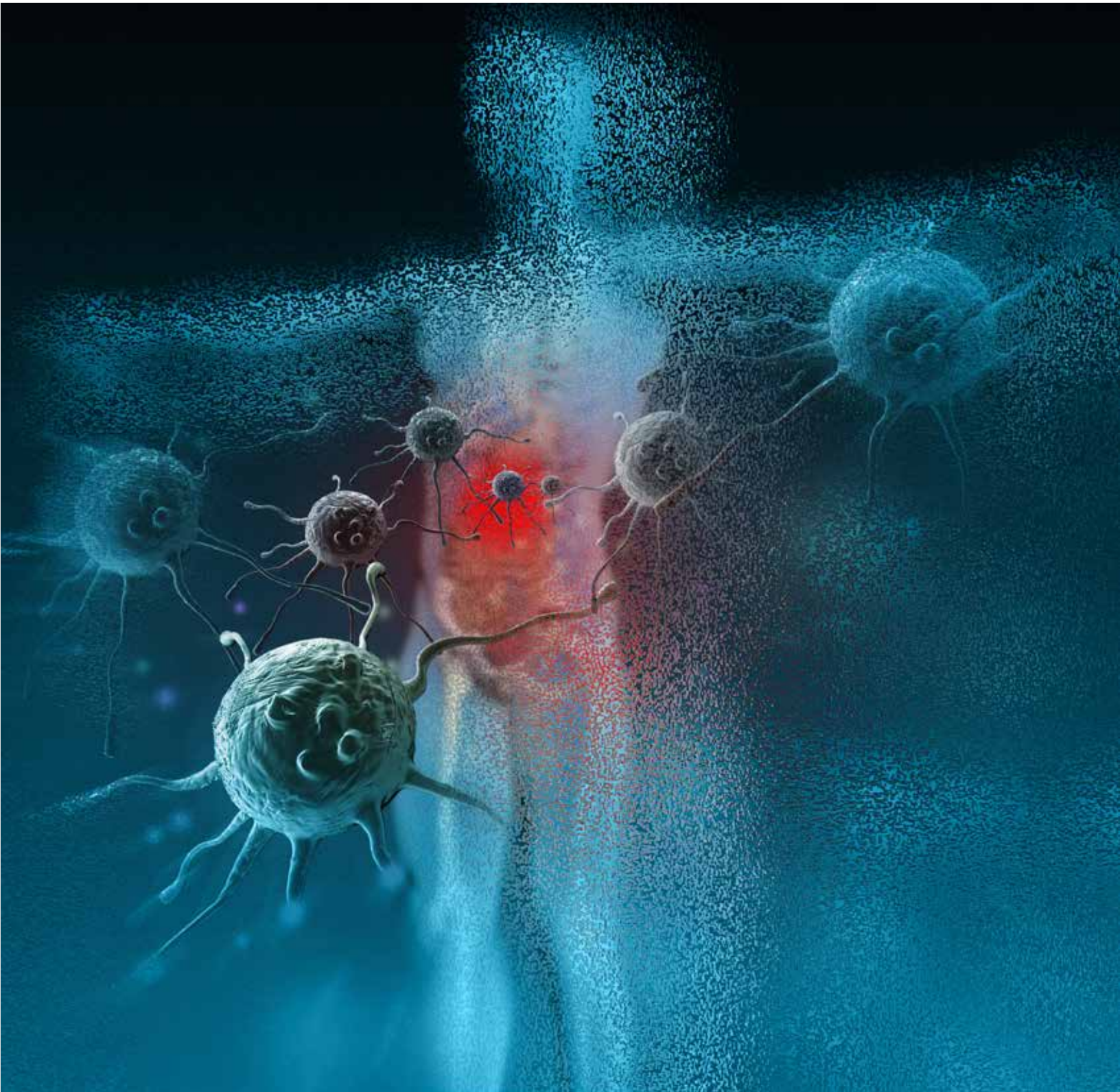
Microbes and Metagenomics in Human Health

An overview of recent publications featuring Illumina® technology



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This document highlights recent publications that demonstrate the use of Illumina technologies in immunology research. To learn more about the platforms and assays cited, visit www.illumina.com.

INTRODUCTION

The study of microbes in human health traditionally focused on identifying and treating pathogens in patients, usually with antibiotics. The rise of antibiotic resistance and an increasingly dense—and mobile—global population is forcing a change in that paradigm.[1, 2, 3] Improvements in high-throughput sequencing, also called next-generation sequencing (NGS), allow a holistic approach to managing microbes in human health.

In and on the human body, microbes outnumber human cells 10 to 1.[4] These microbial cells constitute the human microbiome. Uncovering the members of the human microbiome, and their collective genes and functions, defines the role of our microbial communities in health and disease. This goal can only be accomplished by high-throughput sequencing technologies paired with powerful analytic tools.[5]

Metagenomics is one of the fastest growing scientific disciplines, and it is becoming a central tool for improving the quality of life worldwide. Originally conceived as the collective genomes of nonculturable microorganisms,[6] metagenomics now refers to the use of high-throughput DNA sequencing to provide taxonomic ("Who is there?") and functional ("What are they doing?") profiles of microbial communities without the need to culture the microbes in the laboratory.[7] It is estimated that less than 2% of bacteria can be cultured in the laboratory.[8] Our sudden ability to identify the previously unstudied 98% of microbes—whether they are prokaryotes, eukaryotes, or viruses—is revolutionizing and energizing the field of microbiology.

This document highlights recent publications that apply Illumina sequencing technologies to metagenomics research.

1. Roca I., Akova M., Baquero F., Carlet J., Cavaleri M., et al. (2015) The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect 6: 22-29
2. Shallcross L. J., Howard S. J., Fowler T. and Davies S. C. (2015) Tackling the threat of antimicrobial resistance: from policy to sustainable action. Philos Trans R Soc Lond B Biol Sci 370: 20140082
3. Sharland M., Saroey P. and Berezin E. N. (2015) The global threat of antimicrobial resistance - The need for standardized surveillance tools to define burden and develop interventions. J Pediatr (Rio J) 91: 410-412
4. National Institutes of H. (2012) NIH Human Microbiome Project defines normal bacterial makeup of the body. 2015: www.nih.gov/news/health/jun2012/nhgri-13.htm; accessed September 29, 2015
5. Cho I. and Blaser M. J. (2012) The human microbiome: at the interface of health and disease. Nat Rev Genet 13: 260-270
6. Handelsman J., Rondon M. R., Brady S. F., Clardy J. and Goodman R. M. (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5: R245-249
7. Franzosa E. A., Hsu T., Sirota-Madi A., Shafquat A., Abu-Ali G., et al. (2015) Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. Nat Rev Microbiol 13: 360-372
8. Wade W. (2002) Unculturable bacteria—the uncharacterized organisms that cause oral infections. Journal of the Royal Society of Medicine 95: 81-83
9. Peacock S. (2014) Health care: Bring microbial sequencing to hospitals. Nature 509: 557-559

Reviews

"The application of sequencing technology to microbial genomes will improve patient care and enhance public health. The feasibility and economics are clear." – Peacock 2014[9]

HUMAN MICROBIOME

The human microbiome encompasses the collection of microorganisms associated with the human body, including eukaryotes, archaea, bacteria and viruses.[10] The National Institutes of Health (NIH) Common Fund Human Microbiome Project was established in 2008, with the mission of generating resources to enable the comprehensive characterization of the human microbiome and analyze its role in health and disease.[11] This effort resulted in the characterization of microbial communities in different body parts, e.g., oral, gut, skin, and vaginal microbiomes. In parallel, research has also characterized multiple biomes associated with disease states, highlighting the influence of microbiome dysbiosis in disease development.[12] Some animal-associated microbiomes have also been characterized in efforts to improve animal health and understand the interactions between humans and the environment.[13]

10. Human Microbiome Project C. (2012) A framework for human microbiome research. Nature 486: 215-221
11. Foxman B. and Rosenthal M. (2013) Implications of the human microbiome project for epidemiology. Am J Epidemiol 177: 197-201
12. Morgan X. C., Segata N. and Huttenhower C. (2013) Biodiversity and functional genomics in the human microbiome. Trends Genet 29: 51-58
13. Penders J., Stobberingh E. E., Savelkoul P. H. and Wolffs P. F. (2013) The human microbiome as a reservoir of antimicrobial resistance. Front Microbiol 4: 87

Reviews

DeWeerdt S. (2015) Microbiome: Microbial mystery. Nature 521: S10-11

Franzosa E. A., Hsu T., Sirota-Madi A., Shafquat A., Abu-Ali G., et al. (2015) Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. Nat Rev Microbiol 13: 360-372

Koch L. (2015) Metagenomics: Shaping the gut microbiome. Nat Rev Genet 16: 2

Preidis G. A. and Hotez P. J. (2015) The newest "omics"--metagenomics and metabolomics--enter the battle against the neglected tropical diseases. PLoS Negl Trop Dis 9: e0003382

Zhou J., He Z., Yang Y., Deng Y., Tringe S. G., et al. (2015) High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. MBio 6:

Fricke W. F. and Rasko D. A. (2014) Bacterial genome sequencing in the clinic: bioinformatic challenges and solutions. Nat Rev Genet 15: 49-55

Lasken R. S. and McLean J. S. (2014) Recent advances in genomic DNA sequencing of microbial species from single cells. Nat Rev Genet 15: 577-584

Peacock S. (2014) Health care: Bring microbial sequencing to hospitals. Nature 509: 557-559

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Franzosa E. A., Morgan X. C., Segata N., Waldron L., Reyes J., et al. (2014) Relating the metatranscriptome and metagenome of the human gut. Proc Natl Acad Sci U S A 111: E2329-2338

The majority of microbiome studies require that samples be taken at specific facilities by trained personnel, increasing costs and excluding some subjects from participation. The authors developed a protocol for self-collection of stool and saliva samples, and frozen shipping within 24 hours of collection. They used metagenome and metatranscriptome analysis to assess the accuracy and feasibility of this protocol against 2 other sample handling methods. There was strong within-subject correlation between all 3 methods at gene, species, and transcript levels, indicating that the developed protocol had minimal effect on metagenomic profiles. Oral microbes co-occurring in stool samples displayed a drop in abundance and transcript levels, suggesting that oral microbes that transit into the gut are not stable and do not play a role in gut ecology. Overall, the study provides a robust method for self-collection and distinguishes relationships between metatranscriptomes and metagenomes, highlighting the complementarity of both approaches in microbiome studies.

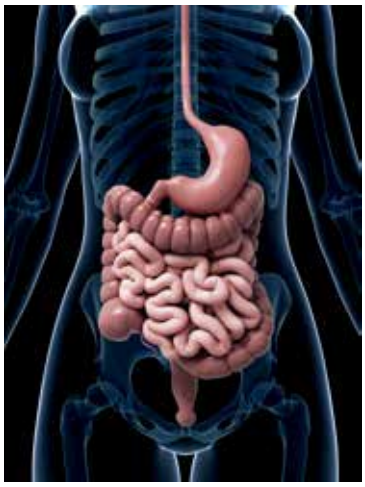
Illumina Technology: HiSeq

Lax S., Smith D. P., Hampton-Marcell J., Owens S. M., Handley K. M., et al. (2014) Longitudinal analysis of microbial interaction between humans and the indoor environment. Science 345: 1048-1052
Humans imprint their residences with their specific microbial communities. These homes are a good proxy to monitor indoor microbial profiles related to their inhabitants. The Home Microbiome Project monitored skin and home-surface microbial communities by 16S rRNA V4 amplicon sequencing. A total of 10 houses were monitored for 6 weeks, including 3 families before and after moving to a new home. Microbial communities were specific to each home/family pair because human inhabitants were the main source of such microbiomes, which lead to acquisition of the family microbial community soon after a home move. Genetically related individuals had insignificant microbiome differentiation, whereas a nonrelated individual sharing common areas displayed some differentiation. Shotgun metagenomic sequencing revealed few pathogens and their virulence genes on kitchen counter surfaces associated with specific house inhabitants. This study highlights the interactions between humans and their environment and how this relationship may reflect and impact health status.

Illumina Technology: Nextera XT DNA Library Prep Kit, HiSeq 2000, MiSeq

Gut Microbiome

In the gut, the host provides whole foods while the microbiota makes nutrients available to the host. This symbiotic relationship also keeps opportunistic pathogens at bay. Microbiota composition is variable among individuals due to the influence of multiple factors, including genetics,[14] birth mode,[15] diet,[16, 17] geographic region,[18, 19] age,[20] and even exercise.[21] This complex microbial biome influences many biological aspects of human health, such as the immune system,[22, 23, 24] diabetes,[25] atherosclerosis,[26] and the interaction with xenobiotics.[27]



In the gut, the host provides whole foods while the microbiota makes nutrients available to the host.

Diet and modern processed foods can alter microbiomes and metabolic pathways in unforeseen ways. For example, certain artificial sweeteners reduce glucose tolerance in mice through intestinal dysbiosis.[28] Significant shifts in microbiota composition can also occur before or during the development of colorectal cancer in humans. These shifts could be potential biomarkers for diagnostics and disease management, and they can place emphasis on gut microbiome characterization.[29] Bioinformatic tools have been developed to identify bacterial species, genes/pathways, and to support inferences associated with clinical variables.[30]

14. Goodrich J. K., Waters J. L., Poole A. C., Sutter J. L., Koren O., et al. (2014) Human genetics shape the gut microbiome. Cell 159: 789-799
15. Dominguez-Bello M. G., Costello E. K., Contreras M., Magris M., Hidalgo G., et al. (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 107: 11971-11975
16. Veiga P., Pons N., Agrawal A., Oozeer R., Guyonnet D., et al. (2014) Changes of the human gut microbiome induced by a fermented milk product. Sci Rep 4: 6328
17. Wu G. D., Chen J., Hoffmann C., Bittinger K., Chen Y. Y., et al. (2011) Linking long-term dietary patterns with gut microbial enterotypes. Science 334: 105-108
18. Nakayama J., Watanabe K., Jiang J., Matsuda K., Chao S. H., et al. (2015) Diversity in gut bacterial community of school-age children in Asia. Sci Rep 5: 8397
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20. Yatsunenko T., Rey F. E., Manary M. J., Trehan I., Dominguez-Bello M. G., et al. (2012) Human gut microbiome viewed across age and geography. Nature 486: 222-227
21. Lambert J. E., Myslicki J. P., Bomhof M. R., Belke D. D., Shearer J., et al. (2015) Exercise training modifies gut microbiota in normal and diabetic mice. Appl Physiol Nutr Metab 40: 749-752
22. Erny D., Hrabe de Angelis A. L., Jaitin D., Wieghofer P., Staszewski O., et al. (2015) Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 18: 965-977
23. Kamada N., Seo S. U., Chen G. Y. and Nunez G. (2013) Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol 13: 321-335
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25. Markle J. G., Mortin-Toth S., Wong A. S., Geng L., Hayday A., et al. (2013) gammadelta T cells are essential effectors of type 1 diabetes in the nonobese diabetic mouse model. J Immunol 190: 5392-5401
26. Org E., Mehrabian M. and Lusis A. J. (2015) Unraveling the environmental and genetic interactions in atherosclerosis: Central role of the gut microbiota. Atherosclerosis 241: 387-399
27. Kim D. H. (2015) Gut Microbiota-Mediated Drug-Antibiotic Interactions. Drug Metab Dispos 43: 1581-1589
28. Suez J., Korem T., Zeevi D., Zilberman-Schapira G., Thaiss C. A., et al. (2014) Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature 514: 181-186
29. Feng Q., Liang S., Jia H., Stadlmayr A., Tang L., et al. (2015) Gut microbiome development along the colorectal adenoma-carcinoma sequence. Nat Commun 6: 6528
30. Nayfach S., Fischbach M. A. and Pollard K. S. (2015) MetaQuery: a web server for rapid annotation and quantitative analysis of specific genes in the human gut microbiome. Bioinformatics

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Dietert R. R. and Silbergeld E. K. (2015) Biomarkers for the 21st century: listening to the microbiome. Toxicol Sci 144: 208-216

Gritz E. C. and Bhandari V. (2015) The human neonatal gut microbiome: a brief review. Front Pediatr 3: 17

Koch L. (2015) Metagenomics: Shaping the gut microbiome. Nat Rev Genet 16: 2

Candela M., Turroni S., Biagi E., Carbonero F., Rampelli S., et al. (2014) Inflammation and colorectal cancer, when microbiota-host mutualism breaks. World J Gastroenterol 20: 908-922

Cenit M. C., Matzaraki V., Tigchelaar E. F. and Zhernakova A. (2014) Rapidly expanding knowledge on the role of the gut microbiome in health and disease. Biochim Biophys Acta 1842: 1981-1992

Salazar N., Arboleya S., Valdes L., Stanton C., Ross P., et al. (2014) The human intestinal microbiome at extreme ages of life. Dietary intervention as a way to counteract alterations. Front Genet 5: 406

Walker A. W., Duncan S. H., Louis P. and Flint H. J. (2014) Phylogeny, culturing, and metagenomics of the human gut microbiota. Trends Microbiol 22: 267-274

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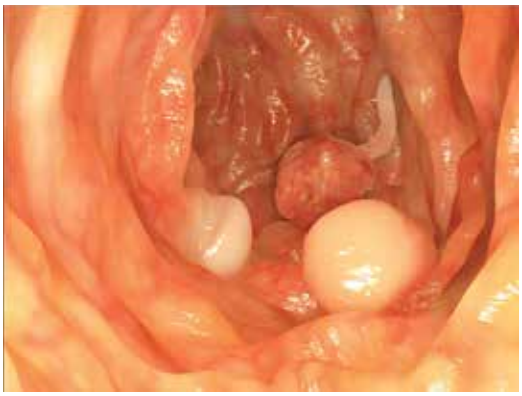
Feng Q., Liang S., Jia H., Stadlmayr A., Tang L., et al. (2015) Gut microbiome development along the colorectal adenoma-carcinoma sequence. Nat Commun 6: 6528

Benign polyps slowly turn to colorectal cancer, but changes in gut microbiota during progression of disease are not well understood. Metagenome-wide association studies on stool samples from adenoma and carcinoma patients and healthy controls were used to assess microbes, genes and functions enriched in each group. Profound shifts in gut microbiota associated with tumors were observed, such as an increase of specific *Bacillus* species in progression from healthy to advanced carcinomas. The authors identified specific gene markers enriched in carcinoma samples as potential biomarkers of disease. A possible risk factor for disease is higher consumption of red meat relative to vegetables, due to the outgrowth of bacteria hostile to the gut environment promoted by amino acid degradation in the colon. Likewise, lactic acid producers enriched in control samples might inhibit amino acid degradation in the colon and inhibit potential pathogens, contributing to a nurturing gut environment.

Illumina Technology: HiSeq 100 bp paired-end (PE) reads



Intestinal polyps.

Martinez I., Stegen J. C., Maldonado-Gomez M. X., Eren A. M., Siba P. M., et al. (2015) The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes. Cell Rep 11: 527-538

The gut microbiota is influenced by factors such as lifestyle and diet; alterations in these factors may lead to health disturbances. Amplicon sequencing of 16S V5 rRNA was used to assess the community differences in fecal samples from rural Papua New Guinea (PNG, nonindustrialized) and US (Westernized) residents. PNG and US residents shared a core of the most abundant phylotypes, although there were substantial differences in the specific microbiome signatures for each population. PNG microbiota was characterized by bacterial homogenizing dispersal processes that resulted in lower interindividual variation, greater bacterial diversity and abundance, and the presence of bacterial species unique to this population. The authors suggest that microbiome differences may result from population heterogeneity and hygiene practices used in Westernized societies that limit bacterial dispersal processes found in nonindustrialized microbiomes. It is likely that a combination of factors contributes to the observed differences.

Illumina Technology: MiSeq

Papua New Guineans harbor a characteristically diverse gut microbiota that distinguishes them from the microbiota found in industrialized societies.

Obregon-Tito A. J., Tito R. Y., Metcalf J., Sankaranarayanan K., Clemente J. C., et al. (2015) Subsistence strategies in traditional societies distinguish gut microbiomes. Nat Commun 6: 6505

Amplicon sequencing of 16S V4 rRNA gene and metagenome sequences were used to compare two traditional populations from Peru (hunter-gatherers and an agricultural community) and a typical US community. Both traditional communities had higher microbial diversity than the US cohort. Available data from similar populations in distant geographic locations support these findings, showing clear separation between traditional and industrial microbiomes. Rural communities were enriched for nonpathogenic *Treponema* similar to the carbohydrate metabolizer *T. succinifaciens*. Similar reports in ancient microbiomes and other traditional populations suggest that these microbes may have been lost through Westernization but remain in traditional populations due to their potential metabolic functions.

Illumina Technology: Nextera DNA Library Prep Kit, HiSeq v4

Arthur J. C., Gharaibeh R. Z., Muhlbauer M., Perez-Chanona E., Uronis J. M., et al. (2014) Microbial genomic analysis reveals the essential role of inflammation in bacteria-induced colorectal cancer. Nat Commun 5: 4724

The complex relationships among microbiota, inflammation, and pathology in colorectal cancer are still undefined. 16S V6 rRNA gene amplicon sequencing and RNA-Seq were used to define gut microbiota composition in a colitis model using ex-germ-free interleukin 10-/- mice. The authors showed that inflammation was essential for development of *E. coli*–induced colorectal cancer in an inflammation-sensitive genetic background. This model illustrates how gut environment can alter microbial gene expression and how inflammation is a defining factor in *E. coli*–induced cancer.

Illumina Technology: TruSeq RNA Library Prep Kit v2, HiSeq 2000

Goodrich J. K., Waters J. L., Poole A. C., Sutter J. L., Koren O., et al. (2014) Human genetics shape the gut microbiome. Cell 159: 789-799

The interplay of host genetics, gut microbiota, and metabolic patterns can impact disease and obesity. Defining this interrelationship could open new avenues for disease prevention, diagnosis, and management. Self-collected fecal samples from 416 adult twin pairs were subjected to amplicon sequencing of 16S V4 rRNA genes. The abundance of specific microbiota members was influenced by host genetics: taxa abundance was more correlated in monozygotic than dizygotic twin pairs. The authors found highly heritable bacterial taxa (*Christensenellaceae*) forming co-occurrence networks with other heritable bacteria and methanogenic archaea. These networks were enriched in individuals with low body mass. Introducing *Christensenellaceae minuta* to obese microbiomes followed by transplantation to germ-free mice reduced weight gain in the recipients, who acquired stable changes in community diversity.

Illumina Technology: MiSeq 250 bp PE reads

Hsiao A., Ahmed A. M., Subramanian S., Griffin N. W., Drewry L. L., et al. (2014) Members of the human gut microbiota involved in recovery from Vibrio cholerae infection. Nature 515: 423-426

Changes in gut microbiota during or after diarrhea episodes, caused by infectious agents, are not well characterized. As a result, their contribution to recovery or susceptibility remains poorly understood. The results from amplicon sequencing of 16S V4 rRNA gene and metagenomic analysis of fecal samples from Bangladeshi adults during and recovering from *V. cholerae* diarrhea were examined. Microbial composition

during recovery parallels the establishment patterns found in healthy Bangladeshi children. Fecal transplant of the recovering microbiota into gnotobiotic mice revealed that *Ruminococcus oleum*, associated with infant microbiota establishment and recovery from *V. cholerae* diarrhea, was able to restrict *V. cholerae* colonization by a quorum-sensing autoinducer-2 mechanism and a novel regulatory pathway. The authors highlight the value of mining microbiota data to identify mechanisms that limit colonization by enteropathogens.

Illumina Technology: MiSeq 250 bp PE reads, Genome Analyzer$_{IIx}$, HiSeq 2000

Cholera has been prevalent in the Ganges delta since ancient times.[31]

Integrative H. M. P. R. N. C. (2014) The Integrative Human Microbiome Project: dynamic analysis of microbiome-host omics profiles during periods of human health and disease. Cell Host Microbe 16: 276-289

The Human Microbiome Project (HMP) generated a wealth of protocols, bioinformatic pipelines, and data. The Integrative HMP (iHMP) Research Network Consortium will carry out the second phase of the HMP, aiming to understand microbiome-host interactions by monitoring microbiome and host changes for a period of 3 years. Phylogenetic composition (amplicon and shotgun sequencing) and functional data from multiple 'omic approaches (transcriptome, proteome) will be generated from 3 longitudinal studies with focus on: i) pregnancy; ii) gut disease onset (inflammatory bowel disease (IBD) model); and iii) respiratory viral infection and onset of type 2 diabetes.

Illumina Technology: Illumina platforms will be used in the study

Li J., Jia H., Cai X., Zhong H., Feng Q., et al. (2014) An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol 32: 834-841

Multiple gut microbiome studies have generated large gene datasets. Unifying data to generate a global representative catalog of reference genes would allow for integrative study of the gut microbiome. A nonredundant catalog comprised of 9,879,896 human gut microbial genes was generated by combining 249 newly sequenced samples, 1018 samples from MetaHit, HMP and a diabetes study, as well as 511 sequenced genomes of gut bacteria and archaea. The integrated gene catalog (IGC) was tested by successful mapping and correlation of metatranscriptomic and metagenomic data from a recent study. IGC was used to demonstrate country-specific gut microbiome signatures in Chinese and Danish cohorts, highlighting its usefulness to facilitate characterization of variation across populations.

Illumina Technology: Genome Analyzer$_{IIx}$ and available Illumina-generated data from previous studies

Moeller A. H., Li Y., Mpoudi Ngole E., Ahuka-Mundeke S., Lonsdorf E. V., et al. (2014) Rapid changes in the gut microbiome during human evolution. Proc Natl Acad Sci U S A 111: 16431-16435

The evolutionary history of the human gut microbiome can be elucidated by studying the microbiomes of nonhuman primates. Amplicon sequencing of the 16S V4 rRNA gene was used to characterize the gut microbiomes of diverse African apes (chimpanzees, bonobos, and gorillas). This information was used to generate a model for evolutionary divergence of the current human microbiome from ancestral populations through reconstruction of microbiome changes. African ape gut microbiomes followed slow compositional changes during diversification, but remain highly diverse in comparison to humans. The authors propose that humans have followed accelerated rates of divergence, characterized by the loss of microbial diversity as an adaptation to animal-based diets.

Illumina Technology: MiSeq

31. Sack D. A., Sack R. B., Nair G. B. and Siddique A. K. (2004) Cholera. Lancet 363: 223-233

Mountain gorilla (*Gorilla gorilla berengeii*) eating leaves, Park du Volcanes, Rwanda. African apes have been used as models of gut microbiome changes during human evolution.

