Mikrobiyotanın En Etkili Analizi:

Yeni Nesil Dizileme Sistemleri

Barış Otlu
İnönü Üniversitesi Tıp Fakültesi
Tıbbi Mikrobiyoloji Anabilimdalı

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Yeni Nesil Dizileme Sistemleri

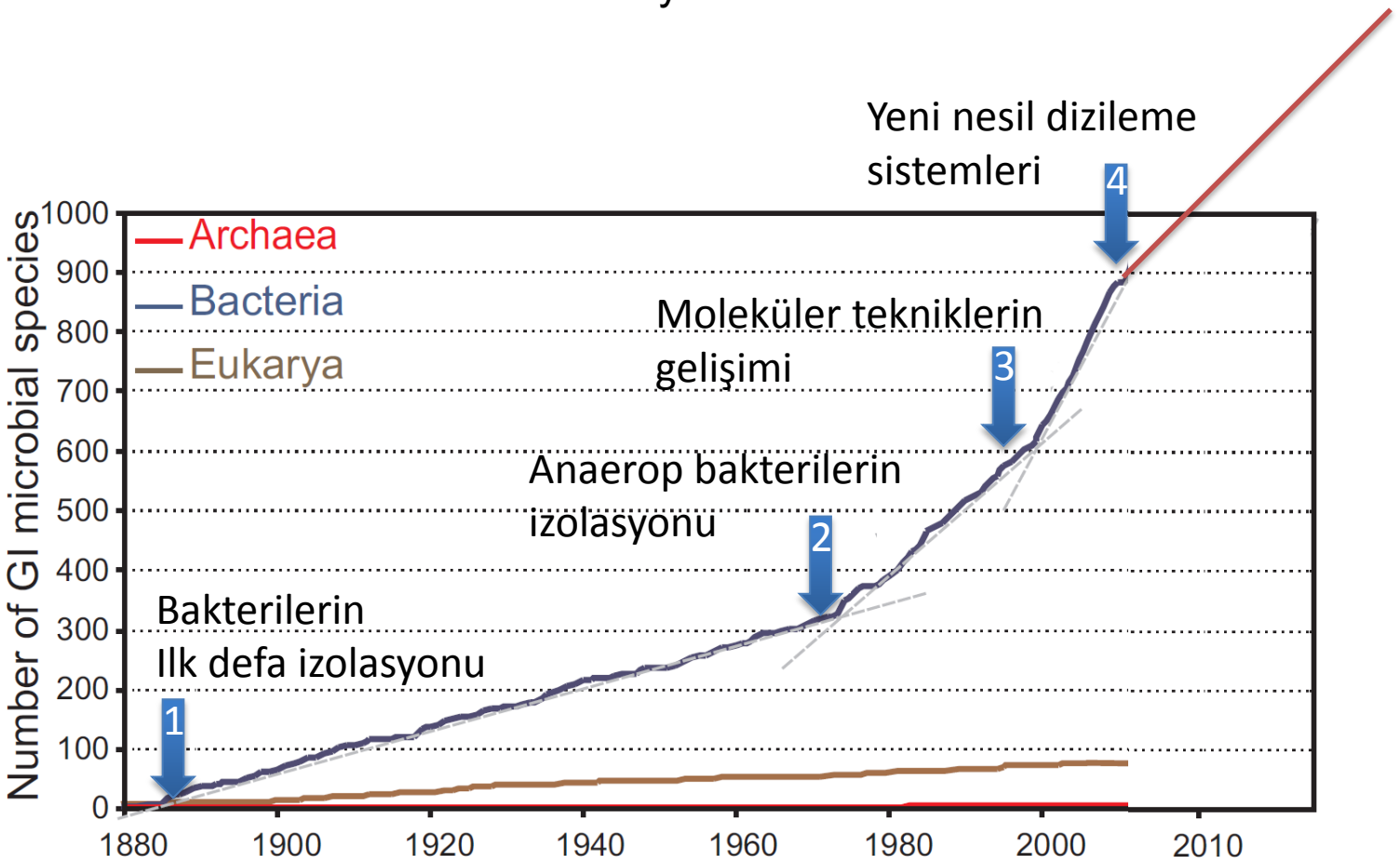
- Dünya Sağlık Örgütü yeni nesil dizileme sistemlerinin mikrobiyolojide potansiyel kullanım alanlarını;
  - tanısal mikrobiyoloji,
  - yeni patojenlerin keşfi,
  - antimikrobiyal duyarlılık ve
  - moleküler epidemiyoloji olmak üzere dört ana başlık altında değerlendirmektedir.

