Microbial Genomics Research Review

An Overview of Publications Featuring Illumina® Technology

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Introduction

Next-generation sequencing[1] lends itself particularly well to the microbial laboratory, where the genomes are small. The appealing difference between sequencing and all other laboratory measurements is that the results can be directly related to a genomic locus and a potential explanation of the biological impact. This represents a quantum step forward in the interpretation of the experimental results and understanding of the biological system. The second advantage is the ability to measure single base changes anywhere in the genome without any prior knowledge. Single base resolution allows us to track microbial adaptation over short periods of time, both in the laboratory and in the environment. The advantages are so profound that in the foreseeable future it will be difficult to imagine a biological laboratory without a sequencer.

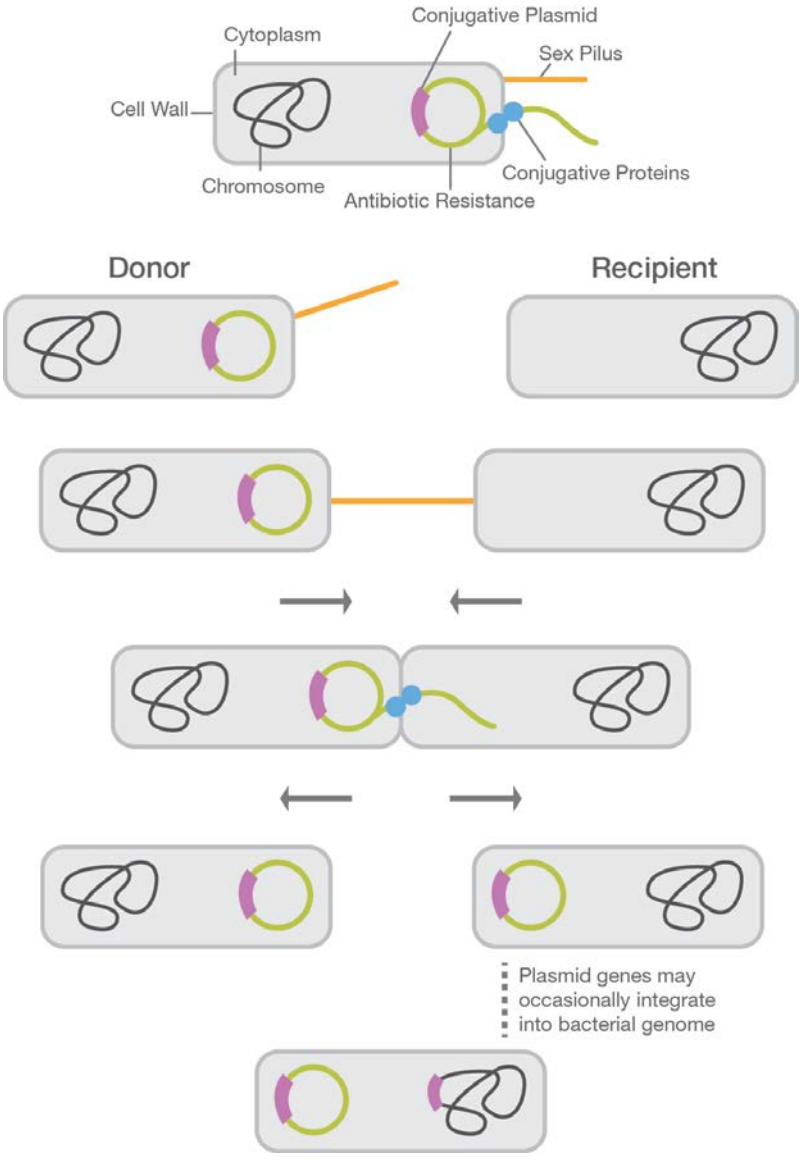
Recent studies have shown that the genomes of biological systems are remarkably active in adapting to the laboratory and clinical environments. Historically the spread of global epidemics was followed over a period of years. With the single base resolution of next-generation sequencing applied to bacterial genomes, it is possible to rapidly track epidemics within a local population, hospital, or even within a family over a period of weeks.

This review highlights recent examples where Illumina sequencing technology is used to track rapid genetic adaptation in nature, the laboratory, and the clinic.

[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.

Mosaicism

Horizontal gene transfer is the movement of genetic material between bacteria other than by descent.[2] This can happen through several mechanisms but the end result is mosaicism, where the majority of the organism's genome is composed of sequences inherited from its predecessors, with some fraction consisting of DNA fragments derived from other organisms in its environment. Since the exchange of genetic material is not by descent, it is unpredictable. This makes the microbial genome particularly challenging to analyze. Sequencing is the only tool that can accurately map mosaic genomes.



Horizontal transfer of functional genes, or even significant genomic rearrangements, may not be reported through methods that use markers representing only a small fraction of the genome.

