The Human Microbiome:
Biomedical Implications and Birth of a Market

Ken Rubenstein, Ph.D.

This report covers the evolution of microbiome research and its growth in the commercial market. Specific areas of study include microbial ecology, systems biology, diet, diagnostics, contributions in health and disease, and infectious disease.

Microbiota have become a center for discussion and investigation after research has unraveled the symbiotic relationship between humans and their microbial counterparts, and the impact of the delicate balance of specific microbiota on various diseases. Such diseases include diabetes, Chronic Obstructive Pulmonary Disease (COPD), and Irritable Bowel Diseases (IBD). Understanding how microbiota act as communities, and thus is relation to disease, has been the topic of choice, known as microbial ecology, for several researchers. It has been demonstrated that when in equilibrium with their environment, microbiota gain benefits from the host, which enables them to provide useful benefits themselves. However, when this balance is disrupted, the repercussions can have a negative impact on human health. For example, the use of antibiotics can lead to vaginal yeast infections in women. This is because antibiotics kill not only the build-up of the infectious bacteria, but also all the other bacteria around it that keeps the control of yeast in check. Destroying these bacteria leads to the overgrowth of yeast and thus yeast infections.

In addition to antibiotics disrupting the microbiome environment, diet is another factor that can pose as a problem. A diet high in carbohydrates can also cause yeast build-up because yeast feed on sugar. Diet can also create an imbalance in the microbiome environment and jeopardize the symbiotic relationship between the microbiota and the host. A simple difference between consuming plant fats vs. animals fats has been demonstrated to have an effect on the abundance of certain types of bacteria in the gut over others, suggesting that these bacteria may be linked to weight loss and/or gain. In fact, researchers concluded there was a link between people who had animal-based dietary fats, bile acids, and irritable bowel diseases. Due to these findings, researchers are further investigating the relation between microbiota and the impact on various immune pathways, and the influence on obesity and diabetes.
In addition to covering several aspects of the microbiome, the report also includes the impact of this rising field on commercial aspects and product development. Companies covered in this space include:

- 4D Pharma
- ActoGenixAOBiome
- AvidBiotics
- CIPAC Limited
- Enterome Bioscience
- Human Longevity
- Metabiomics
- Microbiome Therapeutics
- Miomics
- OmniBiome Therapeutics
- OptiBiotix
- Osel Inc
- OxThera
- PathoGenetix
- Rebiotix
- Ritter Pharmaceuticals
- Second Genome
- Seres Health
- uBiome
- UCB
- UC San Francisco
- Vedanta
- ViThera Pharmaceuticals
- Whole Biome

Final features of the report include:

- In-depth analysis of market data

Exclusive interview transcripts with the following experts:

- Peter DiLaura, President & CEO, Second Genome
- Colleen Cutcliffe, CEO, Whole Biome
- James Brown, PhD; Head, Computational Biology; Head, Microbiome Matrix Team; GlaxoSmithKline
- Jack Gilbert, PhD., Environmental Microbiologist, Assoc. Prof., Department of Ecology & Evolution, Argonne National Laboratory
- Glenn Tillotson, PhD, Senior Partner, Transcrip Partners, USA
- Peter P. Lee, MD, Executive Chairman, Osel, Inc.
- Gregory Kuehn, VP Business Development and Marketing, Metabiomics
- Stephen Elms, CEO, Miomics Bio-Therapeutics
- Sydney M. Finegold, M.D., Emeritus Professor of Medicine, and Emeritus Professor of Microbiology, Immunology, and Molecular Biology, UCLA School of Medicine; Staff Physician, Infectious Disease Section, VA Greater Los Angeles Healthcare System
Executive Summary

Results from the Human Microbiome Project and other massive studies of human bacterial symbionts, which far outnumber human cells in the body, have drawn lots of attention in recent years from both the basic and applied biomedical research communities. During 2009, a PubMed search on the term ‘human microbiome’ yielded 579 citations, but for 2013 that number had increased to 3,324.

A number of new commercial ventures have sprung up in recent years to develop therapies and biomarkers for a number of common chronic conditions, notably inflammatory, metabolic, and autoimmune diseases. Most such programs are based on the notion that disruption of relatively stable microbiome ecosystems results in dysbioses, i.e., imbalances that in turn destabilize homeostasis and create the conditions for diseases to arise and flourish.

