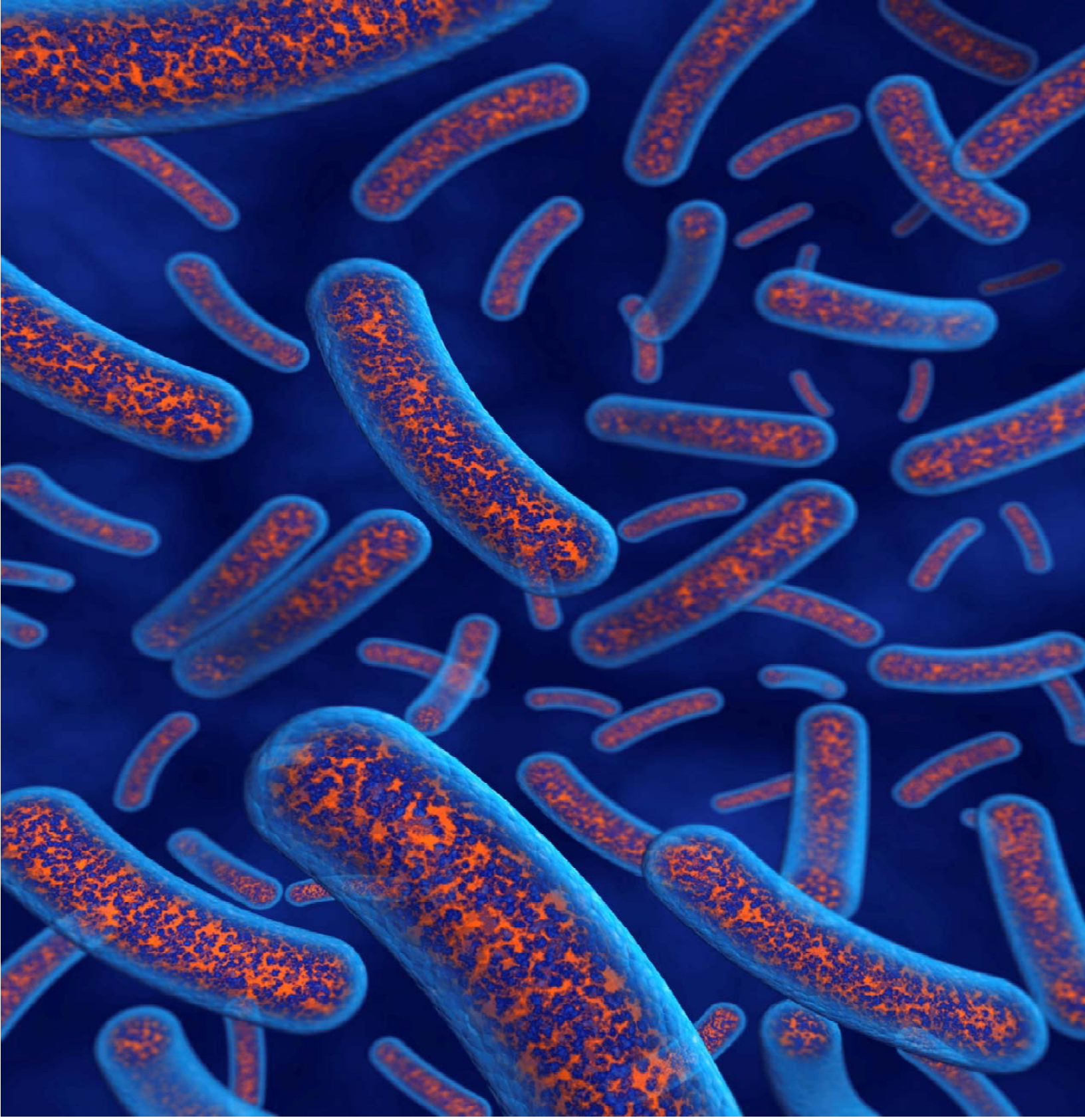

Infectious Disease Review

An Overview of Publications Featuring Illumina technology

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Introduction

The new generation of sequencing technology[1,2] is an extraordinary powerful set of tools that is opening the door to improved diagnosis and treatment of infectious diseases.[3,4] Whole-genome next-generation sequencing can both detect and identify infections agents in one assay without any prior knowledge of the clinical presentation. The same assay will also provide information about the antibiotic resistance, virulence and origin of the infectious agent. A universal test for all microbes provides a simplified and uniform laboratory workflow that is objective, independent of the skill of the operator and can readily be validated and controlled.[5] A more accurate diagnosis and treatment will lead to reduced hospital stays and the more considered use of antibiotics, but the ultimate beneficiary will be the patient who will benefit from the improved level of care.

