**Next-generation sequencing**

Ion AmpliSeq Microbiome Health Research Assay

The Ion AmpliSeq™ Microbiome Health Research Assay (MHRA) is a next-generation sequencing (NGS) assay that allows for cost-effective yet comprehensive profiling of microbial diversity of the human gut microbiome. This assay offers increased resolution and specificity of species-level detection compared with traditional 16S ribosomal RNA (rRNA) sequencing for key organisms associated with immunological response to cancer, diabetes and autoimmune diseases, gastrointestinal (GI) disorders, and infectious diseases.

Most other commercially available 16S panels target two to four hypervariable (HV) regions of the 16S rRNA gene, while the panel included with the Ion AmpliSeq MHRA targets eight out of the nine HV regions, in addition to a set of high-resolution markers for increased species-level identification for highly comprehensive and sensitive microbial profiling research.

This larger targeted NGS panel, combined with fully integrated data analysis tools, creates a complete end-to-end solution to help both expand and simplify your microbiome research. Two versions of the Ion AmpliSeq MHRA are available, one on the **Ion GeneStudio™ System**, and the other on the **Ion Torrent™ Genexus™ System**, and these two versions of the assay take advantage of the different benefits offered by each NGS platform.
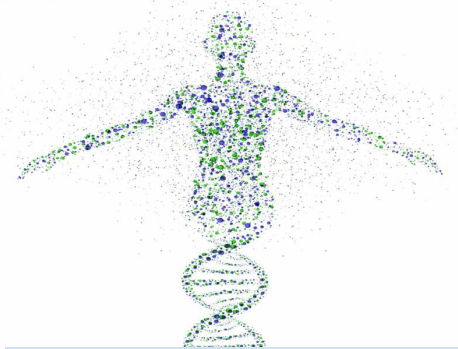
- **Targeted sequencing—** cost-effective approach to investigate human gut microbiome diversity using the Ion AmpliSeq assay protocol

- **Panel targeting 8 out of 9 HV regions—**one of the most comprehensive 16S rRNA gene panels to detect and quantify all microbes of bacterial origin in the sample

- **Species-level resolution—** detection of 73 key bacterial species associated with research in immuno-oncology as well as immunological disorders, GI disorders, and infectious diseases

MHRA on the Ion GeneStudio S5 System

The Ion GeneStudio™ S5 System is a flexible, scalable, and targeted NGS workhorse with wide application breadth and throughput capability. It's ideal for labs looking for a cost-efficient NGS solution (Figure 1). You can perform Ion AmpliSeq MHRA analysis on up to 48 samples per Ion 550™ Chip, or choose the chip that fits your needs with an end-to-end, nucleic-acid-to-report turnaround time of 48 hours.
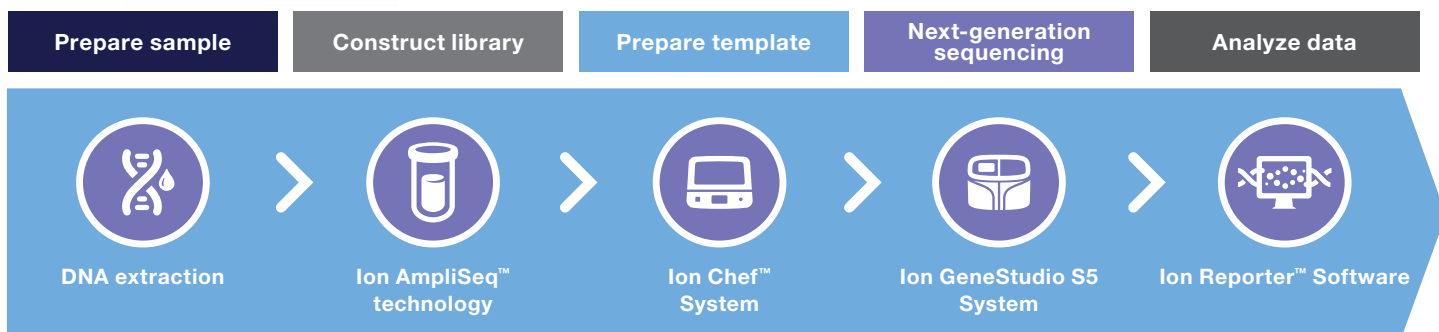
Prepare sample	Construct library	Prepare template	Next-generation sequencing	Analyze data
DNA extraction	Ion AmpliSeq™ technology	Ion Chef™ System	Ion GeneStudio S5 System	Ion Reporter™ Software

Figure 1. The Ion AmpliSeq Microbiome Health Research Assay workflow on the Ion GeneStudio S5 System.

