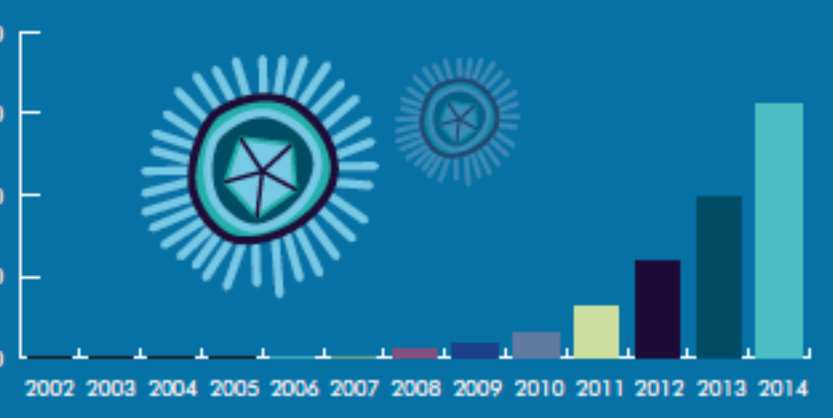
Mining the Microbiome

Trillions of microbes inhabit the human body—more than 10 times the number of our own mammalian cells. While the microbiome of the gut is the most well studied, researchers now recognize that single-celled organisms from all three domains of life form distinctive microbial communities in diverse tissues and organs, including those once thought to be sterile, such as the eye and the reproductive organs. To better understand how our microbial organisms affect our health and influence disease, scientists are now surveying these communities using increasingly affordable genomics techniques.

Typical metagenomics studies focus on the 16S rRNA gene that encodes the small subunit of the ribosome, because this gene is shared by all microbes, but also contains hypervariable regions allowing differentiation of microbial communities. Other studies dive deeper, sequencing entire bacterial genomes or all the genes of a given environmental sample. Whole-community metaproteomic or metabolomic analyses can also yield clues regarding the community's composition and function. Finally, bioinformatic and statistical tools are applied to investigate dysbioses that might cause disease.

NUMBER OF PUBLICATIONS ON MICROBIOME IN PubMed*



*PubMed search of "microbiome"[All Fields] in March 2015 returned 3750 results.

SOIL

Distinct, diverse microbial species populate the soil of temperate, desert, tropical, and Arctic regions. Desert (pH of >8) and tropical forest (pH of <4.5) soils are home to the lowest levels of microbial diversity, while regions with near-neutral soil pH have the highest microbial diversity.[1] Soil biodiversity is also influenced by the chemical composition of soil organic matter within the soil microenvironment.[2]