This report focuses on biomedical aspects of human microbiome research, development, and commerce. It covers necessary background material, evolution of the field, progress in basic research, activities in the developing commercial market, deal activity, interviews with experts, and trends in microbiome research and commerce. Information for the report has been drawn from the scientific literature, discussions with knowledgeable individuals, and an online survey of people working in this area.

Background and evolution

After making significant pioneering contributions to genomics, Craig Venter led an expedition to collect samples of marine bacteria and sequence them en masse using Sanger shotgun methodology. The project identified more than 1,800 species and set a precedent that established metagenomics as a viable field for investigation and commercial participation. The advent of next-generation sequencing technologies and subsequent focus on short hypervariable regions of microbial 16S rRNAs enabled the NIH-sponsored Human Microbiome Project and others of its sort to provide sufficient definition of human microbiomes to stimulate broad and accelerating participation in the field.

Early progress in the microbiome R&D led to identification of several enterotypes that may provide a basis for personalized microbial medicine. Furthermore, particular diets appear to influence individual enterotypes, which can change in accord with dietary alteration. Another provocative observation suggests that a well-behaved microbiome can serve to maintain weight, while dysbioses can result in obesity with attendant metabolic disease consequences. Early research also uncovered a likely mechanism by which dysbioses can trigger atherosclerosis.

Such early research has served to promote a view of humans as superorganisms composed not only of human cells,
tissues, organs, etc., but also large numbers of bacteria, archaea, viruses, and single-celled eukaryotes living with us in symbiotic relationship. Microbiome work also has promoted an alternative to viewing the human immune system through a military metaphor in which antibodies and cells latch onto and destroy invading pathogens. A developing perspective envisions an immune system that serves to maintain equilibrium between microbes and host.

A key observation that’s triggered much of today’s commercial activity came with studies showing that patients suffering recurrent severe diarrhea-inducing C. difficile infections could be cured or improved by receiving a fecal transplant from a healthy matched individual. A common interpretation is that antibiotic treatment temporarily altered the gut microbiome and allowed normally quiescent C. difficile to overgrow and become pathogenic. Restoration of the microbiome via transplant restores balance, and the infectious agent becomes commensal once again.

**Basic microbiome research**

Early work in next-generation metagenomic sequencing centered on Roche 454 pyrosequencing. Speed and cost considerations later shifted attention to faster and cheaper short-fragment sequencing, a field currently dominated by Illumina’s systems. Some microbiome work continues to benefit from applying combinations of sequencing formats. A subset of researchers favors Pacific Biosciences’ technology which provides impressively long reads averaging 5,000 bases.

Data analysis remains an area of vulnerability in the microbiome space. Informatics workflows fall into two classes: gene-centric, favored when addressing high complexity microbiomes; and assembly-based, better for lower diversity applications. Either choice requires further selections downstream from the branch point. While significant improvements in data analysis have been made in recent years, the process will likely remain a bottleneck in metagenomics for some time to come. Results of metagenomic analyses must also be made consistent among laboratories and technology platforms, and early indications suggest that more work is needed in this regard.

Viewing microbiomes as ecosystems with components in dynamic equilibrium suggests that the field can benefit from adopting systems biology methods and perspectives. Leroy Hood has pointed out that the complexity of microbiomes and their interactions with each other and their host suggests that multi-omic analyses may be needed to identify subpopulations of microbes with distinct functions. The goal, as he sees it, is integration of data into metadata structures, such as a network of networks, in order to generate predictive models for use in understanding the function of microbes in communities. These in turn will enable their
molecular reengineering to produce desired results. Research from Chalmers University in Denmark has identified such bacterial groups, defined ways they interact, and provided means to interpret transcriptomic data in a metabolic context. Rob Knight’s lab, which focuses in part on metabolomics of microbiomes, points out that effects of the microbiome on the whole human metabolome are only beginning to be understood, but earlier work has shown clearly that such effects do exist.