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MHRA on the Genexus System

Running the Ion AmpliSeq™ Microbiome Health Research Assay GX on the Ion Torrent™ Genexus™ Integrated Sequencer is ideal for labs that are new to in-house NGS and want to leverage the highly automated workflow (Figure 2). Using the Ion Torrent™ GX7™ Chip and the 48-barcode capabilities on the Genexus Integrated Sequencer, customers can run the Ion AmpliSeq MHRA GX on 24 samples in 2 lanes with only 10 minutes of hands-on time and a total turnaround time of 30 hours.
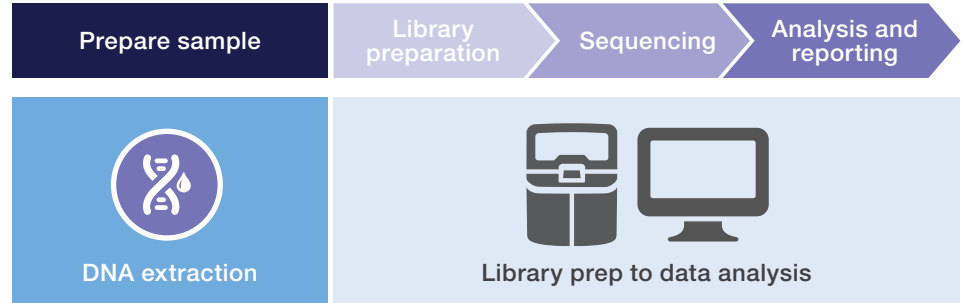

Figure 2. The Ion AmpliSeq Microbiome Health Research Assay GX on the Ion Torrent Genexus System.

While shotgun metagenomics can offer some advantages over targeted NGS, such as higher functional resolution, it is more costly and time-consuming. It also requires more complex bioinformatics tools, making this approach difficult to master and potentially cause issues with data reliability and reproducibility. The Ion AmpliSeq MHRA offers an easy-to-use, cost-effective, and rapid solution to detect and quantify all microbes of bacterial origin in the sample by targeting 8 out of 9 hypervariable regions present in the 16S rRNA gene. The assay also provides species-level specificity to interrogate the presence of a curated list of 73 bacterial species (Table 1) that have strong scientific evidence to be implicated in immuno-oncology (I-O) response, gut health, and autoimmunity, as well as response to infection (Figure 3). This highly curated, species-specific panel allows for 100% specificity and sensitivity.
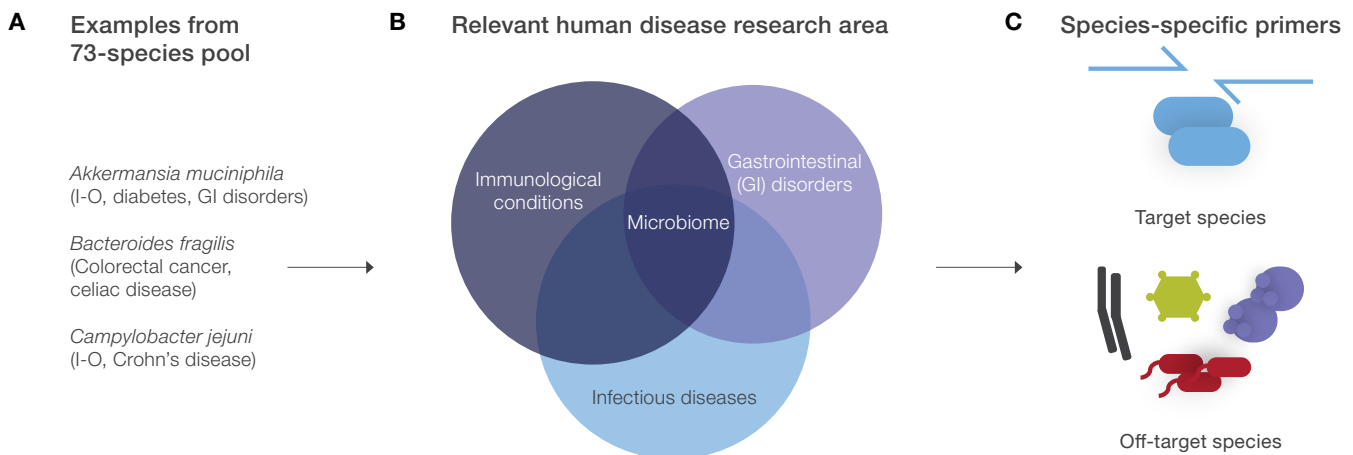

