Rapid sequencing of microorganisms with the versatile Ion S5 next-generation sequencing systems

Ion S5™ Systems and infectious disease research

- Targeted sequencing of viruses, bacteria, or fungi from biological materials without culturing
- Robust sequencing of whole bacterial genomes from isolates or mixed cultures
- Low cost of ownership and low cost of sequencing per sample
- Rapid workflows that allow sequencing results from microbial samples in less than 24 hours
- Integrated software for simple run setup and data analysis

Figure 1. The Ion S5 System and infectious disease research applications. Efficient semiconductor-based sequencing provides rapid results at low cost with up to 80 million DNA fragments sequenced in 7.5 hours on the Ion S5 XL System (sequencing and primary data analysis).

Next-generation sequencing technologies have transformed our understanding of the microbial universe. Complete bacterial genomes can now be sequenced at low cost and often without the need to culture the organism. Approaches that isolate a single bacterial cell followed by whole genome amplification and sequencing further expand the database of unknown and unculturable organisms. In addition, targeted approaches based on the 16S rRNA gene or other specific genes or regions of viral, bacterial, and fungal genomes have provided a highly effective strategy to identify organisms without the requirement for host nucleic acid subtraction.

Sequencing of microbes directly from biological samples is expected to have a significant impact on detection rates in the future. Currently, 20% of pneumonial samples and 50–70% of diarrheal, meningitis, and encephalitis samples cannot be associated with a pathogen by conventional microbial analyses. Furthermore, blood cultures are associated with a low detection rate, <25%, for bloodstream infections.

Targeted or whole genome sequencing (WGS) approaches can be employed effectively on the Ion S5 and Ion S5 XL Systems for research that may be potentially used to help trace the origin of outbreaks, to monitor biologics manufacturing processes, ensure food and beverage safety, and for clinical microbiology applications (Figure 1).

The tunable sequencing output of the Ion S5 System, with 3 million to 80 million reads, enables a broad set of microbial research applications and sample throughputs to be addressed effectively (Table 1).

Sequencing workflow

The sequencing workflow consists of four main steps (Figure 2).

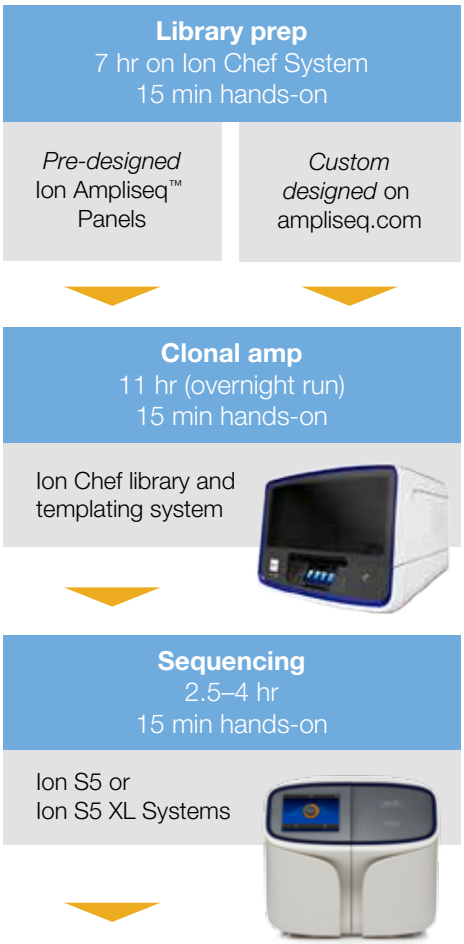


Figure 2. Targeted sequencing workflow. Ion AmpliSeq libraries are prepared manually or with the Ion Chef System. Libraries are then placed in the Ion Chef System for emulsion PCR, enrichment, and loading onto Ion S5 chips. Chips are placed in the Ion S5 System with reagents for sequencing. Primary data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