WATER

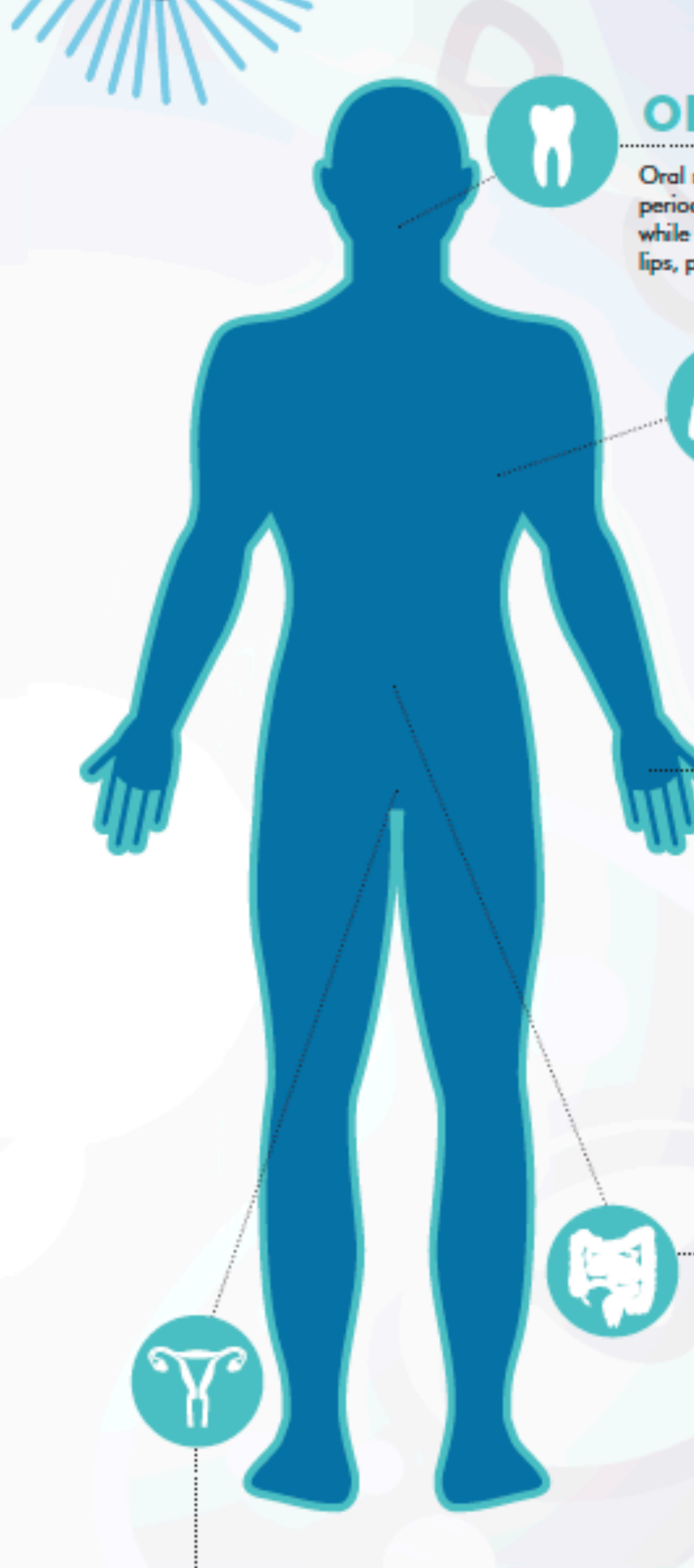
Microbial biodiversity in aquatic environments is subject to natural variation and is influenced by environmental factors—light, temperature, pH, waterflow, salt concentration, and aquatic species. Marine microbes impact the oceanic food web, chemical and nutrient cycling, and aquatic species health. Microbes also drive the degradation of dissolved organic matter in rivers and lakes, playing an important role in carbon cycling and the recycling of organic matter and nutrients.

PLANTS

While both root and leaf microbial communities play a role in plant fitness and adaptability, host-microbe interactions at the root may also be involved in the acquisition of nutrients from the soil. Studying how the microbiota can influence plant health in natural and agricultural ecosystems has implications for crop production, biodiversity management, and responses to climate change.

ARCHITECTURE

Scientists are also mapping microscopic life in urban environments to better understand public health implications of our surrounding microbial ecosystem. Analysis of 1,400 samples from surfaces of the New York City subway system identified more than 15,000 different species, including DNA fragments of bubonic plague and anthrax.[3] Analyzing the relationship between building design and microbial diversity is critical to understand the influence of the urban ecosystem on public health.



ORAL

Oral microflora play an important role in health and oral disease, including periodontitis and caries. The oral cavity is a distinct site for microbiota colonization; while it is warm, nutrient rich, and offers dynamic colonization surfaces such as teeth, lips, palate, and tongue, the antimicrobial properties of saliva inhibit bacterial growth.

LUNGS

While the lung was once thought to be a sterile environment in the absence of infection, recent studies identified diverse microbial communities in the healthy lung. The interactions of microbiota and host cells are being examined in the cystic fibrosis lung, to better understand the impact of pathogens in chronic infection and to guide therapeutic intervention. Alterations in the lung microbiome are observed in response to cigarette smoke and other environmental factors.

SKIN

The skin acts as a protective barrier for the body and is home to diverse commensal microorganisms that play a key role in host immunity. Within an individual, niche microbial colonies form that are dependent on variation in skin characteristics such as temperature, hair, sebaceous glands, and moisture content. The belly button is home to a diverse microbial community—more than 2,000 bacterial phylotypes were identified in a sample of 60 volunteers.[4] Disruption of the commensal skin microbiota has been implicated in cutaneous infections, atopic dermatitis, acne, psoriasis, arthritis, and chronic wounds.

GUT

The human gut is host to diverse microorganisms that form a complex ecosystem involved in host digestion, metabolism, and immunity. Alterations in gastrointestinal microorganisms have been identified in inflammatory bowel disease, irritable bowel syndrome, gastroenteritis, colorectal cancer, and neurological disorders. Scientists are actively studying gut microbial colonization, preservation of the healthy ecology of the gut, and the therapeutic potential of modulating the interplay between microbes and the immune system to better understand the role of this complex ecosystem in health and disease.