This document is intended to highlight recent publications that demonstrate the application and potential of next-generation sequencing technologies to detect and control of infectious agents.

To learn more about Illumina sequencing technology, visit www.illumina.com.

[1] Next Generation Sequencing (NGS) and Massively Parallel Sequencing MPS are often used interchangeably to refer to high throughput sequencing technologies. Sequencing by Synthesis (SBS) refers specifically to Illumina sequencing technology.
[2] Didelot, X., Bowden, R., Wilson, D. J., Peto, T. E. and Crook, D. W. (2012) Transforming clinical microbiology with bacterial genome sequencing. Nat Rev Genet 13: 601-612
[3] Loman, N. J., Misra, R. V., Dallman, T. J., Constantinidou, C., Gharbia, S. E., et al. (2012) Performance comparison of benchtop high-throughput sequencing platforms. Nat Biotechnol 30: 434-439
[4] Sibley, C. D., Peirano, G. and Church, D. L. (2012) Molecular methods for pathogen and microbial community detection and characterization: current and potential application in diagnostic microbiology. Infect Genet Evol 12: 505-521
[5] Didelot, X., Bowden, R., Wilson, D. J., Peto, T. E. and Crook, D. W. (2012) Transforming clinical microbiology with bacterial genome sequencing. Nat Rev Genet 13: 601-612

General Reviews

The following reviews offer a useful introduction to the topic

Didelot, X., Bowden R., Wilson D. J., Peto T. E. and Crook D. W. (2012) Transforming clinical microbiology with bacterial genome sequencing. Nat Rev Genet 13: 601-612

The authors compare the current practices of identifying and typing bacteria to a workflow based on next-generation sequencing. They predict that the application of next-generation sequencing will soon be sufficiently fast, accurate and cheap to be used in routine clinical microbiology practice. It could replace many complex techniques currently used in clinical microbiology practice with a single, more efficient workflow. The review goes on to discuss the changes that will be required in the organization, skill mix and infrastructure of diagnostic laboratories to adopt this technology.

Loman, N. J., Misra R. V., Dallman T. J., Constantinidou C., Gharbia S. E., et al. (2012) Performance comparison of benchtop high-throughput sequencing platforms. Nat Biotechnol 30: 434-439

This review benchmarks the performance of the three commercially-available benchtop sequencing platforms. An isolate of Shiga-toxin-producing E. coli O104:H4 as sequenced and analyzed with commonly used assembly and analysis pipelines. Figure 1 shows the sequence quality produced by the various sequencers.

Loman, N. J., Constantinidou C., Chan J. Z., Halachev M., Sergeant M., et al. (2012) High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity. Nat Rev Microbiol 10: 599-606

Sibley, C. D., Peirano G. and Church D. L. (2012) Molecular methods for pathogen and microbial community detection and characterization: current and potential application in diagnostic microbiology. Infect Genet Evol 12: 505-521

Pathogen Identification

Traditional microbial identification relies on clinical symptoms and some prior knowledge to identify microorganisms. Some cases are atypical and defy identification. With the increase in international travel obscure tropical diseases can appear in unlikely places. To complicate the matter further some organisms are still unknown and many cannot be cultured in the laboratory. In some cases the organism can escape detection altogether.[6] Next-generation sequencing interrogates the genome directly and can detect and identify completely novel, uncultured organisms and at the same time derive information about their epidemiology and biology, such as virulence and antibiotic resistance.[7]

Sequencing does not require prior knowledge to identify microorganisms.

References

Moore, R. A., Warren R. L., Freeman J. D., Gustavsen J. A., Chenard C., et al. (2011) The sensitivity of massively parallel sequencing for detecting candidate infectious agents associated with human tissue. PLoS ONE 6: e19838

Some autoimmune diseases and several types of cancer are associated with pathogens. This study is a proof of principle to determine the detection level of viral transcripts through RNA-seq libraries spiked with decreasing amounts of an RNA-virus. The authors find that viral transcripts can be detected at frequencies less than 1 in 1,000,000 even at a modest depth of sequencing. This shows that NGS is a highly sensitive method for detecting putative infectious agents associated with human tissues.