Figure 3. Target species pool for species relevant to human disease research. (A) To increase assay sensitivity and specificity to key species in human health, we selected 73 species from the literature [1–5] pertinent to research areas including **(B)** immunological conditions, GI disorders, and infectious diseases. **(C)** We generated our target species pool using proprietary software to identify unique genomic targets and primers for the relevant species, resulting in a highly specific panel.

The Ion AmpliSeq MHRA also offers a complete end-to-end, nucleic-acid-to-report solution with an optimized analysis workflow using Ion Reporter Software for analysis of data generated with the GeneStudio system or embedded in Ion Torrent™ Genexus™ Software when using the Genexus System. The analysis workflow enables multisample analysis and visualization, such as heatmap, PCoA, relative abundance, and diversity visualization (Figure 4). The analysis uses up-to-date reference databases such as the curated SILVA database, the Applied Biosystems™ MicroSeq™ database, Greengenes, and the NCBI.
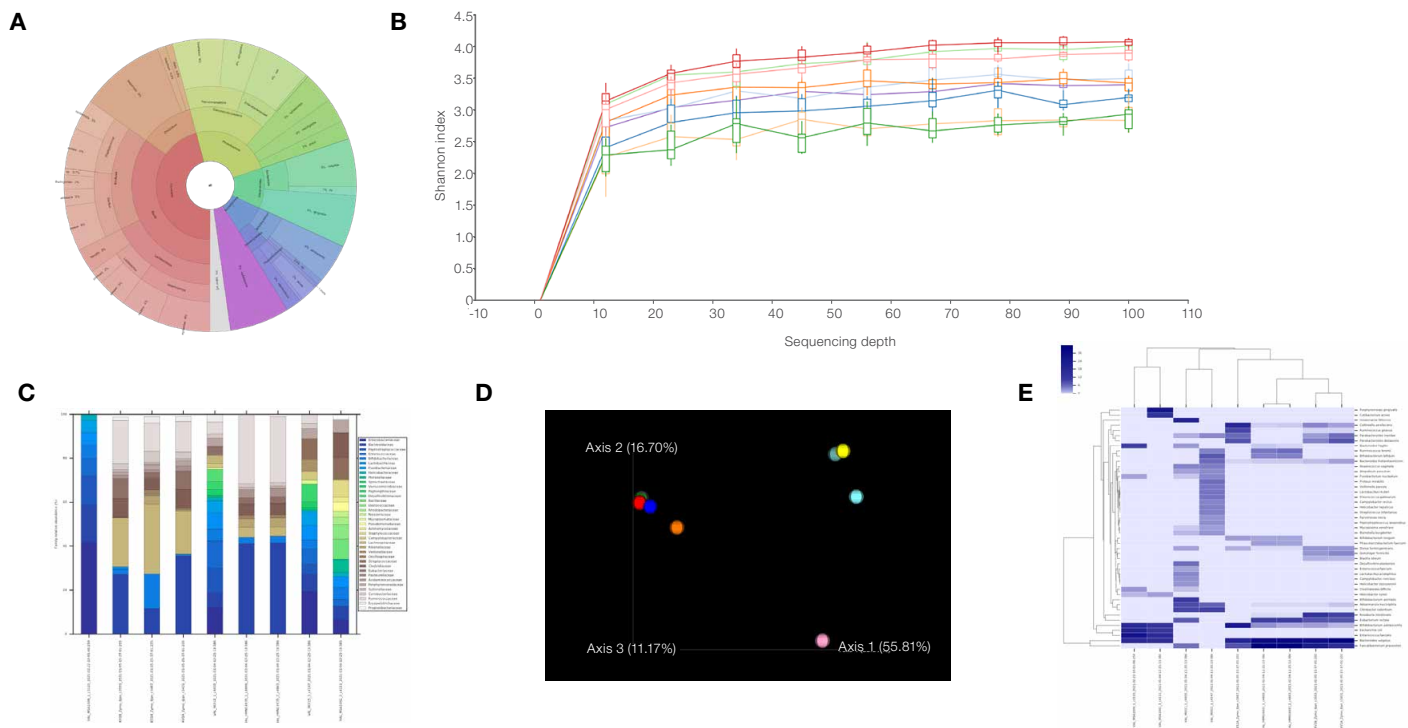
Figure 4. Integrated Ion AmpliSeq MHRA data analysis and reporting tools. (A) Relative abundance (Krona plots). (B) Diversity metrics. (C) Stacked bar plots. (D) PCoA plots. (E) Interactive taxonomy viewer.