UROGENITAL

The urogenital region is host to a variety of bacteria that are influenced by factors such as age, genetics, sexual activity, circumcision, and pregnancy. The urogenital system is now known to host diverse microbiota, even in the absence of infection. Disruption of microbial communities correlates with pelvic infections, bacterial vaginosis, and preterm birth. The role of microbes in the immune response to HIV and sexually transmitted diseases is also being studied.

DNA Sample Preparation

While scientists have studied microbial communities for years, advances in DNA extraction tools combined with more efficient and affordable next-generation sequencing has revolutionized the characterization of different sample types such as tissue, blood, soil, air, etc. Successful downstream analysis is dependent on the preparation of pure, high-quality DNA.

SAMPLE COLLECTION

Good results start with proper sample collection and storage prior to DNA isolation.

*Tips:

Samples should be processed soon after collection or frozen at -20°C for long-term storage.

If using a preservative, be sure that it is compatible with the microbes in your sample and your extraction method. Many preservatives for tissue do not work with environmental samples, e.g. ethanol.

Numbers of microbes vary widely between sample types. Consider maxi kits or combining preps in order to isolate adequate yields of DNA, e.g. when working with low-biomass soil.

CELL LYSIS

Cells can be lysed by mechanical (e.g. bead beating), chemical (e.g. detergent), or enzymatic (e.g. lysozyme, proteinase K) methods. The accuracy of microbial community diversity is impacted by cell lysis methods. Enzymatic lysis may not provide a good representation of the microbial communities in the sample; mechanical lysis via bead beating provides a better representation of microbial diversity.[5]

*Tips:

Optimize bead type to maximize DNA yield and integrity. Use smaller beads (0.1–0.5 mm) for lysis of bacteria, yeast and fungi. Use larger beads (2.0–3.0 mm) for breaking down bulk tissue such as seeds, animal tissue, and plants.

A short heating step (e.g. 10 minutes at 65°C) can assist the cell lysis of more-difficult microbes such as gram+ bacteria and spores.

Optimize the time and speed of homogenization to maximize the lysis of microbes while minimizing DNA degradation.

REMOVAL OF INHIBITORS

Many samples contain PCR inhibitors, released during cell lysis, that can interfere with quantification, inhibit amplification, and result in false negatives in downstream analysis, e.g. humic acids in soil samples; polysaccharides and poylphenolics in plant and seed samples; heme, lipids, and polysaccharides in stool and gut samples.

*Tips:

Remove inhibitors prior to DNA purification to avoid interference with downstream applications.

PURIFICATION

Once inhibitors have been removed from the solution of lysed cells and nucleic acids, DNA is further purified on a silica spin filter or using magnetic beads.

*Tips:

Optimize solutions to bind, wash, and elute purified DNA from silica spin filters. Magnetic bead–based technologies are ideal for high-throughput DNA isolation.

QUALITY CONTROL

DNA should be quantified and checked prior to analysis to confirm that it is free of inhibitors.

*Tips:

Run DNA on an agarose gel to check size and quality.

Quantify DNA (e.g. with a NanoDrop) to determine purity—compare both the A260/280 and A260/230 ratios and measure double-stranded DNA via a PicoGreen® assay to determine an accurate concentration.

Samples free of inhibitors will have a matching NanoDrop and PicoGreen® concentration, while samples containing inhibitors will appear to have a higher concentration via NanoDrop.

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Advancing Microbiome Research

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Saving You Time For Life

MO BIO Laboratories Inc., a privately held company located in the San Diego biotech corridor (Carlsbad, CA, USA), was founded in 1993 by Liz and Mark Brolaski. Today, MO BIO's growth and customer loyalty is challenging some of the world's leading molecular biology tool companies with innovative technologies and a commitment to value.

MO BIO is known as the World Leader in Soil DNA & RNA Isolation®, thanks to its patented Inhibitor Removal Technology®, which enables isolation of pure nucleic acids from challenging samples such as soil, water, plants and stool. Thus, MO BIO kits are the method of choice for microbiome projects, as these studies often require isolation of nucleic acids from challenging samples that contain a variety of PCR inhibitors. Microbiome projects relying on MO BIO products include the Earth Microbiome Project (EMP), the NIH-funded Human Microbiome Project (HMP), and many other related studies.