Illumina Technology: Genome Analyzer$_{IIx}$

Engelthaler, D. M., Bowers J., Schupp J. A., Pearson T., Ginther J., et al. (2011) Molecular investigations of a locally acquired case of melioidosis in Southern AZ, USA. PLoS Negl Trop Dis 5: e1347

A recent case of melioidosis in non-endemic Arizona was determined to be the result of locally acquired infection, as the patient had no travel history to endemic regions and no previous history of disease. Diagnosis of the case was confirmed through multiple microbiologic and molecular techniques. This is a nice example of sequencing to identify a pathogen.

[6] Colvin, J. M., Jaffe, D. M. and Muenzer, J. T. (2012) Evaluation of the precision of emergency department diagnoses in young children with fever. Clin Pediatr (Phila) 51: 51-57

[7] Sibley, C. D., Peirano, G. and Church, D. L. (2012) Molecular methods for pathogen and microbial community detection and characterization: current and potential application in diagnostic microbiology. Infect Genet Evol 12: 505-521

Illumina Technology: Genome Analyzer$_{II}$ 50-bp, paired-end reads

Auburn, S., Campino S., Clark T. G., Djimde A. A., Zongo I., et al. (2011) An effective method to purify Plasmodium falciparum DNA directly from clinical blood samples for whole genome high-throughput sequencing. PLoS ONE 6: e22213

Cejkova, D., Zobanikova M., Chen L., Pospisilova P., Strouhal M., et al. (2012) Whole genome sequences of three Treponema pallidum ssp. pertenue strains: yaws and syphilis treponemes differ in less than 0.2% of the genome sequence. PLoS Negl Trop Dis 6: e1471

Koser, C. U., Niemann S., Summers D. K. and Archer J. A. (2012) Overview of errors in the reference sequence and annotation of Mycobacterium tuberculosis H37Rv, and variation amongst its isolates. Infect Genet Evol 12: 807-810

Virus Identification

The ambiguous symptoms of viral infections make them a diagnostic challenge. In addition their genetic nimbleness[8] and stringent culture requirements have made them a challenge to identify. Recent studies demonstrate the ability of next-generation sequencing to detect and identify viruses that have escaped detection by standard techniques.[9] This capability has led to new interest in searching for viral infections in tumors[10] and chronic diseases.[11]

The combination of high sensitivity without the need for prior information will make next-generation sequencing the primary tool for viral detection and identification.

References

Wylie, K. M., Mihindukulasuriya K. A., Sodergren E., Weinstock G. M. and Storch G. A. (2012) Sequence analysis of the human virome in febrile and afebrile children. PLoS ONE 7: e27735

The authors analyzed 176 nasopharyngeal swabs and plasma samples from children with unidentified fever and afebrile controls. They identified of sequences from 25 viral genera, including expected pathogens, such as adenoviruses, enteroviruses, and roseoloviruses, plus viruses with unknown pathogenicity. Differences between febrile and afebrile groups were most striking in the plasma samples, where detection of viral sequence may be associated with a disseminated infection. These data indicate that virus infection is associated with unidentified fever.

Illumina Technology: Genome Analyzer$_{IIx}$

Yozwiak, N. L., Skewes-Cox P., Stenglein M. D., Balmaseda A., Harris E., et al. (2012) Virus identification in unknown tropical febrile illness cases using deep sequencing. PLoS Negl Trop Dis 6: e1485

The authors used deep sequencing to detect virus sequence in 37% (45/123) of previously negative cases. These included 13 cases with Human Herpesvirus 6 sequences. Other samples contained sequences with similarity to sequences from viruses in the Herpesviridae, Flaviviridae, Circoviridae, Anelloviridae, Asfarviridae, and Parvoviridae families. In some cases, the putative viral sequences were virtually identical to known viruses, and in others they diverged, suggesting that they may derive from novel viruses. By contrast the Virochip analysis produced putative viral hits in 10/123 (8%) of the previously negative samples. These results demonstrate the utility of unbiased approaches in the detection of known and divergent viruses in the study of tropical febrile illness.

Illumina Technology: Genome Analyzer$_{II}$ and HiSeq 2000. Total nucleic acid from 140 ml of serum was extracted using the QIAamp Viral RNA Isolation Kit (Qiagen), which co-purifies RNA and DNA.