Microbial ecology and systems biology, two symbiotic subject areas as it were, are keys to elucidating the role of human microbiomes in health and disease. To paraphrase a recent workshop report on the subject, microorganisms shape their host environments and are, in turned, shaped by it. Microbiomes ideally exist in stable equilibrium with their environment. They gain benefits from the host, while providing useful ‘goods and services.’ Disruption of these equilibria based on perturbation of hosts, microbes, or environmental niches due to changes in diet, use of antibiotics, et al. can result in dysbiosis with consequences for health that microbiome research has only begun to uncover. Microbiome researchers have established associations between dysbiosis and numerous chronic diseases, but have yet to establish whether these relationships are also causal. Still, the dramatic growth in incidence of many such diseases since the start of the antibiotics era combined with observations on geographic variation are highly suggestive of causation in certain instances. Two recent studies in microbial ecology, one for environmental microbiomes in homes and the other in hospitals, provide provocative and surprising insights into how our individual microbiomes affect and are affected by these environments.

Microbiome research has much to say about obesity, inflammation, and insulin resistance, which increase in association with decreases in gut bacterial diversity. Many studies report associations between diet, weight, dysbiosis, and disease, but at present evidence for causation remains largely circumstantial. Observers indicate a need for more longitudinal studies in which large numbers of affected and control individuals have their microbiomes monitored over extended time periods. A recent study showed that antibiotic-induced dysbioses in young mice reversed when the drug was withdrawn. Yet mice so-treated gained weight much more rapidly than controls when put on high-fat diets. Transplanting their microbiota to germ-free mice also transferred the weight gain phenotype so that this study appears to establish causation.

Research activity in pursuit of diagnostic assays is at present largely confined to population studies. At least one private and another public program collect fecal samples from individuals who pay a fee, and in return get results telling how their microbiome data appears in comparison to other populations with and without particular disease phenotypes.
Commercial activity in the microbiome space

Projects now in various stages of commercial development cover a broad spectrum of applications. This report covers descriptions and activities of 23 microbiome companies. The most prevalent applications fall in the category of live biotherapeutic agents (probiotics to some) that deal with various diseases and disorders. These include Crohn’s disease, skin ulcers, acne, diabetes, inflammatory bowel disease, and C. difficile gastroenteritis. Some programs in this category address broader disease or health maintenance issues including: autoimmune disease, cholesterol reduction, metabolic diseases, the immune system, and infectious disease. Two companies work to engineer commensal bacteria that can enter the microbiome and generate therapeutic molecules in situ for treatment of inflammatory bowel disease, mucositis, and infectious diseases. Several organizations are developing microbiome-based diagnostic products, which include biomarkers for Crohn’s disease, colon cancer, and pre-term labor risk.

The report also describes 12 recent microbiome-related deals. Of these, five involve research collaborations between small companies and big phamas. J&J’s Janssen Biotech unit is active in three of these deals, Pfizer in one, and Merck in another. The Mayo Clinic Center for Personalized Medicine has been active in the microbiome space, providing funds and collaborative support to three small companies. In two instances large companies have provided research funds to academic groups. Other arrangements include small companies working together, another collaborating with a large clinical lab services firm, and another which has been granted a technology license.

Our online survey of 63 people active in microbiome work divides about evenly between those in commerce and academia. About three-quarters of respondents are managers or principal investigators. Nearly half are connected to work on inflammation, and a third are involved with metabolic diseases. About three-quarters of respondents feel that levels of microbiome-related activities in their organization would stay about the same or increase during the next two years. Two-thirds of respondents felt that sufficient microbiome-related information is now available to justify translational efforts, while one-third disagreed. Two-thirds felt optimistic that microbiome work will provide major contributions to healthcare, while nearly a third said it’s too early to tell. Nearly two-thirds agreed that big pharma will become heavily involved in microbiome work over the next decade, and only 15% disagreed.
Trends and conclusions

Based on our interviews and survey results, it seems clear that people working in the field are highly bullish about the importance and future success of microbiome R&D in diagnostics and therapeutics, quite possibly with a personalized twist. Nonetheless, it is still early days for the field, and it may be well to remember that many veterans of post-genomic wars can relate tales of great promise with results that fell short of expectations. Still, the microbiome space has a certain compelling air to it that suggests warranted optimism. The C. difficile fecal transplant example alone seems emblematic.

Regarding commercial potential in the microbiome space, the market size is at present negligibly small, and we expect it will take a couple of years more work on products currently in development before estimates can be made with any reasonable degree of confidence. However, it seems also fair to predict that the market size at the end of a decade will likely be well up in the billions of dollars in annual sales if only some of the products in development accomplish their aims.