Table 1. Number of microbial samples that can be run on Ion S5 chips (barcodes are 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
Output	200 bp reads	0.6–1 Gb	3–4 Gb	10–15 Gb
	400 bp reads	1.2–2 Gb	6–8 Gb	–
Samples per run				
Ion 16S Metagenomics Kit*		48	192	–
Ion AmpliSeq TB Research Panel†		36	144	384**
Viral whole genome‡		72	288	384**
Bacterial whole genome§		12	48	96††

* Assumes 1,000x coverage of mid-complexity sample.
† Assumes 1,000x coverage.
‡ Assumes 275 kb genome at 100x coverage.
§ Assumes 5 Mb genome at 30x 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.
†† 200 bp reads only.

1. Library preparation

For targeted sequencing:

Genomic DNA (10–100 ng) isolated from the sample is converted to a sequencing library by selective amplification of regions of interest using the Ion AmpliSeq protocol that enables highly multiplexed PCR amplification of up to 20,000 targets in a single tube. Eliminating primer-dimers a fraction of the target-specific Ion AmpliSeq primers are digested away from the amplified product, followed by a ligation step to add adapter sequences to the amplicons. Pre-designed microbial panels are available, such as the Ion AmpliSeq TB Research Panel and the Ion AmpliSeq Ebola Research Panel, or custom panels can be easily developed using the Ion AmpliSeq Designer online tool.

The Ion AmpliSeq library preparation workflow may be performed manually or can be performed automatically for 8 samples per run of 1- or 2-pool panel designs on the Ion Chef™ System. Requiring less than 2 pipetting steps per sample, Ion AmpliSeq library preparation on the Ion Chef System reduces hands-on time, generating pooled libraries ready for downstream template preparation.

For whole genome sequencing:

The Ion Xpress™ Plus Fragment Library Kit is used to produce a whole genome library from genomic DNA. The kit uses an enzyme-based random fragmentation method followed by ligation of adapters and amplification. Alternatively, the Thermo Scientific™ MuSeek™

Library Preparation Kit provides a fast and simple transposon-based method for preparing high-quality genomic DNA libraries. The kit utilizes MuA transposase enzyme for fragmentation and simultaneous tagging of the target DNA, eliminating the need for separate shearing, end repair, and adaptor ligation steps. This enables library construction in as little as 80 minutes with consistent yields from low DNA sample amounts (100 ng).

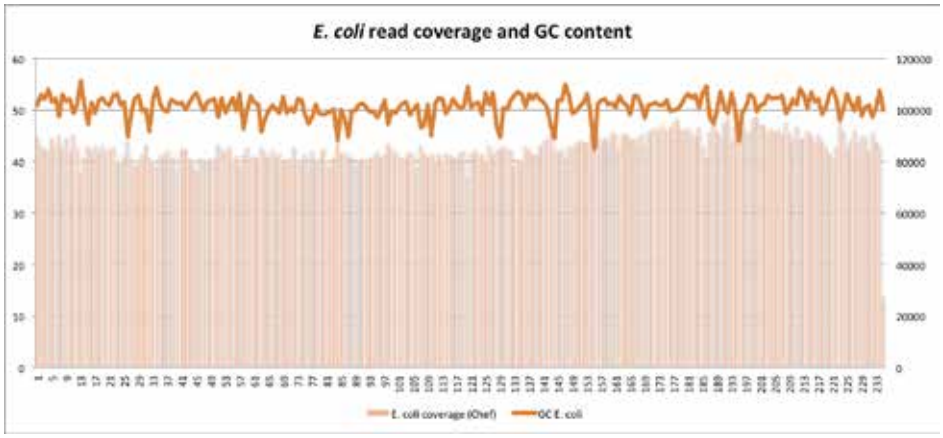
2. Clonal amplification (template preparation)

Libraries that are prepared manually or by automation are then clonally amplified on the Ion Chef System by emulsion PCR of library molecules captured on beads. The Ion Chef System performs all template preparation steps, including creating the emulsion mixture, performing the PCR, carrying out the post-PCR purifications, and finally loading of the purified templated beads onto the Ion S5 chips. The prepared chips are ready for sequencing on the Ion S5 System.