[8] Tapparel, C., Cordey, S., Junier, T., Farinelli, L., Van Belle, S., et al. (2011) Rhinovirus genome variation during chronic upper and lower respiratory tract infections. PLoS ONE 6: e21163
[9] Yozwiak, N. L., Skewes-Cox, P., Stenglein, M. D., Balmaseda, A., Harris, E., et al. (2012) Virus identification in unknown tropical febrile illness cases using deep sequencing. PLoS Negl Trop Dis 6: e1485
[10] Jiang, Z., Jhunjhunwala, S., Liu, J., Haverty, P. M., Kennemer, M. I., et al. (2012) The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. Genome Res 22: 593-601
[11] Kriesel, J. D., Hobbs, M. R., Jones, B. B., Milash, B., Nagra, R. M., et al. (2012) Deep sequencing for the detection of virus-like sequences in the brains of patients with multiple sclerosis: detection of GBV-C in human brain. PLoS ONE 7: e31886

Conway, C., Chalkley R., High A., Maclennan K., Berri S., et al. (2012) Next-generation sequencing for simultaneous determination of human papillomavirus load, subtype, and associated genomic copy number changes in tumors. J Mol Diagn 14: 104-111

This study uses next-generation sequencing to investigate viral infection in a variety of different tumor types stored as FFPE samples. The authors are able to detect HPV subtypes that would not have been detected by traditional methods.

Illumina Technology: Genome Analyzer

Nishijima, N., Marusawa H., Ueda Y., Takahashi K., Nasu A., et al. (2012) Dynamics of hepatitis B virus quasispecies in association with nucleos(t)ide analogue treatment determined by ultra-deep sequencing. PLoS ONE 7: e35052

To characterize the hepatitis B virus (HBV) genetic heterogeneity in association with anti-viral therapy, the authors performed ultra-deep sequencing of full-genome HBV in the liver and serum of 19 patients with chronic viral infection. They found that clones resistant to anti-viral therapy were common in both the liver and serum of treatment-naıve patients, which indicates the putative risk of developing drug resistance.

Illumina Technology: Genome Analyzer$_{II}$

Yongfeng, H., Fan Y., Jie D., Jian Y., Ting Z., et al. (2011) Direct pathogen detection from swab samples using a new high-throughput sequencing technology. Clin Microbiol Infect 17: 241-244

This paper describes the detection and identification of viruses from swabs. The authors could sequence and identify A (H1N1) and seasonal A (H3N2) influenza viruses without any a priori information. This approach could be a valuable tool for diagnosing emerging infectious diseases.

Illumina Technology: Genome Analyzer

Flaherty, P., Natsoulis G., Muralidharan O., Winters M., Buenrostro J., et al. (2012) Ultrasensitive detection of rare mutations using next-generation targeted resequencing. Nucleic Acids Res 40: e2

The authors demonstrate that they can robustly detect mutations at 0.1% fractional representation. This represents accurate detection of one mutant per every 1000 wild-type alleles. The method for detecting rare variants compares the baseline error rate from multiple reference replicates to the sample error rate at each position. To demonstrate the utility of the method they analyzed nine clinical samples of H1N1 influenza A and detected an oseltamivir (antiviral therapy) resistance mutation in the H1N1 neuraminidase gene at a sample fraction of 0.18%.

Illumina Technology: Genome Analyzer

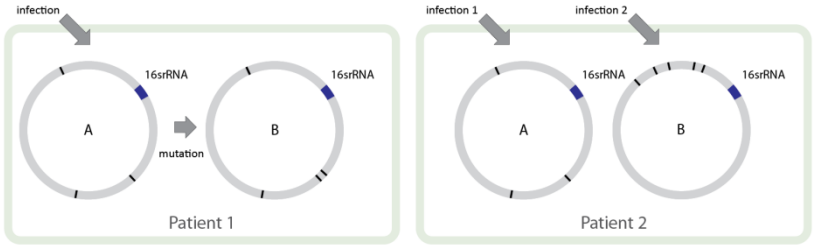
Kriesel, J. D., Hobbs M. R., Jones B. B., Milash B., Nagra R. M., et al. (2012) Deep sequencing for the detection of virus-like sequences in the brains of patients with multiple sclerosis: detection of GBV-C in human brain. PLoS ONE 7: e31886

Jiang, Z., Jhunjhunwala S., Liu J., Haverty P. M., Kennemer M. I., et al. (2012) The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. Genome Res 22: 593-601

Multiple- and Chronic Infections

Recurring infectious disease can be challenging to manage if the underlying cause is unknown.[12] Is the recurring disease due to a reservoir of untreated microbes in the patient or was the patient exposed to an external source of infection? The answer to this question will fundamentally change how the patient will be treated and the infection controlled.