Rosser E. C., Oleinika K., Tonon S., Doyle R., Bosma A., et al. (2014) Regulatory B cells are induced by gut microbiota-driven interleukin-1beta and interleukin-6 production. Nat Med 20: 1334-1339

Regulatory B (B_{reg}) cells prevent excessive immune responses by release of interleukin (IL)-10. These cells differentiate upon inflammation, but the spectrum of signals involved in this process is unknown. The authors treated mice with antibiotics to deplete their gut microbiota. Antigen-induced arthritis of these mice resulted in milder arthritis and reduced numbers of immune cells and proinflammatory cytokines. Similar disturbances were found in splenocytes and mesenteric lymph nodes, along with reduced function and differentiation of Breg cells. In the arthritis-induced model, proinflammatory cytokines IL-1β and IL-6 were produced by conventionally housed mice but not in sterile-housed animals. These cytokines promote Breg cell differentiation and IL-10 production, and their signals are produced in response to gut microbiota and arthritis. The authors suggest that IL-1β, IL-6, and th17 differentiation are signals also required for Breg cell differentiation. In addition, a small variation in commensal microbiota contributes to both proinflammatory and regulatory responses behind these dynamics.

Illumina Technology: MiSeq 250 bp PE reads

Schwab C., Berry D., Rauch I., Rennisch I., Ramesmayer J., et al. (2014) Longitudinal study of murine microbiota activity and interactions with the host during acute inflammation and recovery. ISME J 8: 1101-1114

Gut microbiota functional changes and recovery patterns after colitis have not been addressed in detail. Metatranscriptomics, 16S rRNA amplicon sequencing, and host markers were used to monitor changes in gut microbiota during acute inflammation and recovery in a mouse model of colitis induced by dextran sodium sulfate. Severe dysbiosis was observed during acute colitis, followed by gradual recovery of the original microbiota composition. However, microbial gene expression was more resilient to disturbance and seemed to recover quickly. Host inflammation markers correlated with acute colitis, reduced transcripts of commensal clostridial flagellar genes, and an increase in transcripts related to mucin degradation. The authors propose that the gut microbiota has a remarkable ability to recover and inflammation triggers immune reactions against flagella of commensal bacteria that results in temporary microbiota dysbiosis.

Illumina Technology: HiSeq

Suez J., Korem T., Zeevi D., Zilberman-Schapira G., Thaiss C. A., et al. (2014) Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature 514: 181-186

Noncaloric artificial sweeteners (NAS) are widely used due to their low caloric content; most of these pass the gastrointestinal tract undigested by the host and directly encounter the intestinal microbiota. Amplicon sequencing of 16S V2 rRNA and shotgun metagenomics were used in fecal samples of a small human cohort and mice fed different NAS. Mice developed glucose intolerance after chronic exposure to NAS. Humans developed poor glycemic responses with gut microbiota compositional changes. Glucose tolerance was reinstituted by fecal transplant and abolished by antimicrobial treatment. A shift in microbial composition and abundance further corroborated the contribution of gut dysbiosis in NAS-induced glucose tolerance. Glycan degradation pathways were overrepresented in NAS-fed mice, which likely resulted in increased energy harvest and production of precursors of de novo glucose and lipid synthesis. The authors stress the need to reconsider the effects of NAS in humans.

Illumina Technology: MiSeq, HiSeq

Wang J., Linnenbrink M., Kunzel S., Fernandes R., Nadeau M. J., et al. (2014) Dietary history contributes to enterotype-like clustering and functional metagenomic content in the intestinal microbiome of wild mice. Proc Natl Acad Sci U S A 111: E2703-2710

The existence of enterotypes characterized by specific gut microbiota signature taxa is still a topic of debate. Amplicon sequencing of 16S V1 and V2 rRNA gene, shotgun metagenomics, and diet reconstruction were used to assess enterotypes in wild house mice. The authors identified 2 clusters similar to those identified in humans and other mammals. The clusters displayed differences in gene transcripts involved in carbohydrate and protein metabolism pathways that relate to plant and meat food sources. However, enterotype classification changed quickly upon diet change. In addition, changes associated with different environmental settings and reconstruction of dietary history in wild-caught mice revealed that enterotype-like clustering was highly influenced by diet.

Illumina Technology: HiSeq 2000 100 bp PE reads

Gut Microbiome and Disease

Several recent studies link alterations of the individual microbiome (or dysbiosis) with different diseases of clinical importance. From chronic inflammation to infectious diseases, the gut microbiome has an expanding role in health and disease, including a new possible role in acute graft-versus-host disease.[32]

Restoration of gut microbiota is a promising alternative therapy that addresses intestinal dysbiosis,[33] mimicking normal microbial succession after intestinal events.[34] *Clostridium difficile* colitis, a recurring leading cause of nosocomial-associated infections, is a well-characterized model of antibiotic-related intestinal dysbiosis. The dysbiosis is characterized by reduced taxa diversity that results in uncontrolled proliferation of *C. difficile*. Successful microbiota restoration can reduce the recurrence of *C. difficile*.[35]

The development of diagnostic tests associated with specific microbiome signatures, customized therapeutics, development of new antimicrobials, targeted diets, and the profiling of individual biomes might become future tools for personalized health care. A new generation of nontoxic small molecules, identified by analysis of combined omics approaches, can interfere with pathways or metabolites associated with disease.[36, 37, 38, 39]

Reviews

Gritz E. C. and Bhandari V. (2015) The human neonatal gut microbiome: a brief review. Front Pediatr 3: 17

Shono Y., Docampo M. D., Peled J. U., Perobelli S. M. and Jenq R. R. (2015) Intestinal microbiota-related effects on graft-versus-host disease. Int J Hematol 101: 428-437

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Buffie C. G., Bucci V., Stein R. R., McKenney P. T., Ling L., et al. (2015) Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. Nature 517: 205-208

Clostridium difficile is a major cause of antibiotic-induced diarrhea in hospitalized patients. However, the bacteria involved in sensitivity or resistance to *C. difficile* colonization are not known. Amplicon sequencing of the 16S V4-V5 rRNA gene was used in conjunction with targeted depletion of gut microbiota to evaluate the role of different bacterial populations in *C. difficile* colonization in mice. C. scindens, a bile secondary acid synthesis species common to humans and mice, was associated with resistance to *C. difficile* infection. Enhanced resistance was achieved upon addition of human-derived *C. scindens* in mice and correlated with synthesis of secondary bile acids, which corroborated a functional role in *C. difficile* inhibition. This approach identified single bacteria, out of the rich intestinal microbiota context, that were able to inhibit *C. difficile*. Its potential use in fecal transplants, microbiota manipulation, and an initial antimicrobial approach is considered in light of the biological aspects inherent to complex microbiota interactions.

Illumina Technology: TruSeq DNA Library Prep Kit, MiSeq

He B., Nohara K., Ajami N. J., Michalek R. D., Tian X., et al. (2015) Transmissible microbial and metabolomic remodeling by soluble dietary fiber improves metabolic homeostasis. Sci Rep 5: 10604

Dietary management can help improve metabolic diseases by promoting health benefits not yet totally characterized. Digestion-resistant plant-derived fibers, like maltodextrin (RM), improve glucose and lipid homeostasis and help reduce weight gain. The authors fed RM to obese mice, tested its effects in glucose tolerance, and monitored gut microbiota changes by amplicon sequencing of the 16S V4 rRNA gene. RM improved glycemic control by decreasing fasting glucose levels. RM induced beneficial gut microbiota remodeling by increasing the abundance of beneficial bacteria (*Lactobacillus* and *Bifidobacterium*) and decreasing fat-associated bacteria (*Alistipes*). Fecal transplantation corroborated the positive effects of RM-remodeled gut microbiota, which was accompanied by metabolic changes, such as improved cholesterol and glucose metabolism. The study sheds light on the mechanisms underlying the beneficial effects of RM.

Illumina Technology: MiSeq 250 bp PE reads, CASAVA v1.8.3

Weingarden A., Gonzalez A., Vazquez-Baeza Y., Weiss S., Humphry G., et al. (2015) Dynamic changes in short- and long-term bacterial composition following fecal microbiota transplantation for recurrent *Clostridium difficile* infection. Microbiome 3: 10

Fecal transplants can restore the gut microbiome in patients suffering recurring *C. difficile* infections refractory to antimicrobial therapy. The stability and long-lasting effects of fecal transplantation have not been addressed, despite its increasing use. Amplicon sequencing of the 16S rRNA gene was used to monitor gut microbiota changes in patients with *C. difficile* treated by fecal transplantation for up to 151 days after treatment. Microbial composition shifted from severe dysbiosis to microbiomes similar to the healthy cohort established in the HMP. Upon fecal transplant, the recipient gut microbiota resembled that from the donor but diverged over time in each treated individual. The authors showed a very dynamic picture of variable microbial changes that succeeded each other over time and by individual. This surveillance can only be achieved by frequent sampling and close monitoring of gut microbiomes using high-throughput methods.

Illumina Technology: MiSeq v3 150 bp PE reads

Yin J., M P., Wang S., Liao S. X., Peng X., et al. (2015) Different Dynamic Patterns of beta-Lactams, Quinolones, Glycopeptides and Macrolides on Mouse Gut Microbial Diversity. PLoS One 10: e0126712

The use of oral antimicrobials is a common practice in hospital and nonhospital settings. These compounds come into contact with gut microbiota and have recognizable effects, although fine detail about specific disturbances is still needed. Amplicon sequencing of the 16S V6 rRNA gene was used to monitor gut microbial changes caused by administration of 6 commonly used antimicrobials in BALB/c mice. Ceftriaxone sodium, cefoperazone/sulbactam, meropenem and vancomycin resulted in quick dramatic changes. Azithromycin caused detectable changes, and ofloxacine effect was minimal. β-lactams inhibited *Alistipes* and favored the growth of *Clostridium*, which may influence the development of *C. difficile* infections after β-lactam use. In contrast, vancomycin favored *E. coli* growth and inhibited *Clostridium*. Lastly, azithromycin and meropenem induced the growth of *Enterococcus*. This study sheds light on the effects of commonly used antibiotics in gut microbiota, which can take place swiftly and have long-lasting effects in the microbial community.

Illumina Technology: TruSeq DNA Library Prep Kit, HiSeq 2000 100 bp PE reads

Lukens J. R., Gurung P., Vogel P., Johnson G. R., Carter R. A., et al. (2014) Dietary modulation of the microbiome affects autoinflammatory disease. Nature 516: 246-249

Inflammatory diseases have been on the rise for a few years, and there is evidence that changes in dietary habits may have a role in disease development. In this study, the authors used a mouse osteomyelitis model (*Pstpip2*^{cmo}) and amplicon sequencing of the 16S V4 rRNA gene to assess the contribution of diet to inflammatory disease. Mice with osteomyelitis had enriched abundance of *Prevotella*. Diet-induced protection against osteomyelitis reduced expression of pro-IL-1β in distant neutrophils. The study shows that diet changes can limit inflammation in a mouse osteomyelitis model and thus contribute to the interplay between inflammatory disease and gut microbiota.

Illumina Technology: MiSeq

Morgan A. P., Crowley J. J., Nonneman R. J., Quackenbush C. R., Miller C. N., et al. (2014) The antipsychotic olanzapine interacts with the gut microbiome to cause weight gain in mouse. PLoS One 9: e115225

Olanzapine is prescribed as treatment for schizophrenia, bipolar disorder, and other disorders. However, patients receiving this medication exhibit dramatic weight gain by a yet unknown mechanism. Amplicon sequencing of the 16S v4 rRNA gene was used to survey microbiota changes in mice receiving olanzapine (crossover experimental design). The drug potentiated variable weight gain in 8 different inbred mice strains under a high-fat diet (HFD). This effect was associated to gut microbiota, as demonstrated by crossover experiments where olanzapine and a HFD enriched "obesogenic" bacteria in the gut microbiota of mice. Furthermore, olanzapine was able to impair or delay growth of 2 abundant commensal enteric bacteria *in vitro*. The study shows 3 pieces of evidence that strongly suggest an obesogenic effect of olanzapine by altering gut microbiota composition. It pinpoints the importance of gut microbiota as a biomarker and as a potential therapeutic target in drug-induced weight gain.

Illumina Technology: Nextera DNA Library Prep Kit, MiSeq 250 bp PE reads, CASAVA v1.8.2

Stefka A. T., Feehley T., Tripathi P., Qiu J., McCoy K., et al. (2014) Commensal bacteria protect against food allergen sensitization. Proc Natl Acad Sci U S A 111: 13145-13150

Food allergy may be exacerbated by changes in commensal microbiota, although specific mechanisms or protective/sensitive bacterial taxa have not been addressed in detail. Amplicon sequencing of the 16S V4 rRNA gene and selective colonization of gnotobiotic mice identified a bacterial community enriched with *Clostridia* as allergy-protective. This protective *Clostridia* induced novel regulatory mechanisms of innate lymphoid cell function related to activation of Treg cells, IgA production in the colon, and regulation of allergen access to the bloodstream. Additionally, intestinal epithelial permeability was upregulated by *Clostridia*, and antimicrobial depletion of the neonatal gut microbiota resulted in increased sensitization to food allergens. These findings collectively: i) suggest a role for gut microbiota in food allergy; ii) identify a "protective" community; and iii) raise the possibility of microbiota manipulation to prevent or treat food allergies.

Illumina Technology: Illumina MouseRef-8 array, MiSeq

Gnotobiotic mice are used to determine the role of microbes in food allergen sensitization.

Inflammatory Bowel Disease (IBD)

The MetaHit project aimed to characterize the relationship between microbial genes and intestinal health and disease, with a focus on IBD and obesity.[40] In general, low diversity is a dysbiosis often found in chronic inflammatory diseases and other health disturbances, a defect that might be controlled to some extent by dietary management.[41] Dietary management could also alleviate the intestinal symptoms associated with cancer.[42]

Metagenomic analysis has linked variation in microbial gene copy number, at the strain level, to IBD and obesity.[43] Viral sequences have also been recovered from biopsy samples, suggesting an interplay between viral and bacterial elements.[44] This disturbance plays a role in the immunopathogenesis of IBD, given the reciprocal relationship between the development and performance of the immune system and its associated gut microbiome.[45] The complexity of IBD and the importance of a healthy microbiome demonstrate the need to address gut microbiome management as a reasonable measure to control this and other diseases.

Reviews

Cammarota G., Ianiro G., Cianci R., Bibbo S., Gasbarrini A., et al. (2015) The involvement of gut microbiota in inflammatory bowel disease pathogenesis: potential for therapy. Pharmacol Ther 149: 191-212

Ohman L., Tornblom H. and Simren M. (2015) Crosstalk at the mucosal border: importance of the gut microenvironment in IBS. Nat Rev Gastroenterol Hepatol 12: 36-49

Bringiotti R., Ierardi E., Lovero R., Losurdo G., Di Leo A., et al. (2014) Intestinal microbiota: The explosive mixture at the origin of inflammatory bowel disease? World J Gastrointest Pathophysiol 5: 550-559

Ferreira C. M., Vieira A. T., Vinolo M. A., Oliveira F. A., Curi R., et al. (2014) The central role of the gut microbiota in chronic inflammatory diseases. J Immunol Res 2014: 689492

Gkouskou K. K., Deligianni C., Tsatsanis C. and Eliopoulos A. G. (2014) The gut microbiota in mouse models of inflammatory bowel disease. Front Cell Infect Microbiol 4: 28

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Jones-Hall Y. L., Kozik A. and Nakatsu C. (2015) Ablation of tumor necrosis factor is associated with decreased inflammation and alterations of the microbiota in a mouse model of inflammatory bowel disease. PLoS One 10: e0119441

Chronic inflammation, characteristic of IBD, is closely related to the prolonged secretion of tumor necrosis factor (TNF). The effects of TNF in colitis and gut microbiota are not fully characterized and may explain why anti-TNF therapy is not always successful. Microbial composition was assessed by 16S V3-V4 rRNA amplicon sequencing in an acute colitis model with wild-type (WT) and tnf-/- mice to elucidate TNF effects in colitis and gut microbiota. Inflammation caused significant differences in microbiota composition according to mice genotype; absence of TNF resulted in milder colitis and less microbial α-diversity than in WT mice. The authors conclude that TNF-mediated inflammation modifies gut microbiota and, therefore, combined therapies that inhibit TNF and alter microbial communities could be beneficial.

Illumina Technology: MiSeq 250 bp PE reads

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44. Wang W., Jovel J., Halloran B., Wine E., Patterson J., et al. (2015) Metagenomic analysis of microbiome in colon tissue from subjects with inflammatory bowel diseases reveals interplay of viruses and bacteria. Inflamm Bowel Dis 21: 1419-1427
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Morgan X. C., Kabakchiev B., Waldron L., Tyler A. D., Tickle T. L., et al. (2015) Associations between host gene expression, the mucosal microbiome, and clinical outcome in the pelvic pouch of patients with inflammatory bowel disease. Genome Biol 16: 67

Ileal pouch-anal anastomosis (IPAA) surgery for ulcerative colitis (UC) is often complicated by pouchitis associated with anatomical and microbiota changes that resemble a colon-like environment. How and why these changes take place, and their relation to UC and IBD, is not fully understood. The authors obtained paired host microbiomes (by 16S V4 amplicon sequencing) and transcriptomes from a large cohort of IPAA patients to study the microbiome-host gene expression axis. Microbiomes were variable across individuals and influenced by clinical variables such as antibiotic therapy; host epithelial transcription was influenced by tissue location. Associations between microbiome and host transcription patterns were related to the level of host tissue inflammation: the strongest microbe-host association pattern was enriched complement system and cytokine IL-12 pathways inversely correlated with abundance of *Bifidobacteria* and others. However, it was not possible to generate a pouchitis outcome model based on microbial composition and/or transcriptional activity, suggesting that the role of sectional changes in epithelial transcripts may not be critical for the host-microbiome interface during IPAA.

Illumina Technology: MiSeq v2 175 bp PE reads

Schaubeck M., Clavel T., Calasan J., Lagkouvardos I., Haange S. B., et al. (2015) Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defence.

Gut Intestinal dysbiosis is associated with intestinal inflammatory disease (Crohn's disease or CD), although functional explanation of this frequent observation is still missing. Temporal metaproteomic (LC-MS) and metagenomic (16S rRNA gene) profiles of gut microbiota were obtained from $TNF^{deltaARE}$ mice, a model that resembles CD pathology. Disease severity and location were microbiota-dependent: inflammation was absent in germ-free $TNF^{deltaARE}$ mice and ileitis (but not colitis) was attenuated after antibiotic treatment. Several compositional and functional alterations were observed in microbiota communities in inflamed mice, features that were reproducible through microbiome transplantation that resulted in CD-like ileitis accompanied by loss of Paneth cell function. The study provides evidence of a causal role for gut dysbiosis in the development of chronic ileal inflammatory disease.

Illumina Technology: MiSeq

Knights D., Silverberg M. S., Weersma R. K., Gevers D., Dijkstra G., et al. (2014) Complex host genetics influence the microbiome in inflammatory bowel disease. Genome Med 6: 107

The interplay between host genetics and gut microbiome has been observed in inflammatory diseases, without distinction of specific disease host alleles in relation to specific microbial taxa. Here, biopsies of three IBD cohorts were evaluated through genotyping by Immunochip and 16S rRNA sequencing to identify relationships in host allele-microbial taxa pairs. The authors showed that the risk allele NOD2 consistently associated with the bacterial taxa *Enterobacteriaceae* across the different independent cohorts. They also identified 48 additional IBD single-nucleotide polymorphisms (SNPs) consistently associated with bacterial taxa in 2 or 3 of the cohorts. These SNPs are related to genes involved in immune responses (eg, JAK-STAT). Thus, the pairing of genome-microbiome analysis successfully demonstrates the complex relationships between altered host functional pathways and microbiome structure in IBD.

Illumina Technology: MiSeq, Infinium Immunochip

Rooks M. G., Veiga P., Wardwell-Scott L. H., Tickle T., Segata N., et al. (2014) Gut microbiome composition and function in experimental colitis during active disease and treatment-induced remission. ISME J 8: 1403-1417

IBD patients often receive different individual therapy courses to mitigate disease symptoms and promote remission. The variability of treatments confuses the effective mechanisms responsible for remission, because not much is known about how therapy affects gut microbiota. Whole-shotgun metagenomics and 16S rRNA amplicon sequencing were used to survey the gut microbiome in a mouse model of colitis during active disease and therapy-induced remission. Microbiome differences between both stages, related to carbohydrate-energy metabolism, bacterial pathogenesis (motility and signal transduction pathways), and increased capacity for xenobiotic metabolism were reported. Unlike gentamicin and metronidazole, vancomycin was not useful in promoting symptom amelioration, even though the 3 are often used to treat *C. difficile* infection. Immunomodulatory therapy also caused alteration of the microbial community by enrichment of different bacterial taxa. The study identifies specific bacterial taxa and pathways involved with disease, as well as specific alterations under different therapy regimes in a mouse model of colitis.

Illumina Technology: HiSeq 2000

Metabolic Diseases: Diabetes and Obesity

Type-1 (T1D) and Type-2 (T2D) diabetes are multifactorial metabolic disorders that have been linked to changes in the gut microbiota, which is affected by the same factors that influence development of the disease in genetically predisposed individuals.[46] Although studies on T1D are not conclusive yet, there is cumulative evidence of reduced taxa diversity and altered interactions with epithelial receptors and immune cells. Together, these factors exacerbate autoimmune destruction of pancreatic β cells.[47]

A moderate degree of dysbiosis reduced epithelial permeability and leakage of inflammatory mediators in T2D, along with reduced abundance of bile metabolizing genes.[48] Excessive production and intake of branched-chain and aromatic amino acids by an altered microbiota[49] are events associated with the disease. Altered metabolic pathways may be useful as diabetes markers,[50] due to their contribution to differential dysbiosis signatures.[51]

46. Gulden E., Wong F. S. and Wen L. (2015) The gut microbiota and Type 1 Diabetes. Clin Immunol 159: 143-153
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He C., Shan Y. and Song W. (2015) Targeting gut microbiota as a possible therapy for diabetes. Nutr Res 35: 361-367

Hu C., Wong F. S. and Wen L. (2015) Type 1 diabetes and gut microbiota: Friend or foe? Pharmacol Res 98: 9-15

Parekh P. J., Balart L. A. and Johnson D. A. (2015) The Influence of the Gut Microbiome on Obesity, Metabolic Syndrome and Gastrointestinal Disease. Clin Transl Gastroenterol 6: e91

Gomes A. C., Bueno A. A., de Souza R. G. and Mota J. F. (2014) Gut microbiota, probiotics and diabetes. Nutr J 13: 60

Nielsen D. S., Krych L., Buschard K., Hansen C. H. and Hansen A. K. (2014) Beyond genetics. Influence of dietary factors and gut microbiota on type 1 diabetes. FEBS Lett 588: 4234-4243

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Daft J. G., Ptacek T., Kumar R., Morrow C. and Lorenz R. G. (2015) Cross-fostering immediately after birth induces a permanent microbiota shift that is shaped by the nursing mother. Microbiome 3: 17

Recognizing the influence of microbiota in metabolic diseases with host genetic components is only a small aspect of disease complexity. Manipulating the microbiota toward a stable "healthy" phenotype is desirable and must include gut microbiota establishment. Cross-fostering effects in gut microbiota were surveyed by amplicon sequencing in mouse pups (NOR and NOD strains). Establishment of gut microbiota was quick and determined by the nursing mother microbiota, and was stable at 32 weeks. This method can be extended to current models of inflammatory disease and T1D to normalize commensal microbiota among different strains of mice.

Illumina Technology: MiSeq 250 bp PE reads

Cross-fostering effects in gut microbiota were surveyed by amplicon sequencing in mouse pups.

Bell E. T., Suchodolski J. S., Isaiah A., Fleeman L. M., Cook A. K., et al. (2014) Faecal microbiota of cats with insulin-treated diabetes mellitus. PLoS One 9: e108729

Cats also suffer from T2D and receive insulin treatment just like humans do. This study evaluated the fecal microbiota in a small number of insulin-treated diabetic (n = 10) and nondiabetic (n =2 0) cats by 16S V4 rRNA sequencing and qPCR. The authors did not observe any differences in gut microbiota composition or abundance between insulin-treated diabetic and nondiabetic cats. In fact, no differences were observed according to age, sex, breed, or dietary formulation. Only a decrease in *Faecalibacterium spp* was identified by qPCR in cats aged over 10 years. Additional studies are required to investigate differences in larger cohorts. These studies should compare functional products, transcripts, and metabolic factors that are not reflected by total microbial composition, in diabetic and nondiabetic cats.

Illumina Technology: MiSeq

Obesity

Host and environmental factors that influence metabolic diseases highlight the relationship between diet, microbes, and metabolism. For example, obese mice and humans show reproducible shifts in gut microbial communities. Disturbances in the microbiota enhance energy harvest by enriching metabolic pathways that yield an excess of short-chain fatty acids (later deposited in adipose tissue) and energy gain.[52] Additionally, products of altered microbial metabolism act as host metabolism signals in sites outside the intestines, like brain and liver, affecting the level of obesity and its associated comorbidities.[53]

Gut microbiome studies in diabetic and obese mouse models reveal complex relationships among diet, inflammation, and metabolism.

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Chassaing B., Koren O., Goodrich J. K., Poole A. C., Srinivasan S., et al. (2015) Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature 519: 92-96

Gut microbes are kept at a safe distance from the intestinal epithelium by layers of mucus. Emulsifiers disrupt this mucus layer *in vitro*, and their inclusion in processed foods may be a contributing factor for the increased incidence of metabolic diseases. Gut tissue and microbiomes of mice were exposed to low concentrations of 2 common emulsifiers, carboxymethylcellulose (CMC) or polysorbate-80 (P80), and monitored by 16S V4 rRNA sequencing. Both emulsifiers caused mild inflammation and obesity in WT mice, and colitis in a mouse-sensitive model. Obesity was correlated with increased microbiota invasion of the epithelial layer, altered species composition, and increased proinflammatory potential. Fecal transplants into germ-free mice corroborated the role of these altered gut microbiota in the development of metabolic syndrome. The novel effects of common emulsifiers in the gut environment, and possible association with metabolic syndromes and inflammatory diseases, need to be reconsidered in light of their increased incidence.