- Mikrobiyota analizi

Mikrobiyomunu Keşfet

Mikrobiyota- Tarihsel Gelişimi

- Gastrointestinal sistemin mikrobiyotası.



FEMS Microbiol Rev. 2014 May 27. doi: 10.1111/1574-6976

The First 1,000 Cultured Species of the Human Gastrointestinal Microbiota.

1975-“Plus and Minus” Yöntemi

- Sanger ve Coulson tarafından bu metod, sonraki 30 yıl süresince geliştirilerek modern dizileme yöntemleri için yol gösterici olmuştur.

J. Mol. Biol. (1975) **94**, 441–448

A Rapid Method for Determining Sequences in DNA by Primed Synthesis with DNA Polymerase

F. Sanger and A. R. Coulson

Medical Research Council
Laboratory of Molecular Biology
Hills Road, Cambridge CB2 2QH, England

(Received 20 December 1974)

A simple and rapid method for determining nucleotide sequences in single-stranded DNA by primed synthesis with DNA polymerase is described. It depends on the use of *Escherichia coli* DNA polymerase I and DNA polymerase from bacteriophage T4 under conditions of different limiting nucleoside triphosphates and concurrent fractionation of the products according to size by ionophoresis on acrylamide gels. The method was used to determine two sequences in bacteriophage ϕX174 DNA using the synthetic decanucleotide A-G-A-A-A-T-A-A-A-A and a restriction enzyme digestion product as primers.