Table 1. List of targeted bacterial species.

Species			
Akkermansia muciniphila	Campylobacter rectus	Gardnerella vaginalis	Parvimonas micra
Anaerococcus vaginalis	Chlamydia pneumoniae	Gemmiger formicilis	Peptostreptococcus anaerobius
Atopobium parvulum	Chlamydia trachomatis	Helicobacter bilis	Peptostreptococcus stomatis
Bacteroides fragilis	Citrobacter rodentium	Helicobacter bizzozeronii	Phascolarctobacterium faecium
Bacteroides nordii	Cloacibacillus porcorum	Helicobacter hepaticus	Porphyromonas gingivalis
Bacteroides thetaiotaomicron	Clostridium difficile	Helicobacter pylori	Prevotella copri
Bacteroides vulgatus	Collinsella aerofaciens	Holdemania filiformis	Prevotella histicola
Barnesiella intestinihominis	Collinsella stercoris	Klebsiella pneumoniae	Propionibacterium acnes
Bifidobacterium adolescentis	Desulfovibrio alaskensis	Lactobacillus acidophilus	(Cutibacterium acnes)
Bifidobacterium animalis	Dorea formicigenerans	Lactobacillus delbrueckii	Proteus mirabilis
Bifidobacterium bifidum	Enterococcus faecalis	Lactobacillus johnsonii	Roseburia intestinalis
Bifidobacterium longum	Enterococcus faecium	Lactobacillus murinus	Ruminococcus bromii
Blautia obeum	Enterococcus gallinarum	Lactobacillus reuteri	Ruminococcus gnavus
Borrelia burgdorferi	Enterococcus hirae	Lactobacillus rhamnosus	Slackia exigua
Campylobacter concisus	Escherichia coli	Lactococcus lactis	Streptococcus gallolyticus
Campylobacter curvus	Eubacterium limosum	Mycoplasma fermentans	Streptococcus infantarius
Campylobacter gracilis	Eubacterium rectale	Mycoplasma penetrans	Veillonella parvula
Campylobacter hominis	Faecalibacterium prausnitzii	Parabacteroides distasonis	
Campylobacter jejuni	Fusobacterium nucleatum	Parabacteroides merdae	

Ion AmpliSeq Microbiome Health Research™ Assay   thermofisher.com/mhra

Ordering information—GeneStudio products

Description	Quantity	Cat. No.
Ion AmpliSeq Microbiome Health Research Assay, Library Prep only*	48 samples (manual library)	A46495
Ion AmpliSeq Microbiome Health Research Assay, Ion 540 bundle**	256 samples (manual library), 32 samples/Ion 540 Chip	A46496
Ion AmpliSeq Microbiome Health Research Assay, Ion 550 bundle**	384 samples (manual library), 48 samples/Ion 550 Chip	A46497

* Library prep reagents only.

** Library prep, templating, and sequencing reagents.

Ordering information—Genexus product

Description	Quantity	Cat. No.
Ion AmpliSeq Microbiome Health Research Assay GX (Library prep reagents only)	24 samples—uses 2 lanes on the GX7 Chip	A47022

References
1. Routy B et al. (2018) Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science* 359:91-97.
2. Matson V et al. (2018) The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients. *Science* 359:104-108.
3. Gopalakrishnan V et al. (2018) Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. *Science* 359:97-103.
4. Baruch E et al. (2021) Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* 371:602-609.
5. Mazzarelli A et al. (2021) 16S rRNA gene sequencing of rectal swab in patients affected by COVID-19. *PLoS ONE* 16(2): e0247041.

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

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