Illumina Technology: MiSeq 250 bp PE reads

Frank D. N., Bales E. S., Monks J., Jackman M. J., MacLean P. S., et al. (2015) Perilipin-2 Modulates Lipid Absorption and Microbiome Responses in the Mouse Intestine. PLoS One 10: e0131944

Microbiota and metabolic diseases are related, but only a few molecular mechanisms underlying this relationship have been unveiled. Among them, cytoplasmic lipid drop protein Perilipin-2 (Plin2) is involved in lipid metabolism in mice fed with a HFD and may alter gut microbiota during diet changes. Wild-type and Plin2-null mice were fed a low-fat diet (LFD) or HFD, microbiome composition was monitored by 16S V2–V3 rRNA sequencing, and physiological parameters were recorded. Plin-2 mice displayed significant disturbances in physiological parameters when compared to WT controls. Dietary fat content and Plin-2 were independently associated with microbiome composition, diversity, and functional differences. Plin-2 modulates lipid levels in feces and energy utilization in mice, both of which correlate with specific microbiota communities. The authors conclude that Plin-2 regulates lipid uptake in intestines that leads to gut microbiome changes associated with diet-induced obesity.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq Reagent Kit v3

Yasir M., Angelakis E., Bibi F., Azhar E. I., Bachar D., et al. (2015) Comparison of the gut microbiota of people in France and Saudi Arabia. Nutr Diabetes 5: e153

Dietary habits influence the gut microbiota composition and function, just as an obesogenic microbiota influences the development of metabolic disease. Fecal microbiota of normal and obese Saudi and European individuals, who follow very dissimilar diets, were compared through 16S V3–V4 rRNA sequencing. The differences found between obese and normal cohorts were not conserved between French and Saudi individuals. The obese French microbiome was less diverse and mostly dominated by *Proteobacteria* and *Bacteroidetes* when compared to their normal cohort. Obese Saudis were dominated by *Firmicutes* but were not different from their normal cohort in terms of diversity. Overall, French individuals had higher diversity and richness in their microbiome than Saudis. Differences in gut microbiomes from geographically distant populations, along with contrast between normal and obese microbiomes, can be informative and add to large cohesive efforts to provide integrative views addressing metabolic syndromes.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq 250 bp PE reads

Everard A., Lazarevic V., Gaia N., Johansson M., Stahlman M., et al. (2014) Microbiome of prebiotic-treated mice reveals novel targets involved in host response during obesity. ISME J 8: 2116-2130

The identification of beneficial microbes and the pathways that prevent or counteract inflammatory disturbances and metabolic syndromes can improve management of these cases. Additionally, such studies can support the use of intervention measures, such as prebiotics. Prebiotic treatment was evaluated in mice under normal and HFD through shotgun metagenomics and antimicrobial peptide analysis. HFD and prebiotics significantly altered gut microbiota composition and potential functionality at several phylum levels relative to normal controls. The authors identified several taxa with differential abundance in both groups, as well as enriched pathways associated with these taxa. HFD decreased production of Reg3g and PLA2g2 in the jejunum, whereas prebiotics increased levels of Reg3g and intectin, both related to intestinal epithelium health and turnover, partially ameliorating the development of metabolic syndrome in mice. This study shows specific beneficial mechanisms performed by prebiotic-modified gut microbiota, which are interesting candidates for dietary intervention in metabolic syndromes.

Illumina Technology: TruSeq SBS Kit v3, HiSeq 2000, CASAVA v1.8.2

Walters W. A., Xu Z. and Knight R. (2014) Meta-analyses of human gut microbes associated with obesity and IBD. FEBS Lett 588: 4223-4233

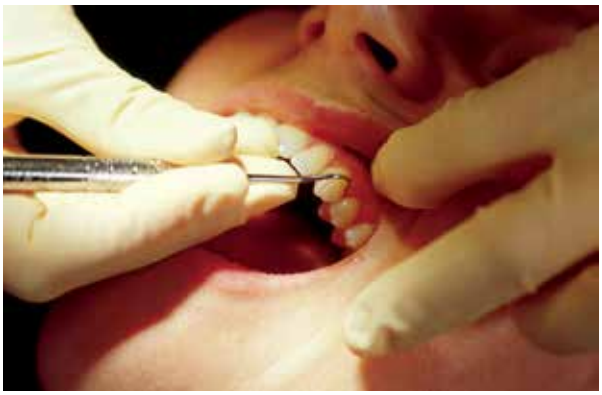
Several recent publications indicate an intimate relationship between the gut microbiota and metabolic diseases. The authors surveyed available literature that had employed high-throughput 16S rRNA gene amplicon sequencing to characterize the gut microbiota in IBD and obesity. IBD showed consistent microbiome signatures across different studies, and these microbiomes could distinguish IBD patients from healthy individuals. On the contrary, obesogenic microbiota signatures were not consistent across studies, despite reproducing the disease by fecal transplantation and being statistically significant in each study. This study highlights the importance and effect of cohort selection and analysis strategies in different clinical conditions, as they may not have the same microbiota-disease effect size.

Illumina Technology: 16S rRNA gene amplicon sequencing obtained with Illumina technology

Oral Microbiome

The community of microbes living in the oral cavity performs multiple functions. While the microbial composition for individuals is relatively stable, it can vary substantially among individuals.[54] Altered oral microbiomes are informative of health status, such as nutritional deficiencies[55] and cardiovascular disease.[56]

The microbial communities in the oral cavity are organized in compartments and biofilms. Excessive buildup can form plaque and cause oral diseases. Metagenomic studies have identified specific metabolic pathways of oral disease.[57] During oral diseases—such as chronic periodontitis, dental caries, and gingivitis—the subgingival microbiome undergoes dynamic changes that are associated with infection of dental implants by oral pathogens[58] and smoking.[59] These changes can be monitored to assess disease progression and therapy effectiveness.[60]



During oral diseases—such as chronic periodontitis, dental caries, and gingivitis—the subgingival microbiome undergoes dynamic changes that can be monitored to assess disease progression and therapy effectiveness.

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Diaz P. I., Strausbaugh L. D. and Dongari-Bagtzoglou A. (2014) Fungal-bacterial interactions and their relevance to oral health: linking the clinic and the bench. Front Cell Infect Microbiol 4: 101

McLean J. S. (2014) Advancements toward a systems level understanding of the human oral microbiome. Front Cell Infect Microbiol 4: 98

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Zaura E., Nicu E. A., Krom B. P. and Keijser B. J. (2014) Acquiring and maintaining a normal oral microbiome: current perspective. Front Cell Infect Microbiol 4: 85

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Edlund A., Yang Y., Yooseph S., Hall A. P., Nguyen D. D., et al. (2015) Meta-omics uncover temporal regulation of pathways across oral microbiome genera during *in vitro* sugar metabolism. ISME J

Bacteria-mediated pH drops and subsequent recovery occur upon addition of a carbohydrate source in supragingival plaque. These events are important in dental caries development, but fully characterized metabolic or transcriptomic details are still missing. The authors developed an *in vitro* model inoculated with supragingival plaque microbial communities and analyzed metatranscriptomic and metabolomic profiles of these biofilms. The authors found microbial taxa (*Lactobacillus, Streptococcus*) and their upregulated pathways involved in acid neutralization in response to a pH drop. These taxa were also important during pH recovery of the plaque. Specific metabolites and corresponding transcripts/taxa were identified along the pH gradient. These signatures, for healthy and disease states, could be used to compare oral microbiome studies.

Illumina Technology: HiSeq 2000

Sato Y., Yamagishi J., Yamashita R., Shinozaki N., Ye B., et al. (2015) Inter-Individual Differences in the Oral Bacteriome Are Greater than Intra-Day Fluctuations in Individuals. PLoS One 10: e0131607

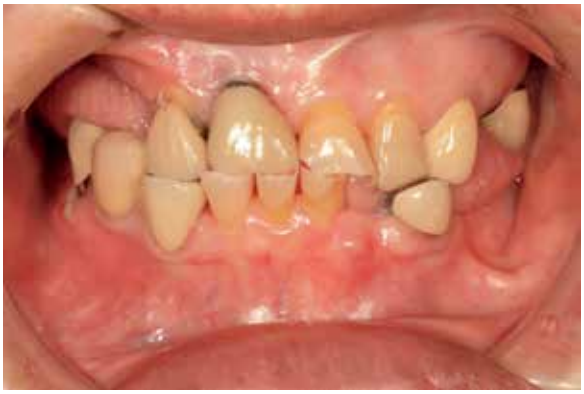
In this study, oral supragingival microbiomes were obtained from healthy individuals at 3 time points during the day for 3 days and studied by 16S V4 rRNA gene sequencing. The authors showed that oral microbiomes from healthy individuals were stable throughout the day. In contrast, interindividual variation was common at the species level. The within-individual stability allowed them to identify 40 co-occurrences among rare bacteria.

Illumina Technology: MiSeq Reagent Kit v2 250 bp PE reads

Duran-Pinedo A. E., Chen T., Teles R., Starr J. R., Wang X., et al. (2014) Community-wide transcriptome of the oral microbiome in subjects with and without periodontitis. ISME J 8: 1659-1672

Periodontitis is a polymicrobial inflammation disease. Metatranscriptomes and metagenomes were obtained from subgingival samples from healthy and periodontitis-positive individuals to identify community composition and transcriptome profile differences between both states. The most abundant species with higher expression of virulence genes found in periodontitis were the known pathogens *P. gingivalis*, *T. forsythia*, and *T. denticola*. The correlation between abundance and expression was not conserved in other microorganisms, and some “healthy” bacteria expressed putative virulence factors. The community displayed upregulated pathways consistent with periodontitis related to flagellar motility, peptide transport, iron acquisition, β-lactam degradation, and lipid A biosynthesis. These results support the integrated actions of the community toward disease progression.

Illumina Technology: Nextera XT DNA Library Prep Kit, cBOT, MiSeq v2 150 PE reads, HiSeq 2500



Periodontitis.

Other Human Biomes

High-throughput technologies and lower costs have enabled research to characterize various complex and dynamic human biomes.

The skin is the first surface to come into contact with the environment. Its microbiota is complex and defined by microenvironments in specific topographical regions of the body. These microenvironments contribute to particular chemical signatures influenced by gender[61] and external compounds, such as hygiene products.[62] The skin microbiome might be a tool for therapies against cutaneous diseases, skin cancer, and monitoring health threats or environmental disturbances. The development of cosmetic and hygiene products may benefit from specific gender- and topographic-based signatures that, for example, influence body odor.

The development of cosmetic and hygiene products may benefit from specific gender- and topographic-based signatures that, for example, influence body odor.[63]

The vaginal microbiome is highly dynamic throughout life and influenced by age, reproductive state, ethnicity, pH, and other factors.[64] Disturbances in taxa abundance and diversity are associated with increased risk of preterm birth,[65] gynecologic infections, cancer, and side-effects from chemotherapy and radiation in cancer patients.[66]

Recently, airway microbiome dynamics have been related to chronic obstructive pulmonary disease,[67] asthma,[68] and cystic fibrosis.[69, 70, 71, 72, 73, 74]

Reviews

Grice E. A. (2014) The skin microbiome: potential for novel diagnostic and therapeutic approaches to cutaneous disease. Semin Cutan Med Surg 33: 98-103

Oh J., Byrd A. L., Deming C., Conlan S., Program N. C. S., et al. (2014) Biogeography and individuality shape function in the human skin metagenome. Nature 514: 59-64

Payne M. S. and Bayatibojakhi S. (2014) Exploring preterm birth as a polymicrobial disease: an overview of the uterine microbiome. Front Immunol 5: 595

Salava A. and Lauerma A. (2014) Role of the skin microbiome in atopic dermatitis. Clin Transl Allergy 4: 33

van de Wijgert J. H., Borgdorff H., Verhelst R., Crucitti T., Francis S., et al. (2014) The vaginal microbiota: what have we learned after a decade of molecular characterization? PLoS One 9: e105998

61. Troccaz M., Gaia N., Beccucci S., Schrenzel J., Cayeux I., et al. (2015) Mapping axillary microbiota responsible for body odours using a culture-independent approach. Microbiome 3: 3
62. Bouslimani A., Porto C., Rath C. M., Wang M., Guo Y., et al. (2015) Molecular cartography of the human skin surface in 3D. Proc Natl Acad Sci U S A 112: E2120-2129
63. Oh J., Byrd A. L., Deming C., Conlan S., Program N. C. S., et al. (2014) Biogeography and individuality shape function in the human skin metagenome. Nature 514: 59-64
64. Zhou X., Brown C. J., Abdo Z., Davis C. C., Hansmann M. A., et al. (2007) Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. ISME J 1: 121-133
65. Hyman R. W., Fukushima M., Jiang H., Fung E., Rand L., et al. (2014) Diversity of the vaginal microbiome correlates with preterm birth. Reprod Sci 21: 32-40
66. Chase D., Goulder A., Zenhausern F., Monk B. and Herbst-Kralovetz M. (2015) The vaginal and gastrointestinal microbiomes in gynecologic cancers: a review of applications in etiology, symptoms and treatment. Gynecol Oncol 138: 190-200
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70. Hoffman L. R., Pope C. E., Hayden H. S., Heltshe S., Levy R., et al. (2014) Escherichia coli dysbiosis correlates with gastrointestinal dysfunction in children with cystic fibrosis. Clin Infect Dis 58: 396-399
71. Lopez-Collazo E., Jurado T., de Dios Caballero J., Perez-Vazquez M., Vindel A., et al. (2015) In vivo attenuation and genetic evolution of a ST247-SCCmecI MRSA clone after 13 years of pathogenic bronchopulmonary colonization in a patient with cystic fibrosis: implications of the innate immune response. Mucosal Immunol 8: 362-371
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73. Waters V. (2014) Editorial commentary: how can understanding the phenotype of Pseudomonas aeruginosa lead to more successful eradication strategies in cystic fibrosis? Clin Infect Dis 59: 632-634
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Bouslimani A., Porto C., Rath C. M., Wang M., Guo Y., et al. (2015) Molecular cartography of the human skin surface in 3D. Proc Natl Acad Sci U S A 112: E2120-2129

The skin is the largest organ in the human body. Its chemical makeup is likely influenced by skin cells and the microbes that inhabit it. The authors developed a highly integrated 3D molecular cartography map of the human body by integrating chemical data with proteomic data (UPLC-QTOF, MALDI-TOF) and microbiome composition (16S rRNA sequencing) of the skin according to body site. This 3D map associated specific taxa to body site, metabolites, and chemical compounds. It allowed co-occurrence analysis, and revealed gender-associated characteristics and the impact of hygiene products on human skin. This map constitutes a detailed panorama of the human skin, and it is potentially useful in research and customized product development.

Illumina Technology: HiSeq 2000 100 bp PE reads

Boutin S., Graeber S. Y., Weitnauer M., Panitz J., Stahl M., et al. (2015) Comparison of microbiomes from different niches of upper and lower airways in children and adolescents with cystic fibrosis. PLoS One 10: e0116029

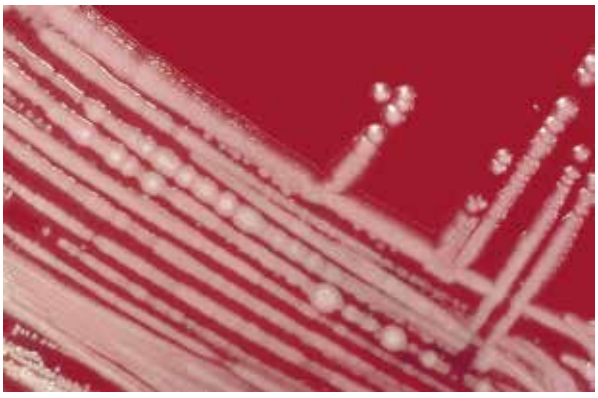
The relationship between the respiratory tract microbiome and onset/progression of cystic fibrosis (CF) is not fully understood, despite chronic infections in CF caused by characteristic pathogens. NGS was used to compare the microbiome of the nasal cavity, throat, and sputum in young CF patients with mild to moderate lung disease. The sputum microbiome between children and adult CF patients was similar, implying that an adult-like microbiome is established early in life. The high interindividual variation in sputum microbiomes may be due to an increased abundance of pathogens, such as *Pseudomonas aeruginosa*. This study shows the effects of potential pathogens in CF airway microbiota. It also demonstrates that throat swabs may be an appropriate proxy if sputum sampling is not feasible.

Illumina Technology: HiSeq

Darch S. E., McNally A., Harrison F., Corander J., Barr H. L., et al. (2015) Recombination is a key driver of genomic and phenotypic diversity in a Pseudomonas aeruginosa population during cystic fibrosis infection. Sci Rep 5: 7649

Pseudomonas aeruginosa causes chronic lung infections in CF patients, with diverging phenotypes in isolates from the same individuals over time. Whole-genome sequencing (WGS) of *P. aeruginosa* isolates collected from the same individual at different time points was used to uncover mechanisms responsible for this phenotypic variation. This study showed intraindividual diversity related to SNPs ranging from 5 to 64, with evidence of recombination as the main event driving genetic and phenotypic diversity of *P. aeruginosa* during *in vivo* chronic CF infection. The authors discuss these findings and their implications in important phenotypes, such as antimicrobial resistance patterns.

Illumina Technology: HiSeq 2000 150 bp PE reads



Pseudomonas aeruginosa colonies.

Kramer R., Sauer-Heilborn A., Welte T., Jauregui R., Brettar I., et al. (2015) High individuality of respiratory bacterial communities in a large cohort of adult cystic fibrosis patients under continuous antibiotic treatment. PLoS One 10: e0117436

Routine clinical diagnosis of microbial infections in CF patients is restricted to a few known pathogens, despite growing molecular evidence of polymicrobial infections. Amplicon sequencing (16S V3 rRNA gene) was applied to sputum samples from adults under continuous antibiotic treatment and compared to routine

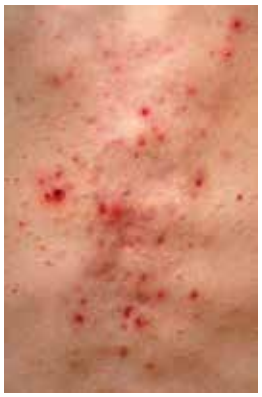
culture methods. Communities were diverse and highly individual. Bacteria associated with CF were revealed in finer detail only by NGS, while other methods showed a similar prevalence of known pathogens. There were no associations between host factors and the *CFTR* genotype, although non-ΔF508 mutations in the *CFTR* gene often showed low abundances of *P. aeruginosa*. Continuous antibiotic treatment may be the cause of the high individuality and lack of correlation to clinical host factors.

Illumina Technology: Genome Analyzer$_{IIx}$

Liu J., Yan R., Zhong Q., Ngo S., Bangayan N. J., et al. (2015) The diversity and host interactions of Propionibacterium acnes bacteriophages on human skin. ISME J 9: 2078-2093

Phages are able to regulate microbial populations by inducing lysis, lysogeny, or resistance. This regulation may also occur in human skin, but relatively little information is available. This study focused on the diversity and host interactions of the bacteriophages of *Propionibacterium* acnes. The authors sequenced 48 phages recovered from acne patients and healthy individuals and analyzed healthy skin metagenomes looking for *P. acnes* phages. One phage strain at a time dominates skin, although some phages are common among related and unrelated individuals, which may suggest the existence of a common core of phages in the human population and transmission of phages. Furthermore, CRISPR sequences may not be sufficient to confer resistance against phages in type II *P. acnes* strains. This study shows how phages can regulate microbial populations, resulting in individualized microbiomes.

Illumina Technology: MiSeq



Skin with acne. Phages are able to regulate microbial populations by inducing lysis, lysogeny, or resistance. The authors sequenced 48 phages recovered from acne patients and healthy individuals and analyzed healthy skin metagenomes looking for *P. acnes* phages.[75]

MacIntyre D. A., Chandiramani M., Lee Y. S., Kindinger L., Smith A., et al. (2015) The vaginal microbiome during pregnancy and the postpartum period in a European population. Sci Rep 5: 8988

Most vaginal microbiome studies have focused on Northern American populations and have often been associated with specific pregnancy outcomes. The vaginal microbiomes of pregnant British women were monitored periodically during uncomplicated pregnancy and after partum by 16S V1-V2 rRNA gene sequencing. Significant changes were observed after partum, characterized by less dominance of *Lactobacillus spp.* with increased α-diversity. Some women, mostly Asian and Caucasian, had a *L. jensenii*-dominated microbiome; whereas *L. gasseri* was absent in African women. This study revealed biogeography and ethnicity as factors in pregnancy and the postpartum vaginal microbiome, contributing to an understanding of the relationships between the microbiome, health, and pregnancy outcomes.

Illumina Technology: MiSeq 300 bp PE reads

Misic A. M., Davis M. F., Tyldsley A. S., Hodkinson B. P., Tolomeo P., et al. (2015) The shared microbiota of humans and companion animals as evaluated from Staphylococcus carriage sites. Microbiome 3: 2

Staphylococcus species are common commensal residents of the skin in humans and animals. *S. epidermidis* can be a reservoir for antimicrobial resistance genes, and methicillin-resistant *S. aureus* (MRSA) can cause serious skin and soft-tissue infections (MRSA SSTI). The authors evaluated the skin microbiome of MRSA SSTI patient households and their companion animals through 16S V4 rRNA gene sequencing. Habitants in households were more similar to each other in the absence of pets, suggesting that animals contribute to the household microbial profile. There were no differences in microbiota associated with MRSA SSTI, or carriage of other *Staphylococcus*. The study highlights how the microbiota is shared between pets and humans, adding to diversity and influencing stability over time.

Illumina Technology: MiSeq 150 bp PE reads

75. Liu J., Yan R., Zhong Q., Ngo S., Bangayan N. J., et al. (2015) The diversity and host interactions of Propionibacterium acnes bacteriophages on human skin. ISME J 9: 2078-2093

Silva P. E., Costa P. S., Avila M. P., Suhadolnik M. L., Reis M. P., et al. (2015) Leprous lesion presents enrichment of opportunistic pathogenic bacteria. Springerplus 4: 187

M. leprae causes leprosy, a chronic disease that affects skin. Changes in skin microbiota composition caused by the disease have not been characterized in the past, but now high-throughput sequencing can address this question. Skin microbiota of healthy skin (previously published) and leprous lesions were obtained by 16S V3-V4 rRNA gene and Sanger sequencing. Profound shifts in skin microbiota taxa composition and abundance were associated with leprous lesions. These shifts favored overgrowth of potentially pathogenic bacteria not usually associated with normal skin (*Burkhordelia, Pseudomonas* and *Bacillus*) at the expense of normal flora (*Propionibacterium, Staphylococcus* and *Corynebacterium*). The results suggest that potentially pathogenic bacteria may have gained a competitive advantage over normal resident microbes in the environment provided by leprous lesions.

Illumina Technology: MiSeq

Ghartey J. P., Smith B. C., Chen Z., Buckley N., Lo Y., et al. (2014) *Lactobacillus crispatus* dominant vaginal microbiome is associated with inhibitory activity of female genital tract secretions against *Escherichia coli*. PLoS One 9: e96659

Female genital tract secretions can inhibit growth or prevent colonization of potential pathogens. These cooperative activities can yield biomarkers of a healthy microbiome. Vaginal secretions from nonpregnant and near-term pregnant women were sampled and characterized by microbiome sequencing (16S V6 amplicon) and inhibitory activity against *E. coli*. There was no overall difference in microbiome composition between pregnant and nonpregnant women. *E. coli* inhibitory secretions were enriched in *L. crispatus*, and culture supernatants reproduced this effect *in vitro* with variation among different *L. crispatus* strains. This study shows the protective effects against *E. coli* colonization in microbiomes rich in *L. crispatus*, suggesting that promoting these environments may prevent negative pregnancy outcomes.

Illumina Technology: HiSeq 2000 100 bp PE reads

Walther-Antonio M. R., Jeraldo P., Berg Miller M. E., Yeoman C. J., Nelson K. E., et al. (2014) Pregnancy's stronghold on the vaginal microbiome. PLoS One 9: e98514

Influence of the vaginal microbiome in pregnancy outcome has been suggested by previous studies with some limitations as to length of experimental observation. In this study, the vaginal microbiomes of women carrying uncomplicated pregnancies were monitored at intervals throughout the complete pregnancy period by 16S V3-V5 rRNA sequencing. Vaginal microbiomes were stable, exhibited low diversity, and were dominated by *Lactobacillus* genus, specifically *L. crispatus*, in both Caucasian and African-American women. Very few samples were dominated or shifted towards *L. liners*. However, some observed differences were attributed to ethnicity. Caucasian microbiomes clustered by trimester and progressed toward a common attractor, whereas African-American microbiomes clustered by individual. The study supports previous observations about vaginal microbiome characteristics and speculates about the influence of ethnicity on microbiome differences contributing to the risk for complications of pregnancy.

Illumina Technology: MiSeq 250 bp PE reads

Vaginal microbiomes are dominated by the genus *Lactobacillus*. Disturbances influence pregnancy outcomes and may contribute to the risk for complications of pregnancy.

VIROMES AND HUMAN HEALTH

Viral Populations

Direct sequencing, also called shotgun metagenomics, is used to identify viruses and determine their abundance in microbial communities.[76] It has the distinctive advantages of being able to detect novel viruses and avoid viral mutations during cell culture adaptation, a common concern for standard isolation and propagation techniques.[77] Viruses do not have a phylogenetic marker analogous to the bacterial 16S rRNA gene, so targeted sequencing based on the 16S rRNA gene will not detect viruses.