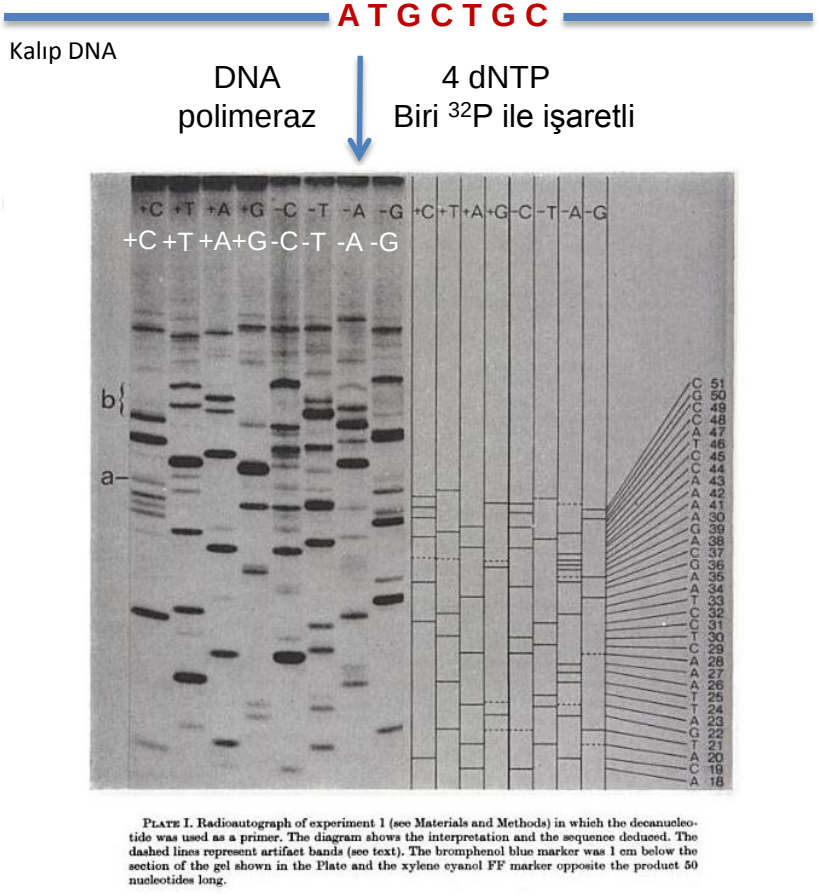


Plate I. Radioautograph of experiment 1 (see Materials and Methods) in which the decanucleotide was used as a primer. The diagram shows the interpretation and the sequence deduced. The dashed lines represent artifact bands (see text). The bromphenol blue marker was 1 cm below the section of the gel shown in the Plate and the xylene cyanol FF marker opposite the product 50 nucleotides long.

1975-“Plus and Minus” Yöntemi

- ΦX174 Bakteriyofajının “tümü genomu” dizilendi.

Nature Vol. 265 February 24 1977

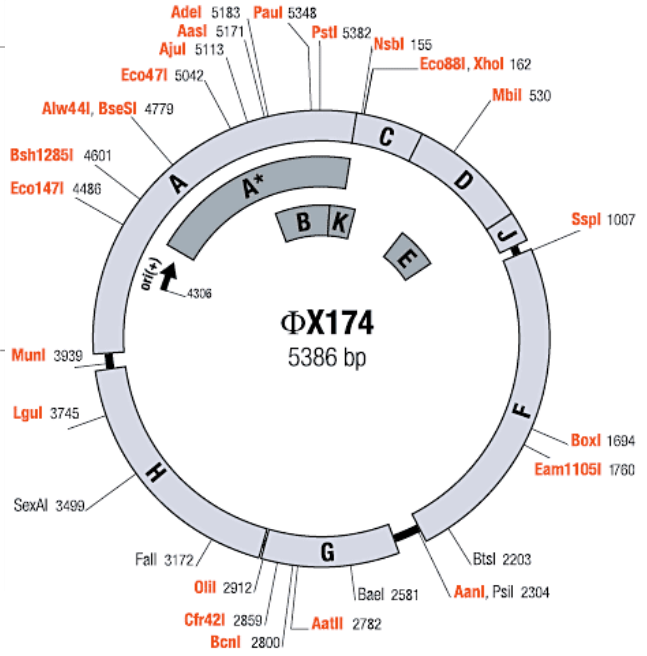
articles

Nucleotide sequence of bacteriophage ΦX174 DNA

F. Sanger, G. M. Air*, B. G. Barrell, N. L. Brown†, A. R. Coulson, J. C. Fiddes, C. A. Hutchison III‡, P. M. Slocombe§ & M. Smith‖

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

A DNA sequence for the genome of bacteriophage ΦX174 of approximately 5,375 nucleotides has been determined using the rapid and simple 'plus and minus' method. The sequence identifies many of the features responsible for the production of the proteins of the nine known genes of the organism, including initiation and termination sites for the proteins and RNAs. Two pairs of genes are coded by the same region of DNA using different reading frames.