In the course of an infection microbes acquire mutations as they adapt to the host responses and clinical interventions,[13] such as the development of antibiotic resistance during a chronic infection.[14] As a result naturally acquired infections can consist of several clones. Alternatively clones may also represent recurring infections.[15] Next generation sequencing provides a high resolution map of the microbe that can easily distinguish between those two possibilities.[16] The microbial sequence also provides insight in the development of antibiotic resistance and virulence.[17]



In patient 1 a single infection may lead to two clonal types due to evolution of the microbe. These clones may be highly similar and vary by as little as a single nucleotide mutation anywhere in the genome. In patient 2 there are several unrelated differences between the clones. This may be due to multiple infections. Multiple clones are of clinical interest for infection control

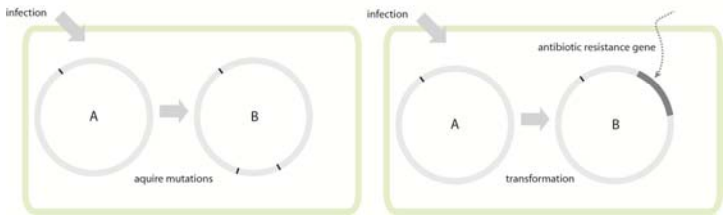
Some important human pathogens, both Gram positive and negative, can undergo transformation.[18] Genetic transformation is the process by which cells take up DNA from the environment and integrate the sequence into their genome.[19] Transformation is a clinically important mechanism because it allows bacteria to rapidly adapt in response to clinical interventions; examples include facilitating vaccine evasion and the development of penicillin resistance in the major respiratory pathogen Streptococcus pneumoniae.[20]

Clones are of clinical interest as they may harbor drug resistance genes that may lead to enhanced survival of the resistant clone after treatment and the possibility of subsequent therapeutic failure.

---

[12] Gysin, C., Alothman, G. A. and Papsin, B. C. (2000) Sinonasal disease in cystic fibrosis: clinical characteristics, diagnosis, and management. Pediatr Pulmonol 30: 481-489
[13] Levert, M., Zamfir, O., Clermont, O., Bouvet, O., Lespinats, S., et al. (2010) Molecular and evolutionary bases of within-patient genotypic and phenotypic diversity in Escherichia coli extraintestinal infections. PLoS Pathog 6: e1001125
[14] Gao, W., Chua, K., Davies, J. K., Newton, H. J., Seemann, T., et al. (2010) Two novel point mutations in clinical Staphylococcus aureus reduce linezolid susceptibility and switch on the stringent response to promote persistent infection. PLoS Pathog 6: e1000944
[15] Reeves, P. R., Liu, B., Zhou, Z., Li, D., Guo, D., et al. (2011) Rates of mutation and host transmission for an Escherichia coli clone over 3 years. PLoS ONE 6: e26907
[16] McAdam, P. R., Holmes, A., Templeton, K. E. and Fitzgerald, J. R. (2011) Adaptive evolution of Staphylococcus aureus during chronic endobronchial infection of a cystic fibrosis patient. PLoS ONE 6: e24301
[17] Sibley, C. D., Peirano, G. and Church, D. L. (2012) Molecular methods for pathogen and microbial community detection and characterization: current and potential application in diagnostic microbiology. Infect Genet Evol 12: 505-521
[18] Johnsborg, O., Eldholm, V. and Havarstein, L. S. (2007) Natural genetic transformation: prevalence, mechanisms and function. Res Microbiol 158: 767-778
[19] Griffith, F. (1928) The Significance of Pneumococcal Types. J Hyg (Lond) 27: 113-159
[20] Croucher, N. J., Harris, S. R., Barquist, L., Parkhill, J. and Bentley, S. D. (2012) A high-resolution view of genome-wide pneumococcal transformation. PLoS Pathog 8: e1002745

Current approaches require prior knowledge of the markers that can identify each clone and PCR frequently fail to detect low-abundance clones.



In patient 1 a single infection may progressively acquire mutations in response to clinical intervention. These clones may be highly similar and vary by as little as a single nucleotide mutation anywhere in the genome. In patient 2 the microbe actively acquires antibiotic resistance or virulence genes from donors in the environment. The process allows rapid changes in drug resistance or virulence.