In clinical samples, various protocols are used to increase nucleic acid yield for discovery and detection of viruses.[78] Enrichment and cDNA synthesis are particularly important to detect RNA viruses[79, 80] such as Ebola virus (EBOV), severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory coronavirus (MERS-CoV), rabies, and many others that dwell in the environment within vector hosts.[81, 82] Analysis of metagenomic data from environmental and human sources shows the presence of giant viruses of amoebae, which may be linked to pneumonia of unknown etiology in humans.[83]

In humans, eukaryotic and prokaryotic viruses shape the intestinal microbiota,[84] and a human virome is recognized as characteristically persistent and particular to each individual.[85]

Reviews

Sridhar S., To K. K., Chan J. F., Lau S. K., Woo P. C., et al. (2015) A systematic approach to novel virus discovery in emerging infectious disease outbreaks. J Mol Diagn 17: 230-241

McElroy K., Thomas T. and Luciani F. (2014) Deep sequencing of evolving pathogen populations: applications, errors, and bioinformatic solutions. Microb Inform Exp 4: 1

Quinones-Mateu M. E., Avila S., Reyes-Teran G. and Martinez M. A. (2014) Deep sequencing: becoming a critical tool in clinical virology. J Clin Virol 61: 9-19

Virgin H. W. (2014) The virome in mammalian physiology and disease. Cell 157: 142-150

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Rosseel T., Ozhelvaci O., Freimanis G. and Van Borm S. (2015) Evaluation of convenient pretreatment protocols for RNA virus metagenomics in serum and tissue samples. J Virol Methods 222: 72-80

A thorough evaluation of pretreatment methods is needed to provide uniform guidelines for RNA virus metagenomics. In this study, an RNA virus was spiked, in low and high concentrations, in serum and lung tissue samples to evaluate common pretreatment and enrichment methods for viral sequences. The authors found that virion enrichment strategies (filtering and DNase treatment) are beneficial. In serum samples, DNase treatment coupled to rRNA depletion provided the best gain in viral sequences. In tissue samples, rRNA depletion obtained the biggest gain. The authors highlight that different samples benefit from different enrichment protocols to obtain optimal nucleic acid concentration and quality for metagenomic analysis.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq v3 300 bp PE reads

76. Allen H. K., Bunge J., Foster J. A., Bayles D. O. and Stanton T. B. (2013) Estimation of viral richness from shotgun metagenomes using a frequency count approach. Microbiome 1: 5
77. Sridhar S., To K. K., Chan J. F., Lau S. K., Woo P. C., et al. (2015) A systematic approach to novel virus discovery in emerging infectious disease outbreaks. J Mol Diagn 17: 230-241
78. Rosseel T., Ozhelvaci O., Freimanis G. and Van Borm S. (2015) Evaluation of convenient pretreatment protocols for RNA virus metagenomics in serum and tissue samples. J Virol Methods 222: 72-80
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81. Qin X. C., Shi M., Tian J. H., Lin X. D., Gao D. Y., et al. (2014) A tick-borne segmented RNA virus contains genome segments derived from unsegmented viral ancestors. Proc Natl Acad Sci U S A 111: 6744-6749
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83. Colson P., La Scola B. and Raoult D. (2013) Giant viruses of amoebae as potential human pathogens. Intervirology 56: 376-385
84. Kernbauer E., Ding Y. and Cadwell K. (2014) An enteric virus can replace the beneficial function of commensal bacteria. Nature advance online publication:
85. Abeles S. R., Robles-Sikisaka R., Ly M., Lum A. G., Salzman J., et al. (2014) Human oral viruses are personal, persistent and gender-consistent. ISME J 8: 1753-1767

Xu G. J., Kula T., Xu Q., Li M. Z., Vernon S. D., et al. (2015) Viral immunology. Comprehensive serological profiling of human populations using a synthetic human virome. Science 348: aaa0698
Serological tests are performed based on clinical presumption about the identity of a single (or small number of) infectious agent(s). Assumption-free tests that can determine serological responses to current and past pathogens can be useful in clinical settings and epidemiology studies at the population level. The authors have developed Systematic Viral Epitope Scanning or VirScan, a high-throughput method to analyze the inventory of antiviral antibodies using single drop of blood. The method relies on immunoprecipitation and massively parallel sequencing of phage libraries displaying viral peptides from all currently known human viruses, representing 206 human viral species. Tested in 569 individuals from around the world, VirScan identified antibodies to an average of 10 viruses per person and 84 viral species in 2 individuals. Some peptides were consistently targeted by antibodies and may represent "public epitopes."

Illumina Technology: HiSeq 2000 50 bp PE reads

Bzhalava D., Muhr L. S., Lagheden C., Ekstrom J., Forslund O., et al. (2014) Deep sequencing extends the diversity of human papillomaviruses in human skin. Sci Rep 4: 5807
Human papillomaviruses (HPVs) are abundant in and on skin. Previous metagenomic analyses have shown 273 cutaneous HPVs, including 47 previously unknown types. The authors used Illumina sequencing to obtain deeper sequencing, without prior PCR amplification, in pools of skin lesions. They identified 23 known HPV types, 3 novel putative HPVs, and 4 non-HPV viruses. They detected 385 HPV types, including 226 previously unknown types, in 326 PCR-amplified samples of skin lesions. The authors conclude that the number of HPV types will likely increase as deep sequencing technologies improve in performance and cost.

Illumina Technology: TruSeq Nano DNA Library Prep Kit, Nextera DNA Library Prep Kit, MiSeq v2 250 bp PE reads, MiSeq v3 300 bp PE reads

Viral Zoonotic Reservoirs

Zoonotic reservoirs play a significant role in the dissemination of pathogens. With NGS, it is now possible to screen reservoir animals to predict and prevent viral pathogen outbreaks.

Bat viromes are highly variable within and among species, but colonies maintain viral species in the wild.[86] The bat virome also contains vertebrate pathogens such as influenza A, hepaciviruses, hepadnaviruses, hantaviruses, cedar virus, SARS-like betacoronaviruses,[87] papillomavirus, picornavirus, and polyomavirus.[88] Bats are important reservoirs for highly pathogenic emerging viruses (Table 1).

Bat stew.

86. Dacheux L., Cervantes-Gonzalez M., Guigon G., Thiberge J. M., Vandenbogaert M., et al. (2014) A preliminary study of viral metagenomics of French bat species in contact with humans: identification of new mammalian viruses. PLoS One 9: e87194
87. Quinones-Mateu M. E., Avila S., Reyes-Teran G. and Martinez M. A. (2014) Deep sequencing: becoming a critical tool in clinical virology. J Clin Virol 61: 9-19
88. Wang J., Moore N. E., Murray Z. L., McInnes K., White D. J., et al. (2015) Discovery of novel virus sequences in an isolated and threatened bat species, the New Zealand lesser short-tailed bat (Mystacina tuberculata). J Gen Virol 96: 2442-2452

Table 1: Pathogenic Viruses in Bats

Common Name	Scientific Name	Virus	Reference
Chinese horsehoe bats	Genus *Rhinolophus*	SARS-CoV	89
Egyptian tomb bat	*Taphozous perforatus*	MERS-CoV	90
Fruit bats	*Hypsignathus monstrosus, Epomops franqueti,* and *Myonycteris torquata*	EBOV	91, 92

Arthropods play important roles in ecology and transmission of infectious diseases.[93] They harbor a diversity of pathogens that affect mammals, along with nonpathogen symbionts in primitive gut compartments.[94] Unlike bats, some mosquitoes are actively attracted to humans and may have evolved this preference based on expression of human-specific odorant receptors. In turn, human body odor is influenced by skin microbiota. This is an extraordinary example of behavioral specialization.[95] Understanding the biology of these interactions is critical when designing rational measures to control infectious diseases transmitted by vectors. This approach can certainly be invaluable for molecular epidemiology studies that track changes in viral communities in wildlife populations to identify and predict outbreaks.

Reviews

Han H. J., Wen H. L., Zhou C. M., Chen F. F., Luo L. M., et al. (2015) Bats as reservoirs of severe emerging infectious diseases. Virus Res 205: 1-6

Huang J. H., Jing X. and Douglas A. E. (2015) The multi-tasking gut epithelium of insects. Insect Biochem Mol Biol

Liang G., Gao X. and Gould E. A. (2015) Factors responsible for the emergence of arboviruses; strategies, challenges and limitations for their control. Emerg Microbes Infect 4: e18

Narasimhan S. and Fikrig E. (2015) Tick microbiome: the force within. Trends Parasitol 31: 315-323

Bichaud L., de Lamballerie X., Alkan C., Izri A., Gould E. A., et al. (2014) Arthropods as a source of new RNA viruses. Microb Pathog 77: 136-141

O'Shea T. J., Cryan P. M., Cunningham A. A., Fooks A. R., Hayman D. T., et al. (2014) Bat flight and zoonotic viruses. Emerg Infect Dis 20: 741-745

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Chandler J. A., Thongsripong P., Green A., Kittayapong P., Wilcox B. A., et al. (2014) Metagenomic shotgun sequencing of a Bunyavirus in wild-caught Aedes aegypti from Thailand informs the evolutionary and genomic history of the Phleboviruses. Virology 464-465: 312-319

The *Aedes aegypti* mosquito, a vector for many viral diseases in human health, remains apparently unaffected by the many viruses it carries. RNA metagenomic shotgun sequencing coupled to de novo assembly of wild *A. aegypti* samples yielded the nearly complete genome of a novel member of the *Phlebovirus* genus. This particular virus lacks a gene necessary for virulence in vertebrates that may restrict it exclusively to arthropod hosts. This study sheds light on the evolution of viral host tropicity, as well as the utility of deep metagenomic sequencing for viral identification.

Illumina Technology: TruSeq Stranded RNA Library Prep Kit, Genome Analyzer$_{IIx}$ 50 bp single-end (SE) reads

89. Ge X. Y., Li J. L., Yang X. L., Chmura A. A., Zhu G., et al. (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 503: 535-538

90. Milne-Price S., Miazgowicz K. L. and Munster V. J. (2014) The emergence of the Middle East respiratory syndrome coronavirus. Pathog Dis 71: 121-136

91. Leroy E. M., Epelboin A., Mondonge V., Pourrut X., Gonzalez J. P., et al. (2009) Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. Vector Borne Zoonotic Dis 9: 723-728

92. Leroy E. M., Kumulungui B., Pourrut X., Rouquet P., Hassanin A., et al. (2005) Fruit bats as reservoirs of Ebola virus. Nature 438: 575-576

93. Bichaud L., de Lamballerie X., Alkan C., Izri A., Gould E. A., et al. (2014) Arthropods as a source of new RNA viruses. Microb Pathog 77: 136-141

94. Ponnusamy L., Gonzalez A., Van Treuren W., Weiss S., Parobek C. M., et al. (2014) Diversity of Rickettsiales in the microbiome of the lone star tick, Amblyomma americanum. Appl Environ Microbiol 80: 354-359

95. McBride C. S., Baier F., Omondi A. B., Spitzer S. A., Lutomiah J., et al. (2014) Evolution of mosquito preference for humans linked to an odorant receptor. Nature 515: 222-227

Dacheux L., Cervantes-Gonzalez M., Guigon G., Thiberge J. M., Vandenbogaert M., et al. (2014) A preliminary study of viral metagenomics of French bat species in contact with humans: identification of new mammalian viruses. PLoS One 9: e87194

Studying the bat virome might be the key to preventing outbreaks of viral diseases transmitted to humans by these vectors. In this study, tissue samples (brain, liver, and lung) were collected from a small cohort of carcasses of insectivorous bats that were found dead or died shortly after collection as part of a rabies surveillance program. Samples were subjected to high-throughput sequencing to obtain the total viromes. Among the viruses found were several known viruses able to infect mammals (*Retroviridae*, *Herpesviridae*, and *Flaviviridae*). New mammalian viruses were also identified, including rotaviruses, bornaviruses, bunyaviruses, and a novel bat nairovirus. The high diversity and abundance of known and novel mammalian viruses in such a small sample cohort, which was in contact with humans, demonstrate the importance of bats as viral reservoirs.

Illumina Technology: TruSeq DNA Library Prep Kit v2, TruSeq PE Cluster Kit v3, TruSeq SBS Kit v3, HiSeq 2000 100 bp PE reads

He B., Zhang Y., Xu L., Yang W., Yang F., et al. (2014) Identification of diverse alphacoronaviruses and genomic characterization of a novel severe acute respiratory syndrome-like coronavirus from bats in China. J Virol 88: 7070-7082

Several severe acute respiratory syndrome-like coronaviruses (SARS-like CoVs) have been identified in bats previously, but none of their receptor-binding domains are similar to human SARS-CoV, implying that they are unlikely progenitors of this virus. Here, the authors performed metagenomic analysis of 268 bat rectal swabs in China and identified α- and β-coronavirus sequences. Full genomic analysis of a SARS-like CoV (LYRa11) revealed 91% nucleotide identity with human SARS-CoV. The highest identity was found in the S gene, especially in the receptor-binding domain region. SARS-convalescent human sera recognized the S1 domain of this virus, showing antigenic similarity. The authors suggest that LYRa11 is likely a recombinant descended from parental lineages that evolved into a number of bat SARS-like CoVs.

Illumina Technology: Genome Analyzer

McBride C. S., Baier F., Omondi A. B., Spitzer S. A., Lutomiah J., et al. (2014) Evolution of mosquito preference for humans linked to an odorant receptor. Nature 515: 222-227

Aedes aegypti mosquitoes carry several pathogenic viruses that infect humans. For unknown reasons, some of these mosquitoes prefer humans ("domestic form") instead of nonhuman animals ("forest form"). These 2 mosquito forms were raised in laboratory colonies. RNA-Seq of antennae tissue, the major olfactory organ, showed that preference for humans is linked to increased expression and sensitivity of the odorant receptor AaegOr4, which recognizes the abundant human odorant sulcatone. This sensitivity to a human odor compound is an evolutionary adaptation that has enabled mosquitoes to detect and prefer human hosts. However, neither odorant receptor nor sulcatone alone are likely to be the only factors that contribute to this behavior.

Illumina Technology: TruSeq RNA Library Prep Kit v2, Illumina GEX and HiSeq 2000

The preference of some mosquitos for humans is linked to increased expression and sensitivity of the odorant receptor AaegOr4, which recognizes the abundant human odorant sulcatone.

Woo P. C., Lau S. K., Teng J. L., Tsang A. K., Joseph M., et al. (2014) Metagenomic analysis of viromes of dromedary camel fecal samples reveals large number and high diversity of circoviruses and picobirnaviruses. Virology 471-473: 117-125

The emergence of MERS-CoV, and the presence of neutralizing antibodies against this virus in dromedaries, prompts questions about the role of these animals in spread of infectious viral diseases. This study focused on the fecal virome obtained by metagenomic sequencing of 203 dromedaries in Dubai. The authors identified a wide array of mammalian viruses (*Picobirnaviridae*, *Circoviridae*, *Picornaviridae*, *Parvoviridae*, *Astroviridae*, and *Herpesviridae*), some of which had not been described previously in dromedaries, and generated 14 complete *Circoviridae* genomes. The large diversity of viruses in dromedary feces demands further studies to establish their incidence and effects on dromedary and human health.

Illumina Technology: Nextera XT DNA Library Prep Kit, Nextera XT Index Kit, HiSeq 2500 150 bp PE reads

Yang L., Wu Z., Ren X., Yang F., Zhang J., et al. (2014) MERS-related betacoronavirus in *Vespertilio superans* bats, China. Emerg Infect Dis 20: 1260-1262

To control transmission of MERS-CoV to humans, it is necessary to find natural viral reservoirs. The authors collected 32 anal swab samples from *Vespertilio superans* bats in China that were pooled and processed by virus particle–protected nucleic acid purification and NGS. They identified and generated a draft genome of a novel betacoronavirus lineage C that shares 60-97% amino acid identity with MERS-CoV, being closely related to human and camel MERS-CoV. PCR amplicon sequencing showed that 5 samples were positive for the virus; these samples shared over 98% nucleotide identity with each other. This new lineage is the closest resembling MERS-CoV at the time of the study, adding to other investigations that place bats as the natural reservoirs of MERS-CoV.

Illumina Technology: Genome Analyzer$_{IIx}$

Zhuang L., Zhang Z., An X., Fan H., Ma M., et al. (2014) An efficient strategy of screening for pathogens in wild-caught ticks and mosquitoes by reusing small RNA deep sequencing data. PLoS One 9: e90831

Small RNAs may have regulatory roles in viral-host interactions. The authors set out to reanalyze small RNA–derived sequencing data, obtained from ticks and mosquitoes in Beijing, to screen for viral, prokaryotic, and eukaryotic pathogens. A novel bioinformatic pipeline was developed in house to search for putative pathogen sequences within the data, and nested PCR was used to confirm some of the results. This method identified a novel *Rickettsia spp* in H. *longicornis* ticks, as well several other putative prokaryotic and eukaryotic pathogens of interest. Reanalysis of sRNA deep sequencing data could be potentially useful to discover novel agents or track the origin of pathogens.

Illumina Technology: Genome Analyzer$_{IIx}$

Wu Z., Yang L., Ren X., He G., Zhang J., et al. (2015) Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. ISME J

Identifying the catalog of viruses that reside in bats might be useful to understand emerging infectious diseases in humans. Here, complete viromes were obtained by high-throughput sequencing of pharyngeal and anal swabs from 4440 bats of 40 major bat species throughout China. The authors show, in the context of bat ecological and biological diversity, an in-depth genetic survey that reveals novel bat viruses as well as viruses closely related to human or animal pathogens. This survey also provides a basis for the origin or evolution of certain viruses and ecological information for predicting and tracing emerging pathogens.

Illumina Technology: Genome Analyzer$_{IIx}$

The virome of bats, like the fruit bats depicted above, harbors a wide array of viruses able to infect humans.

DNA Viruses

High-throughput sequencing of environmental and clinical samples reveals a diverse virosphere, where DNA viruses present a broad host range, from hyperthermophilic archaea to mammal vertebrates.[96] NGS is an important tool for discovering novel viruses, new genes, and associated functions and niches.

96. DiMaio F., Yu X., Rensen E., Krupovic M., Prangishvili D., et al. (2015) Virology. A virus that infects a hyperthermophile encapsidates A-form DNA. Science 348: 914-917

References

Jensen R. H., Mollerup S., Mourier T., Hansen T. A., Fridholm H., et al. (2015) Target-dependent enrichment of virions determines the reduction of high-throughput sequencing in virus discovery. PLoS One 10: e0122636

High-throughput sequencing usually requires virion concentration and enrichment for viral sequence recovery. The sensitivity and variability of these approaches have not been fully assessed and may be variable. This study evaluated different library preparation techniques, targeting both RNA and DNA, with and without virion enrichment. Recovery of DNA and RNA viral sequences benefited from virion enrichment, especially for DNA viruses, resulting in reduced sequencing efforts. Despite this finding, it was not evident that a lower detection level was achieved when compared to direct metagenomic sequencing.

Illumina Technology: Nextera XT DNA Library Prep Kit, ScriptSeq v2 RNA-Seq Library Prep Kit, HiSeq 2000 100 bp PE reads

Krupovic M., Zhi N., Li J., Hu G., Koonin E. V., et al. (2015) Multiple layers of chimerism in a single-stranded DNA virus discovered by deep sequencing. Genome Biol Evol 7: 993-1001

Single stranded DNA (ssDNA) viruses can infect many organisms, are highly abundant and diverse, can tolerate high nucleotide substitution rates, and perform recombination. A group of ssDNA viruses appears genomically chimeric because it combines capsid and replicative genes inherited from RNA and other ssDNA viruses. A new layer of chimerism was exposed in this study by NGS of spin-column–associated CHIV14 ssDNA virus. The replicative genes of this putative new virus are chimeras of functional domains inherited from viruses of different families (RNA and DNA). These chimeric genes may be the product of horizontal gene transfer and domain shuffling, giving rise to this unusual type of chimeric ssDNA virus.

Illumina Technology: HiSeq 2000

Markus A., Lebenthal-Loinger I., Yang I. H., Kinchington P. R. and Goldstein R. S. (2015) An *in vitro* model of latency and reactivation of varicella zoster virus in human stem cell-derived neurons. PLoS Pathog 11: e1004885

Varicella zoster virus latent infection in ganglia can cause herpes zoster upon reactivation. However, there are no *in vitro* models to study this latency-reactivation mechanism. The authors present an *in vitro* model based on human neurons derived from embryonic stem cells that undergo stable acyclovir-induced latency, followed by reactivation upon withdrawal of growth factors or inhibition of phosphoinositide-3 kinase activity. RNA-Seq was used to monitor gene expression during latency and reactivation, showing preferential transcription of specific genome regions. Interestingly, reducing temperature to 34°C resulted in enhanced viral activation. This system could prove useful for modeling latency-activation and as a tool to assess therapeutic options.

Illumina Technology: TruSeq Stranded Total RNA LT Library Prep Kit, HiSeq 2500

Chen J., Xue Y., Poidinger M., Lim T., Chew S. H., et al. (2014) Mapping of HPV transcripts in four human cervical lesions using RNAseq suggests quantitative rearrangements during carcinogenic progression. Virology 462-463: 14-24

Human papillomavirus (HPV) 16 is associated with high risk of cervical cancer, while HPV6b is associated with benign lesions in the anogenital tract. However, the drivers for these phenotypic differences are not fully understood. This study used RNA-Seq to generate complete transcriptomes of both viruses in clinical lesions. Both viral types, in their associated lesions, showed intrinsic transcriptomic differences, particularly in promoter usage. In case of HPV16, defined transcriptomic signatures characterized progression of disease that were mostly related to regulatory proteins, such as E7, E2, E1, and E5. These specific signatures could potentially be used as biomarkers for disease progression and prognosis.

Illumina Technology: TruSeq RNA Library Prep Kit v2, HiSeq 2000 v3 50 bp PE reads

RNA Viruses

This group of viruses is characterized by a constrained genome size as well as host, vector, and genomic diversity. RNA viruses also have a striking ability to undergo rapid evolution by recombination, reassortment, and conservative amino acid substitution in changing environments. Many important viral infections are due to RNA viruses, and NGS is becoming an important tool for viral discovery and epidemiology. This is especially true for unknown illnesses from unidentified microorganisms or unsuspected sources. For example, a metagenomic approach incorporating NGS showed recent transmission of Bornavirus VSBV-1 from variegated squirrels to human hosts who had developed fatal encephalitis.[97]

A metagenomic approach incorporating NGS showed recent transmission of Bornavirus VSBV-1 from variegated squirrels to human hosts who had developed fatal encephalitis.

Some ancient RNA viruses have positive effects in human health and development. Endogenous retroviruses constitute about 8% of the human genome.[98] They are mostly silenced, except during certain cancers, human immunodeficiency virus (HIV) infections, and during early embryogenesis when expression may protect the developing embryo from viral infections.[99]

References

Andersen K. G., Shapiro B. J., Matranga C. B., Sealfon R., Lin A. E., et al. (2015) Clinical Sequencing Uncovers Origins and Evolution of Lassa Virus. Cell 162: 738-750

The authors generated a genomic catalog of almost 200 Lassa virus (LASV) sequences from clinical and rodent reservoir samples. LASV infections mainly result from reservoir-to-human infections. They followed the spread of LASV across West Africa and showed that this migration was accompanied by changes in LASV genome abundance, fatality rates, codon adaptation, and translational efficiency.

Illumina Technology: HiSeq 2000 and Nextera XT libraries

Brown J. R., Morfopoulou S., Hubb J., Emmett W. A., Ip W., et al. (2015) Astrovirus VA1/HMO-C: an increasingly recognized neurotropic pathogen in immunocompromised patients. Clin Infect Dis 60: 881-888

When conventional methods fail to identify the cause, encephalopathy of unknown etiology is a great concern in clinical settings. Fast and reliable tools are needed to address these cases. Brain biopsy obtained from an immunosuppressed child with encephalopathy of unknown origin was subjected to RNA-Seq, identifying VA1/HMO-C astrovirus. The finding was confirmed as the cause of encephalopathy by immunohistochemistry, and the virus was also found in other bodily fluids. These results prompted a survey of 680 stool and 349 cerebrospinal fluid samples that identified a similar virus in another immunosuppressed child. The authors conclude that this type of astrovirus is neuropathic and that RNA-Seq is a valuable diagnostic tool in unexplained encephalitis cases.

Illumina Technology: MiSeq

97. Hoffmann B., Tappe D., Hoper D., Herden C., Boldt A., et al. (2015) A Variegated Squirrel Bornavirus Associated with Fatal Human Encephalitis. N Engl J Med 373: 154-162
98. Belshaw R., Pereira V., Katzourakis A., Talbot G., Paces J., et al. (2004) Long-term reinfection of the human genome by endogenous retroviruses. Proc Natl Acad Sci U S A 101: 4894-4899
99. Grow E. J., Flynn R. A., Chavez S. L., Bayless N. L., Wossidlo M., et al. (2015) Intrinsic retroviral reactivation in human preimplantation embryos and pluripotent cells. Nature 522: 221-225

Grow E. J., Flynn R. A., Chavez S. L., Bayless N. L., Wossidlo M., et al. (2015) Intrinsic retroviral reactivation in human preimplantation embryos and pluripotent cells. Nature 522: 221-225

Human endogenous retrovirus type K (HERV-K) is the most recently acquired endogenous retrovirus that has retained multiple copies of complete open reading frames in human cells. The virus is mostly silenced in cells, except under specific conditions such as HIV-1 infection. This study used RNA-Seq and individual-nucleotide resolution cross-linking and immunoprecipitation (iCLIP) analysis to show that hypomethylation of long terminal repeats and transactivation by OCT4 induce expression of HERV-K transcripts in early embryos. Expression of some HERV-K products (e.g., Rec) seems to inhibit viral infection in pluripotent cells through expression of interferon-induced transmembrane protein 1 (IFITM1) on the cell surface and it also regulates a set of cellular RNAs. These results indicate that HERV-K may have a protective antiviral role in early embryo development and interact with host cell factors to regulate embryogenesis.

Illumina Technology: HiSeq 2000, HiSeq 2500

Stenglein M. D., Jacobson E. R., Chang L. W., Sanders C., Hawkins M. G., et al. (2015) Widespread recombination, reassortment, and transmission of unbalanced compound viral genotypes in natural arenavirus infections. PLoS Pathog 11: e1004900

Arenaviruses are segmented RNA viruses (L and S segments) that infect snakes—and, occasionally, rodents and humans—causing hemorrhagic fever. Events like mutation, recombination, and reassortment occur in segmented RNA viruses and may be the origin of the emergence of mammalian arenaviruses. These events have not been determined in snakes. Metagenomic sequencing was used to determine arenavirus diversity in 48 naturally infected captive snakes. A total of 23 L and 11 S genotypes were found, and snakes commonly had multiple infections. The S/L ratio was always imbalanced, with L segment genotypes outnumbering the S genotypes. Genomic evidence of recombination and reassortment events were common and even atypical structures, able to replicate and transmit, were observed. The authors speculate that human intervention, by commingling snakes previously infected with these viruses, has resulted in this great viral diversity.

Illumina Technology: HiSeq 2500 135 bp PE reads

Arenaviruses are segmented RNA viruses that infect snakes and, occasionally, rodents and humans.