1977-Maxam-Gilbert Yöntemi

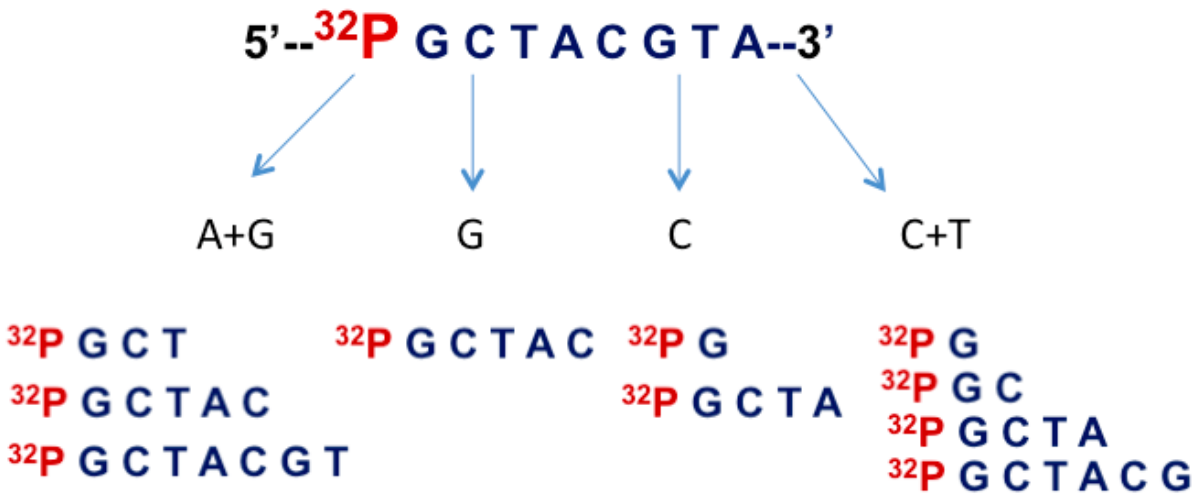
- DNA'nın kimyasal modifikasyonunu takiben DNA'nın özgül bölgelerden kırılması prensibine dayanır.

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 2, pp. 560–564, February 1977
Biochemistry

A new method for sequencing DNA

(DNA chemistry/dimethyl sulfate cleavage/hydrazine/piperidine)

ALLAN M. MAXAM AND WALTER GILBERT



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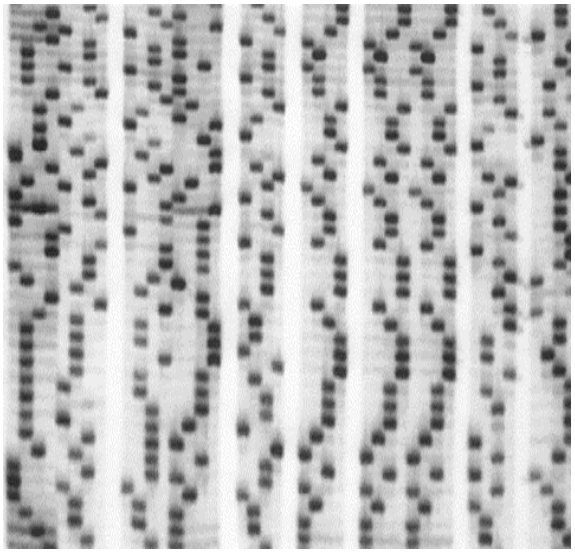


1977- Sanger Zincir Sonlandırma Yöntemi

DNA sequencing with chain-terminating inhibitors

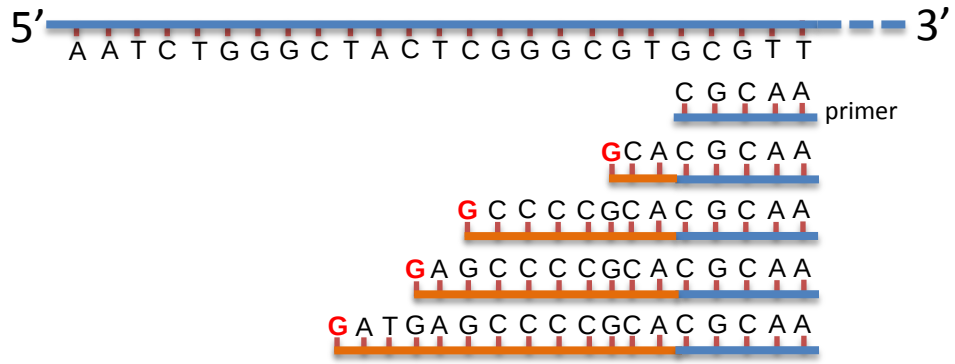
(DNA polymerase/nucleotide sequences/bacteriophage ϕX174)

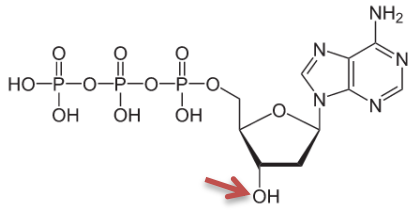
F. SANGER, S. NICKLEN, AND A. R. COULSON

Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, England

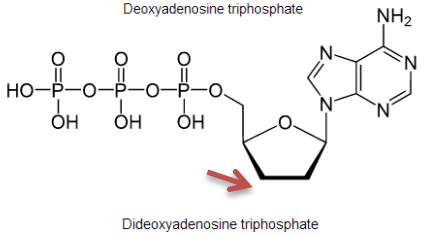
Contributed by F. Sanger, October 3, 1977

ABSTRACT A new method for determining nucleotide sequences in DNA is described. It is similar to the "plus and minus" method [Sanger, F. & Coulson, A. R. (1975) *J. Mol. Biol.* 94, 441–448] but makes use of the 2′,3′-dideoxy and arabinonucleoside analogues of the normal deoxynucleoside triphosphates, which act as specific chain-terminating inhibitors of DNA polymerase. The technique has been applied to the DNA of bacteriophage ϕX174 and is more rapid and more accurate than either the plus or the minus method.



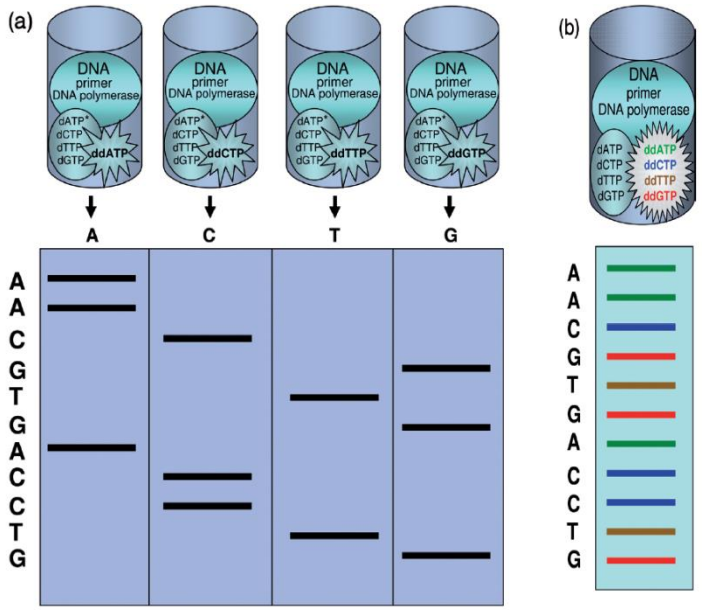


dNTP's



ddNTP's P^{32}

A T G C



1977- Filogentik analizler için rRNA dizileri

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 11, pp. 5088–5090, November 1977
Evolution

Phylogenetic structure of the prokaryotic domain: The primary kingdoms

(archaebacteria/eubacteria/urkaryote/16S ribosomal RNA/molecular phylogeny)

CARL R. WOESE AND GEORGE E. FOX*

Department of Genetics and Development, University of Illinois, Urbana, Illinois 61801

Communicated by T. M. Sonneborn, August 18, 1977

ABSTRACT **A phylogenetic analysis based upon ribosomal RNA sequence characterization reveals that living systems represent one of three aboriginal lines of descent: (*i*) the eubacteria, comprising all typical bacteria; (*ii*) the archaebacteria, containing methanogenic bacteria; and (*iii*) the urkaryotes, now represented in the cytoplasmic component of eukaryotic cells.**