[2] Maiden, M. C. (1998) Horizontal genetic exchange, evolution, and spread of antibiotic resistance in bacteria. Clin Infect Dis 27 Suppl 1: S12-20

Reviews:

Joseph, S. J. and Read T. D. (2012) Genome-wide recombination in Chlamydia trachomatis. Nat Genet 44: 364-366

Rajendhran, J. and Gunasekaran P. (2011) Microbial phylogeny and diversity: small subunit ribosomal RNA sequence analysis and beyond. Microbiol Res 166: 99-110

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 AnalyzerII

Wang, D., Wang H., Zhou Y., Zhang Q., Zhang F., et al. (2011) Genome sequencing reveals unique mutations in characteristic metabolic pathways and the transfer of virulence genes between V. mimicus and V. cholerae. PLoS ONE 6: e21299

Vibrio mimicus, the species most similar to V. cholerae, is a microbe present in the natural environment. It is naturally not particularly virulent and sometimes causes diarrhea and internal infections in humans. Horizontal transfer of virulence-related genes from an uncommon clone of V. cholera has resulted in the pathogenic V. mimicus strain carrying cholera toxin genes. This is an outstanding example of horizontal gene transfer (mosaicism) and the value of whole-genome sequencing.

Illumina Technology: Genome Analyzer with 130x coverage.

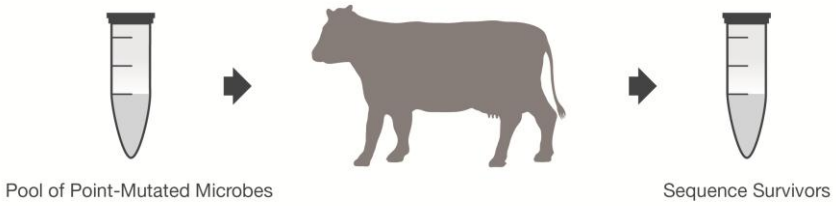
Gonzalez-Escalona, N., Strain E. A., De Jesus A. J., Jones J. L. and Depaola A. (2011) Genome sequence of the clinical O4:K12 serotype Vibrio parahaemolyticus strain 10329. J Bacteriol 193: 3405-3406

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

Fitness

There is a trade-off in microbial populations between the optimal adaption of a homogeneous population and the maintenance of less-optimal variants that will survive when conditions change.[3,4] For example, in a microbial population under antibiotic pressure, microbes that are antibiotic resistant will survive even though they may grow slower and appear less fit.[5,6] The fitness landscape describes the possible mutational trajectories by which lineages evolve in a stepwise manner from genotypes that lie in regions of low fitness to ones of higher fitness.[7]

A more systematic approach uses site-directed mutagenesis to introduce mutations into a pool of microbes before culturing or passage through an animal, followed by high throughput sequencing to detect the mutations in the survivors. This approach uses the strengths of high-throughput sequencing and is highly efficient.[8,9]



Use site-directed mutagenesis to introduce mutations into a pool of microbes before culturing or passage through an animal, followed by high throughput sequencing to detect the mutations in the survivors.[10]

References:

Goodman, A. L., Wu M. and Gordon J. I. (2011) Identifying microbial fitness determinants by insertion sequencing using genome-wide transposon mutant libraries. Nat Protoc 6: 1969-1980

This is a detailed description of insertion sequencing (INSeq), a method for determining the insertion site and relative abundance of large numbers of transposon mutants in a mixed population of isogenic mutants of a sequenced microbial species. The protocol is easy to scale up, amenable to automation, and useful for a variety of samples.

Illumina Technology: Protocol specific for GA/HiSeq

[3] Gerstein, A. C. and Otto, S. P. (2011) Cryptic fitness advantage: diploids invade haploid populations despite lacking any apparent advantage as measured by standard fitness assays. PLoS ONE 6: e26599
[4] Bachmann, H., Starrenburg, M. J., Molenaar, D., Kleerebezem, M. and van Hylckama Vlieg, J. E. (2012) Microbial domestication signatures of Lactococcus lactis can be reproduced by experimental evolution. Genome Res 22: 115-124
[5] Comas, I., Borrell, S., Roetzer, A., Rose, G., Malla, B., et al. (2012) Whole-genome sequencing of rifampicin-resistant Mycobacterium tuberculosis strains identifies compensatory mutations in RNA polymerase genes. Nat Genet 44: 106-110
[6] 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
[7] Kvitek, D. J. and Sherlock, G. (2011) Reciprocal sign epistasis between frequently experimentally evolved adaptive mutations causes a rugged fitness landscape. PLoS Genet 7: e1002056
[8] Moses, A. M. and Davidson, A. R. (2011) In vitro evolution goes deep. Proc Natl Acad Sci U S A 108: 8071-8072
[9] Han, T. X., Xu, X. Y., Zhang, M. J., Peng, X. and Du, L. L. (2010) Global fitness profiling of fission yeast deletion strains by barcode sequencing. Genome Biol 11: R60
[10] Eckert, S. E., Dziva, F., Chaudhuri, R. R., Langridge, G. C., Turner, D. J., et al. (2011) Retrospective application of transposon-directed insertion site sequencing to a library of signature-tagged mini-Tn5Km2 mutants of Escherichia coli O157:H7 screened in cattle. J Bacteriol 193: 1771-1776

Coffey, L. L., Beeharry Y., Borderia A. V., Blanc H. and Vignuzzi M. (2011) Arbovirus high fidelity variant loses fitness in mosquitoes and mice. Proc Natl Acad Sci U S A 108: 16038-16043

This study demonstrates the tradeoff between fitness and diversity in viral genomes.