Stremlau M. H., Andersen K. G., Folarin O. A., Grove J. N., Odia I., et al. (2015) Discovery of novel rhabdoviruses in the blood of healthy individuals from West Africa. PLoS Negl Trop Dis 9: e0003631

The authors used NGS to discover RNA viruses in blood samples of healthy (n = 328) and unexplained febrile individuals (n = 195) in Nigeria. Febrile individuals carried sequences from a variety of viruses (eg, HIV-1, Lassa virus). More interestingly, 2 healthy individuals carried 2 novel rhabdoviruses (Ekpoma virus [EKV]-1 and EKV-2) that were highly divergent from other known rhabdoviruses and each other. The closest known virus is the Bas-Congo virus. Serological surveys in healthy individuals showed that seroprevalence of EKV-2 was higher than that of EKV-1, although cross-reactivity against other rhabdoviruses could not be ruled out.

Illumina Technology: HiSeq 2500 100 bp PE reads

Bhat R. K., Rudnick W., Antony J. M., Maingat F., Ellestad K. K., et al. (2014) Human endogenous retrovirus-K(II) envelope induction protects neurons during HIV/AIDS. PLoS One 9: e97984

HERV-K is mostly silenced, except under specific conditions. This study aimed to elucidate the role of HERV-K Env expression in the brain during HIV-1 infection. RNA sequences encoded by HERV-K were among the most abundant in brain, particularly in cultured human neurons and in HIV-1–infected brains. Expression of Env in neuronal cells increased cellular viability and prevented neurotoxicity mediated by HIV-1 Vpr. Expression in neural stem cells suppressed TNF-α expression and microglial activation, and improved neurobehavioral deficits in mice. Collectively, these findings suggest a protective role for HERV-K during pathological stress; this role may be the reason behind its conservation within the human genome.

Illumina Technology: Genome Analyzer$_{IIx}$

Dennis F. E., Fujii Y., Haga K., Damanka S., Lartey B., et al. (2014) Identification of novel Ghanaian G8P[6] human-bovine reassortant rotavirus strain by next generation sequencing. PLoS One 9: e100699
Group A rotaviruses are the leading cause of gastroenteritis in small children. The vaccine Rotarix was introduced in Ghana in 2012, even though viral diversity was not available at the time. Genome sequencing was performed in 2 G8P[6] Ghanaian strains from before vaccine introduction to obtain phylogeny and diversity information. The authors show that both strains exhibited an unusual unreported genotype: G8-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H3. Additionally, 10 out 11 genes were identical, with VP1 being the only exception. Reassortment and transmission events with bovine/ovine/caprine rotaviruses were evident, highlighting the need to monitor circulating animal and human strains that add diversity to Ghanaian rotavirus.

Illumina Technology: MiSeq Reagent Kit v2, MiSeq

Mohammadi P., di Iulio J., Munoz M., Martinez R., Bartha I., et al. (2014) Dynamics of HIV latency and reactivation in a primary CD4+ T cell model. PLoS Pathog 10: e1004156
HIV latency strategy allows the virus to prevent curation or being effectively purged from infected cells, even when latency-reverting agents (eg, Vorinostat) inhibit histone deacetylases. A combination of RNA-Seq of latently infected cells and a viral-encoded reporter were used in a CD4+ T-cell model to investigate the characteristics of latency and reactivation. The authors showed persistent presence of viral transcripts with very limited translation during latency. Reactivating agents did increase viral transcription but failed to enhance viral translation, suggesting that some posttranslational blocks not targeted by activating agents may lead to HIV latency. These blocks need to be identified and addressed in order to clear cells from latent infection.

Illumina Technology: TruSeq RNA Library Prep Kit, TruSeq Cluster Generation Kit, HiSeq 2000

Viral Small RNAs and Host Interactions

RNA viruses produce double-stranded RNA intermediates (virus-derived small RNA) within cells and activate RNA interference (RNAi) mechanisms in insects and plants. These antiviral responses seem linked to antiviral interferon responses. Cell-derived small RNAs are, in turn, also disturbed during viral infections. However, some viruses can interfere with these host cell responses by encoding suppressors of the RNAi pathway.[100, 101] Virus small RNAs could possibly have regulatory functions in viral replication, as observed in Dengue virus-2.[102]

References

Jiang P., Zhou N., Chen X., Zhao X., Li D., et al. (2015) Integrative analysis of differentially expressed microRNAs of pulmonary alveolar macrophages from piglets during H1N1 swine influenza A virus infection. Sci Rep 5: 8167
H1N1 swine influenza A virus carries pandemic potential, and it also serves as a model to study pathogenesis in humans. This study aimed to uncover differential expression of microRNA (miRNA) in pulmonary alveolar macrophages of piglets infected with H1N1. The study showed that host miRNAs followed a downregulation trend during the acute phase of infection that gradually returned to normal during disease recovery to avoid severe lung damage. Additionally, miRNA-target regulatory networks could serve to identify functions and regulatory mechanisms of miRNAs during influenza infection.

Illumina Technology: HiSeq 2000, Genome Analyzer$_{IIx}$

Zhou Z., Li X., Liu J., Dong L., Chen Q., et al. (2015) Honeysuckle-encoded atypical microRNA2911 directly targets influenza A viruses. Cell Res 25: 39-49
Honeysuckle (HS, *Lonicera japonica*) is a traditional Chinese herb with reported activity against replication of influenza virus. However, its specific mode of action has not been described. The authors used high-throughput sequencing to identify miRNA MIR2911 encoded by HS that targets a broad spectrum of Influenza A viruses. MIR2911 was found in lungs and blood of mice given a HS decoction, suggesting it is heat-stable. Synthetic and decoction preparations of M IR2911 inhibited PB2 and NS1 protein expression and also replication of H1N1. The inhibitory effects of HS decoction were abolished by mutagenesis of PB2 and NS1, and by anti-MIR2911 antagomir, suggesting that the concentration of MIR2911 in HS decoction was sufficient to induce viral inhibitory effects. MIR2911 also inhibited H5N1 and H7N9 replication *in vitro* and *in vivo*. The authors present the first active component identified in HS that effectively suppresses influenza virus A.

Illumina Technology: HiSeq 2000

100. Parameswaran P., Sklan E., Wilkins C., Burgon T., Samuel M. A., et al. (2010) Six RNA viruses and forty-one hosts: viral small RNAs and modulation of small RNA repertoires in vertebrate and invertebrate systems. PLoS Pathog 6: e1000764
101. van Mierlo J. T., Overheul G. J., Obadia B., van Cleef K. W., Webster C. L., et al. (2014) Novel Drosophila viruses encode host-specific suppressors of RNAi. PLoS Pathog 10: e1004256
102. Hussain M. and Asgari S. (2014) MicroRNA-like viral small RNA from Dengue virus 2 autoregulates its replication in mosquito cells. Proc Natl Acad Sci U S A 111: 2746-2751

Honeysuckle tea used in traditional Chinese medicine encodes a miRNA with inhibitory effects against influenza A virus.

Cooper D. A., Jha B. K., Silverman R. H., Hesselberth J. R. and Barton D. J. (2014) Ribonuclease L and metal-ion-independent endoribonuclease cleavage sites in host and viral RNAs. Nucleic Acids Res 42: 5202-5216

Ribonuclease L (RNase L) is a metal-ion–independent endoribonuclease thought to contribute to immune responses and cancer. However, little is known about the cellular and viral targets for this enzyme. The authors validated 2',3'-cyclic phosphate cDNA synthesis and Illumina sequencing methods to determine viral and cellular RNA targets for RNAse L. The study identified regions of hepatitis C virus and poliovirus RNA genomes susceptible to RNAse L. The results also showed RNAse L-dependent and -independent cellular targets within ribosomal RNAs and evidence of 2',3'-cyclic phosphates at the ends of 5S rRNA.

Illumina Technology: MiSeq, Genome Analyzer$_{IIx}$

Li D. J., Verma D., Mosbruger T. and Swaminathan S. (2014) CTCF and Rad21 act as host cell restriction factors for Kaposi's sarcoma-associated herpesvirus (KSHV) lytic replication by modulating viral gene transcription. PLoS Pathog 10: e1003880

The mechanism for reactivation and lytic cycle of Kaposi's sarcoma-associated herpes virus (KSHV) requires transcription of about 80 lytic cycle genes and viral DNA replication. CTCF and cohesin are cellular proteins that may be involved in cycle regulation by binding to specific sites in herpes virus genomes, although the mechanisms are not fully characterized. RNAi depletion of both CTCD and cohesin coupled to chromatin immunoprecipitation sequencing (ChIP-Seq) showed that both proteins are restriction factors for viral KSHV replication. Dissociation from the herpes viral genome led to increased viral yield. Both factors initially activated transcription of the KSHV genome but later inhibited lytic transcription to prevent viral RNA accumulation. The authors show that RNAi is an efficient way to knock down cellular proteins to evaluate their effects in viral genomes.

Illumina Technology: ChIP-Seq DNA Library Prep Kit, TruSeq RNA Library Prep v2 Kit, HiSeq 2000

Shrinet J., Jain S., Jain J., Bhatnagar R. K. and Sunil S. (2014) Next generation sequencing reveals regulation of distinct *Aedes* microRNAs during chikungunya virus development. PLoS Negl Trop Dis 8: e2616

The roles of virus-derived small RNAs (vsRNAs) on various cellular processes in insects has prompted research on their role in disease transmission. This study focused on *Aedes albopictus* (Singh's) cell line carrying chikungunya virus (CHIKV). Compared to uninfected controls (n = 88), infected cells expressed 79 miRNAs. Closer analysis showed that 77 miRNAs common to both libraries were differentially expressed, and that 8 specific miRNAs were altered by CHIKV infection by upregulation (n = 4) and downregulation (n = 4). Predicted targets for these miRNAs were clustered in 17 different pathways associated with immune responses, amino acid degradation, and viral entry, among others. The authors provide pathways and interactions for the differentially expressed miRNAs that may shed light on cellular modification exerted by miRNAs during CHIKV infection.

Illumina Technology: TruSeq Small RNA Library Prep Kit, Genome Analyzer$_{IIx}$

Aedes albopictus, Asian tiger mosquito.

Weng K. F., Hung C. T., Hsieh P. T., Li M. L., Chen G. W., et al. (2014) A cytoplasmic RNA virus generates functional viral small RNAs and regulates viral IRES activity in mammalian cells. Nucleic Acids Res 42: 12789-12805

The details of vsRNAs action against RNA viruses have not been explored in mammalian cells. NGS and Northern blots identified 4 vsRNAs (1 through 4) in cells infected with enterovirus-71. vsRNA1 was highly abundant in infected cells and associated with Dicer. vsRNA1 overexpression resulted in inhibition of viral translation and activity of internal ribosomal entry site (IRES) in infected cells. This mechanism may be mediated by targeting the stem-loop II of the viral 5'-UTR that prevents interaction with IRES. Thus, vsRNA1 inhibition led to enhanced viral replication and protein synthesis. The authors describe a potential novel mechanism for viral regulation mediated by interaction between vsRNA and IRES.

Illumina Technology: HiSeq 2000

Human Viral Pathogens

Human incursion into wild habitats, climate change, international trade, globalization, and international travel are key factors in the emergence and transmission of viral infectious diseases. Combining human mobility data and viral sequences proves how these factors contribute to and help predict transmission dynamics, as demonstrated in models for human influenza H3N2.[103]

References

Nordahl Petersen T., Rasmussen S., Hasman H., Caroe C., Baelum J., et al. (2015) Meta-genomic analysis of toilet waste from long distance flights; a step towards global surveillance of infectious diseases and antimicrobial resistance. Sci Rep 5: 11444

Globalization and massive transport systems contribute to the rapid spread of diseases around the world. Monitoring worldwide transmission is a difficult task that can be helped by fast and reliable high-throughput methods. The authors performed shotgun sequencing of toilet waste from 18 international flights arriving in Copenhagen, Denmark from 9 cities in 3 regions of the world. Resistance genes against the antimicrobials tetracycline, macrolides, and β-lactams were the most abundant in all samples. Flights originating in South Asia contained higher abundance and diversity of these genes when compared to US flights. Norovirus and *Salmonella enterica* were also more abundant from South Asia, whereas *C. difficile* was more common in US flights. This study shows the usefulness of shotgun sequencing for global surveillance, providing pathogen detection, and monitoring antimicrobial resistance profiles.

Illumina Technology: HiSeq 2000

103. Lemey P., Rambaut A., Bedford T., Faria N., Bielejec F., et al. (2014) Unifying viral genetics and human transportation data to predict the global transmission dynamics of human influenza H3N2. PLoS Pathog 10: e1003932

Metagenomic surveys of toilet waste from air transport systems uncover the potential use of NGS in global health surveillance.

Briese T., Mishra N., Jain K., Zalmout I. S., Jabado O. J., et al. (2014) Middle East respiratory syndrome coronavirus quasispecies that include homologues of human isolates revealed through whole-genome analysis and virus cultured from dromedary camels in Saudi Arabia. MBio 5: e01146-01114

Antibodies to (and nucleic acids of) MERS-CoV have been identified in dromedaries, suggesting they are the viral reservoirs of MERS-CoV human infections. In this study, whole virus genome sequences were recovered from nasal swabs of dromedaries in Saudi Arabia through direct sequencing. Dromedary MERS-CoV consensus sequences were identical to human MERS-CoV sequences. More than 1 genomic variant was found in dromedaries, implying that they can be infected by more than 1 strain of MERS-CoV, in contrast to single clonal sequences found in humans. These findings support the role of dromedaries as reservoirs of MERS-CoV in human infections. Based on clonality differences, the authors suggest the possibility that specific genotypes of MERS-CoV in dromedaries can pass bottleneck selection and achieve interspecies transmission.

Illumina Technology: HiSeq 2500

Dromedaries may be the viral reservoirs of MERS-CoV human infections.

Brown B. A., Nix W. A., Sheth M., Frace M. and Oberste M. S. (2014) Seven Strains of Enterovirus D68 Detected in the United States during the 2014 Severe Respiratory Disease Outbreak. Genome Announc 2: e01201

Enterovirus D68 (EV-D68) was first identified in California in 1962, causing bronchiolitis and pneumonia. Rarely detected for many years, it reappeared in 2009 and it is now causing the most widespread outbreak to date. Here, the CDC generated 7 genome sequences of cocirculating representative EV-D68 strains. The authors described the genomic structure of these 7 strains and concluded that, based on their VP1 gene, the strains are closely related to those previously isolated in the US, Europe, and Asia.

Illumina Technology: HiSeq 2500 v2

Chan B. K., Wilson T., Fischer K. F. and Kriesel J. D. (2014) Deep sequencing to identify the causes of viral encephalitis. PLoS One 9: e93993

Clinical cases of encephalitis of unknown etiology abound and require non a priori techniques to determine their etiology. To this end, 14 frozen normal and 7 frozen encephalitis brain samples were examined with deep RNA sequencing and pathogen-specific PCR. Among encephalitis samples, measles and herpes simplex virus type-1 sequences were found in a total of 2 and 3 brain samples, respectively. These results were in agreement with clinical and pathogen-specific PCR estimations, showing that metagenomic sequencing can correctly identify viral infections in frozen brain tissue.

Illumina Technology: TruSeq Library Prep Kit, HiSeq 2000

Escalera-Zamudio M., Nelson M. I., Cobian Guemes A. G., Lopez-Martinez I., Cruz-Ortiz N., et al. (2014) Molecular epidemiology of influenza A/H3N2 viruses circulating in Mexico from 2003 to 2012. PLoS One 9: e102453

The H1N1 2009 influenza pandemic highlights the need to address the nature of viral reservoirs through molecular epidemiology approaches. The authors sequenced 19 influenza A/H3N2 strains isolated in Mexico between 2003 and 2012, analyzed their phylogenetic relationships, and compared antigenic determination. The study showed that many lineages cocirculate within the same flu season. Some of these lineages persist across different seasons, becoming possible reservoirs for reassortment events. Phylogenetic characterization did not necessarily correlate with antigenic identity, underscoring the need to use genomic determination tools, in addition to antigenic data, for surveillance of influenza virus.

Illumina Technology: Genome Analyzer$_{IIx}$

Peng X., Alfoldi J., Gori K., Eisfeld A. J., Tyler S. R., et al. (2014) The draft genome sequence of the ferret (*Mustela putorius furo*) facilitates study of human respiratory disease. Nat Biotechnol 32: 1250-1255

The domestic ferret is considered the "gold standard" model for human influenza virus infection and transmission; however, its full genome has not been established, limiting inferences based on this animal model. This study obtained the draft genome sequence of 1 female ferret, selected for its low heterozygosity, and then determined the total RNA sequence of different tissues obtained from 24 ferrets. Similarly, transcriptomic profiles in 42 ferrets infected with 1918 and 2009 pandemic influenza strains showed different transcriptomic signatures in lung and trachea tissues. Microarray data from 16 ferret samples reflecting CF disease progression showed that *CFTR* knockout animals have pathways of disease that go undetected in infant samples.

Illumina Technology: HiSeq 100 bp PE reads

Sikora D., Rocheleau L., Brown E. G. and Pelchat M. (2014) Deep sequencing reveals the eight facets of the influenza A/HongKong/1/1968 (H3N2) virus cap-snatching process. Sci Rep 4: 6181

NAs and uses these capped fragments as primers for viral mRNA synthesis in a process called "cap snatching." The authors deep sequenced all the 5' ends of viral mRNAs in human and mouse cells infected with influenza A/HongKong/1/1968 (H3N2). These regions are variable in length, motif nucleotides, and hijacked host mRNAs. Mapping the reads to known transcription start sites showed that the virus targets the most abundant cellular mRNAs. Overall findings suggest that viral polymerase-viral mRNA complexes compete minimally for host mRNA targets. Other details about cap snatching are also provided.

Illumina Technology: HiSeq 2000

Wylie K. M., Wylie T. N., Orvedahl A., Buller R. S., Herter B. N., et al. (2015) Genome sequence of enterovirus D68 from St. Louis, Missouri, USA. Emerg Infect Dis 21: 184-186

As of October 2014, CDC had confirmed 594 cases of human EV-D68 in 43 states, an unprecedented level for this particular virus. Only 5 viral sequences were available until 2014, limiting molecular epidemiology studies. The authors of this study provide 1 full genome and 8 partial sequences of clinical strains of EV-D68 isolated in St. Louis. These sequences were compared to other 7 sequences from the Midwest generated by the CDC. All the St. Louis sequences were highly similar, sharing up to 99% nucleotide identity. The full St. Louis genome was similar to the Midwest viruses, and they clustered together with European and Asian isolates from the past several years. This sequence is another tool for genomic comparison of this rapidly spreading virus, although detailed characterization would be useful to associate genomic changes with phenotypic traits.

Illumina Technology: HiSeq 2500 100 bp PE reads

Ebola

Outbreaks of EBOV, influenza virus, MERS-CoV, and Chikunguya virus exemplify the complex social and scientific challenges posed by emergent pathogens. Rapid and reliable tools for viral identification are required to monitor outbreaks in support of epidemiological studies nearly in real-time.[104] NGS has become a powerful tool that meets these demands.[105] Its use in metagenomic analysis of toilet waste from long-distance flights illustrates its potential use in global health and threat surveillance.[106]

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Carroll M. W., Matthews D. A., Hiscox J. A., Elmore M. J., Pollakis G., et al. (2015) Temporal and spatial analysis of the 2014-2015 Ebola virus outbreak in West Africa. Nature 524: 97-101

Complementing multiple efforts to support epidemic studies of the current EBOV outbreak, this study resulted in deep sequencing of 179 samples processed by the European Mobile Laboratory in Guinea to reveal an epidemiological and evolutionary history of the epidemic from March 2014 to January 2015. The analysis concluded that EBOV was introduced from Guinea into Sierra Leone, most likely in April or early May 2014. Sequences corresponding to August, September, and October 2014 indicated that this lineage evolved independently in Guinea. Along with similar efforts in Sierra Leone, these studies inform the effectiveness of control measures and describe the ongoing history of the outbreak.

Illumina Technology: ScriptSeq v2 RNA-Seq Library Prep Kit, HiSeq 2500 v4 125bp PE reads

Park D. J., Dudas G., Wohl S., Goba A., Whitmer S. L., et al. (2015) Ebola Virus Epidemiology, Transmission, and Evolution during Seven Months in Sierra Leone. Cell 161: 1516-1526

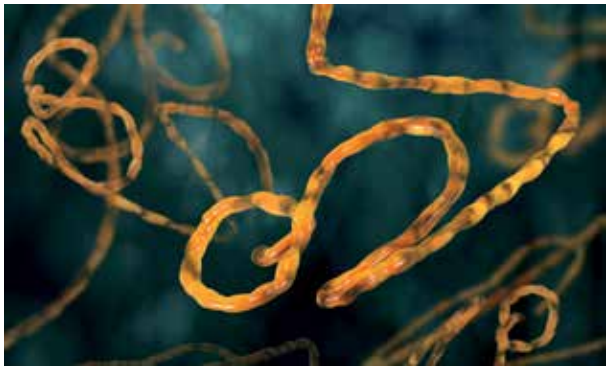
The Makona variant of EBOV continues to cause damage in African countries. Insight into virus transmission and dynamics are important to create intervention measures. The authors sequenced 232 new genomes that were sampled over 7 months in Sierra Leone and compared them to data obtained earlier in the epidemic. Sustained human-to-human transmission within Sierra Leone was confirmed by this analysis, without evidence of import of EBOV across borders after initial introduction. The emergence of intrahost genetic variants, host-to-host transmission, effective purifying selection suppression of nonsynonymous mutations, and changes in the mucin-like domain over this longer timescale were detected. Importantly, the authors noted that the estimated viral evolution rate was lower and closer to the long-term rate than to the rate estimated early in the outbreak. This study provides insightful details about epidemic dynamics in a longer timescale and highlights the importance of continued sampling and sequencing throughout the epidemic.

Illumina Technology: Nextera XT DNA Library Prep Kit, HiSeq 2500 100 bp PE reads

Simon-Loriere E., Faye O., Faye O., Koivogui L., Magassouba N., et al. (2015) Distinct lineages of Ebola virus in Guinea during the 2014 West African epidemic. Nature 524: 102-104

The authors sequenced 85 EBOV samples from patients infected from July to November 2014 in Guinea. Sequence analysis showed sustained transmission and cocirculation of 3 viral lineages: One was unique to Guinea and closely related to the earliest sampled viruses; the second contained viruses probably reintroduced from Sierra Leone; and the third was later spread to Mali. Each lineage contained specific sets of mutations, including the mucin-like domain of the viral glycoprotein. The authors discuss the implication of glycoprotein mutations in phenotypic variation.

Illumina Technology: ScriptSeq v2 RNA-Seq Library Prep Kit, HiSeq 2500 v4 125 bp PE reads



Ebola virus.

104. Kugelman J. R., Wiley M. R., Mate S., Ladner J. T., Beitzel B., et al. (2015) Monitoring of Ebola Virus Makona Evolution through Establishment of Advanced Genomic Capability in Liberia. Emerg Infect Dis 21: 1135-1143

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Zaraket H., Baranovich T., Kaplan B. S., Carter R., Song M. S., et al. (2015) Mammalian adaptation of influenza A(H7N9) virus is limited by a narrow genetic bottleneck. Nat Commun 6: 6553

Human infection with avian influenza A (H7N9) virus is mostly associated with contact with infected poultry, although transmission between humans in limited. This study reported that H7N9 was highly diverse and asymptomatic in poultry. In ferrets, the opposite occurred: diversity was highly restricted and was not fully transmissible among these species. A series of mutations in different viral genes were associated with ferret infection, but they came with a fitness cost that limited further host-to-host transmission. This mechanism limits species jumps and provides a tool for risk assessment in pandemic preparedness.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq 150 bp PE reads

Gire S. K., Goba A., Andersen K. G., Sealfon R. S., Park D. J., et al. (2014) Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 345: 1369-1372

The authors were able to recover genome sequences in the early stages of the African Ebola epidemic. A total of 99 EBOV genomes from 78 patients in Sierra Leone were generated using NGS. These genomes showed accumulated intrahost and interhost genetic variation. The results showed that this West Africa variant probably diverged from central African lineages around 2004 and sustained human-to-human transmission upon entering Guinea in May 2004. The authors point out that many viral genes show distinct mutations that should be monitored to evaluate their impact on intervention measures.

Illumina Technology: Hybridase Thermostable RNase H, Nextera XT DNA Library Prep Kit, HiSeq 100 bp PE reads

HIV

Viruses like HIV-1 continue to cause concern due to their ability to establish latency and evade therapy.[107, 108] There is also the emergence of aggressive variants that progress quickly to AIDS.[109] The use of NGS in HIV research has shown viral contribution to dysbiosis of the intestinal microbiome, shifting toward increased abundance of *Prevotella*, association with immune activation in nontreated patients,[110] and in cohorts treated with antiretroviral therapy (ART).[111]

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Cotten M., Oude Munnink B., Canuti M., Deijs M., Watson S. J., et al. (2014) Full genome virus detection in fecal samples using sensitive nucleic acid preparation, deep sequencing, and a novel iterative sequence classification algorithm. PLoS One 9: e93269

Metagenomic sequencing of fecal viromes can provide clues about pathogens in a sample without a priori assumptions about its identity. The authors set out to develop a full-genome detection process by combining nucleic acid extraction, library preparation, and virus identification using Illumina sequencing and bioinformatic algorithms. De novo assembly was used to generate full viral genomes. The procedure was tested in fecal samples from patients infected with HIV-1. This cohort exhibited an array of viruses, yielding 12 complete viral genomes from 6 virus families, including opportunistic enteropathic viruses. This method is useful in viral detection and may improve the analysis of changes associated with HIV-1 progression.

Illumina Technology: MiSeq

Dillon S. M., Lee E. J., Kotter C. V., Austin G. L., Dong Z., et al. (2014) An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal Immunol 7: 983-994

HIV-1 infection is known to cause intestinal immune system disturbance, leading to microbial translocation, lipopolysaccharide (LPS) leakage, and systemic activation of the immune system. 16S V4 rRNA gene amplicon sequencing of colon biopsies was used to assess gut microbiota changes. The authors showed gut microbiota shifts in HIV-1–infected individuals. Greater abundance of *Prevotella* species and decreased numbers of *Bacteroides* suggested disruption of the gut microbial community, which was accompanied by activated colonic T cells and dendritic cells, microbial translocation, and blood T-cell activation. Thus, HIV-1 infections lead to gut microbiota disruption and downstream effects in local and systemic immune activation.