3. Sequencing

A sequencing run on the Ion S5 and Ion S5 XL Systems is initiated by loading a reagent cartridge, buffer, cleaning solution, and waste container. The Ion S5 chip is then loaded and the run is started. The addition of bases by the DNA polymerase results in the production of hydrogen ions, changing the pH which is coverted to sequencing signal through ion-sensitive wells that hold the templated beads. Read lengths of up to 400 bp can be produced with three available semiconductor chips that produce up to 5, 20, or 80 million reads per chip (Table 1).

A



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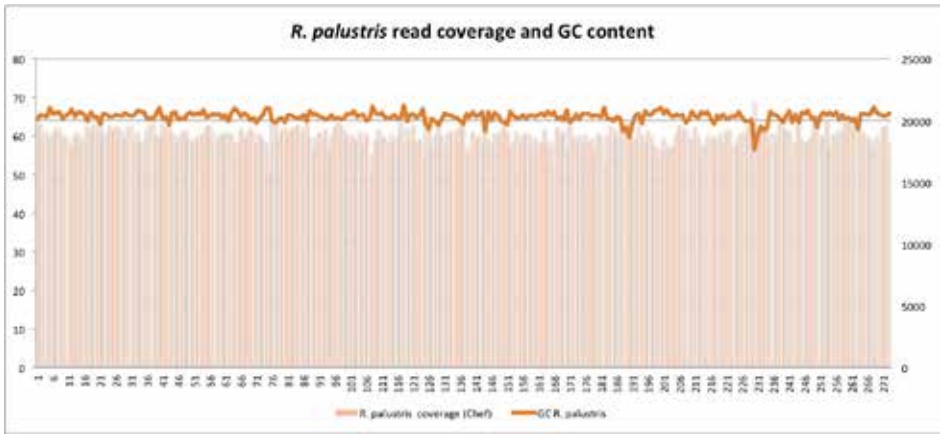

Figure 3. Whole genome sequencing on the Ion S5 System. Fragment libraries were sequenced on the Ion 520 Chip. Coverage plots for libraries prepared with the Ion Chef System (20 Kb window) compared with GC content indicate high levels of coverage uniformity across the genome. **(A)** *E. coli* genome with balanced GC content. **(B)** *R. palustris* genome with high GC content.

4. Data analysis

Torrent Suite Software coordinates all the experiment planning, from run setup to data processing, and primary analysis of microbial sequencing data, with plugins for assembly and microbial-specific analysis. Furthermore, 16S rRNA profiling data can be uploaded to Ion Reporter Software for further analysis with a suite of data analysis and visualization tools.

Results

The flexibility, simple workflows, and cost effectiveness of the Ion S5 Systems enable highly efficient sequencing-based microbial analysis.

Sequencing bacterial whole genomes

For epidemiological research applications such as outbreak investigations, surveillance, and determining disease etiologies, whole genome sequencing provides the highest data resolution.

As an example of rapid WGS, fragment libraries were generated from *Escherichia coli* and *Rhodopseudomonas palustris* with

the Ion Xpress Plus Fragment Kit followed by clonal amplification and chip loading on the Ion Chef System, sequencing on the Ion S5 System, and *de novo* assembly using the SPAdes v3.1 plugin. Data from the rapid whole genome sequencing workflow is shown in Figure 3.

Metagenome sequencing research

16S rRNA profiling is an efficient method for characterizing bacterial populations at low cost and with minimal bioinformatics effort. The Ion 16S™ Metagenomics Kit has been designed to provide comprehensive identification (to >80% of Greengenes sequences) by simultaneously sequencing 7 hypervariable regions in the bacterial 16S rRNA gene. When applied to ground beef samples, the 16S sequencing results showed high-resolution identification of bacterial species. Species diversity was substantially reduced on culture (Figure 4).