A subject of great interest to participants and observers in the space is whether big pharma will embrace the microbiome in a big way. Early participation as evidenced by the level of dealing-making at such an early stage in the field’s development supports optimism. In this regard, our interview with James Brown, the head of GlaxoSmithKline’s Microbiome Matrix Team reveals further reason for optimism. The very existence of such a team and GSK’s willingness to commit resources in that way reveals serious interest. Regulatory policy in the U.S. and Europe will no doubt play an important role in the future of microbiome-related commerce. The FDA’s behavior in the fecal transplant matter may be instructive in this regard. Initial concern over pathogenic microbes in transplant material led the agency initially to require an IND for each instance of that activity. Very recently, the FDA changed direction and now allows physicians to do the procedure without need for an IND.
Report Contents

Executive Summary
Background and evolution
Basic microbiome research
Commercial activity in the microbiome space
Trends and conclusions

CHAPTER 1
Introduction
Scope and organization of the report

CHAPTER 2
Background and Evolution of Human Microbiome R&D 16
  Exhibit 2.1 Estimated numbers of species and genes found in various body sites
  Exhibit 2.2 PubMed citations for the search term ‘human microbiome’

CHAPTER 3
Research on the Human Microbiome
Culture
Systems Biology
Microbial Ecology
Diet
Virome
Diagnostics
The microbiome in health and disease
Infectious disease
CHAPTER 4
Commercial Aspects of Microbiome Research and Development

Companies active in the microbiome space

Exhibit 4.1
4D Pharma
ActoGenix
Alma BioTherapeutics
AObiome
AvidBiotics
CIPAC Limited
Enterome Bioscience SA
Human Longevity, Inc.
Metabiomics Corp.
Microbiome Therapeutics
Miomics
OmniBiome Therapeutics
OptiBiotix
OseI Inc.
OxThera
PathoGenetix
Rebiotix
Ritter Pharmaceuticals
Second Genome
Seres Health
uBiome
Vedanta Biosciences
ViThera Pharmaceuticals
Whole Biome

Deal Activity

Exhibit 4.2
Actogenix and Merck  
CIPAC Ltd. and University of Minnesota  
Enterome Biosciences and Synthetic Biologics  
Enterome Bioscience and Mayo Clinic  
Metabonomics and Kindstar  
Second Genome and Pfizer  
Second Genome and Janssen Biotech  
Seres Health and Mayo Clinic  
UCB and Harvard University  
University of California, San Francisco and Janssen Research & Development  
Vedanta Biosciences and Janssen Research and Development  
Whole Biome and Mayo Clinic

CHAPTER 5  
Market Survey  
Exhibit 5.1 Participants’ subject matter category in microbiome R&D  
Exhibit 5.2 Type of organization where respondent work  
Exhibit 5.3 Further categorization of workplace  
Exhibit 5.4 Position titles of respondents  
Exhibit 5.5 Type of work currently performed by respondents  
Exhibit 5.6 Type of work likely to be performed by respondents during the next year  
Exhibit 5.7 Disease focus for respondents’ current or impending work  
Exhibit 5.8 Respondents’ expectation of change in their organization’s microbiome involvement during the next two years  
Exhibit 5.9 Opinion regarding academic participation in developing new concepts for Rx and/or Dx  
Exhibit 5.10 Level agreement with statement: Current methods are adequate for quantitative characterization of microbiomes for personalized medicine  
Exhibit 5.11 Level agreement with statement: Sufficient information about the composition and function of human microbiomes has been gathered to justify translational R&D  
Exhibit 5.12 My work on detecting dysbiosis uses the following techniques  
Exhibit 5.13 Respondents’ view on the potential of microbiome R&D to provide major contributions to healthcare  
Exhibit 5.14 Agreement with: Recent microbiome findings will enable an important new generation of therapies for chronic diseases  
Exhibit 5.15 Agreement with: Recent microbiome findings will enable an important new generation of measures to maintain good health
Exhibit 5.16 Agreement with: It is still early days for microbiome-based translational medicine and years of basic research are required to establish its potential
Exhibit 5.17 Agreement with: Sequencing technology will remain dominant over microarrays for most microbiome diagnostic applications
Exhibit 5.18 Agreement with: Big pharma will become heavily involved in microbiome related R&D over the next decade
Exhibit 5.19 Agreement with: We can expect to see a flood of new rationally-designed and/or personalized prebiotics to emerge during the next decade