Illumina Technology: MiSeq Reagent Kit v2

Dudley D. M., Bailey A. L., Mehta S. H., Hughes A. L., Kirk G. D., et al. (2014) Cross-clade simultaneous HIV drug resistance genotyping for reverse transcriptase, protease, and integrase inhibitor mutations by Illumina MiSeq. Retrovirology 11: 122

Viral resistance to ART commonly arises in the *pol* gene of HIV-1 virus. Current genotyping methods are optimized for subtype B virus and identify resistance to protease and reverse transcriptase inhibitors, but not to integrase inhibitors. The authors developed a universal primer set targeting the *pol* gene of M HIV subtypes

107. Elliott J. H., Wightman F., Solomon A., Ghneim K., Ahlers J., et al. (2014) Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. PLoS Pathog 10: e1004473
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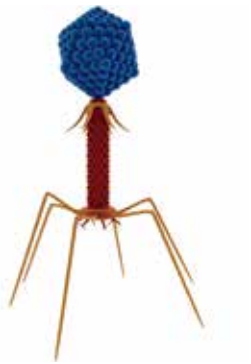
110. Dillon S. M., Lee E. J., Kotter C. V., Austin G. L., Dong Z., et al. (2014) An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal Immunol 7: 983-994
111. Mutlu E. A., Keshavarzian A., Losurdo J., Swanson G., Siewe B., et al. (2014) A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. PLoS Pathog 10: e1003829

that, when coupled to Illumina MiSeq sequencing, could detect resistance against the 3 types of antivirals. This genotyping method was tested on 62 samples, being consistent and sensitive at detecting drug resistance mutations. This tool could aid in quick detection and tracking of antiviral resistance variants.

Illumina Technology: Nextera XT DNA Library Prep, MiSeq

Phages

Bacteriophages represent an absolute majority of all organisms in the biosphere.[112] These organisms are viruses that infect bacteria and play a prominent role in shaping microbial populations.[113] The genetic diversity of the population is very high, and it appears that phages have been actively evolving for billions of years. Frequent horizontal genetic exchange among phages results in pervasive mosaicism in their architectures and the emergence of novel bacterial pathogens.[114] The current crisis with antibiotic-resistant bacteria has renewed interest in phage therapy and biocontrol approaches in infection control.[115]



Bacteriophage.

Bacteria are equipped to resist phage infection by encoding CRISPR sequences, although resistance is variable because it occurs in *P. acnes* strains in human skin.[116] The use of phages as fomite decontamination agents against MRSA[117] exemplifies promising alternatives to antimicrobial use. In addition, phage typing of pathogenic bacteria is a resource for surveillance and epidemiologic investigation of enteropathogen outbreaks.[118]

The bacterial virome may have a profound impact in health and the environment.[119] An average of 5 bacteriophage taxa exist in variable abundance in the human gut. Some of these taxa are characteristic of specific human populations, confirming specific host-phage associations.[120] Changes in signature viromes could be early markers of disturbance and could provide noninvasive diagnostic tests in the future.

Virophages are satellite viruses that inhibit or impair the reproduction of the auxiliary virus. Virophage sequences are globally distributed, including the human[121] and animal gut.[122] Their role is still largely unexplored.

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120. Waller A. S., Yamada T., Kristensen D. M., Kultima J. R., Sunagawa S., et al. (2014) Classification and quantification of bacteriophage taxa in human gut metagenomes. ISME J 8: 1391-1402
121. Zhou J., Zhang W., Yan S., Xiao J., Zhang Y., et al. (2013) Diversity of virophages in metagenomic data sets. J Virol 87: 4225-4236
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Cowley L. A., Beckett S. J., Chase-Topping M., Perry N., Dallman T. J., et al. (2015) Analysis of whole genome sequencing for the *Escherichia coli* O157:H7 typing phages. BMC Genomics 16: 271

E. coli O157:H7 produces hemolytic uremic syndrome and bloody diarrhea. Phage typing helps public health surveillance efforts, given that certain phages associate with niches, age groups, and severity. The authors sequenced 16 typing phages (T4 and T7) and showed that T7 phages differ in only 3 nucleotides whereas T4 phages fall into 3 different groups of similar genomic sequences.

Illumina Technology: TruSeq DNA Library Prep Kit, MiSeq 150 bp PE reads, Nextera DNA Library Prep Kit, Genome Analyzer$_{IIx}$

Jakhetia R. and Verma N. K. (2015) Identification and Molecular Characterisation of a Novel Mu-Like Bacteriophage, SfMu, of Shigella flexneri. PLoS One 10: e0124053

S. flexneri is a common cause of dysentery in developing countries, and several of its phages have been described. The authors characterized the genomic features and phenotypic characteristics of the Mu-like phage SfMu by NGS. O antigen was identified as a SfMu receptor. Prophages and remnants were found in different *S. flexneri* serotypes, indicating that such transposable elements may be common in *S. flexneri*. Understanding phage-bacteria interactions will help to elucidate their roles in evolution and pathogenesis.

Illumina Technology: MiSeq 250 bp PE reads

Moon B. Y., Park J. Y., Hwang S. Y., Robinson D. A., Thomas J. C., et al. (2015) Phage-mediated horizontal transfer of a Staphylococcus aureus virulence-associated genomic island. Sci Rep 5: 9784

The pathogenic island vSaβ, found in almost all *Staphylococcus aureus* strains, differs greatly in virulence gene content. Diversity and mobilization of the island remain poorly understood. In this study, vSaβ and an associated prophage were sequenced and characterized. The mobility of vSaβ to human and animal S. *aureus* is mediated by this prophage through multiple conversions of transducible particles that may also be involved in diversity acquisition. These findings highlight the role of phages in the pathogenic evolution of S. *aureus*.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq v2

Sangster W., Hegarty J. P. and Stewart D. B., Sr. (2015) Phage tail-like particles kill Clostridium difficile and represent an alternative to conventional antibiotics. Surgery 157: 96-103

Recurrent *C. difficile* infections (CDI) are difficult to control with antibiotic courses. As a result, alternative therapies like phage tail-like particles (PTLP)—proteins morphologically similar to phages and produced by *C. difficile*—are being explored. Purified PTLP from a clinical *C. difficile* sample was tested against patient-derived *C. difficile* of varying ribotypes. The PTLP genomic cluster was sequenced and annotated. The bactericidal activity of PTLP was ribotype- and species-dependent, and the putative genes required for killing were identified. These *in vitro* results are promising for the development of alternative therapies against CDI.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq 150 bp PE reads

Hare J. M., Ferrell J. C., Witkowski T. A. and Grice A. N. (2014) Prophage induction and differential RecA and UmuDAb transcriptome regulation in the DNA damage responses of Acinetobacter baumannii and Acinetobacter baylyi. PLoS One 9: e93861

The SOS response requires RecA to relieve LexA transcriptional repression. In *Acinetobacter* species, *umuDAb* is instead required for induction after DNA damage, suggesting it might be a LexA analog. RNA-Seq defined the DNA damage transcriptome of WT, *recA*, and *umuDAb* mutant strains of *A. baylyi* and *A. baumannii*, which had differential reliance on *recA*- and *umuDAb*-induced SOS genes. *A. baumannii* genes were clustered in 3 prophage regions, and bacteriophage particles were observed after mitomycin C treatment. These results suggest that both species possess a robust and complex DNA damage response involving both recA-dependent and recA-independent regulons. They also demonstrate that, although *umuDAb* has a specialized role in repressing error-prone polymerases, additional regulators participate in these species' transcriptional response to DNA damage.

Illumina Technology: Genome Analyzer$_{IIx}$

Virus Vaccine Development

Smallpox was successfully eradicated through massive immunization programs in the 1980s.[123] Substantial progress has since been made toward poliovirus eradication, despite the presence of WT poliovirus pockets in a few countries.[124] After polio virus, human enterovirus 71 (HEV71) is the most threatening neurotropic enterovirus.

123. World Health Organization (2015) Smallpox.
124. Wassilak S. G., Oberste M. S., Tangermann R. H., Diop O. M., Jafari H. S., et al. (2014) Progress toward global interruption of wild poliovirus transmission, 2010-2013, and tackling the challenges to complete eradication. J Infect Dis 210 Suppl 1: S5-15

Effective vaccines remain unavailable despite its recent emergence in the Asia-Pacific region,[125] a situation shared with other serious emerging pathogens, like SARS.

A different picture has characterized the search for an HIV-1 vaccine. The large efforts devoted to vaccine development against HIV-1 have yielded only modest success, due to factors such as latency and antigenic variation.[126, 127] Paradoxically, technological improvements from these efforts have boosted other vaccine development programs. For example, engineered cytokine-like adjuvants attached to virus-like particles (VLPs) improved immunogenicity in HIV and have been applied to other pathogens.[128] Some cancers have been associated with virus infection. The viral sequences of HPV-18 have been identified in ovarian cancer samples, implying that current vaccine strategies that include this strain might help to reduce the number of cases of this type of neoplasia.[129]

Many issues are inherent to vaccine development. For example, WT strains are able to replace vaccine strains over time, and genetic instability has been reported. These cases impact vaccine effectiveness and make genetic monitoring of live and attenuated vaccines an important tool in immunization strategies.[130]

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129. Roos P., Orlando P. A., Fagerstrom R. M. and Pepper J. W. (2015) In North America, some ovarian cancers express the oncogenes of preventable human papillomavirus HPV-18. Sci Rep 5: 8645
130. Gladstone R. A., Jefferies J. M., Tocheva A. S., Beard K. R., Garley D., et al. (2015) Five winters of pneumococcal serotype replacement in UK carriage following PCV introduction. Vaccine 33: 2015-2021

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Bande F., Arshad S. S., Bejo M. H., Moeini H. and Omar A. R. (2015) Progress and challenges toward the development of vaccines against avian infectious bronchitis. J Immunol Res 2015: 424860

Beasley D. W., McAuley A. J. and Bente D. A. (2015) Yellow fever virus: genetic and phenotypic diversity and implications for detection, prevention and therapy. Antiviral Res 115: 48-70

Salazar-Gonzalez J. A., Angulo C. and Rosales-Mendoza S. (2015) Chikungunya virus vaccines: Current strategies and prospects for developing plant-made vaccines. Vaccine 33: 3650-3658

Sanchez-Sampedro L., Perdiguero B., Mejias-Perez E., Garcia-Arriaza J., Di Pilato M., et al. (2015) The evolution of poxvirus vaccines. Viruses 7: 1726-1803

Archin N. M., Sung J. M., Garrido C., Soriano-Sarabia N. and Margolis D. M. (2014) Eradicating HIV-1 infection: seeking to clear a persistent pathogen. Nat Rev Microbiol 12: 750-764

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Fulton B. O., Sachs D., Beaty S. M., Won S. T., Lee B., et al. (2015) Mutational Analysis of Measles Virus Suggests Constraints on Antigenic Variation of the Glycoproteins. Cell Rep 11: 1331-1338

The mechanisms behind measles virus (MeV) antigenic stability have not been fully explored. In this study, MeV was subjected to transposon insertional mutagenesis, and viruses that tolerated insertions were rescued. NGS was used to identify insertion sites and the evenness of the MeV transposon library. Insertions in the glycoproteins of MeV (F and H proteins) were severely underrepresented when compared to previous work on other viruses, such as influenza. This study shows that changes in F and H glycoproteins of MeV are not well tolerated, limiting the sequence variation required to escape antibody neutralization. This finding allows for durable immunity after infection and vaccination.

Illumina Technology: TruSeq DNA LT Library Prep Kit, HiSeq 2000 100 bp SE reads

Marsh A. K., Ambagala A. P., Perciani C. T., Russell J. N., Chan J. K., et al. (2015) Examining the species-specificity of rhesus macaque cytomegalovirus (RhCMV) in cynomolgus macaques. PLoS One 10: e0121339

Cytomegalovirus (CMV) is highly species-specific, which may be a limiting factor for its use as a vaccine vector. In this study, cynomolgus macaques were infected with a recombinant, laboratory-adapted rhesus macaque cytomegalovirus (RhCMV). Sequencing was used to determine the viral genetic integrity and enhanced green fluorescent protein (eGFP) insertion site. Infected macaques did not show signs of disease,

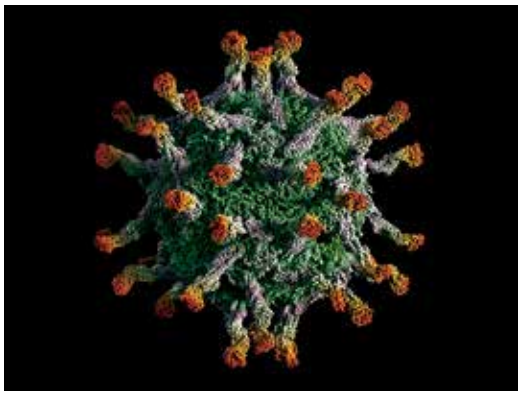
presence of the virus in tissue samples, or eGFP expression. Collectively, the data suggest that cynomolgus were not productively infected with RhCMV. The authors discuss the use of lab-adapted and primary viruses in vaccine development.

Illumina Technology: MiSeq 150 bp PE reads

Mee E. T., Minor P. D. and Martin J. (2015) High resolution identity testing of inactivated poliovirus vaccines. Vaccine 33: 3533-3541

Quality control is a critical component of vaccine development. Thus, reliable methods are required to identify vaccine strains and distinguish biological contaminants. Random RT-PCR and high-throughput sequencing were used to recover full-length genomes from monovalent and trivalent poliovirus vaccines at various stages of manufacturing. The method correctly identified expected strains, discriminating them from Sabin 1 and Mahoney strains. Additionally, slight variations can be introduced to characterize minor variants and distinguish closely related products.

Illumina Technology: Nextera XT Library Prep Kit, MiSeq v2 250 bp PE reads



Poliovirus capsid.

Ang L., Arboleya S., Lihua G., Chuihui Y., Nan Q., et al. (2014) The establishment of the infant intestinal microbiome is not affected by rotavirus vaccination. Sci Rep 4: 7417

The effects of oral vaccination against rotavirus on gut microbiota establishment in 3 infants was assessed through metagenomics, PCR-denaturing gel gradient electrophoresis (DGGE), and short chain fatty acids analyses of prevaccination and postvaccination fecal samples. Microbiomes were similar at the functional level, and vaccination did not seem to have a detectable effect in the establishment of gut microbiota.

Illumina Technology: HiSeq 2000 100 bp PE reads, CASAVA v1.8.2

Beck A., Tesh R. B., Wood T. G., Widen S. G., Ryman K. D., et al. (2014) Comparison of the live attenuated yellow fever vaccine 17D-204 strain to its virulent parental strain Asibi by deep sequencing. J Infect Dis 209: 334-344

The characterization of attenuated live vaccines, relative to their parental strains, reveals the mechanisms of viral attenuation, vaccine stability, and the risk of reversion to virulence. The authors applied deep sequencing to a live attenuated yellow fever virus strain, 17D-204, and its parental strain, Asibi. They found lack of quasispecies diversity and discrete attenuating mutations in the vaccine strain, features that might correlate in the attenuation of live viral vaccines.

Illumina Technology: HiSeq 1000 50 bp PE reads

Zhou W., Gao S., Podgorska K., Stadejek T., Qiu H. J., et al. (2014) Rovac is the possible ancestor of the Russian lapinized vaccines LK-VNIVViM and CS strains but not the Chinese strain (C-strain) vaccine against classical swine fever. Vaccine 32: 6639-6642

Lapinized vaccines are attenuated by passing through rabbits. The origins of lapinized vaccines against classical swine fever virus (CSFV) are lost in history. The authors performed NGS to elucidate vaccine origins by phylogenetic analysis of the historical Russian Rovac strain and its lapinized vaccines, LK-VNIVViM and CS. They also compared it to the lapinized Chinese strain (C-strain). Genetic analysis confirmed a close relationship between the Russian strains Rovac and LK-VNIVViM, and showed how the latter had an ancestry role in relation to the CS strain and its derivative, RUCSFPLUM. The authors suggest that the Rovac vaccine is the possible ancestor of the Russian vaccine strains but not the C-strain vaccine.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq

MICROBIAL PATHOGENESIS

Of the vast number of environmental microbes, a very small percentage cause infectious diseases. Microbial genomes are dynamic and can employ processes such as horizontal gene transfer, SNP, gene duplication, gene loss, and others. Bacterial pathogenomics offers snapshots of these constant changes.[131]

Culture and isolation methods require a priori knowledge about the identity of the causative agents. NGS does not require assumptions about microbial identity and allows functional inferences about the microorganism(s) in question, uncovering aspects of pathogen biology and evolution. These advantages are fundamental in understanding pathogenesis. They can support epidemiological studies, identify public health threats, and detect the emergence of strains not controlled by existing vaccines.[132]

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Angelakis E. and Raoult D. (2014) Methods for the discovery of emerging pathogens. Microb Pathog 77: 114-118

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Bruggemann H., Brzuszkiewicz E., Chapeton-Montes D., Plourde L., Speck D., et al. (2015) Genomics of *Clostridium tetani*. Res Microbiol 166: 326-331

Only 2 genomes of *C. tetani* are available. The authors used NGS to obtain the genomes of 3 additional *C. tetani* strains and compared them with the 2 previously sequenced genomes. Major features include tetanus-toxin–encoding plasmids and a core genome that contains about 85% of all genes. The non–core chromosome includes elements for environmental interaction, defenses, and fitness functions.

Illumina Technology: Genome Analyzer$_{IIx}$

Guerra-Assuncao J. A., Houben R. M., Crampin A. C., Mzembe T., Mallard K., et al. (2015) Recurrence due to relapse or reinfection with Mycobacterium tuberculosis: a whole-genome sequencing approach in a large, population-based cohort with a high HIV infection prevalence and active follow-up. J Infect Dis 211: 1154-1163

Tuberculosis (TB) recurrence may be due to relapse with the original strain or reinfection with a new strain. WGS was performed in all available cultures from a TB cohort actively followed after end of treatment in northern Malawi. Relapse was defined by a difference of ≤ 10 SNPs, while reinfections had a difference of ≥ 100 SNPs. Reinfection was associated with HIV infection, while relapses associated with lineage-3 strains and isoniazid resistance. Several factors contribute to TB recurrence; however, differences in the *M. tuberculosis* genome also play a role in relapse and infection.

Illumina Technology: HiSeq 2000 100 bp PE reads

Springer D. J., Billmyre R. B., Filler E. E., Voelz K., Pursall R., et al. (2014) *Cryptococcus gattii* VGIII isolates causing infections in HIV/AIDS patients in Southern California: identification of the local environmental source as arboreal. PLoS Pathog 10: e1004285

The *C. gatii* fungal pathogen affects immunocompromised patients. Ongoing outbreaks in the US Northwest have called attention to the need for finding a link between clinical isolates and environmental sources in California. The authors isolated active *C. gatii* VGIII from soil debris near residences of infected patients. Multilocus sequence typing (MLST) and WGS showed that these environmental isolates were the source of human infections. This study highlights that environmental sources present a risk of *C. gatii* infection in HIV patients, a risk that has previously been underestimated.

Illumina Technology: HiSeq 2500 100 bp PE reads

Important Microorganisms in Human Health

Some microorganisms have a long history of causing infections in humans and animals,[133] whereas some bacteria are host-specific and others have a wide range of tropism.[134] However, changes in lifestyle, globalization,[135] vaccination, and the rise of antibiotic resistance are redefining the landscape of those microorganisms we consider important. The molecular mechanisms of disease are rooted in the genome, but interactions between microbial communities and their changing ecosystems are important drivers in the evolution of bacterial transmission and pathogenesis.[136, 137, 138]

Mycobacteria

Slow-growing mycobacteria are responsible for a wide range of diseases in human and animal hosts (Table 2).

Table 2: Mycobacteria and Diseases.

Name	Host	Disease	Reference
Mycobacterium abscessus	Humans	A major pathogen in CF patients; has been associated with poor clinical outcomes	139
Mycobacterium abscessus **subsp. massiliense**	Humans	CF patients and soft-tissue infections	140
Mycobacterium avium **Subsp. hominissuis**	Pigs, humans	Disseminated disease in immunocompromised hosts, such as individuals infected by HIV or pulmonary disease in individuals without systemic immunosuppression	141
Mycobacterium lepromatosis	Humans	Diffuse lepromatous leprosy and a reactional state known as Lucio's phenomenon	142
Mycobacterium terrae **complex**	Humans	Rare but severe pulmonary disease	143
Mycobacterium tuberculosis	Humans	Tuberculosis	144
Mycobacterium ulcerans	Humans	Buruli ulcer, an ulcerative skin infection	145

The *M. tuberculosis* Beijing lineage is causing a global TB epidemic, often found with increased levels of drug resistance. WGS is a tool to analyze the evolution of this pathogen, support epidemiological studies that place isolates in a global context,[146] and rapidly determine some drug resistance patterns.[147]

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134. Bos K. I., Harkins K. M., Herbig A., Coscolla M., Weber N., et al. (2014) Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. Nature 514: 494-497
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137. Ochman H. and Moran N. A. (2001) Genes Lost and Genes Found: Evolution of Bacterial Pathogenesis and Symbiosis. Science 292: 1096-1099
138. van Boven M., Mooi F. R., Schellekens J. F., de Melker H. E. and Kretzschmar M. (2005) Pathogen adaptation under imperfect vaccination: implications for pertussis. Proc Biol Sci 272: 1617-1624
139. Harris K. A., Underwood A., Kenna D. T., Brooks A., Kavaliunaite E., et al. (2015) Whole-genome sequencing and epidemiological analysis do not provide evidence for cross-transmission of mycobacterium abscessus in a cohort of pediatric cystic fibrosis patients. Clin Infect Dis 60: 1007-1016
140. Tettelin H., Davidson R. M., Agrawal S., Aitken M. L., Shallom S., et al. (2014) High-level relatedness among Mycobacterium abscessus subsp. massiliense strains from widely separated outbreaks. Emerg Infect Dis 20: 364-371
141. Uchiya K., Takahashi H., Nakagawa T., Yagi T., Moriyama M., et al. (2015) Characterization of a novel plasmid, pMAH135, from Mycobacterium avium subsp. hominissuis. PLoS One 10: e0117797
142. Singh P., Benjak A., Schuenemann V. J., Herbig A., Avanzi C., et al. (2015) Insight into the evolution and origin of leprosy bacilli from the genome sequence of Mycobacterium lepromatosis. Proc Natl Acad Sci U S A 112: 4459-4464
143. Ngeow Y. F., Wong Y. L., Tan J. L., Hong K. W., Ng H. F., et al. (2015) Identification of new genomospecies in the Mycobacterium terrae complex. PLoS One 10: e0120789
144. Kumar V. and Robbins S. L. (2007) Robbins basic pathology. xiv, 946 p.
145. Ablordey A. S., Vandelannoote K., Frimpong I. A., Ahortor E. K., Amissah N. A., et al. (2015) Whole genome comparisons suggest random distribution of Mycobacterium ulcerans genotypes in a Buruli ulcer endemic region of Ghana. PLoS Negl Trop Dis 9: e0003681
146. Eldholm V., Monteserin J., Rieux A., Lopez B., Sobkowiak B., et al. (2015) Four decades of transmission of a multidrug-resistant Mycobacterium tuberculosis outbreak strain. Nat Commun 6: 7119
147. Coll F., McNerney R., Preston M. D., Guerra-Assuncao J. A., Warry A., et al. (2015) Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med 7: 51

Early efforts to develop TB drugs. Spores bred from the master strain of tuberculosis are made into a "suspension" with distilled water and then injected into the prepared liquor.

Review

Galagan J. E. (2014) Genomic insights into tuberculosis. Nat Rev Genet 15: 307-320

Lechartier B., Rybniker J., Zumla A. and Cole S. T. (2014) Tuberculosis drug discovery in the post-post-genomic era. EMBO Mol Med 6: 158-168

References

Ali A., Hasan Z., McNerney R., Mallard K., Hill-Cawthorne G., et al. (2015) Whole genome sequencing based characterization of extensively drug-resistant Mycobacterium tuberculosis isolates from Pakistan. PLoS One 10: e0117771

The spread of extensively drug-resistant (XDR) *M. tuberculosis* calls for improved methods to detect these strains and its variants in a reliable manner. In this study, WGS was applied to 37 XDR strains from Pakistan to investigate genes related to drug resistance. A variable number of SNPs and mutations in genes previously identified as part of the resistance complex were observed, although several additional changes also took place. The authors estimate that the concordance between phenotypic and genotypic testing using commercial assays would be variable, which argues for inclusion of expanded targets for drug resistance detection in *M. tuberculosis*.

Illumina Technology: HiSeq 2000

Colman R. E., Schupp J. M., Hicks N. D., Smith D. E., Buchhagen J. L., et al. (2015) Detection of Low-Level Mixed-Population Drug Resistance in *Mycobacterium tuberculosis* Using High Fidelity Amplicon Sequencing. PLoS One 10: e0126626

Low-levels of drug-resistant (DR) subpopulations in *M. tuberculosis* infections are often undetected and untreated, and may lead to DR tuberculosis. The authors developed and tested a method combining "single-molecule-overlapping reads" (SMOR) analysis with NGS for determination of ultrarare target alleles from clinical sputum samples. This method enables evaluation of the clinical relevance of previously undetected, low-level DR subpopulations and may support interventions to suppress them before they become clinically relevant.

Illumina Technology: MiSeq v3 300 bp PE reads

Curtis J., Luo Y., Zenner H. L., Cuchet-Lourenco D., Wu C., et al. (2015) Susceptibility to tuberculosis is associated with variants in the ASAP1 gene encoding a regulator of dendritic cell migration. Nat Genet 47: 523-527

TB is a major health problem in developing countries, and host genetic factors may be important in determining susceptibility to the disease. Expression arrays analysis of healthy and pulmonary TB cohorts showed an association between TB and variants in introns of the *ASAP1* gene on chromosome 8q24. Experimentally, depletion of *ASAP1* in DCs showed impaired matrix degradation and migration. The *ASAP1* protein is involved in actin and membrane remodeling and has been associated with podosomes. Thus, a potential mechanism of predisposition to TB might involve impaired migration of DCs, due to excessive reduction of *ASAP1* expression.