Illumina Technology: Genome AnalyzerIIx

Eckert, S. E., Dziva F., Chaudhuri R. R., Langridge G. C., Turner D. J., et al. (2011) Retrospective application of transposon-directed insertion site sequencing to a library of signature-tagged mini-Tn5Km2 mutants of Escherichia coli O157:H7 screened in cattle. J Bacteriol 193: 1771-1776

Lee, C. Y., Kam Y. W., Fric J., Malleret B., Koh E. G., et al. (2011) Chikungunya virus neutralization antigens and direct cell-to-cell transmission are revealed by human antibody-escape mutants. PLoS Pathog 7: e1002390

Hietpas, R. T., Jensen J. D. and Bolon D. N. (2011) Experimental illumination of a fitness landscape. Proc Natl Acad Sci U S A 108: 7896-7901

Nair, D., Memmi G., Hernandez D., Bard J., Beaume M., et al. (2011) Whole-genome sequencing of Staphylococcus aureus strain RN4220, a key laboratory strain used in virulence research, identifies mutations that affect not only virulence factors but also the fitness of the strain. J Bacteriol 193: 2332-2335

The Core Genome

Within a taxonomic group there is an evolutionarily conserved set of core genes that is shared by all members of the group. This core genome is critical for survival. Individual members of the group also have accessory genes that contribute niche-specific phenotypes such as virulence and drug-resistance. It is important to distinguish between the core and accessory genes if we want to classify or modify the microbes. The core genome of conserved genes can be identified by comparing members of a taxonomic group. The core genome can also be determined experimentally by introducing random mutations and tracking the ability of the mutants to survive. If a critical gene is disabled, the microorganism will fail to survive under the culture conditions. This approach is very flexible and can be highly informative.

Reviews

Laing, C. R., Zhang Y., Thomas J. E. and Gannon V. P. (2011) Everything at once: comparative analysis of the genomes of bacterial pathogens. Vet Microbiol 153: 13-26

References:

Laing, C., Villegas A., Taboada E. N., Kropinski A., Thomas J. E., et al. (2011) Identification of Salmonella enterica species- and subgroup-specific genomic regions using Panseq 2.0. Infect Genet Evol 11: 2151-2161

In this study, the authors used PanSeq 2.0 to analyze 39 Salmonella enterica genomes (16 closed, 23 draft). Salmonella is a species that contains two human-specific serovars that cause typhoid fever, as well as a large number of zoonotic serovars that cause gastroenteritis in humans. The authors achieved high levels of discrimination, even amongst the most closely related strains of S. enterica Typhi. This information can then be used to determine the potential core genes of the species.

Evolution in the Laboratory

In the laboratory, the genomes of model organisms accumulate mutations over time.[11,12] As a result, the genomes of the same model organism may diverge over time and experiments will become progressively more difficult to replicate. Whole-genome resequencing has become a powerful tool to routinely monitor genomes and treat them just like any other experimental variable that needs to be recorded and controlled.

Based on the model of neutral evolution we expect bacteria to accumulate neutral mutations at a steady rate over time in a stable laboratory environment. In reality, the interaction between adaptive and neutral evolution is much more complex. A long-range study over 40,000 generations from a laboratory population of Escherichia coli showed that almost all the mutations appear to be beneficial and that there were sharp changes in neutral mutation over time in the apparently stable laboratory population.[13] It is also notable, and somewhat unsettling, that there appears to be no saturation in the number of mutations accumulated even after 40,000 generations.[14]

References:

Lee, D. H., Feist A. M., Barrett C. L. and Palsson B. O. (2011) Cumulative number of cell divisions as a meaningful timescale for adaptive laboratory evolution of Escherichia coli. PLoS ONE 6: e26172

The authors show that in short-term adaptive laboratory evolution (up to 40-50 days), Escherichia coli, under growth rate selection pressure, was found to undergo approximately 1011.2 total cumulative cell divisions within the population, producing a new stable growth phenotype that results from two to eight mutations. Continuous exposure to a low level of the mutagen can accelerate this timescale.

Illumina Technology: Genome Analyzer$_{II}$ 36 bp reads

Kashiwagi, A. and Yomo T. (2011) Ongoing phenotypic and genomic changes in experimental coevolution of RNA bacteriophage Qβ and Escherichia coli. PLoS Genet 7: e1002188

This paper describes the "arms race" between a bacterium and a phage when they are copropagated. E. coli first adapted by developing partial resistance to infection and later by increasing specific growth rate. The phage counter-adapted by improving release efficiency with a change in host specificity and a decrease in virulence.