CHAPTER 6
Trends and Conclusions
Microbiome market potential
Microbial ecology and systems level views of the human microbiome
Big pharma’s involvement with the human microbiome
Food industry involvement with the human microbiome
Regulatory considerations
Consistency of results in analyzing microbiomes
Future directions

CHAPTER 7
Interview Transcripts
Peter DiLaura, President & CEO, Second Genome
Colleen Cutcliffe, CEO, Whole Biome
James Brown, PhD; Head, Computational Biology; Head, Microbiome Matrix Team; GlaxoSmithKline
Jack Gilbert, PhD., Environmental Microbiologist, Assoc. Prof., Department of Ecology & Evolution, Argonne National Laboratory
Glenn Tillotson, PhD, Senior Partner, Transcrip Partners, USA
Peter P. Lee, MD, Executive Chairman, Osel, Inc.
Gregory Kuehn, VP Business Development and Marketing, Metabiomics
Stephen Elms, CEO, Miomics Bio-Therapeutics
Sydney M. Finegold, M.D., Emeritus Professor of Medicine, and Emeritus Professor of Microbiology, Immunology, and Molecular Biology, UCLA School of Medicine; Staff Physician, Infectious Disease Section, VA Greater Los Angeles Healthcare System

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Introduction

The microbiome has captured a great deal of attention in the past few years, not least because of reports suggesting that restoration of the microbial gut ecosystem via fecal transplantation comprises an effective treatment for recurrent C. difficile infection. No doubt the large amount of scientific, commercial, and media attention rendered in the past year to antibiotic-resistant bacteria has, arguably, wafted over into enthusiasm for microbiome R&D, which no doubt has also received a tremendous boost from initial reports from the Human Microbiome Project and MetaHIT. Indeed, the C. difficile example links antibiotic resistance and the microbiome since many of these infections develop consequent to antibiotic-driven dysbioses (microbial imbalances on or inside the body).

Increased attention to this subject becomes especially apparent when one compares PubMed citations for ‘human microbiome’ between the years 2009, which predates the aforementioned mass study publications, and 2013. The latter count, 3,324 papers, dwarfs the 2009 total of 579. Venture capital firms and angel investors have taken notice, as indicated by their funding a number of microbiome startups (See Chapter 4), given the field’s relatively short history. Furthermore, several of these companies have already established collaborative ties with big phamas, most of which appear to be keeping a close watch on this potentially explosive new field of endeavor. We find it intriguing that a report published in *Science* in late August 2014 by members of the Home Microbiome Project team (see Chapter 3) had generated 966 Google News citations by September 1.

Dysbioses have now been associated with many common inflammatory, metabolic, neurogenic, and even infectious diseases. Whether many of these correlative associations will ultimately prove causative has yet to be determined, but it appears highly likely that many will.

It is worth noting here that members of the diverse microbial communities living in and on our bodies interact by exchanging nucleic acids and absorbing various molecular signals from each other. Developing a broad understanding of how microbiota act as communities lies in the realm of microbial ecology, which is now entering a new age of discovery, enabled in large measure by continuing rapid advances in DNA sequencing technology. Indeed Craig Venter’s well publicized 2003 Global Ocean Sampling Expedition and subsequent metagenomic analysis of microbes collected therefrom provided a strong impetus for later large-scale studies of human microbiomes.

In show business patois, the microbiome very likely ‘has legs,’ and will succeed in creating a new paradigm for understanding, curating, and maintaining the human organism.
Scope and organization of the report

This report aims to provide individuals and organizations with research or commercial interest, who have already entered the microbiome space, or may wish to do so, with knowledge of how the field has developed, its detailed nature at present, how participants feel about progress and prospects, and how top managers and experts view the microbiome and its potential.