References

Robinson, T., Campino S. G., Auburn S., Assefa S. A., Polley S. D., et al. (2011) Drug-resistant genotypes and multi-clonality in Plasmodium falciparum analysed by direct genome sequencing from peripheral blood of malaria patients. PLoS ONE 6: e23204[21]

The authors sequenced 5 non-propagated Plasmodium falciparum parasite isolates taken directly from 4 different patients treated for clinical malaria. The sequenced isolates contained both known and novel gene deletions and amplifications. Genome-wide analysis of clone multiplicity revealed that each patient harbored at least 3 clones of P. falciparum by this analysis. This demonstrates that genome sequencing of peripheral blood P. falciparum taken directly from malaria patients provides high quality data useful for drug resistance studies, genomic structural analyses and population genetics, and also robustly represents clonal multiplicity.

Illumina technology: Genome AnalyzerII generated 54- or 76bp PE sequence reads from 200–300bp DNA fragments

Avasthi, T. S., Devi S. H., Taylor T. D., Kumar N., Baddam R., et al. (2011) Genomes of two chronological isolates (Helicobacter pylori 2017 and 2018) of the West African Helicobacter pylori strain 908 obtained from a single patient. J Bacteriol 193: 3385-3386

The authors describe the genome sequences of the two serial isolates, H. pylori 2017 and 2018. The genome sequences reveal genomic alterations relevant to virulence optimization or host-specific adaptation.

Illumina technology: Genome Analyzer$_{IIx}$

Manske, M., Miotto O., Campino S., Auburn S., Almagro-Garcia J., et al. (2012) Analysis of Plasmodium falciparum diversity in natural infections by deep sequencing. Nature 487: 375-379

Antibiotic Resistance and Virulence

[21] Robinson, T., Campino, S. G., Auburn, S., Assefa, S. A., Polley, S. D., et al. (2011) Drug-resistant genotypes and multi-clonality in Plasmodium falciparum analysed by direct genome sequencing from peripheral blood of malaria patients. PLoS ONE 6: e23204

Antibiotic resistance is a serious consequence of evolutionary pressure in a bacterial population. Serotyping is commonly used as a marker for virulence. However, there is little or no functional connection between the genes that encode the bacterial capsule to determine the serotype and the genes associated with antibiotic resistance. The bacteria can change its capsule while retaining its virulence and antibiotic resistance.[22] Next-generation sequencing is proving to be a powerful tool to track the evolution of antibiotic resistance and virulence.

Four paper discs containing different antibiotics are placed on to a thin layer of Staphylococcus aureus bacteria in a Petri dish. The effects of antibiotic become visible by producing a reacting zone free of bacterial growth around the disc.

References

Harris, S. R., Clarke I. N., Seth-Smith H. M., Solomon A. W., Cutcliffe L. T., et al. (2012) Whole-genome analysis of diverse Chlamydia trachomatis strains identifies phylogenetic relationships masked by current clinical typing. Nat Genet 44: 413-419, S411

This paper presents a detailed phylogeny based on whole-genome sequencing of representative strains of C. trachomatis from both trachoma and lymphogranuloma venereum (LGV) biovars. It shows that predicting phylogenetic structure using ompA, which is traditionally used to classify Chlamydia, is misleading because extensive recombination in this region masks the true relationships.

Illumina technology: Genome Analyzer$_{II}$

Chua, K. Y., Seemann T., Harrison P. F., Monagle S., Korman T. M., et al. (2011) The dominant Australian community-acquired methicillin-resistant Staphylococcus aureus clone ST93-IV [2B] is highly virulent and genetically distinct. PLoS ONE 6: e25887

The comparisons of geographically and genetically diverse CA-MRSA genomes suggest that apparent convergent evolution in CA-MRSA may be better explained by the rapid dissemination of a highly conserved accessory genome from a common source. This is a good example of how misleading clinical and epidemiological profiles can be and how important it is to sequence whole bacterial genomes when tracking epidemics.

