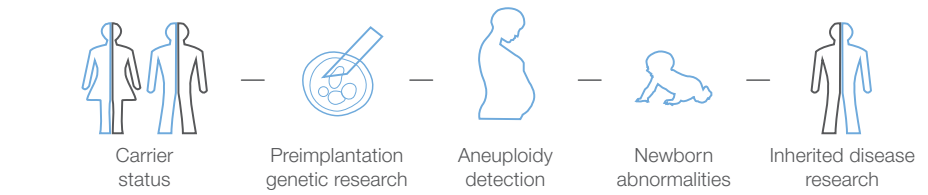

Figure 1. Ion S5™ systems support flexible workflows that enable multiple applications in inherited disease research. The combination of targeted Ion AmpliSeq™ panels with new components, including easy-to-use cartridge-based reagents, enables comprehensive inherited disease research. Three chips are available for scalable sequencing output from 5 million to 80 million reads.

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Using Ion AmpliSeq™ targeted assay technology, thousands of amplicons, including the complete exome, can be rapidly amplified by PCR followed by sequencing using the Ion S5™ or Ion S5™ XL System. Ready-to-use Ion AmpliSeq assays are available to target genetic loci of interest for carrier screening, newborn screening, and inherited disease research (Tables 1 and 2). In addition, structural variations such as aneuploidies and large CNVs can be detected in preimplanted embryos by sequencing the genome at low depth.

Table 1. Number of samples that can be multiplexed per chip on Ion S5 systems (barcodes available for up to 96 samples per run).

	Ion 520™ Chip	Ion 530™ Chip	Ion 540™ Chip
			
Reads (millions/chip)	3–5	15–20	60–80
Maximum read length (bp)	400	400	200
Samples per run			
Ion AmpliSeq™ Pharmacogenomics Research Panel (119 amplicons)*	96	384†	NA
Ion AmpliSeq™ Cardiovascular Research Panel (404 genes; 10,430 amplicons)‡	2	8	32
Ion AmpliSeq™ Exome Kit (~294,000 amplicons)	NA	NA	2

* Assumes 250x coverage.
† Upon availability of 384 barcodes. The content provided herein may relate to products that have not been officially released and is subject to change without notice.
‡ Assumes 175x coverage.

Sequencing workflow

Detecting variants and determining copy number changes for genetic disease research can be accomplished in 5 steps (Figure 2).

1. Target selection

Ready-to-use panels are available; for a complete list for inherited disease research, visit **thermofisher.com/ionampliseq**

Panels and targeted regions can be customized using a free online assay design tool such as Ion AmpliSeq Designer (ampliseq.com). Primer pools can be created to target any customer-defined region of interest within a reference sequence.

2. Integration of library preparation with the Ion Chef System

Ion AmpliSeq technology combines the simplicity and speed of PCR with the advantages of low DNA input (as low as 10 ng) and allows analysis of difficult samples, including formalin-fixed, paraffin-embedded (FFPE) samples.

Ion AmpliSeq™ libraries may be prepared automatically for 8 samples per run of 1- or 2-pool panel designs on the Ion Chef™ System. Requiring no more than two pipetting steps per sample,** Ion AmpliSeq library preparation on the Ion Chef System helps reduce hands-on time, resulting in pooled libraries ready for downstream template preparation.

3. Clonal amplification (template preparation)

The Ion Chef System automates emulsion PCR to clonally amplify library molecules. Requiring only 15 minutes of hands-on time, the Ion Chef System combines automated template preparation and chip loading for users at any experience level.

4. Sequencing

Setup of sequencing runs on the Ion S5 and Ion S5 XL instruments is simple and rapid, with less than 15 minutes of hands-on time to install the ready-to-use nucleotide cartridge, wash buffer, cleaning solution, and waste container. The user then installs the chip and initiates a run using simple touch-screen commands.

5. Data analysis

Raw data, base calling, and variant calling are processed on the Ion S5 sequencer or, in the case of the Ion S5 XL System, transferred to a dedicated Ion S5™ Torrent Server for faster analysis. Torrent Suite™ Software is used to plan runs and for plugin analysis.