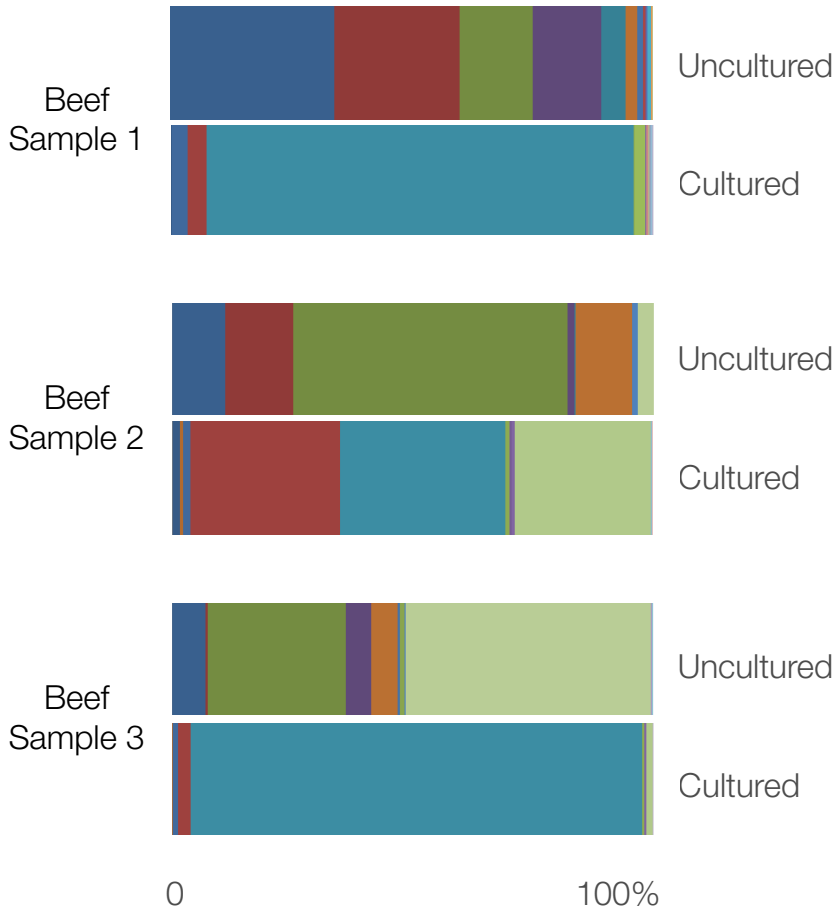

Figure 4. 16S rRNA sequence analysis of ground beef samples. Libraries generated with the Ion 16S Metagenomics Kit were generated from ground beef samples and sequenced on the Ion 530 Chip. Automated analysis, annotation, and taxonomical assignment were generated using Ion Reporter Software. Uncultured ground beef samples show a greater diversity of genera compared to cultured samples, suggesting that culturing introduces bias. Of note, the number and diversity of bacterial genera differ in ground beef from different sources as shown by color..

Sequencing targeted regions of bacteria

Targeted sequencing approaches that query specific genes or regions of interest can be used for species or subtype identification, or for the identification of known variants associated with drug resistance or virulence markers. Sequencing output requirements can be substantially reduced by targeted sequencing, especially for complex samples with a background of large amounts of host nucleic acids (e.g., characterization of microbes directly from blood or sputum samples). Optimized Ion S5 System workflows can also offer rapid data generation capabilities of less than 24 hours for research into culture-free identification. Ion AmpliSeq technology, with its capability to amplify and sequence thousands of amplicons simultaneously, offers a robust approach for microbial characterization such as drug resistance in *Mycobacterium tuberculosis*.