[22] Pillai, D. R., Shahinas, D., Buzina, A., Pollock, R. A., Lau, R., et al. (2009) Genome-wide dissection of globally emergent multi-drug resistant serotype 19A Streptococcus pneumoniae. BMC Genomics 10: 642

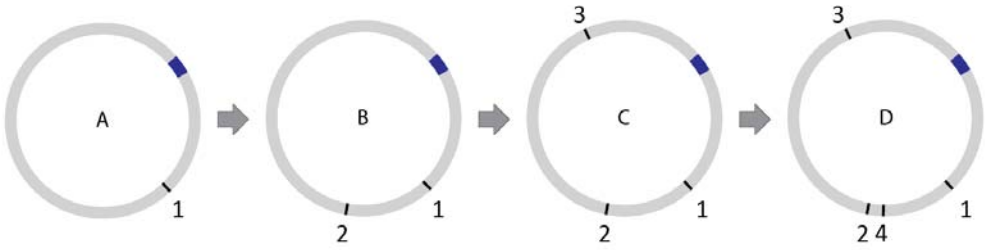
Illumina technology: Genome Analyzer$_{IIx}$ 36bp paired-end reads

Gonzalez-Escalona, N., Strain E. A., De Jesus A. J., Jones J. L. and Depaola A. (2011) Genome sequence of the clinical O4:K12 serotype Vibrio parahaemolyticus strain 10329. J Bacteriol 193: 3405-3406

Izumiya, H., Sekizuka T., Nakaya H., Taguchi M., Oguchi A., et al. (2011) Whole-genome analysis of Salmonella enterica serovar Typhimurium T000240 reveals the acquisition of a genomic island involved in multidrug resistance via IS1 derivatives on the chromosome. Antimicrob Agents Chemother 55: 623-630

Epidemics and Transmission

Epidemics and microbial transmission have traditionally been tracked with serological or other markers that monitor only a small, arbitrary part of the microbial genome. Due to the mosaic nature of the microbial genome any approach that monitors only a part of the genome will be relatively unreliable and insensitive. By contrast next-generation sequencing can track every base in the genome, which has led to a revolution in our understanding of these processes.[23,24] Transmission can now be tracked over relatively short periods of time even within families[25] or hospitals[26] and the source of the outbreak can be determined.[27,28,29] This allows a much more rapid and targeted response to outbreaks.[30] An additional benefit is that the mutations that accumulate during an outbreak can provide information about the potential development of antibiotic resistance and changes in virulence.[31,32]



As microbes accumulate mutations it is possible to track the sequence of transmission. The numbers indicate the addition of mutations in this hypothetical case. To track transmission events over short periods of time, such as weeks, the sequencing technology must be able to detect, with confidence, single base mutations anywhere in the genome.

Reviews

Lenski, R. E. (2011) Chance and necessity in the evolution of a bacterial pathogen. Nat Genet 43: 1174-1176

Editorial, N. B. (2011) Outbreak genomics. Nat Biotechnol 29: 769

[23] Croucher, N. J. (2009) From small reads do mighty genomes grow. Nat Rev Microbiol 7: 621
[24] He, M., Sebaihia, M., Lawley, T. D., Stabler, R. A., Dawson, L. F., et al. (2010) Evolutionary dynamics of Clostridium difficile over short and long time scales. Proc Natl Acad Sci U S A 107: 7527-7532
[25] Reeves, P. R., Liu, B., Zhou, Z., Li, D., Guo, D., et al. (2011) Rates of mutation and host transmission for an Escherichia coli clone over 3 years. PLoS ONE 6: e26907
[26] Harris, S. R., Feil, E. J., Holden, M. T., Quail, M. A., Nickerson, E. K., et al. (2010) Evolution of MRSA during hospital transmission and intercontinental spread. Science 327: 469-474
[27] Hendriksen, R. S., Price, L. B., Schupp, J. M., Gillece, J. D., Kaas, R. S., et al. (2011) Population genetics of Vibrio cholerae from Nepal in 2010: evidence on the origin of the Haitian outbreak. MBio 2: e00157-00111
[28] Mutreja, A., Kim, D. W., Thomson, N. R., Connor, T. R., Lee, J. H., et al. (2011) Evidence for several waves of global transmission in the seventh cholera pandemic. Nature 477: 462-465
[29] Grad, Y. H., Lipsitch, M., Feldgarden, M., Arachchi, H. M., Cerqueira, G. C., et al. (2012) Genomic epidemiology of the Escherichia coli O104:H4 outbreaks in Europe, 2011. Proc Natl Acad Sci U S A 109: 3065-3070
[30] Eyre, D. W., Golubchik, T., Gordon, N. C., Bowden, R., Piazza, P., et al. (2012) A pilot study of rapid benchtop sequencing of Staphylococcus aureus and Clostridium difficile for outbreak detection and surveillance. BMJ Open 2:
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[32] Tse, H., Bao, J. Y., Davies, M. R., Maamary, P., Tsoi, H. W., et al. (2012) Molecular characterization of the 2011 Hong Kong scarlet fever outbreak. J Infect Dis 206: 341-351