Illumina Technology: Genome AnalyzerIIx 51 bp single reads

[11] Conrad, T. M., Joyce, A. R., Applebee, M. K., Barrett, C. L., Xie, B., et al. (2009) Whole-genome resequencing of Escherichia coli K-12 MG1655 undergoing short-term laboratory evolution in lactate minimal media reveals flexible selection of adaptive mutations. Genome Biol 10: R118

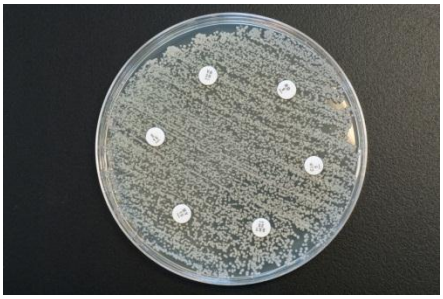
[12] Charusanti, P., Conrad, T. M., Knight, E. M., Venkataraman, K., Fong, N. L., et al. (2010) Genetic basis of growth adaptation of Escherichia coli after deletion of pgi, a major metabolic gene. PLoS Genet 6: e1001186

[13] Barrick, J. E. and Lenski, R. E. (2009) Genome-wide mutational diversity in an evolving population of Escherichia coli. Cold Spring Harb Symp Quant Biol 74: 119-129

[14] Harris, D. R., Pollock, S. V., Wood, E. A., Goiffon, R. J., Klingele, A. J., et al. (2009) Directed evolution of ionizing radiation resistance in Escherichia coli. J Bacteriol 191: 5240-5252

Antibiotic Resistance and Virulence

The development of antibiotic resistance can be considered as a special case of directed evolution. It has been the subject of intense research due to the increasing prevalence of antibiotic resistant strains.



A culture of a multiresistant bacterium. This pathogen is tested for the following antibiotics: VA: vancomycin, RD: rifampicin, CIP: ciprofloxacin, STX: cotrimoxazole, MY: clindamycin, E: erythromycin.
9004039

Due to the mosaic nature of the microbial genome, it can rapidly acquire antibiotic resistance genes or virulence-associated genes from the environment. The acquisition of these genes is often independent of serotype or gene markers. Next-generation sequencing of the whole genome has proved to be the ideal tool to comprehensively and unambiguously track antibiotic resistance- and virulence-associated genes.

The classic approach to studying the changes in antibiotic resistance and virulence is to compare isolates obtained from outbreaks and epidemics.[15,16] A new approach follows the microbial adaptation to sustained antibiotic pressure in the laboratory. It takes advantage of the ability of next-generation sequencing to detect single nucleotide changes rapidly and cost-effectively. The advantages of this approach are that all variables can be carefully controlled in a laboratory environment and the results can be directly related to biological functions.[17]

References:

Toprak, E., Veres A., Michel J. B., Chait R., Hartl D. L., et al. (2012) Evolutionary paths to antibiotic resistance under dynamically sustained drug selection. Nat Genet 44: 101-105

The authors analyzed the evolution of resistance in Escherichia coli under selection with single drugs, including chloramphenicol, doxycycline and trimethoprim. Over a period of approximately 20 days, resistance levels increased dramatically with parallel populations showing similar phenotypic trajectories. Whole-genome sequencing of the evolved strains identified mutations both specific to resistance of a particular drug and shared in resistance to multiple drugs. This is an example of the power that routine sequencing can bring to a microbiology laboratory.

Illumina Technology: Genome Analyzer$_{IIx}$ 75 bp single-end reads

[15] 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
[16] Howden, B. P., McEvoy, C. R., Allen, D. L., Chua, K., Gao, W., et al. (2011) Evolution of multidrug resistance during Staphylococcus aureus infection involves mutation of the essential two component regulator WalKR. PLoS Pathog 7: e1002359
[17] Toprak, E., Veres, A., Michel, J. B., Chait, R., Hartl, D. L., et al. (2012) Evolutionary paths to antibiotic resistance under dynamically sustained drug selection. Nat Genet 44: 101-105

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 the 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.

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

Howden, B. P., McEvoy C. R., Allen D. L., Chua K., Gao W., et al. (2011) Evolution of multidrug resistance during Staphylococcus aureus infection involves mutation of the essential two component regulator WalKR. PLoS Pathog 7: e1002359

Peleg, A. Y., Miyakis S., Ward D. V., Earl A. M., Rubio A., et al. (2012) Whole genome characterization of the mechanisms of daptomycin resistance in clinical and laboratory derived isolates of Staphylococcus aureus. PLoS ONE 7: e28316

Lieberman, T. D., Michel J. B., Aingaran M., Potter-Bynoe G., Roux D., et al. (2011) Parallel bacterial evolution within multiple patients identifies candidate pathogenicity genes. Nat Genet 43: 1275-1280

Thomas, J. C., Figueira M., Fennie K. P., Laufer A. S., Kong Y., et al. (2011) Streptococcus pneumoniae clonal complex 199: genetic diversity and tissue-specific virulence. PLoS ONE 6: e18649

Harvey, R. M., Stroeher U. H., Ogunniyi A. D., Smith-Vaughan H. C., Leach A. J., et al. (2011) A variable region within the genome of Streptococcus pneumoniae contributes to strain-strain variation in virulence. PLoS ONE 6: e19650

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.[18] Transmission can now be tracked over relatively short periods of time, even within families[19] or hospitals[20] and the source of the outbreak can be determined.[21,22,23] This allows a much more rapid and targeted response to outbreaks. 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.[24]

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

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 Chlamydia 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 presence of the true relationships.