In 2014, MO BIO established the Microbiome Awards (www.microbiomeproject.com), which provide young, extraordinary scientists with funding and recognition to carry out scientific work in the field of microbiome research. The annual awards are meant to support and bring to light the many variations of studies in this field.

MO BIO's founders and employees are dedicated to the preservation of the environment and to bettering the quality of the Earth through science. MO BIO's team of scientists is committed to developing innovative tools for researchers that require pure, high-quality nucleic acids for use in next-generation sequencing and other downstream applications. Information about our kits for microbiome, soil, water, plants, stool, biofilm, microbial cultures, and more can be found at www.mobio.com.

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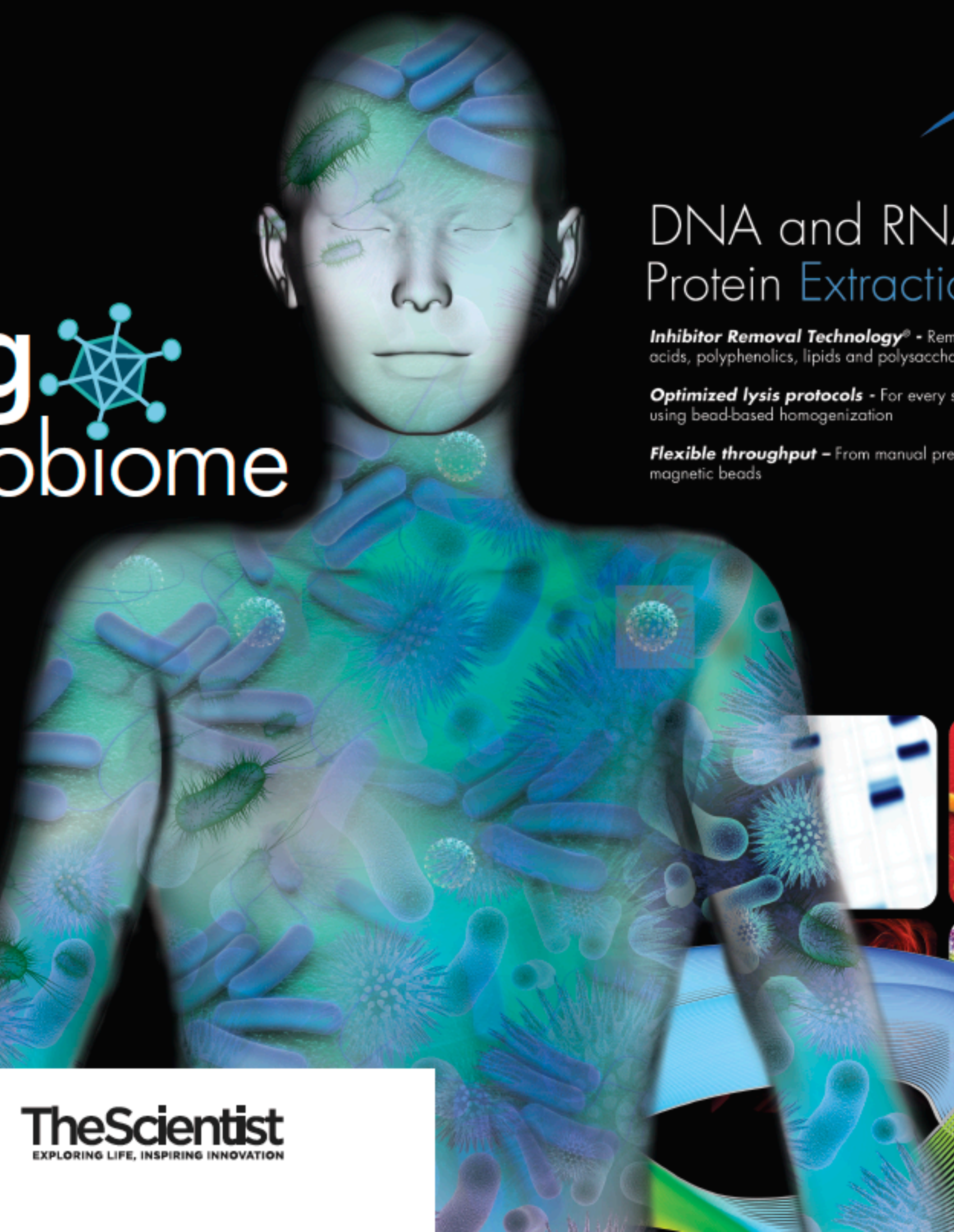

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