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Eyre, D. W., Golubchik T., Gordon N. C., Bowden R., Piazza P., et al. (2012) A pilot study of rapid benchtop sequencing of Staphylococcus aureus and Clostridium difficile for outbreak detection and surveillance. BMJ Open 2:

The authors successfully sequenced and analysed 26 MRSA and 15 C difficile isolates within 5 days of culture. Both MRSA clusters were identified as outbreaks, with most sequences in each cluster indistinguishable and all within three single nucleotide variants (SNVs). Epidemiologically unrelated isolates of the same spa-type were genetically distinct (≥21 SNVs). In both C difficile clusters, closely epidemiologically linked cases (in one case sharing the same strain type) were shown to be genetically distinct (≥144 SNVs). A reconstruction applying rapid sequencing in C difficile surveillance provided early outbreak detection and identified previously undetected probable community transmission. A useful observation in this study is that the same technology and procedures can be used regardless of microbe.

Illumina Technology: MiSeq 150bp paired-end reads

McAdam, P. R., Templeton K. E., Edwards G. F., Holden M. T., Feil E. J., et al. (2012) Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant Staphylococcus aureus. Proc Natl Acad Sci U S A 109: 9107-9112

Phylogeographic analysis indicates that EMRSA-16 spread within the United Kingdom by transmission from hospitals in large population centers in London and Glasgow to regional health-care settings, implicating patient referrals as an important cause of nationwide transmission.

Illumina Technology: Genome Analyzer II

Gardy, J. L., Johnston J. C., Ho Sui S. J., Cook V. J., Shah L., et al. (2011) Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. N Engl J Med 364: 730-739

An outbreak of M. tuberculosis in British Columbia in 2006 demonstrates how sequencing can be used to unravel a complex epidemic. When the results of mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR) genotyping and traditional contact tracing failed to identify a source of the M. tuberculosis epidemic, the authors used whole-genome sequencing and social-network analysis to describe the outbreak dynamics at a higher resolution. To do this, they used an Illumina Genome Analyzer II to sequence a total of 36 M. tuberculosis isolates (32 of the 37 outbreak isolates and 4 historical isolates with identical MIRU-VNTR patterns). This yielded an average of 99.21% of the reference genome being covered by at least one 50bp read. The higher-resolution SNP patterns afforded by whole-genome sequencing revealed that the outbreak was the coalescence of two outbreaks, each with its own causative lineage of M. tuberculosis. The simultaneous reappearance of two extant lineages suggests that a social or environmental factor, not a genetic change in the organism, most likely triggered the outbreak. A rise in crack cocaine use that peaked within the community at the outbreak of the epidemic may have been this trigger.

Illumina Technology: Genome Analyzer$_{II}$

Reeves, P. R., Liu B., Zhou Z., Li D., Guo D., et al. (2011) Rates of mutation and host transmission for an Escherichia coli clone over 3 years. PLoS ONE 6: e26907

The authors report the genome sequences of 14 isolates of a uropathogenic E. coli clone that persisted for 3 years within a household, including a dog. The host data imply at least 6 host transfer events over the 3 years, with 2 lineages present over much of that period. An earlier study using traditional typing techniques could not resolve the transmission.[33]

Illumina technology: Genome Analyzer$_{II}$

Reeves et al.[34] report the transmission of a a uropathogenic E. coli clone that persisted for 3 years within a household, including a dog. The data imply at least 6 host transfer events.

Hendriksen, R. S., Price L. B., Schupp J. M., Gillece J. D., Kaas R. S., et al. (2011) Population genetics of Vibrio cholerae from Nepal in 2010: evidence on the origin of the Haitian outbreak. MBio 2: e00157-00111

Comparison of the whole-genome sequences of Vibrio cholerae isolates from Haiti and Nepal showed that 24 Vibrio cholerae isolates from Nepal belonged to a single monophyletic group that also contained isolates from Bangladesh and Haiti. One cluster contained three Nepalese isolates and three Haitian isolates that were almost identical, with only 1- or 2-bp differences. Results in this study are consistent with Nepal as the origin of the Haitian outbreak.

Illumina technology: Genome Analyzer$_{IIx}$ multiplexed 76bp paired-end reads

Casali, N., Nikolayevskyy V., Balabanova Y., Ignatyeva O., Kontsevaya I., et al. (2012) Microevolution of extensively drug-resistant tuberculosis in Russia. Genome Res 22: 735-745

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