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 three years within a household, including a dog. The host data imply at least six host transfer events occurred over the three years, with two lineages present over much of that period. An earlier study using traditional typing techniques did not resolve the transmission.[25]

Illumina Technology: Genome Analyzer$_{II}$

[18] Croucher, N. J. (2009) From small reads do mighty genomes grow. Nat Rev Microbiol 7: 621
[19] 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
[20] 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
[21] 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
[22] 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
[23] 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
[24] Howden, B. P., McEvoy, C. R., Allen, D. L., Chua, K., Gao, W., et al. (2011) Evolution of multidrug resistance during Staphylococcus aureus infection involves mutation of the essential two component regulator WalKR. PLoS Pathog 7: e1002359
[25] Johnson, J. R., Clabots, C. and Kuskowski, M. A. (2008) Multiple-host sharing, long-term persistence, and virulence of Escherichia coli clones from human and animal household members. J Clin Microbiol 46: 4078-4082

Reeves et al.[26] Next-generation sequencing was able to resolve multiple host transfer events within a family and their dog over several years where traditional techniques had previously failed.

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 bp 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 76 bp paired-end reads

Omer, H., Rose G., Jolley K. A., Frapy E., Zahar J. R., et al. (2011) Genotypic and phenotypic modifications of Neisseria meningitidis after an accidental human passage. PLoS ONE 6: e17145

This is a very interesting study of an accidental infection that occurred in the lab while working with Z5463, a Neisseria meningitidis serogroup A strain. The authors estimate that 25 bacterial divisions occurred in the infected human body. The in vivo passage, despite the small number of divisions, permitted the selection of numerous genomic modifications, which may account for the strain's high capacity to spread.

Illumina Technology: Genome Analyzer giving a 154-, 84- and 78-fold coverage.

Fittipaldi, N., Beres S. B., Olsen R. J., Kapur V., Shea P. R., et al. (2012) Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A Streptococcus. Am J Pathol 180: 1522-1534

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

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

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

[26] 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

Microbial Identification

Traditional microbial identification relies on clinical symptoms and some prior knowledge to identify microorganisms. Some cases are atypical and defy identification. The agnostic nature of microbial detection and sequencing with next-generation sequencing makes it a very useful tool in those cases.

Sequencing does not require prior knowledge to identify microorganisms.

References:

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 using sequencing to identify a pathogen.

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

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

Directed Evolution and Bioengineering

Directed evolution is emerging as a promising new supplement to standard bioengineering techniques. Microbes adapt remarkably quickly to changes in the environment. By systematically changing the environment, researchers can track the changes in gene expression and the mutations incorporated by the organisms to achieve desirable characteristics, for example, adaptive evolution to grow on galactose. In replicated experiments, the organism may achieve the same desirable characteristics by modifying different pathways. This provides a spectrum of possible bioengineering solutions.

References:

Bachmann, H., Starrenburg M. J., Molenaar D., Kleerebezem M. and van Hylckama Vlieg J. E. (2012) Microbial domestication signatures of Lactococcus lactis can be reproduced by experimental evolution. Genome Res 22: 115-124

This paper demonstrates the adaptation of a Lactococcus lactis strain isolated from a plant to a dairy niche by propagating it for 1000 generations in milk. Two out of three independently evolved strains displayed significantly increased acidification rates and biomass yields in milk. Reproducing the transition from the plant to the dairy niche through experimental evolution revealed several genome, transcriptome, and phenotype signatures that resemble those seen in strains isolated from either niche. This is an interesting type of experiment that uses next-generation sequencing effectively to understand the pathways involved in adaptive evolution and genetic engineering.

Illumina Technology: Genome Analyzer

Hong, K. K., Vongsangnak W., Vemuri G. N. and Nielsen J. (2011) Unravelling evolutionary strategies of yeast for improving galactose utilization through integrated systems level analysis. Proc Natl Acad Sci U S A 108: 12179-12184

This paper tracks metabolic changes occurring in the yeast Saccharomyces cerevisiae as a result of its adaptive evolution to grow on galactose. The study demonstrates that adaptive evolution represents a valuable alternative to rational design in the bioengineering of improved strains, and that it is possible to identify mutations in evolved strains that can serve as unforeseen metabolic engineering targets for improving microbial strains for production of biofuels and chemicals.

Illumina Technology: Genome Analyzer$_{IIx}$ with 38 bp paired-end reads

Gibbons, H. S., Broomall S. M., McNew L. A., Daligault H., Chapman C., et al. (2011) Genomic signatures of strain selection and enhancement in Bacillus atrophaeus var. globigii, a historical biowarfare simulant. PLoS ONE 6: e17836

Kvitek, D. J. and Sherlock G. (2011) Reciprocal sign epistasis between frequently experimentally evolved adaptive mutations causes a rugged fitness landscape. PLoS Genet 7: e1002056

Biofuels and Bioremediation

The search for new microbes for the creation of biofuels and bioremediation is usually carried out with metagenomic approaches. However, evolutionary pressure and manipulation in the laboratory can be very effective in improving newly-discovered candidates.

A sewage treatment plant

References:

Summers, Z. M., Ueki T., Ismail W., Haveman S. A. and Lovley D. R. (2012) Laboratory evolution of Geobacter sulfurreducens for enhanced growth on lactate via a single-base-pair substitution in a transcriptional regulator. ISME J 6: 975-983

The authors demonstrated that a single base-pair mutation in a transcriptional regulator can have a significant impact on the capacity for substrate utilization and suggest that adaptive evolution should be considered as a potential response of microorganisms to environmental change(s) imposed during bioremediation.