Illumina Technology: Illumina HT-12 expression array

Merker M., Blin C., Mona S., Duforet-Frebourg N., Lecher S., et al. (2015) Evolutionary history and global spread of the *Mycobacterium tuberculosis* Beijing lineage. Nat Genet 47: 242-249

The authors tracked the evolutionary history and biogeographical structure of *M. tuberculosis* of the Beijing lineage by genetic analysis of 4987 isolates from 99 countries and WGS of 110 representative isolates, the largest data set ever analyzed. They conclude that this lineage originated in the Far East and then irradiated worldwide in several waves, coinciding with the Industrial Revolution, the First World War, and HIV epidemics. Two MDR clones spread throughout Asia and Russia concomitantly with the collapse of public health systems in the former Soviet Union. The study identifies mutations associated with virulence that may have favored successful expansion across the world.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq

Periwal V., Patowary A., Vellarikkal S. K., Gupta A., Singh M., et al. (2015) Comparative whole-genome analysis of clinical isolates reveals characteristic architecture of Mycobacterium tuberculosis pangenome. PLoS One 10: e0122979

Comparative genome analysis is a useful technique to determine the genetic diversity of species considered fairly homogeneous, such as *M. tuberculosis*. The authors integrated previously obtained genome assemblies and included newly sequenced Indian *M. tuberculosis* isolates. They found a "hard-core genome" shared among all tested isolates, a "soft-core genome" shared by at least 95% of strains, and an accessory genome. The study suggests that *M. tuberculosis* has an open pangenome, and that this approach becomes critical when identifying and selecting appropriate targets for isolate typing.

Illumina Technology: Genome Analyzer$_{IIx}$

Rock J. M., Lang U. F., Chase M. R., Ford C. B., Gerrick E. R., et al. (2015) DNA replication fidelity in Mycobacterium tuberculosis is mediated by an ancestral prokaryotic proofreader. Nat Genet 47: 677-681

DNA replication fidelity relies on proofreading activity of the ε-exonuclease of the replisome in *E. coli* systems. However, this unit is dispensable in *M. tuberculosis*. NGS and other techniques were used in *Mycobacterium* to show that the PHP domain of the DnaE1 polymerase performs the exonuclease function, and its absence significantly increases the mutation rate. Phylogenetic analysis showed that the PHP domain–mediated activity is conserved and may be the ancestral prokaryotic proofreader. These findings are relevant for the development of antimicrobials that target replication fidelity.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq v2 100 bp PE reads

Venkatasubramanian S., Dhiman R., Paidipally P., Cheekatla S. S., Tripathi D., et al. (2015) A rho GDP dissociation inhibitor produced by apoptotic T-cells inhibits growth of Mycobacterium tuberculosis. PLoS Pathog 11: e1004617

The mechanisms underlying latent *M. tuberculosis* infection remain poorly understood. The authors identified a subpopulation of CD4 cells ($CD4^+CD25^+$) that produced a soluble factor (D4GDI) able to inhibit the growth of *M. tuberculosis* in human macrophages. These cells enhanced production of IL-1β, TNF-α, and ROS, increased apoptosis of infected cells, and induced expression of dormancy survival regulators in *M. tuberculosis*. Overall, this study provides evidence of enhanced immunity against *M. tuberculosis* by $CD4^+CD25^+D4GDI^+$ cells.

Illumina Technology: HumanHT-12_V4 Expression BeadChip arrays

Wada T., Iwamoto T., Tamaru A., Seto J., Ahiko T., et al. (2015) Clonality and micro-diversity of a nationwide spreading genotype of Mycobacterium tuberculosis in Japan. PLoS One 10: e0118495

M. tuberculosis "M strain" has spread recently throughout Japan. The authors performed deep sequencing of 10 M strain isolates to uncover the history of its spread. All the isolates possessed mutations common to those of referential strains, and accumulated single-nucleotide variants (SNVs) with evidence of high clonality of these isolates. Further analysis revealed distribution into 3 subclonal groups with a bias toward geographical origins. These efforts inform public health actions against TB by providing efficient strategies for big data analysis.

Illumina Technology: Genome Analyzer$_{IIx}$75 bp PE reads

Ablordey A. S., Vandelannoote K., Frimpong I. A., Ahortor E. K., Amissah N. A., et al. (2015) Whole genome comparisons suggest random distribution of Mycobacterium ulcerans genotypes in a Buruli ulcer endemic region of Ghana. PLoS Negl Trop Dis 9: e0003681

Harris K. A., Underwood A., Kenna D. T., Brooks A., Kavaliunaite E., et al. (2015) Whole-genome sequencing and epidemiological analysis do not provide evidence for cross-transmission of mycobacterium abscessus in a cohort of pediatric cystic fibrosis patients. Clin Infect Dis 60: 1007-1016

Ngeow Y. F., Wong Y. L., Tan J. L., Hong K. W., Ng H. F., et al. (2015) Identification of new genomospecies in the Mycobacterium terrae complex. PLoS One 10: e0120789

Singh P., Benjak A., Schuenemann V. J., Herbig A., Avanzi C., et al. (2015) Insight into the evolution and origin of leprosy bacilli from the genome sequence of Mycobacterium lepromatosis. Proc Natl Acad Sci U S A 112: 4459-4464

Anderson S. T., Kaforou M., Brent A. J., Wright V. J., Banwell C. M., et al. (2014) Diagnosis of childhood tuberculosis and host RNA expression in Africa. N Engl J Med 370: 1712-1723

There is a need for improved diagnostic tests for TB in children. This study defined the transcriptional signatures of host blood samples from children in South Africa, Malawi, and Kenya, distributed according to culture status. The authors identified a 51-transcript signature distinguishing TB from other diseases in South African and Malawian children. These results were validated in the Kenyan cohort to determine the sensitivity and specificity against culture-confirmed and culture-negative diagnoses. RNA expression signatures helped distinguish TB from other diseases in African children with and without HIV infection.

Illumina Technology: HumanHT-12_V4 Expression BeadChip arrays

Li D., Gao G., Li Z., Sun W., Li X., et al. (2014) Profiling the T-cell receptor repertoire of patient with pleural tuberculosis by high-throughput sequencing. Immunol Lett 162: 170-180

Pleural effusion is a common manifestation of pulmonary TB. However, the T-cell receptor (TCR) role and repertories of pleural effusion mononuclear cells (PEMCs) have not been elucidated. In this study, NGS was applied to PEMCs and peripheral blood mononuclear cells (PBMCs) collected from a single patient who had developed pleural effusion. The authors describe the genetic profile of TCR β-chain receptors, including different CDR3 clonotypes and patterns, in both PEMCs and PBMCs.

Illumina Technology: Genome Analyzer$_{IIx}$

Lin P. L., Ford C. B., Coleman M. T., Myers A. J., Gawande R., et al. (2014) Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. Nat Med 20: 75-79

Some individuals remain latently infected with TB, although the reasons for this inactive infection are still obscure. A similar pattern is found in macaques experimentally infected with individually tagged bacteria. This study showed how each lung lesion is likely founded by a single cell. However, the fate of each lesion was different in each individual, i.e., some active lesions were sterilized by the host whereas others progressed. This study suggests that critical responses at the lesion level determine the outcome of infection.

Illumina Technology: Genome Analyzer$_{IIx}$

Escherichia coli

Commonly found in the environment, food products, and intestines of people and animals, most strains of *E. coli* are commensals. However, some strains can be pathogens that enjoy high transmissibility and often cause foodborne outbreaks. The pathotype Shiga toxin-producing *E. coli* (STEC) is perhaps the most common example of this group, although 5 other pathotypes are recognized. Rapid identification is essential to track and control foodborne outbreaks to their sources.[148]

Review

Franz E., Delaquis P., Morabito S., Beutin L., Gobius K., et al. (2014) Exploiting the explosion of information associated with whole genome sequencing to tackle Shiga toxin-producing *Escherichia coli* (STEC) in global food production systems. Int J Food Microbiol 187: 57-72

References

Griffing S. M., MacCannell D. R., Schmidtke A. J., Freeman M. M., Hyytia-Trees E., et al. (2015) Canonical Single Nucleotide Polymorphisms (SNPs) for High-Resolution Subtyping of Shiga-Toxin Producing *Escherichia coli* (STEC) O157:H7. PLoS One 10: e0131967

The authors aimed to develop a SNP panel for subtyping of STEC O157:H7 that would be consistent with other available discrimination methods. An initial screening of a large SNP panel failed to resolve some bacterial clusters accurately, forcing a second screening using comparative DNA resequencing from the clusters with poor resolution. This method resulted in increase of SNP panel size, with 63 SNPs previously identified and 438 occurring across multiple clusters. The authors conclude that linkage disequilibrium limits the number of informative SNPs and that these SNPs are inefficient for effective subtyping.

Illumina Technology: TruSeq DNA Library Prep Kit, MiSeq

148. Lambert D., Carrillo C. D., Koziol A. G., Manninger P. and Blais B. W. (2015) GeneSippr: a rapid whole-genome approach for the identification and characterization of foodborne pathogens such as priority Shiga toxigenic Escherichia coli. PLoS One 10: e0122928

Lambert D., Carrillo C. D., Koziol A. G., Manninger P. and Blais B. W. (2015) GeneSippr: a rapid whole-genome approach for the identification and characterization of foodborne pathogens such as priority Shiga toxigenic *Escherichia coli*. PLoS One 10: e0122928

The authors describe a WGS approach that enables same-day identification of colony isolates recovered from investigative food samples. The method used 21-nt reads and 4-fold coverage followed by custom data analysis software, called GeneSippr. Pathogens were correctly identified in all isolates tested, and single colonies were identified within 9 hours.

Illumina Technology: Nextera XT DNA Library Prep Kit, Nextera XT Index Kit, MiSeq v2

Zhang Y., Rowehl L., Krumsiek J. M., Orner E. P., Shaikh N., et al. (2015) Identification of Candidate Adherent-Invasive *E. coli* Signature Transcripts by Genomic/Transcriptomic Analysis. PLoS One 10: e0130902

Adherent-invasive *E. coli* (AIEC) is frequently found in mucosal lesions of patients with Crohn's disease. RNA-Seq was used to identify differentially expressed genes in an AIEC strain relative to a noninvasive *E. coli* (NIEC) strain that may account for difference in phenotypes. The results showed that 224 and 241 genes were upregulated and downregulated in AIEC, respectively. The authors describe the functions of these enriched genes and pathways, as well as genes and CRISPR-Cas loci exclusive to AIEC strains. These findings were confirmed in IBD and non-IBD fecal RNA samples that demonstrated enrichment of CRISPR-Cas in IBD. This approach is useful to identify candidate AIEC signature transcripts.

Illumina Technology: MiSeq 150 bp SE reads

Salipante S. J., Roach D. J., Kitzman J. O., Snyder M. W., Stackhouse B., et al. (2015) Large-scale genomic sequencing of extraintestinal pathogenic *Escherichia coli* strains. Genome Res 25: 119-128

von Mentzer A., Connor T. R., Wieler L. H., Semmler T., Iguchi A., et al. (2014) Identification of enterotoxigenic *Escherichia coli* (ETEC) clades with long-term global distribution. Nat Genet 46: 1321-1326

Enterotoxigenic *E. coli* (ETEC) is an important cause of diarrhea by producing heat-stable and/or heat-labile toxins, along with several colonization factors that are encoded in plasmids. The authors performed WGS of a collection of globally distributed ETEC strains that carry distinct colonization factors and enterotoxin profiles. Challenging previous notions, these recent lineages might contain chromosome and plasmids combinations that optimize fitness and transmissibility, with implications for tracking ETEC outbreaks.

Illumina Technology: HiSeq 2000 100 bp PE reads

Bielaszewska M., Schiller R., Lammers L., Bauwens A., Fruth A., et al. (2014) Heteropathogenic virulence and phylogeny reveal phased pathogenic metamorphosis in *Escherichia coli* O2:H6. EMBO Mol Med 6: 347-357

Petty N. K., Ben Zakour N. L., Stanton-Cook M., Skippington E., Totsika M., et al. (2014) Global dissemination of a multidrug resistant *Escherichia coli* clone. Proc Natl Acad Sci U S A 111: 5694-5699

Xu H., Zhu X., Hu Y., Li Z., Zhang X., et al. (2014) DNA methylome in spleen of avian pathogenic Escherichia coli-challenged broilers and integration with mRNA expression. Sci Rep 4: 4299

Staphylococcus aureus

This Gram-positive pathogen has gained notoriety because of its antimicrobial resistance capabilities. Life-threatening infections caused by this agent in hospital settings are difficult to control. *S. aureus* encodes 4 (methicillin-susceptible, MSSA) or 5 (methicillin-resistant, MRSA) penicillin-binding proteins (PBP) involved in cell-wall peptidoglycan (PG) synthesis. The extra PBP2A in MRSA is a determinant in resistance against β-lactams.[149, 150]

References

Reed P., Atilano M. L., Alves R., Hoiczyk E., Sher X., et al. (2015) *Staphylococcus aureus* Survives with a Minimal Peptidoglycan Synthesis Machine but Sacrifices Virulence and Antibiotic Resistance. PLoS Pathog 11: e1004891

Peptidoglycan is the main component of the cell wall, and a machinery of 9 proteins is required for its synthesis in *S. aureus*. This study examined the genomic and transcriptomic effects of deleting 7 of the 9 proteins of this machinery. The authors identified PBP1 and PBP2 as the minimal machinery required for PG synthesis *in vitro*. However, the rest of the complex seems to be required under more challenging environments, as the minimal machinery is susceptible to a variety of conditions. Thus, the complex could have a role in pathogenesis and antibiotic resistance.

Illumina Technology: MiSeq, HiSeq

149. Hartman B. J. and Tomasz A. (1984) Low-affinity penicillin-binding protein associated with beta-lactam resistance in Staphylococcus aureus. J Bacteriol 158: 513-516
150. Pinho M. G., de Lencastre H. and Tomasz A. (2001) An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. Proc Natl Acad Sci U S A 98: 10886-10891

Tong S. Y., Holden M. T., Nickerson E. K., Cooper B. S., Koser C. U., et al. (2015) Genome sequencing defines phylogeny and spread of methicillin-resistant *Staphylococcus aureus* in a high transmission setting. Genome Res 25: 111-118

WGS was used to establish the flux and genetic diversity of MRSA in 2 intensive-care units where transmission was common. A total of 79 isolates were sequenced, belonging to distinct ST 239 clades that carried different plasmids encoding determinants for antibiotic resistance. Inter- and intra-ward transmission events and multiple colonizations by more than 1 clade were identified. WGS provides insights into the transmission and spread of MRSA, and this approach can be applied in different settings.

Illumina Technology: Genome Analyzer$_{IIx}$ 100 bp PE reads, HiSeq 75 bp PE reads

Brady R. A., Bruno V. M. and Burns D. L. (2015) RNA-Seq Analysis of the Host Response to Staphylococcus aureus Skin and Soft Tissue Infection in a Mouse Model. PLoS One 10: e0124877

Ellington M. J., Reuter S., Harris S. R., Holden M. T., Cartwright E. J., et al. (2015) Emergent and evolving antimicrobial resistance cassettes in community-associated fusidic acid and meticillin-resistant Staphylococcus aureus. Int J Antimicrob Agents 45: 477-484

Everitt R. G., Didelot X., Batty E. M., Miller R. R., Knox K., et al. (2014) Mobile elements drive recombination hotspots in the core genome of Staphylococcus aureus. Nat Commun 5: 3956

Genetic exchange in the core genomes of clonal bacteria like *S. aureus* is not fully understood, despite increasing evidence about mobile elements. The authors performed WGS of MRSA and MSSA historical, international, human, and animal strains to gain insight into core genomes. They found recombination hotspots in close proximity to known mobile elements. This type of mobile element–driven core genome transfer broadens our understanding of clonal bacteria, with a possible impact on phenotype-genotype mapping.

Illumina Technology: Genome Analyzer$_{IIx}$ HiSeq 2000 100 bp PE reads

Salmonella

Salmonella enterica serovar Enteritidis and serovar Typhimurium are important leading causes of foodborne gastroenteritis in humans, although many other serovars also pose health risks for humans and animals. Serovar Enteritidis is prevalent in the poultry industry. It is particularly efficient at replicating within chicken eggs, making identification and origin tracing crucial for surveillance and to control foodborne-associated outbreaks. *Salmonella* is a genetically homogeneous bacterium, and genome sequencing provides accurate differentiation among strains.[151]

Food-borne outbreaks of *Salmonella* Enteritidis are often associated with chicken eggs and poultry products. NGS supports public health surveillance and allows differentiation among genetically homogeneous strains.

Review

Park S. H., Aydin M., Khatiwara A., Dolan M. C., Gilmore D. F., et al. (2014) Current and emerging technologies for rapid detection and characterization of Salmonella in poultry and poultry products. Food Microbiol 38: 250-262

151. den Bakker H. C., Allard M. W., Bopp D., Brown E. W., Fontana J., et al. (2014) Rapid whole-genome sequencing for surveillance of Salmonella enterica serovar enteritidis. Emerg Infect Dis 20: 1306-1314

References

Inns T., Lane C., Peters T., Dallman T., Chatt C., et al. (2015) A multi-country *Salmonella* Enteritidis phage type 14b outbreak associated with eggs from a German producer: 'near real-time' application of whole genome sequencing and food chain investigations, United Kingdom, May to September 2014. Euro Surveill 20:

The authors report an outbreak of *Salmonella* Enteritidis phage type 14b (PT14b) in the UK. The outbreak was related to a specific, multiple-locus variable number tandem repeat analysis (MLVA) pattern that occurred simultaneously in other countries in the European Union. Epidemiological data, MLVA patterns, and WGS demonstrated that the outbreaks were linked to chicken eggs that originated from a German company. In this case, the use of genome sequence information was useful to trace back foodborne outbreaks to their source in a timely manner to implement control measures.

Illumina Technology: HiSeq 2500

Feasey N. A., Gaskell K., Wong V., Msefula C., Selemani G., et al. (2015) Rapid emergence of multidrug resistant, H58-lineage Salmonella typhi in Blantyre, Malawi. PLoS Negl Trop Dis 9: e0003748

Hamidian M., Holt K. E. and Hall R. M. (2015) The complete sequence of Salmonella genomic island SGI1-K. J Antimicrob Chemother 70: 305-306

Schreiber F., Kay S., Frankel G., Clare S., Goulding D., et al. (2015) The Hd, Hj, and Hz66 flagella variants of Salmonella enterica serovar Typhi modify host responses and cellular interactions. Sci Rep 5: 7947

Deng X., Desai P. T., den Bakker H. C., Mikoleit M., Tolar B., et al. (2014) Genomic epidemiology of *Salmonella* enterica serotype Enteritidis based on population structure of prevalent lineages. Emerg Infect Dis 20: 1481-1489

Salmonella enterica is a leading cause of foodborne gastroenteritis worldwide, and surveillance is critical to implement control measures during outbreaks. However, it is genetically homogeneous and, thus, subtyping is challenging. The authors used WGS to characterize 125 S. *enterica* Enteritidis and 3 S. *enterica* Nitra strains, together with SNP analysis to identify 4887 loci that distinguished all isolates from each other. This approach identified 5 major genetic lineages, possibly related to geographic and epidemiologic patterns that may have spawned during the 17th–18th centuries and diversified during the 1920s and 1950s.

Illumina Technology: HiSeq 2000

Leekitcharoenphon P., Nielsen E. M., Kaas R. S., Lund O. and Aarestrup F. M. (2014) Evaluation of whole genome sequencing for outbreak detection of Salmonella enterica. PLoS One 9: e87991

Reliable subtyping of *Salmonella enterica* is essential for surveillance and foodborne outbreak responses. Here, 34 S. Typhimurium, 8 S. Enteritidis, and 5 S. Derby isolates were sequenced to evaluate WGS as an epidemiological typing tool. Four different phylogenetic analyses (pan-genome, K-mer, SNP, nucleotide difference trees) were applied to the S. Typhimurium data and compared to data from pulse-field gel electrophoresis. The results showed that WGS achieved greater performance than traditional typing methods. SNP and nucleotide-difference analytic approaches for WGS data were better methods for epidemiological typing compared to other approaches. However, combination of epidemiological data and WGS is still necessary to relate strains to outbreaks.

Illumina Technology: Genome Analyzer$_{IIx}$

Maudet C., Mano M., Sunkavalli U., Sharan M., Giacca M., et al. (2014) Functional high-throughput screening identifies the miR-15 microRNA family as cellular restriction factors for *Salmonella* infection. Nat Commun 5: 4718

Antimicrobial Resistance

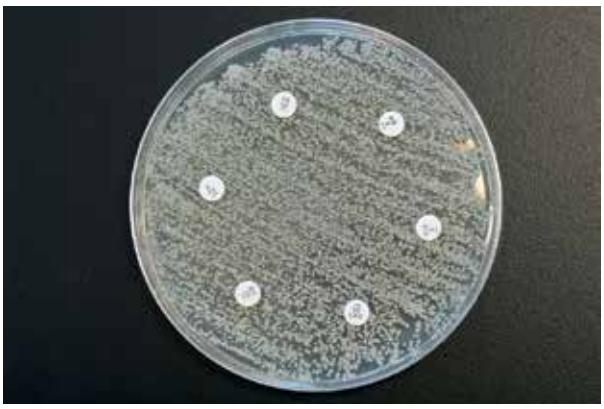
Resistance to antimicrobial compounds is an urgent public health concern, with increased patient mortality and health care costs.[152] Multiple genetically encoded mechanisms play a role in resistance and can be transferred via mobile elements between bacteria, leading to the spread of resistance to single or multiple antimicrobials.

Antibiotic-resistance genes are ancient and ubiquitous,[153] but interaction with people, animals, and the environment may have facilitated their selection and spread.[154] Agricultural practices also impact the spread of antibiotic-resistance genes in soil

152. de Castro A. P., Fernandes Gda R. and Franco O. L. (2014) Insights into novel antimicrobial compounds and antibiotic resistance genes from soil metagenomes. Front Microbiol 5: 489
153. Bhullar K., Waglechner N., Pawlowski A., Koteva K., Banks E. D., et al. (2012) Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS One 7: e34953
154. Li B., Yang Y., Ma L., Ju F., Guo F., et al. (2015) Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. ISME J

and food animals.[155] Aquaculture practices use extreme amounts of antibiotics in certain salmon-producing countries,[156] whereas global consumption of antimicrobials is expected to increase by 67% in livestock animals by 2030.[157] Culture-independent *in silico* antimicrobial susceptibility testing to support surveillance efforts and provide tracking of drug resistant pathogens[158] can be achieved by using information provided by genomic analyses.

Genomic approaches can help reveal many of these genes, identify new ones, unveil *in situ* resistance mechanisms, and potentially identify genes encoding antimicrobial compounds. Soil is probably the richest source of antimicrobials and antimicrobial-resistance genes,[159, 160] with many compounds still locked in the unculturable bacterial world.[161]



Genomic information of antimicrobial resistance in health care systems can inform implementation of control measures and evaluate their effectiveness.

Reviews

Hughes D. and Andersson D. I. (2015) Evolutionary consequences of drug resistance: shared principles across diverse targets and organisms. Nat Rev Genet 16: 459-471

Mather A. E. (2015) There's no place like home. Nat Rev Microbiol 13: 331

Nesme J. and Simonet P. (2015) The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. Environ Microbiol 17: 913-930

van Schaik W. (2015) The human gut resistome. Philos Trans R Soc Lond B Biol Sci 370: 20140087doi: 10.1098/rstb.2014.0087

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MRSA ST239 has seen some replacement with ST22 clones that are more susceptible to antimicrobial treatment over the past few years. To understand the historical and evolutionary features, the authors performed WGS and phylogenetic analysis of ST239 and ST22 in Singapore hospitals. They found that

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161. Ling L. L., Schneider T., Peoples A. J., Spoering A. L., Engels I., et al. (2015) A new antibiotic kills pathogens without detectable resistance. Nature 517: 455-459

ST239 was introduced from adjacent regions and then spread in the health care system. ST22 was introduced around the year 2000, supplanting ST239. The genetic diversity of ST239 appeared to decrease as ST22 replaced it. However, diversity increased once more, around the year 2007 and later. The study shows the importance of hospital practices and competition between clones to drive pathogen evolution.

Illumina Technology: Genome Analyzer$_{IIx}$, HiSeq

Pecora N. D., Li N., Allard M., Li C., Albano E., et al. (2015) Genomically Informed Surveillance for Carbapenem-Resistant *Enterobacteriaceae* in a Health Care System. MBio 6: e01030

WGS allows refined tracking of chromosomal traits and associated mobile genetic elements that harbor resistance genes. This information can be used to alert hospitals to prevent the spread of infections. Isolates of *Klebsiella pneumoniae* and *Enterobacter cloacae* with resistance to carbapenem antibiotics were sequenced. This study identified multiple carbapenemase genes residing in distinct mobile elements that provided strain heterogeneity. The authors were able to draft a timeline of carbapenemase entry and movement in a hospital setting. This type of study supports infection control resources and the development of resistance gene repositories at an institutional level.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq v1 150 bp PE reads, MiSeq v3 300 bp PE reads

Bacterial Vaccines

Vaccines against *C. tetani* are highly effective, and their use is routine. On the other end of the spectrum, for microorganisms such as *H. pylori*, vaccine development is still elusive, despite the potential utility in prevention of ulcer disease and gastric adenocarcinoma.[162]

Long-dreaded microbes now have a chance to contribute to immunotherapy as antigen-expressing platforms. Attenuated *Salmonella* Typhimurium expressing *B. mallei* LPS O antigen has been successful at protecting mice against lethal challenges.[163] Similarly, *Salmonella* T3SS has been used to deliver tumoral antigens, enhance antitumor immunity, and induce tumor regression in the colon carcinoma mouse model.[164] Some pre-existing immunity against these vectors could actually enhance immunogenicity.[165]

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S. pneumoniae PMEM14 serotype 19F is targeted by the PCV7 heptavalent vaccine. Unfortunately, there is an emergence of vaccine-escape variants. The authors performed WGS of 173 PMEM14 or relatives to characterize its evolution. PMEM14 is a single lineage with 4 serotype switches to 19A, all with different resistance to β-lactams through homologous recombinations that also occur in absence of vaccination. These findings suggest that pneumococcal genotypes generate pools of standing variation that result in the emergence of multiple mutants, in parallel, upon changes in selection pressure.

Illumina Technology: Genome Analyzer$_{IIx}$, HiSeq

Cohen-Gihon I., Israeli O., Beth-Din A., Levy H., Cohen O., et al. (2014) Whole-Genome Sequencing of the Nonproteolytic *Bacillus anthracis* V770-NP1-R Strain Reveals Multiple Mutations in Peptidase Loci. Genome Announc 2: e00075-14.

Anthrax is a severe disease caused by inhalation/ingestion of, or contact of skin abrasions with, *B. anthracis* spores. The spores produce vegetative bacilli, resulting in massive bacteremia and toxemia. The authors generated a draft whole-genome sequence of the nonproteolytic *Bacillus anthracis* V770-NP1-R strain. Compared to those of other *B. anthracis* strains, the genome exhibits unique mutations in multiple targets potentially affecting proteolytic functions. One of these mutations is a deletion that disrupts the NprR quorum-sensing regulator of the NprA protease.