Data generated on the Ion S5 System can be automatically uploaded from Torrent Suite Software to Ion Reporter™ Software for workflows typical of inherited disease research, which include read mapping, variant detection, multi-sample analysis, and annotation. Identified variants can be filtered based on technical criteria

such as read coverage, *P* value, allele frequency, and annotations. After you classify and interpret the variants, Ion Reporter Software displays a summary in an easily readable report.

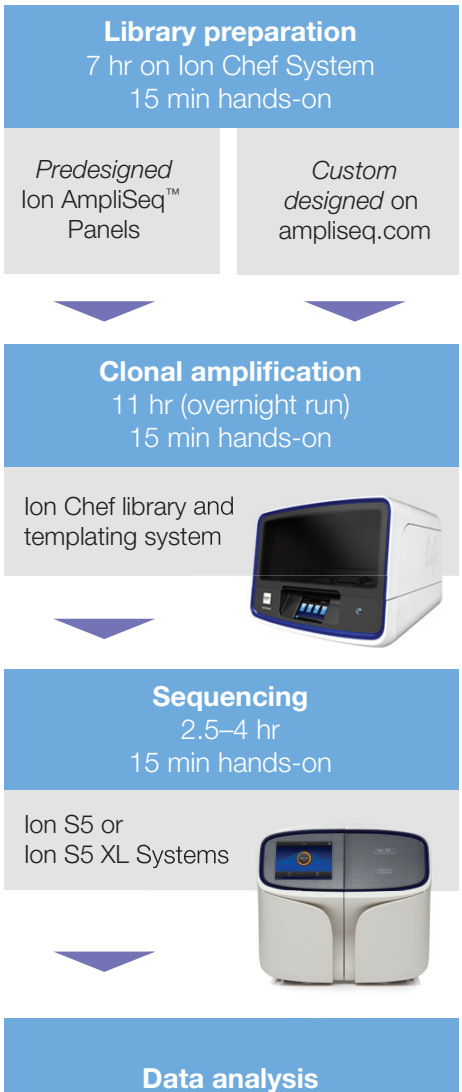

Figure 2. Targeted sequencing workflow. Ion AmpliSeq libraries are prepared manually or with the Ion Chef System. Libraries are then placed in the Ion Chef System for emulsion PCR, enrichment, and loading onto Ion chips. Chips are placed in the Ion S5 System with reagents for sequencing. Primary data analysis is performed by Torrent Suite Software.

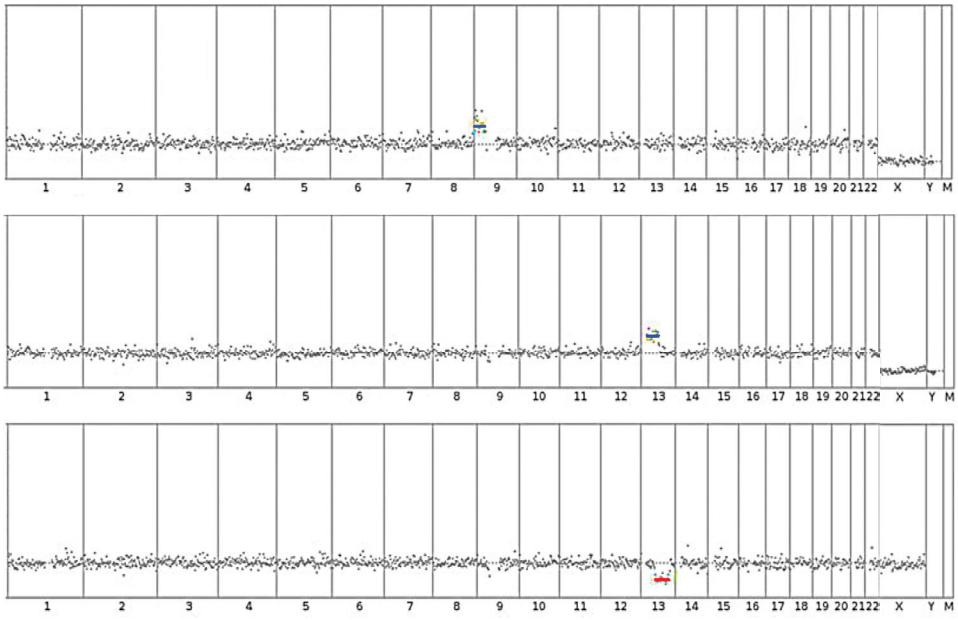

Figure 3. Aneuploidy detection in single-cell samples using an Ion ReproSeq PGS Kit.* Single FACS-sorted cells from 3 reference CNV cell lines were sequenced, for a total of 72 sequenced libraries, using the Ion 530™ Chip. The normalized coverage ratio plots across the entire genome and the determined ploidy are shown. Gains (trisomies) are indicated in blue, and loss (monosomy) in red.