Illumina Technology: Genome Analyzer$_{II}$

Minty, J. J., Lesnefsky A. A., Lin F., Chen Y., Zaroff T. A., et al. (2011) Evolution combined with genomic study elucidates genetic bases of isobutanol tolerance in Escherichia coli. Microb Cell Fact 10: 18

The authors apply experimental evolution followed by genome resequencing and a gene expression study to elucidate genetic bases of adaptation to exogenous isobutanol stress. The evolved lineages exhibit adaptation to isobutanol stress based on remodeling the cell envelope and, surprisingly, stress response attenuation.

Illumina Technology: Genome Analyzer 36 bp single-end and paired-end sequencing with 125x and 500x read depth, respectively.

Radakovits, R., Jinkerson R. E., Fuerstenberg S. I., Tae H., Settlage R. E., et al. (2012) Draft genome sequence and genetic transformation of the oleaginous alga Nannochloropis gaditana. Nat Commun 3: 686

Tremblay, P. L., Summers Z. M., Glaven R. H., Nevin K. P., Zengler K., et al. (2011) A c-type cytochrome and a transcriptional regulator responsible for enhanced extracellular electron transfer in Geobacter sulfurreducens revealed by adaptive evolution. Environ Microbiol 13: 13-23

Viruses

The unique replication strategies of viruses make it particularly challenging to track their evolution in the laboratory. In most living cells misincorporations occur once every billion bases, while in some viruses errors can occur as often as once every thousand bases copied. This results from the use of enzymes without proofreading activity (RdRp or RT) or limited repair activity.[27] Not only do mutations occur more frequently, many copies are made very quickly. A virus may be copied hundreds or even thousands of times in a single life cycle compared to the two progeny cells that result from a single cell cycle.[28]

Virus Detection and Identification

The ambiguous symptoms of viral infections have made them a diagnostic challenge. In addition, their genetic nimbleness has made them a challenge to identify. A recent study by Yozwiak et al. demonstrates the ability of next-generation sequencing to detect and identify viruses that have escaped detection by standard techniques.[29] This capability has generated new interest is searching for viral infections in tumors[30] and chronic diseases.[31]

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:

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 viral sequences in 37% (45/123) of previously negative cases. These included 13 cases with human herpesvirus 6 sequences. Other samples contained sequences similar to viral sequences found 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 be derived 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.

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[30] 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
[31] 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 human papillomavirus subtypes that would not have been detected by traditional methods and show that this approach could be applied to any tumor and any virus.

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 perform 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-naïve patients, which indicates the putative risk of developing drug resistance.

Illumina Technology: Genome Analyzer$_{II}$

Blasdell, K. R., Voysey R., Bulach D., Joubert D. A., Tesh R. B., et al. (2012) Kotonkan and Obodhiang viruses: African ephemeroviruses with large and complex genomes. Virology 425: 143-153

Dunowska, M., Biggs P. J., Zheng T. and Perrott M. R. (2012) Identification of a novel nidovirus associated with a neurological disease of the Australian brushtail possum (Trichosurus vulpecula). Vet Microbiol 156: 418-424

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

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

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

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

Vaccine Production

The manufacture of live viral vaccines requires rigorous quality control to ensure vaccine safety. One of the major risks is that the intrinsic genetic instability of RNA viruses may lead to the accumulation of virulent revertants during manufacture.

The human cytomegalovirus is a good example of the challenges researchers face in the laboratory. Commonly used variants of human cytomegalovirus (HCMV) strains – Towne and AD169 – have been distributed widely and developed as vaccine candidates. Over the years their detailed histories have become obscure and it has become clear that the biological properties of these strains are not conserved between stocks. These genetic differences may affect the interpretation of experimental studies and will obviously significantly impact their use in developing vaccines.[32]

References:

Neverov, A. and Chumakov K. (2010) Massively parallel sequencing for monitoring genetic consistency and quality control of live viral vaccines. Proc Natl Acad Sci U S A 107: 20063-20068

In a recent evaluation of MPS platforms at the Center for Biologics Evaluation and Research at the Food and Drug Administration, the authors found that MPS offered significant advantages over standard quality control tool in vaccine production. MPS "… may represent the ultimate tool for monitoring genetic consistency of live viral vaccines." The currently used mutant analysis by PCR and restriction enzyme cleavage (MAPREC) method measures the frequency of neurovirulent mutations at the 5' untranslated region (UTR) of the viral genome. This region correlates with the level of neurovirulence determined by the monkey neurovirulence test. However, MAPREC can only monitor mutations at a few genomic loci and misses mutations at other sites that could adversely affect vaccine quality. A critical advantage of MPS over MAPREC is that it allows all nucleotide positions in complete viral genomes to be screened in one assay and therefore addresses concerns that mutations at unknown genomic loci could emerge but remain undetected and thus compromise vaccine quality. In this evaluation the MPS results were in perfect agreement with MAPREC results. An unexpected benefit was that the authors were able to distinguish patterns of mutations in the MPS data that were characteristic for the specific seed virus. This would allow the tracking of vaccine lots based on the mutation pattern in the seed.