Illumina Technology: Genome Analyzer$_{IIx}$

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MICROBIAL POPULATIONS

To determine the identity and abundance of microbes in a defined sample, there are 2 sequencing approaches: amplicon sequencing and whole-genome shotgun sequencing (Table 3). Amplicon sequencing relies on PCR amplification and sequencing of the 16S rRNA gene as a single informative marker.[166] Whole-genome shotgun sequencing refers to random shearing of whole extracted DNA and then sequencing the small DNA fragments. Both approaches offer distinct advantages and trade-offs. Selecting an appropriate approach depends on the type of information required.

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Table 3: Amplicon and Whole-Genome Shotgun Sequencing.

Feature	16s rRNA (Amplicon Sequencing)[167]	Whole-Genome Shotgun Sequencing [168, 169]
Type of information produced	Taxonomic composition and community structure as operational taxonomic units[170]	Potentially functional characterization of the whole community; draft genome reconstruction of individual community members
Application	Monitor populations[171]	Detect new members, new genes, resolve complex populations
Ability to detect rare members of the community	Highly sensitive to single target representing 80% of total bacterial RNA	Requires deeper sequencing to achieve the same level of sensitivity
Biases	Probe and amplification bias; unable to represent the whole genomic content[172]	Sequence-content bias
Gene inventory	Functionality is largely unknown	Extensive inventories and partial genomes; discover new genes and biological pathways

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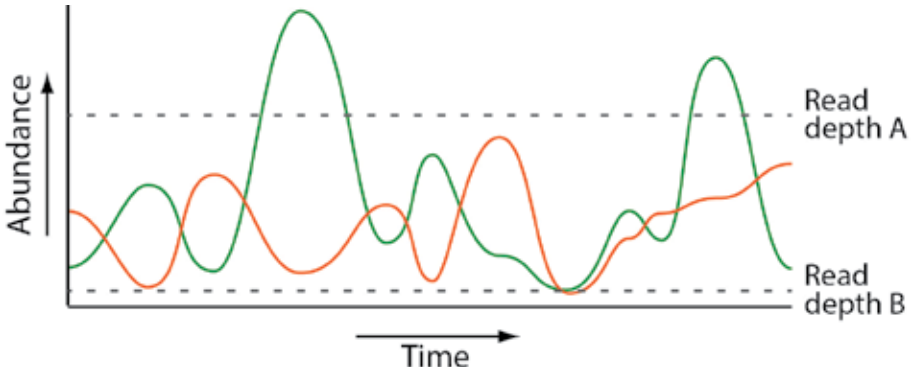
Amplicon Sequencing: 16S Ribosomal RNA

The characterization of a single genetic marker, such as the 16S rRNA gene, has been extensively used for assessing the diversity of microbial populations for phylogenetic and taxonomic studies. The 16S rRNA gene exists in all bacteria and is composed of interspersed variable regions flanked by relatively conserved regions.[173] Probes hybridize to the conserved regions for PCR amplification and sequencing of the variable regions, which cluster into operational taxonomic units (OTUs) according to degree of similarity.[174] This classical approach has yielded comprehensive databases for comparison of sequences in an ecosystem and evolutionary analyses applicable to large projects, such as the Human Microbiome Project.[175]

As a single marker, 16S rRNA sequencing is able to capture broad changes in bacterial community diversity, an attractive feature due to the lower cost of sequencing only a small part of the genome. However, amplicon sequencing does not provide information about the potential genomic functions of the community, nor does it detect viruses.

Deep Sequencing to Characterize Complex Populations

Deep sequencing refers to the resequencing of a genomic region up to thousands of times. This method provides improved representation of all the members of the community, including species that are present in low abundance. In nature, bacteria live in highly diverse populations that are able to change over time and space. Changes can also occur within and among individuals from the same or different species.[176] Within a community, some microorganisms exist in low abundance, but they may have significant effects in the community and health status. This phenomenon usually becomes apparent if their abundance or functions adjust to changes in the ecosystem.[177, 178] The discrimination power of deep sequencing is critical to characterize heterogeneous communities fully and to provide better interpretation of subtle microbiological changes.



Abundant (Green) and poorly represented (Orange) microbial populations sampled at different depths. Deep sequencing (Depth B) captures fluctuations in abundance in both types of populations. These changes are missed when abundance drops below the detection limit in Depth A.

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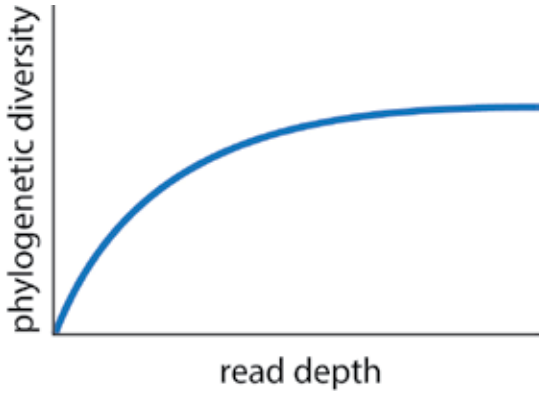

The discovery of most high taxa from environmental surveys might be a reality by the end of this decade.[179] Phylogenetic diversity increases with read depth, and the optimal read depth is when diversity no longer increases.

Amplification Bias

The determination of microbial diversity is hampered due to PCR amplification and cloning-associated biases. Accuracy depends on primer choice (Table 4).

Table 4: Amplification Bias.

Factor	Impact
Primer choice	Coverage Primer-template binding affinity Spurious products Differential annealing capabilities and amplification efficiency[180]
Lack of consensus for universal primers	Prevents comparison between studies and comprehensive coverage of bacterial diversity[181]
Template concentration	Biases the determination of composition and the abundance of microbial communities[182]

Cloning Bias

Metagenomic clone libraries can be created by cloning large genomic fragments into cosmids or fosmids that are propagated in well-characterized host vectors, like *E. coli.*[183] These libraries often exhibit GC biases, poor transcriptional activity of the cloned DNA,[184] and occasional toxicity from expressed genes.[185] Long-term storage favors overgrowth of some clones at the expense of others, skewing the overall library diversity.[186]

Intragenomic Heterogeneity

Variable copy numbers, and the diversity of 16S rRNA sequences across different bacterial taxa and within species, are common. Bacteria contain an average of 4 copies of the 16S rRNA gene per genome, reaching a maximum of 15 copies in some *Fusobacteria*, with sequence variability increasing with copy number.[187] Due to the variability of 16S rRNA copy numbers, amplicon sequencing is limited in its

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ability to estimate bacterial community richness and abundance accurately. More importantly, amplicon sequencing cannot provide information on the genetic potential of the community.[188]

Horizontal Gene Transfer and Mosaicism

With 16S rRNA amplicon sequencing, it is not possible to assess horizontal gene transfer or genomic rearrangements outside or within the 16S rRNA genes. Interspecies exchange of complete 16S rRNA genes, and mosaicism within the operon by horizontal gene transfer, are tolerated in natural and experimental conditions due to conservation of secondary and tertiary ribosomal structures that provide functional plasticity.[189] The identification of species by amplicon sequencing may lead to incorrect taxa assignment and abundance biases.[190]

Metagenome Sequencing: Whole-Genome Shotgun Metagenomics

Metagenome sequencing, or whole-genome shotgun sequencing (Table 5), is the massively parallel sequencing of DNA fragments obtained from the complete gene repertoire in a microbial population. A major advantage of shotgun WGS is the ability to get an accurate phylogenetic tree, as well as the functional profiling of the community, in a single experiment. In functional profiling, the biological pathways represented in the community are reconstructed to determine in which primary activities the community is engaged.

CRISPR/Cas elements are critical in the bacteria's defense against foreign DNA. The use of CRISPR/Cas elements in bioengineering is now receiving attention for its potential use in medicine,[191] from regulating gene expression[192, 193] to eliminating antibiotic-resistance genes in bacteria.[194, 195]

Table 5: Applications for Metagenome Sequencing.

Applications	Reference
Discover rare microorganisms	196
Characterize unculturable microorganisms ("microbial dark matter")	197
Study epidemiology of transmission events	198
Discover immune-escape variants and drug-resistance networks	199
Track evolutionary dynamics and reconstruct genome sequences	200
Discover gene products that are functional within microbial communities but inactive or undetectable in monocultures	201
Characterize biological pathways to discover new chemical compounds	202
Discover compounds from bacterial communities in their natural habitats with high-throughput *in situ* cultivation	203, 204
Identify CRISPR/Cas systems	205

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Eukaryotes

Micro- and macro-eukaryotes are abundant, diverse, and ubiquitous in the environment.[206] In the mammalian gut, these organisms impact the bacterial environment, forming complex communities that impact health and disease.[207] Culture-based methods, and even microscopy techniques, are usually unsuccessful in identifying micro-eukaryotes due to their cryptic phenotypes.[208] Fortunately, NGS is not limited by phenotype, and it is improving our understanding about this enigmatic group, which still has many unknown sequences of uncharacterized genes. [209]

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Hugerth L. W., Muller E. E., Hu Y. O., Lebrun L. A., Roume H., et al. (2014) Systematic design of 18S rRNA gene primers for determining eukaryotic diversity in microbial consortia. PLoS One 9: e95567
There is a need to generate adequate primer sets for broad taxonomy-range identification by 18S rRNA gene high-throughput sequencing. The authors designed degenerate primers at every alignment position of annotated eukaryotic 18S rRNA sequences. Primers were tested in silico, and the best-performing pairs were further tested by amplicon sequencing of DNA from different sources. Regions V4 and V5 were the most informative and provided good agreement between tested samples and previous data from similar sources. Primer combinations can generate highly discriminatory sequences by NGS. However, the best-performing set reaches 79% accuracy at genus-level assignment, whereas 18S copy-number variation limits its use in semiquantitative approaches. Nevertheless, these primers can provide qualitative estimation of eukaryotic communities in complex samples.

Illumina Technology: Nextera DNA Library Prep Kit, MiSeq

Calo S., Shertz-Wall C., Lee S. C., Bastidas R. J., Nicolas F. E., et al. (2014) Antifungal drug resistance evoked via RNAi-dependent epimutations. Nature 513: 555-558

Single-Cell Sequencing (SCS)

SCS can reveal functional differences that may be fundamental for pathogenicity, transmissibility, and antibiotic resistance.[210] These differences may be hidden in mixed-cell populations. In nonculturable microorganisms, where pure cultures are unavailable, SCS can produce complete genome, transcriptome, or plasmid data from a single cell. This technique can provide insights into the evolution, phylogeny, and biological functions of "microbial dark matter" in human health.

In transcriptomics, single-cell RNA-Seq has the key advantage of resolving biases introduced by a few cells with high gene expression that skew the average expression level in the population.[211] The main challenges of SCS include capturing single cells, amplifying minimal amounts of nucleic acid, and analyzing data. There has been rapid progress in the development of techniques and bioinformatic tools to meet these challenges.[212, 213]

For comprehensive literature on single-cell DNA sequencing methods, refer to:

- All Illumina publication reviews (http://www.illumina.com/science/publications/publications-review.html)
- Illumina Methods Review (http://www.illumina.com/content/dam/illumina-marketing/documents/products/research_reviews/sequencing-methods-review.pdf)
- Illumina Single-Cell Sequencing Review (http://www.illumina.com/content/dam/illumina-marketing/documents/products/research_reviews/single-cell-sequencing-research-review.pdf)

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Wang Y. and Navin N. E. (2015) Advances and applications of single-cell sequencing technologies. Mol Cell 58: 598-609

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210. Franzosa E. A., Hsu T., Sirota-Madi A., Shafquat A., Abu-Ali G., et al. (2015) Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. Nat Rev Microbiol 13: 360-372
211. Wu A. R., Neff N. F., Kalisky T., Dalerba P., Treutlein B., et al. (2014) Quantitative assessment of single-cell RNA-sequencing methods. Nat Methods 11: 41-46
212. Ning L., Liu G., Li G., Hou Y., Tong Y., et al. (2014) Current challenges in the bioinformatics of single cell genomics. Front Oncol 4: 7
213. Macaulay I. C. and Voet T. (2014) Single cell genomics: advances and future perspectives. PLoS Genet 10: e1004126

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Wu A. R., Neff N. F., Kalisky T., Dalerba P., Treutlein B., et al. (2014) Quantitative assessment of single-cell RNA-sequencing methods. Nat Methods 11: 41-46

Accurate single-cell RNA-Seq is of interest to uncover expression patterns from precious samples and to avoid biases introduced by nonrepresentative cells. The authors evaluated single-cell transcriptomes (from human cultured cells) obtained with 2 different RNA amplification methods, followed by Nextera library prep and sequencing with a HiSeq instrument. Single-cell transcriptomes were compared against qPCR and RNA-Seq of the bulk sample. Pooling single-cell data resembled the real bulk sample transcriptome. The authors conclude that single-cell transcriptomes obtained by microfluidics yield accurate transcriptomes.

Illumina Technology: Nextera XT DNA Library Prep Kit, HiSeq 2000 150 bp PE reads

Plasmidome

Plasmids are self-replicating extrachromosomal genetic elements that may horizontally transfer genes among microbes in a population.[214, 215, 216] Metagenomes contain DNA from the hosts but also the plasmids they carry. Due to the small size of plasmids, their DNA is overwhelmingly less abundant than host DNA. As a result, the DNA extraction methods and plasmid copy number are critical for obtaining an accurate and complete plasmidome. Systems such as transposon-aided capture of plasmids (TRACA)[217] and multiple displacement amplification (MDA), coupled with NGS, have improved the resolution of these elements.[218]

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Dib J. R., Wagenknecht M., Farias M. E. and Meinhardt F. (2015) Strategies and approaches in plasmidome studies-uncovering plasmid diversity disregarding of linear elements? Front Microbiol 6: 463

Krupovic M. and Koonin E. V. (2015) Polintons: a hotbed of eukaryotic virus, transposon and plasmid evolution. Nat Rev Microbiol 13: 105-115

Jorgensen T. S., Kiil A. S., Hansen M. A., Sorensen S. J. and Hansen L. H. (2014) Current strategies for mobilome research. Front Microbiol 5: 750

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Fu Y., Liu L., Li X., Chen Y., Jiang Y., et al. (2015) Spread of a common blaNDM-1-carrying plasmid among diverse Acinetobacter species. Infect Genet Evol 32: 30-33

Carbapenem resistance is mediated by the gene New Delhi metallo-β-lactamase-1 (*NDM-1*). The gene is carried by plasmids that may have been transmitted by *Acinetobacter* to serious enteropathogens. The authors characterized *Acinetobacter* isolates carrying the *blaNDM-1* gene using PCR, 16S rRNA, and *blaOXA-5* gene sequencing and biochemical methods. They identified a common novel plasmid widely dispersed in diverse *Acinetobacter* species. The common backbone structure indicates that this plasmid is vital in transfer of the *blaNDM-1* gene among these different species.

Illumina Technology: HiSeq 2000

Nigro S. J., Holt K. E., Pickard D. and Hall R. M. (2015) Carbapenem and amikacin resistance on a large conjugative Acinetobacter baumannii plasmid. J Antimicrob Chemother 70: 1259-1261

The authors used NGS to obtain the complete sequence of the extensively resistant *A. baumannii* isolate D46 and its plasmid pD46-3, which simultaneously transfers resistance to carbapenems and aminoglycosides. The backbone of this plasmid resembles that of pD72-2 and pAb-G7-2 (repAci6 plasmids). It has acquired the blaOXA-23 gene, indicating a role in dissemination of carbapenem resistance in *A. baumannii*.

Illumina Technology: HiSeq

Yamaichi Y., Chao M. C., Sasabe J., Clark L., Davis B. M., et al. (2015) High-resolution genetic analysis of the requirements for horizontal transmission of the ESBL plasmid from *Escherichia coli* O104:H4. Nucleic Acids Res 43: 348-360

Relatively little is known about regulatory mechanisms for the dissemination of extended-spectrum beta-lactamases (ESBL). The authors used transposon insertion-site sequencing analysis (TnSeq)[219] to identify

214. Bleicher A., Schofl G., Rodicio Mdel R. and Saluz H. P. (2013) The plasmidome of a Salmonella enterica serovar Derby isolated from pork meat. Plasmid 69: 202-210
215. Brolund A., Franzen O., Melefors O., Tegmark-Wisell K. and Sandegren L. (2013) Plasmidome-analysis of ESBL-producing escherichia coli using conventional typing and high-throughput sequencing. PLoS One 8: e65793
216. Song X., Sun J., Mikalsen T., Roberts A. P. and Sundsfjord A. (2013) Characterisation of the plasmidome within Enterococcus faecalis isolated from marginal periodontitis patients in Norway. PLoS One 8: e62248
217. Jones B. V. and Marchesi J. R. (2007) Transposon-aided capture (TRACA) of plasmids resident in the human gut mobile metagenome. Nat Methods 4: 55-61
218. Brown Kav A., Benhar I. and Mizrahi I. (2013) A method for purifying high quality and high yield plasmid DNA for metagenomic and deep sequencing approaches. J Microbiol Methods 95: 272-279
219. van Opijnen T. and Camilli A. (2013) Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. Nat Rev Microbiol 11: 435-442

genes that enable the maintenance and spread of pESBL, a plasmid isolated from *E. coli* O104:H4. Two short regions constituted a novel regulatory system controlling the spread of this plasmid. Further, a methyltransferase encoded by pESBL protected pESBL from restriction in new hosts, suggesting it may play a role in expansion of host range.

Illumina Technology: MiSeq

Uchiya K., Takahashi H., Nakagawa T., Yagi T., Moriyama M., et al. (2015) Characterization of a novel plasmid, pMAH135, from Mycobacterium avium subsp. hominissuis. PLoS One 10: e0117797

Mycobacterium avium subsp. hominissuis causes pulmonary disease in nonimmunosuppressed individuals and in pigs. The authors used NGS to obtain the complete genome sequence of plasmid pMAH135, derived from *M. avium* isolated from an HIV-negative patient with pulmonary disease. pMAH135 contains sequence homology to mycobactin biosynthesis proteins, type VII secretion system proteins, and putative domains for multidrug efflux transporter. Genes carried by this plasmid were present more frequently in clinical pulmonary disease isolates and absent in pigs. The authors conclude that this plasmid influences both pathology and host specificity.

Illumina Technology: HiSeq 2000 100 bp PE reads

Hamidian M., Kenyon J. J., Holt K. E., Pickard D. and Hall R. M. (2014) A conjugative plasmid carrying the carbapenem resistance gene blaOXA-23 in AbaR4 in an extensively resistant GC1 Acinetobacter baumannii isolate. J Antimicrob Chemother 69: 2625-2628

NGS sequencing was applied to the Australian *A. baumannii* A85 isolate (Global Clone 1), an extensively resistant microbe harboring bla_{OXA-23} in Tn*2006*. Genes for resistance to older antibiotics are in the chromosome, in an AbaR3 resistance island. A second copy of the *ampC* gene in Tn6168 confers cephalosporin resistance, and the *gyrA* and *parC* genes have mutations leading to fluoroquinolone resistance. An 86,335 bp repAci6 plasmid (pA85-3, carrying bla_{OXA-23} in Tn*2006* in AbaR4) was shown to transfer resistance to imipenem, meropenem, and ticarcillin/clavulanate into a susceptible recipient. A85 also contains 2 small cryptic plasmids of 2.7 and 8.7 kb and carries a novel KL15 capsule locus and the OCL3 outer core locus.

Illumina Technology: HiSeq

Skarin H. and Segerman B. (2014) Plasmidome interchange between *Clostridium botulinum*, *Clostridium novyi* and *Clostridium haemolyticum* converts strains of independent lineages into distinctly different pathogens. PLoS One 9: e107777

Clostridium novyi sensu lato contains the closely related species *C. botulinum*, *C. novyi*, and *C. haemolyticum* with different pathogenic phenotypes associated with extrachromosomal mobile elements. WGS was performed in 24 representative strains of *Clostridium novyi sensu lato* and their respective plasmidomes. In addition, 4 *Clostridium novyi sensu lato* lineages and a large variable plasmidome composed of 13 plasmid groups (PG1 to PG13) were obtained. PG1 are unstable prophages encoding the BoNT gene cluster and the C3 toxin. The plasmids PG3, PG4, and PG6 can carry genes for C2 toxin, phospholipase C, and epsilon toxin B, while PG8-PG11 contains α-toxin-encoding prophages. There was modest correlation between species and lineages, perhaps due to the limited mobility of toxin-encoding plasmids and phages between lineages. This observation implies complex genetic relationships between these species: plasmids and phages, which may have been modularly exchanged, can create the pathogenic characteristics of these lineages.

Illumina Technology: TruSeq DNA Library Prep Kit, HiSeq 2000 100 bp PE reads

Transcriptome Sequencing

Transcriptome sequencing provides information on the activation of genes and biological pathways. This information provides insight into what activities the microbes are engaged in, to answer the question: “What are they doing?” When transcriptome sequences are compared to their genome sequences, it is possible to map and characterize genes, regulatory elements, and operon structures. For example, metatranscriptomic analysis revealed that small RNA (sRNA) encoded in intergenic regions can have multiple regulatory functions in archaea and bacteria.[220] Metatranscriptomic analysis can reveal complex interactions between community members to make efficient use of shared resources.

220. Kopf M. and Hess W. R. (2015) Regulatory RNAs in photosynthetic cyanobacteria. FEMS Microbiol Rev 39: 301-315

Combining transcriptomics with additional experimental techniques can provide detailed insight into pathogenic processes and the specific pathways involved in transmission and disease.[221] This approach is of great utility in genetically homogeneous microbes, such as *Salmonella enterica* serovar Enteritidis, where transcriptomic profiles can discern pathways associated with different pathogenic phenotypes.[222] There is a growing interest in the role of pathogens in cancer research, sparked by the discovery of pathogen transcriptomic signatures in tumors. The discovery highlights the role of the immune response and inflammation in host-pathogen interactions.[223]

221. Rosinski-Chupin I., Sauvage E., Sismeiro O., Villain A., Da Cunha V., et al. (2015) Single nucleotide resolution RNA-seq uncovers new regulatory mechanisms in the opportunistic pathogen Streptococcus agalactiae. BMC Genomics 16: 419
222. Shah D. H. (2014) RNA sequencing reveals differences between the global transcriptomes of Salmonella enterica serovar enteritidis strains with high and low pathogenicities. Appl Environ Microbiol 80: 896-906
223. Chen Y. and Wei J. (2015) Identification of Pathogen Signatures in Prostate Cancer Using RNA-seq. PLoS One 10: e0128955

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Miyakoshi M., Chao Y. and Vogel J. (2015) Regulatory small RNAs from the 3' regions of bacterial mRNAs. Curr Opin Microbiol 24: 132-139

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Yost S., Duran-Pinedo A. E., Teles R., Krishnan K. and Frias-Lopez J. (2015) Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis. Genome Med 7: 27

Periodontal disease is common worldwide, and current models hold oral microbiome dysbiosis accountable for periodontitis progression. The authors used metatranscriptomic and metagenomic analysis of microbial communities in subgingival biofilms to identify changes in community composition and expression networks. Global metabolic signatures were consistent with disease progression by upregulation of virulence factors from organisms not associated with disease and from recognized periodontal pathogens. Disease progression cannot be solely attributed to recognized pathogen virulence factors but to changes in community metabolic signatures that favor the expression of disease-causing genes. This study highlights the importance of transcriptomic studies in understanding periodontal disease.

Illumina Technology: Nextera XT DNA Library Prep Kit, MiSeq v2 150 bp PE reads

Shah D. H. (2014) RNA sequencing reveals differences between the global transcriptomes of Salmonella enterica serovar enteritidis strains with high and low pathogenicities. Appl Environ Microbiol 80: 896-906

S. enterica serovar Enteritidis (SE) causes bacterial food-borne gastroenteritis mainly transmitted by chicken eggs and poultry products. Strains are phenotypically diverse and vary in their virulence potential, despite their genetic homogeneity. RNA-Seq was used to characterize *in vitro* differences in global transcriptomes of SE strains with high and low pathogenicity at avian body temperature. The study identified 252 differentially expressed genes, mostly downregulated and related to transcriptional regulators, virulence, and motility in low-pathogenicity strains. This study provides a concise view of transcriptional signatures associated with pathogenicity levels in SE. These signatures may be targets of functional characterization studies, due to their potential role in virulence.

Illumina Technology: TruSeq RNA Library Prep Kit, HiSeq 2000 v3

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Kopf M., Klahn S., Scholz I., Hess W. R. and Voss B. (2015) Variations in the non-coding transcriptome as a driver of inter-strain divergence and physiological adaptation in bacteria. Sci Rep 5: 9560

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GLOSSARY OF TERMS

AL	anthropogenic litter
antagomir	A small synthetic RNA that is perfectly complementary to the specific miRNA
As	arsenic
Cryosols	Frozen soil within 1 meter of the surface and characterized by waterlogging during periods of thaw
DDT	dichlorodiphenyltrichloroethane.
Endosymbionts	Smaller symbiotic partners living inside a host organism
HMP	Human Microbiome Project
HPV	Human papillomavirus
iHMP	Integrative Human Microbiome Project
MERS-CoV	Middle East respiratory syndrome coronavirus
MMETSP	Marine Microbial Eukaryote Transcriptome Sequencing Project
NGS	Next-generation sequencing, also called high-throughput sequencing
PIT	particle interceptor traps
reassortment	Recombination of genomic material, especially as it occurs naturally in related viruses
SARS	Severe acute respiratory syndrome
SARS-CoV	SARS-associated coronavirus
Sb	antimony
tropism	Viral tropism is the specificity of a virus for a particular host tissue
WBD	White Band disease

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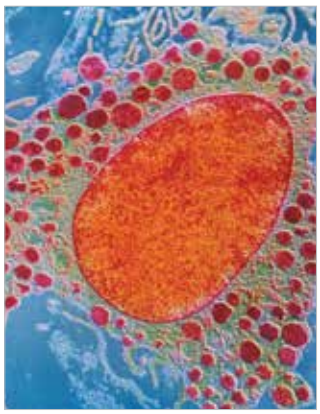

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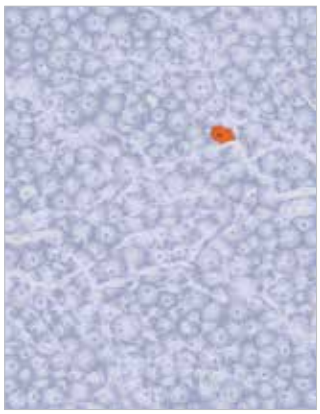

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