Results

Detection of aneuploidy in embryonic cells

The aim of preimplantation genetic screening (PGS) is to help determine which embryos to proceed with to help achieve a successful pregnancy. This requires screening a single cell removed from each developing embryo at day 3, or ~5–10 cells at day 5, for aneuploidies. However, time-to-results is critical when determining which embryos have a normal chromosomal complement, since the embryos are growing and dividing and need to be transferred to help ensure effective implantation.

To this end, an Ion ReproSeq™ PGS Kit protocol* was developed to enable a rapid 8–10 hour workflow. Through the use of whole-genome amplification (WGA), genomic DNA from a small number of cells—even a single cell—can be used for low-pass sequencing of the entire genome. Sequencing of single cells shows high sensitivity and specificity, with all aneuploidies positively identified using the Ion Reporter 5.0 analysis pipeline (Figure 3).

Newborn screening, inherited disease research, and carrier screening

For inherited disease research, the flexible chip compatibility of the Ion S5 System, in combination with the scalability of Ion AmpliSeq technology, enables sequencing of small to large gene panels (including the entire exome) for newborn screening, inherited disease research, and carrier screening. To this end, 18 Mendeliome research panels are available, targeting an average of 1.2 Mb (range of 144 kb–3.6 Mb) per panel representing an average of 294 genes (range of 41–751 genes) (Table 2). The panels are organized by organ system or disorder classification, and the utility of these panels has been demonstrated to be equivalent to exome sequencing but at a potentially lower cost [1].

* The Ion ReproSeq PGS Kit protocol is currently validated and supported only for the Ion PGM™ System.

Ion AmpliSeq Exome RDY Kit

The Ion AmpliSeq™ Exome RDY Kit targets ~33 Mb of coding exons representing >97% of coding regions as described by Consensus Coding Sequence (CCDS) annotation. Multiplexing two samples on an Ion 540™ Chip, the analysis of 16 runs resulted in an average of 44 million reads per sample, of which an average of 94.3% were on target. Excellent targeting efficiency was observed with a mean coverage depth across 32 samples of 125x, with an average of 94.6% targeted bases covered at 20x and an average uniformity of base coverage of 94.3%.

Predicted variants in Coriell sample NA12878 were compared to the overlapping region of high-confidence NIST variant genotypes (total: 27,051 SNVs and 611 indels). Across 32 samples, the average sensitivity for SNVs and indels combined was 97.5%. Demonstrating high accuracy for detecting exome variants, the overall positive predictive value (PPV) was 98.5% while the proportion of wrongly identified variants was low, with average false-positive rates of 9.5 and 2.4 per Mb for SNVs and indels, respectively.

Ion AmpliSeq pharmacogenomics (PGx) research solution

The role that genetic variation plays in drug metabolism can be investigated using the Ion AmpliSeq™ PGx research solution, which assesses 136 SNV and indel hotspots in a single primer pool. The primer pool targets 40 known drug metabolism enzyme (DME) genes, enabling CNV assessment of *CYP2D6* at the gene level and for exon 9. Genotyping and copy number information is provided in Torrent Suite Software by a plugin, and star allele translation through simple transfer to existing tools.

Libraries were prepared using 32 Coriell cell line samples using low-throughput automation on the Ion Chef System (4 runs with 8 samples at a time). Requiring less than 2 pipetting steps per sample, low-throughput automation of library preparation using the Ion Chef System helps reduce hands-on time and user interactions, thereby minimizing potential sources of user error and sequencing variability.

A 96-sample Coriell cell line library and a 96-sample buccal swab library were also prepared by high-throughput automation using the Tecan Freedom EVO™ liquid handling robot. Three Ion 530 Chip runs of 96 samples (two runs of the Coriell library and one run of the buccal swab library) resulted in an average of 83.8% on-target reads with an average mean depth of coverage of 1,070x and an average uniformity of 98.2%. The single Ion 520 Chip run of 32 samples had 86.7% on-target reads with a mean depth of 1,228x and a uniformity of 98.7%.