Illumina Technology: Genome Analyzer$_{IIX}$

Szpara, M. L., Tafuri Y. R., Parsons L., Shamim S. R., Verstrepen K. J., et al. (2011) A wide extent of inter-strain diversity in virulent and vaccine strains of alphaherpesviruses. PLoS Pathog 7: e1002282

The authors present full genome sequence comparisons of the veterinary herpesvirus, pseudorabies vaccine strain Bartha, and two virulent veterinary herpesvirus, pseudorabies isolates, Kaplan and Becker. These data add to growing evidence that even plaque-purified stocks of stable DNA viruses exhibit limited sequence heterogeneity, which likely seeds future strain evolution.

Illumina Technology: Genome Analyzer$_{II}$ 38 bp reads

Checkley, A. M., Wyllie D. H., Scriba T. J., Golubchik T., Hill A. V., et al. (2011) Identification of antigens specific to non-tuberculous mycobacteria: the Mce family of proteins as a target of T cell immune responses. PLoS ONE 6: e26434

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Yeast

The yeast genome is capable of remarkably complex genetic changes when under environmental pressure. A strain isolated after approximately 188 generations of a sulfate-limited continuous culture of Saccharomyces cerevisiae strain DBY10147 was sequenced by MPS. The authors found both single nucleotide polymorphisms and copy number amplifications that were not found by previous array-based studies.[33]

Sequencing does not rely on the reference genome, which is an advantage in the many cases where the reference genome may be unavailable or incorrect. For example, when two strains of yeast S288C (12x coverage) and RM11 (15x coverage) were sequenced the authors found 803 and 1104 errors, respectively, in the public sequences.[34] Any errors and omissions in the reference genomes will be designed into microarrays or tiling arrays that are based on those genomes.

References:

Ma, X., Rogacheva M. V., Nishant K. T., Zanders S., Bustamante C. D., et al. (2012) Mutation hot spots in yeast caused by long-range clustering of homopolymeric sequences. Cell Rep 1: 36-42

The authors use high-coverage whole-genome sequencing of a conditional mismatch repair mutant line of diploid yeast to identify mutations that accumulated after 160 generations of growth. The vast majority of the mutations accumulated as insertion/deletions (indels) in homopolymeric [poly(dA:dT)] and repetitive DNA tracts. Surprisingly, the likelihood of an indel mutation in a given poly(dA:dT) tract is increased by the presence of nearby poly(dA:dT) tracts in up to a 1,000 bp region centered on the given tract.

Illumina Technology: Genome Analyzer 101 bp reads

Hong, K. K., Vongsangnak W., Vemuri G. N. and Nielsen J. (2011) Unravelling evolutionary strategies of yeast for improving galactose utilization through integrated systems level analysis. Proc Natl Acad Sci U S A 108: 12179-12184

This paper tracks metabolic changes occurring in the yeast Saccaromyces cerevisiae to increase its specific growth rate on galactose. The study demonstrates that adaptive evolution represents a valuable alternative to rational design in bioengineering and that it is possible to identify mutations in evolved strains that can serve as unforeseen metabolic engineering targets for improving microbial strains for production of biofuels and chemicals.

Illumina Technology: Genome Analyzer$_{IIX}$ 38 bp paired-end reads

Berry, D. B., Guan Q., Hose J., Haroon S., Gebbia M., et al. (2011) Multiple means to the same end: the genetic basis of acquired stress resistance in yeast. PLoS Genet 7: e1002353

Libkind, D., Hittinger C. T., Valerio E., Goncalves C., Dover J., et al. (2011) Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc Natl Acad Sci U S A 108: 14539-14544

Parts, L., Cubillos F. A., Warringer J., Jain K., Salinas F., et al. (2011) Revealing the genetic structure of a trait by sequencing a population under selection. Genome Res 21: 1131-1138

Warringer, J., Zorgo E., Cubillos F. A., Zia A., Gjuvsland A., et al. (2011) Trait variation in yeast is defined by population history. PLoS Genet 7: e1002111

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[34] Qi, J., Wijeratne, A. J., Tomsho, L. P., Hu, Y., Schuster, S. C., et al. (2009) Characterization of meiotic crossovers and gene conversion by whole-genome sequencing in Saccharomyces cerevisiae. BMC Genomics 10: 475

Escherichia Coli

It has been observed that isolates from some bacterial infections exhibit within-species diversity. Several lines of evidence suggest that this micro-heterogeneity is due to diversification during the infection process rather than an infection by multiple isolates. The observed diversity resembles results obtained in experimental evolution studies. Whatever the mechanisms leading to diversity, the results emphasize the need for more extensive isolate testing before deciding on antibiotic therapies.