Targeted sequencing of *M. tuberculosis*

The Ion AmpliSeq TB Research Panel was developed to enable rapid genotyping of known variants that may be associated with antibiotic resistance. Eight genes (*embB*, *eis*, *gyrA*, *inhA*, *katG*, *pncA*, *rpoB*, *rpsL*) in *M. tuberculosis* are targeted by 109 amplicons that cover protein coding regions and additional upstream and downstream regions to maximize discovery of known and novel variants. Sequencing of 6 wild type (H37Rv) and 6 rifampicin-resistant (mutation in *rpoB*) strains using the Ion AmpliSeq TB Research Panel on a single Ion 520 Chip detected all mutations associated with resistance (Table 2).

Detection of virions directly from CSF

To demonstrate the utility of Ion S5 sequencing for the detection of virions directly from biological samples, experiments were performed by sequencing bulk nucleic acids from CSF on the Ion 540 Chip. Reads that mapped to the human genome were bioinformatically filtered out and remaining reads were analyzed for viral sequences (Table 3).

Table 2. Variants identified from DNA isolated from archived pure *M. tuberculosis* cultures. WT indicates wild type. A red circle indicates presence of mutation S531L (TCG/TTG) 761155.

	WT1..6	*R1*	*R2*	*R3*	*R4*	*R5*	*R6*
embB							
eis							
gyrA							
inhA							
katG							
pncA							
rpoB		●	●	●	●	●	●
rpsL							

Table 3. Detection of virions in CSF.

Sample	Spiked-in virus	Viral reads	Total reads
1	La Crosse virus	311,259	39,320,183
2	Human adenovirus C	36,649	44,845,792

ion torrent

Ordering information

Product	Description	Quantity	Cat. No.
Microbial targeted sequencing panels			
Ion 16S Metagenomics Kit	Two primer pools to amplify seven hypervariable regions (V2, V3, V4, V6, V7, V8, and V9) of bacterial 16S rRNA	100 reactions	A26216
Ion AmpliSeq TB Research Panel	Targets genes associated with antimicrobial resistance in *M. tuberculosis* (TB). The research panel assesses 109 amplicons (two pools) from 8 genes (*embB*, *eis*, *gyrA*, *inhA*, *katG*, *pncA*, *rpoB*, *rpsL*). The panel is designed to be used on cultured sputum extract.	Custom order	ampliseq.com
Ion AmpliSeq Ebola Research Panel	Targets genes associated with the of Ebola virus. The panel assesses 145 amplicons across the Ebola virus genome.	Custom order	ampliseq.com
PathAmp FluA Reagents	A set of highly specific, universal influenza primers combined with a high-fidelity master mix for the amplification of all eight influenza A genomic segments in a single tube	50 preps	4485019
Ion AmpliSeq 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 supplied with IonCode barcodes	4 x 8 reactions	A29024
Microbial whole genome sequencing			
Ion Xpress Plus Fragment Library Kit	Enzymatic fragment library construction method	10 reactions	4471269
Ion TrueMate™ Plus Library Kit	Generation of mate-pair libraries (2–8 kb inserts) from any genomic DNA	10 reactions	A25656
Template preparation			
Ion Chef System	Automates template preparation and chip loading	1 system	4484177
Ion 520/530 Kit-Chef	Template preparation for Ion 520 and Ion 530 Chips on the Ion Chef System	8 reactions	A27757
Ion 540 Kit-Chef	Template preparation for Ion 540 Chips on the Ion Chef System	8 reactions	A27759
Sequencing			
Ion S5 System	Ion S5 next-generation sequencing system	1 system	A27212
Ion S5 XL System	Ion S5 XL next-generation sequencing system	1 system	A27214
Ion 520 Chip Kit	Sequencing reagents including an Ion 520 Chip	8 reactions	A27762
Ion 530 Chip Kit	Sequencing reagents including an Ion 530 Chip	8 reactions	A27764
Ion 540 Chip Kit	Sequencing reagents including an Ion 540 Chip	8 reactions	A27766
Sequencing			
Ion Reporter Software	16S Metagenomics Analysis		thermofisher.com/ionreporter

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

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