The average genotyping call rate was 100% for the 32-sample library and the 96-sample libraries, with 100% sensitivity and specificity. Across all runs and samples, the gene-level CNV detection for *CYP2D6* had a call rate of 100% with 100% accuracy (Figure 4A). At the exon level, detection of the exon 9 CNV in *CYP2D6* had a 97.1% call rate with 100% accuracy for all sample types and run conditions (Figure 4B).

Reference

1. Saudi Mendeliome Group (2015) Comprehensive gene panels provide advantages over clinical exome sequencing for Mendelian diseases. *Genome Biology* 16:134.

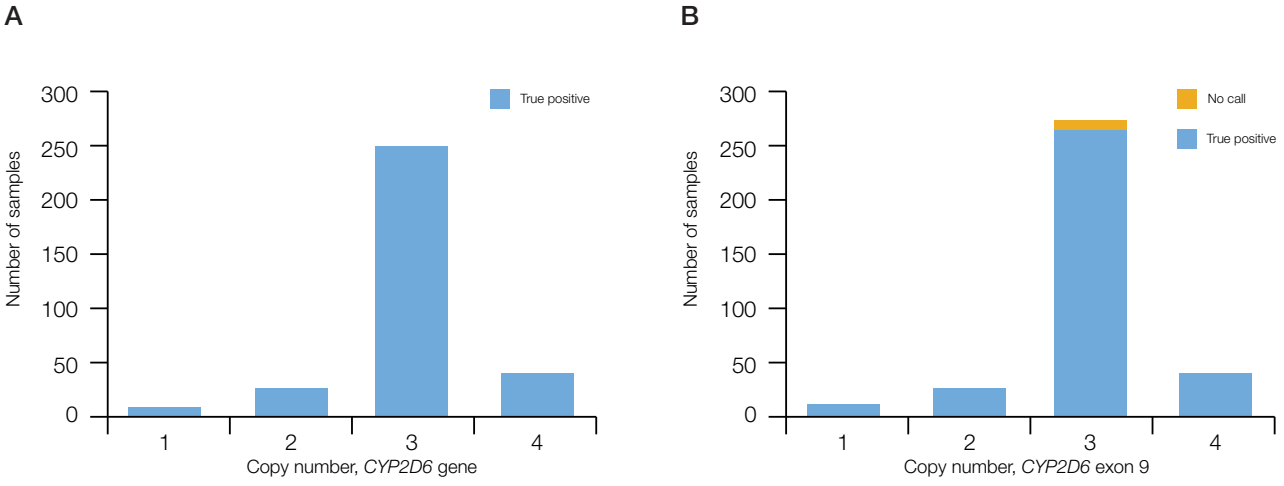

Figure 4. CNV detection in *CYP2D6*. (A) Gene-level CNV detection in *CYP2D6* had a call rate and accuracy of up to 100% for buccal swab and cell line sample libraries. **(B)** Exon-level CNV detection in *CYP2D6* had a call rate of 97.1% with up to 100% accuracy for both buccal and cell line sample libraries.

Table 2. Sequencing products for inherited disease research.