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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

Benson, R. W., Norton M. D., Lin I., Du Comb W. S. and Godoy V. G. (2011) An active site aromatic triad in Escherichia coli DNA Pol IV coordinates cell survival and mutagenesis in different DNA damaging agents. PLoS ONE 6: e19944

Sahl, J. W., Steinsland H., Redman J. C., Angiuoli S. V., Nataro J. P., et al. (2011) A comparative genomic analysis of diverse clonal types of enterotoxigenic Escherichia coli reveals pathovar-specific conservation. Infect Immun 79: 950-960

Staphylococcus Aureus

The spread of antibiotic resistance is usually tracked over long periods. For example, DNA sequences of methicillin-resistant Staphylococcus aureus (MRSA) (ST225) serially sampled through time led the authors to estimate that ST225 had diverged since approximately 1990 (1987 to 1994), and that expansion of the European clade began in 1995 (1991 to 1999), several years before the new clone was recognized.[35]

The spread of antibiotic resistance is also complicated by the potential of horizontal gene transfer. Some clinical MRSA strains are deficient in type III-like restriction endonuclease systems and are therefore hypersusceptible to the horizontal transfer of DNA from other species, such as Escherichia coli. For example, susceptible Staphylococcus aureus strains could easily acquire a vancomycin-resistance gene from enterococci.[36]

A host jump represents one of the most dramatic examples of genetic adaptation. The majority of S. aureus isolates from broiler chickens are the descendants of a single human-to-poultry host jump that occurred approximately 38 years ago (range, 30 to 63 years ago) by a subtype of the worldwide human ST5 clonal lineage unique to Poland. This represents the evolutionary history of a major new animal pathogen that has undergone rapid avian host adaptation and intercontinental dissemination and is a new paradigm for the study of the impact of human activities on the emergence and spread of animal pathogens.[37]

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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

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

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Streptococcus

Streptococcus is a Gram-positive bacteria belonging to the phylum Firmicutes with a genome size of approximately two million bases[38]

References:

Thomas, J. C., Figueira M., Fennie K. P., Laufer A. S., Kong Y., et al. (2011) Streptococcus pneumoniae clonal complex 199: genetic diversity and tissue-specific virulence. PLoS ONE 6: e18649

Harvey, R. M., Stroeher U. H., Ogunniyi A. D., Smith-Vaughan H. C., Leach A. J., et al. (2011) A variable region within the genome of Streptococcus pneumoniae contributes to strain-strain variation in virulence. PLoS ONE 6: e19650

Shea, P. R., Beres S. B., Flores A. R., Ewbank A. L., Gonzalez-Lugo J. H., et al. (2011) Distinct signatures of diversifying selection revealed by genome analysis of respiratory tract and invasive bacterial populations. Proc Natl Acad Sci U S A 108: 5039-5044

Mycobacterium

The fungus-like mycobacteria are responsible for a wide range of diseases, from tuberculosis to leprosy. With a genome size of four million base pairs with 3959 genes it is easily sequenced by next-generation sequencers.

References:

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 Mycobacterium 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 sequenced a total of 36 Mycobacterium tuberculosis isolates (32 of the 37 outbreak isolates and four historical isolates with identical MIRU-VNTR patterns). This yielded an average of 99.21% of the reference genome being covered by at least one 50 bp 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 Mycobacterium 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 within the community, which peaked at the outbreak of the epidemic, may have been this trigger.

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Ghosh, P., Hsu C., Alyamani E. J., Shehata M. M., Al-Dubaib M. A., et al. (2012) Genome-wide analysis of the emerging infection with Mycobacterium avium subspecies paratuberculosis in the Arabian camels (Camelus dromedarius). PLoS ONE 7: e31947

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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

Checkley, A. M., Wyllie D. H., Scriba T. J., Golubchik T., Hill A. V., et al. (2011) Identification of antigens specific to non-tuberculous mycobacteria: the Mce family of proteins as a target of T cell immune responses. PLoS ONE 6: e26434

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Ford, C. B., Lin P. L., Chase M. R., Shah R. R., Iartchouk O., et al. (2011) Use of whole genome sequencing to estimate the mutation rate of Mycobacterium tuberculosis during latent infection. Nat Genet 43: 482-486

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Saunders, N. J., Trivedi U. H., Thomson M. L., Doig C., Laurenson I. F., et al. (2011) Deep resequencing of serial sputum isolates of Mycobacterium tuberculosis during therapeutic failure due to poor compliance reveals stepwise mutation of key resistance genes on an otherwise stable genetic background. J Infect 62: 212-217

Salmonella

The sequencing of Salmonella enterica serovars has demonstrated many of the benefits of genome-wide sequencing.[39,40,41] It also serves as a cautionary tale of how deceptive current phenotypic characterization, such as serovars and phage types, can be. Based on mutational changes, phage type DT104 is heterogeneous and represented in multiple sequence types. This observation should not be surprising. The serotype is coded by a small part of the genome that is under very different selection pressure than the genes that determine drug resistance and virulence phenotypes. Using serotypes as a surrogate marker for virulence or other characteristic is severely limited. This limitation is significant because the multidrug-resistant variant of DT104 is the cause of epidemics in many parts of the world. Sequencing is a direct observation of the genome and the only definitive method to classify these types.

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Deng, X., Li Z. and Zhang W. (2012) Transcriptome sequencing of Salmonella enterica serovar Enteritidis under desiccation and starvation stress in peanut oil. Food Microbiol 30: 311-315

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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

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Pub. No. 077-2012-030 Current as of 13 September 2012

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