Following this brief introduction, Chapter 2 provides relevant historical and background material to provide context for later discussion of the current state and future prospects of human microbiome research, development, and commerce. The third chapter is devoted to exploring important basic microbiome research as applied mainly to human health and disease. Chapter 4 covers commercial aspects of microbiome R&D, including profiles of companies active in the field. The chapter also includes a section describing deals and collaborative arrangements in the microbiome space. The fifth chapter presents and discusses results of an extensive online survey of individuals active in the field. Chapter 6 provides a compendium of trends and conclusions including relevant extracts of expert interviews conducted for the report. The concluding chapter contains transcripts of these interviews with nine researchers, managers, and others active in connection with the human microbiome.
CHAPTER 4

Commercial Aspects of Microbiome Research and Development

This first section of this chapter contains segments on companies active in the microbiome space. The second section is devoted to consideration of microbiome-related deal activity.

Companies active in the microbiome space

Companies described in this section along with brief summaries of their main activities are tabulated in Exhibit 4.1.

<table>
<thead>
<tr>
<th>Company</th>
<th>Product/Project</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>4D Pharma</td>
<td>Thetanix</td>
<td>Gram-positive anaerobe commensal in development for Crohn’s disease</td>
</tr>
<tr>
<td>4D Pharma</td>
<td>Rosburix</td>
<td>‘Live biotherapeutic’ in development for treatment of pediatric ulcerative colitis</td>
</tr>
<tr>
<td>ActoGenix</td>
<td>AG013</td>
<td>Engineered bacterium secretes IL-10 for Rx of mucositis, ready for Phase 2B clinicals</td>
</tr>
<tr>
<td>ActoGenix</td>
<td>AG013</td>
<td>For oral treatment of IBD, to enter Phase 2B clinicals in 2015</td>
</tr>
<tr>
<td>AOBiome</td>
<td>Ammonia-oxidizing bacteria</td>
<td>Pipeline of skin therapeutics for Rx of ulcers, acne, and atopic dermatitis</td>
</tr>
<tr>
<td>AvidBiotics</td>
<td>Avidocin and Purocin</td>
<td>R-type pyocin proteins modified with phage tail fibers to target bacterial pathogens, in development</td>
</tr>
<tr>
<td>CIPAC Limited</td>
<td>Cleaner fecal transplant</td>
<td>Bacteria separated from donor feces, frozen for later transplant vs C. difficile infection</td>
</tr>
<tr>
<td>Enterome Bioscience</td>
<td>Metagenomic-based biomarkers</td>
<td>Lead product is personalized biomarker for Crohn’s disease monitoring, in clinical validation; research on therapeutic agents</td>
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</table>
CHAPTER 5

Market Survey

This chapter contains results of an online market survey conducted in July and August of 2014. Each respondent currently works actively in connection with microbiomes.

The survey began by asking respondents to indicate their participation in microbiome-based therapeutics, diagnostics, or basic research. Multiple responses were permitted. Nearly two-thirds indicated that they participate in basic microbiome research. Half said their work involves therapeutics, and nearly as many (41%) claim diagnostics (Exhibit 5.1).

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of responses</th>
<th>Percent of respondents</th>
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<tbody>
<tr>
<td>Therapeutics</td>
<td>28</td>
<td>50%</td>
</tr>
<tr>
<td>Diagnostics</td>
<td>23</td>
<td>41%</td>
</tr>
<tr>
<td>Basic research</td>
<td>35</td>
<td>63%</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>13%</td>
</tr>
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The next item sought to determine type of organization. Only single responses were permitted in this instance (Exhibit 5.2). The 63 individuals who responded divide near equally between academia and commerce. Academic, government, or private research institutions accounted for 48% of responses. Drug R&D garnered 27%, and diagnostics accounted for 17% of responses. The remaining 8% work in consulting organizations.

<table>
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<tr>
<th>Category</th>
<th>Number of responses</th>
<th>Percent of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial drug discovery and/or development organization</td>
<td>17</td>
<td>27%</td>
</tr>
<tr>
<td>Commercial diagnostic products or services organization</td>
<td>11</td>
<td>17%</td>
</tr>
<tr>
<td>Academic, government, or private research institution</td>
<td>30</td>
<td>48%</td>
</tr>
<tr>
<td>Consulting organization</td>
<td>5</td>
<td>8%</td>
</tr>
</tbody>
</table>

A further breakdown of organization type (Exhibit 5.3) revealed that 44% of respondents to this question work in academia, 8% in consultancies, and 8% in some ‘other’ category than those described. Pharma and biotech companies were categorized according to annual