Product	Description	Quantity	Cat. No.
Predesigned panels available in trial packs			
Ion AmpliSeq Pharmacogenomics Research Panel	40 genes; 119 amplicons	16 reactions	A29250
		96 reactions	A29251
Ion AmpliSeq Hearing Loss Research Panel v1	295 kb targeting 63 genes; 2,064 amplicons	8 reactions	UP1001
Ion AmpliSeq Ophthalmic Research Panel	820.5 kb targeting 316 genes; 7,223 amplicons	8 reactions	A29689
		32 reactions	A29997
Ion AmpliSeq Neurological Research Panel	3.6 Mb targeting 751 genes; 18,077 amplicons	8 reactions	A29688
		32 reactions	A29996
Ion AmpliSeq Hematology Research Panel	826.2 kb targeting 394 genes; 7,434 amplicons	8 reactions	A29687
		32 reactions	A29995
Ion AmpliSeq Cardiovascular Research Panel	1.19 Mb targeting 404 genes; 10,430 amplicons	8 reactions	A29686
		32 reactions	A29994
Ion AmpliSeq Inherited Disease Panel	962.3 kb targeting 325 genes; 10,309 amplicons	8 reactions	4477686
Ion AmpliSeq CFTR Research Panel	8.5 kb targeting 1 gene; 102 amplicons	8 reactions	CP1002
Ion AmpliSeq Exome RDY Kit	~33 Mb of coding exons; ~294,000 amplicons	4 x 2 reactions	A27193
Community panels			
Ion AmpliSeq Deafness Research Panel v2	0.7 Mb targeting 126 genes; 3,520 amplicons	Custom order at **ampliseq.com**	
Ion AmpliSeq Dermatology Research Panel v2	1.0 Mb targeting 222 genes; 4,964 amplicons		
Ion AmpliSeq Dysmorphia-Dysplasia Research Panel v2	2.5 Mb targeting 519 genes; 12,558 amplicons		
Ion AmpliSeq Endocrinal Research Panel v2	1.2 Mb targeting 340 genes; 6,359 amplicons		
Ion AmpliSeq Gastrointestinal Research Panel v2	0.8 Mb targeting 194 genes; 4,103 amplicons		
Ion AmpliSeq Inborn Errors of Metabolism Research Panel v2	1.9 Mb targeting 594 genes; 9,681 amplicons		
Ion AmpliSeq Primary Immune Deficiency Research Panel v2	1.0 Mb targeting 264 genes; 5,241 amplicons		
Ion AmpliSeq Pulmonary Research Panel v2	0.7 Mb targeting 131 genes; 3,365 amplicons		
Ion AmpliSeq Renal Research Panel v2	0.8 Mb targeting 154 genes; 4,332 amplicons		
Ion AmpliSeq Cardiac Arrythmias and Cardiomyopathy Research Panel	410 kb targeting 92 genes; 2,198 amplicons		
Ion AmpliSeq Autism Research Panel	1.3 Mb targeting 236 genes; 7,194 amplicons		
Ion AmpliSeq Epilepsy Research Panel	1.6 Mb targeting 385 genes; 8,875 amplicons		
Ion AmpliSeq Inherited Cancer Research Panel	0.6 Mb targeting 134 genes; 3,340 amplicons		
Ion AmpliSeq Dementia Research Gene Panel	21.7 kb targeting 17 genes; 214 amplicons		
Ion AmpliSeq Noonan Research Panel	27 kb targeting 14 genes; 268 amplicons		
Ion AmpliSeq BRCA 1 & 2 Research Panel	16.3 kb targeting 2 genes; 167 amplicons		
Ion AmpliSeq Ovarian Cancer Research Panel	144 kb targeting 41 genes; 1,331 amplicons		

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(continued)

Product	Description	Quantity	Cat. No.
Manual library preparation			
Ion AmpliSeq Library Kit 2.0	Manual Ion AmpliSeq library preparation	8 reactions	4475345
		96 reactions	4480441
		384 reactions	4480442
Ion Xpress Barcode Adapters 1-96 Kit	96 unique barcode adapters	1 kit	4474517
Ion Library Equalizer Kit	Bead-based solution replacing the need for library quantification and library dilutions for library normalization	96 reactions	4482298
Automated library preparation			
Ion AmpliSeq Kit for Chef DL8	Automated Ion AmpliSeq library preparation kit supplied with IonCode barcodes	4 x 8 reactions	A29024
Template preparation			
Ion 520/530 Kit-Chef	Template preparation kit for Ion 520 or Ion 530 Chips on the Ion Chef System	8 reactions	A27757
Ion 540 Kit-Chef	Template preparation kit for Ion 540 Chips on the Ion Chef System	8 reactions	A27759
Instruments			
Ion S5 Instrument	Ion S5 Sequencer	1 instrument	A27212
Ion S5 XL System	Ion S5 XL Sequencer and Ion S5 Torrent Server	1 system	A27214
Sequencing			
Ion 520 Chip Kit	Sequencing reagents plus an Ion 520 Chip	8 reactions	A27762
Ion 530 Chip Kit	Sequencing reagents plus an Ion 530 Chip	8 reactions	A27764
Ion 540 Chip Kit	Sequencing reagents plus an Ion 540 Chip	8 reactions	A27766
Data analysis			
Ion Reporter Software	Cloud-based hosted data storage or on-site server options available	Find out more at **thermofisher.com/ionreporter**	

Find out more at **thermofisher.com/ions5**

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