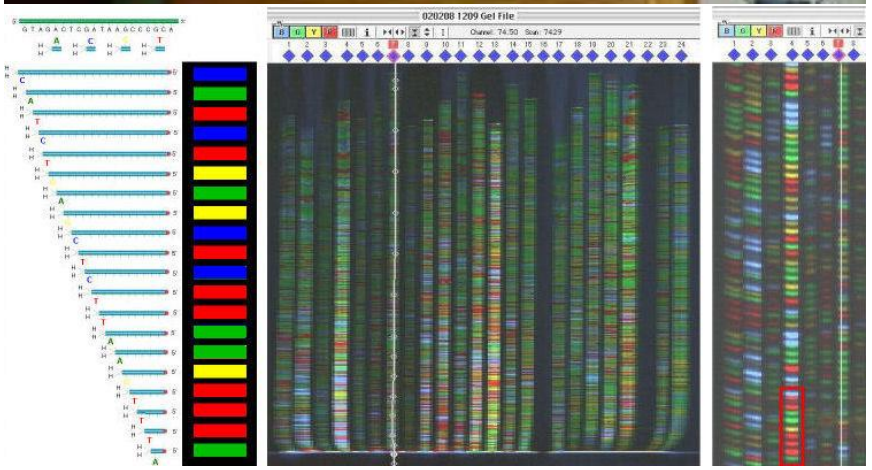
The biologist has customarily structured his world in terms of certain basic dichotomies. Classically, what was not plant was animal. The discovery that bacteria, which initially had been considered plants, resembled both plants and animals less than plants and animals resembled one another led to a reformulation of the issue in terms of a yet more basic dichotomy, that of eukaryote versus prokaryote. The striking differences between eukaryotic and prokaryotic cells have now been documented in endless molecular detail. As a result, it is generally taken for granted that all extant life must be of these two basic types.

Thus, it appears that the biologist has solved the problem of the primary phylogenetic groupings. However, this is not the case. Dividing the living world into *Prokaryotae* and *Eukaryotae* has served, if anything, to obscure the problem of what to construct phylogenetic classifications between domains: Prokaryotic kingdoms are not comparable to eukaryotic ones. This should be recognized by an appropriate terminology. The highest phylogenetic unit in the prokaryotic domain we think should be called an "urkingdom"—or perhaps "primary kingdom." This would recognize the qualitative distinction between prokaryotic and eukaryotic kingdoms and emphasize that the former have primary evolutionary status.

The passage from one domain to a higher one then becomes a central problem. Initially one would like to know whether this is a frequent or a rare (unique) evolutionary event. It is traditionally assumed—without evidence—that the eukaryotic domain has arisen but once; all extant eukaryotes stem from a common ancestor, itself eukaryotic (2). A similar prejudice holds for the prokaryotic domain (2). [We elsewhere argue (6) that a hypothetical domain of lower complexity, that of "progenotes," may have preceded and given rise to the prokaryotes.] The present communication is a discussion of recent findings that relate to the urkingdom structure of the prokaryotic domain and the question of its unique as opposed to multiple origin.

1986- İlk Otomatize Cihaz

- Leroy hood ve Aplied Biosystem tarafından tanıtıldı.



1990-16S rRNA Dizileme

JOURNAL OF BACTERIOLOGY, Jan. 1991, p. 697–703
0021-9193/91/020697-07$02.00/0
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Vol. 173, 1

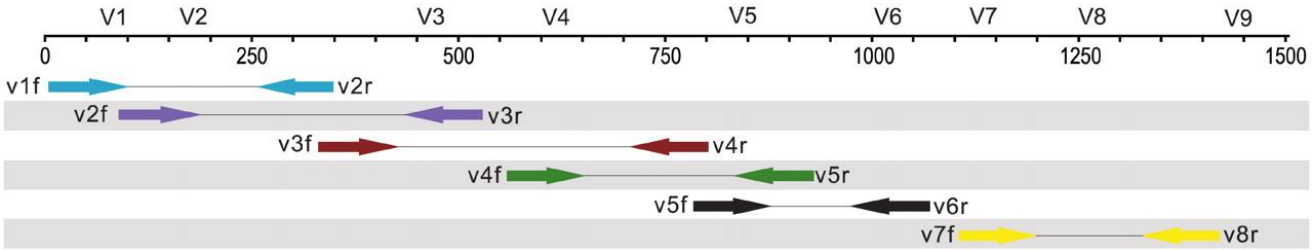
16S Ribosomal DNA Amplification for Phylogenetic Study

WILLIAM G. WEISBURG,* SUSAN M. BARNS, DALE A. PELLETIER, AND DAVID J. LANE

GENE-TRAK Systems, 31 New York Avenue, Framingham, Massachusetts 01701

Received 16 April 1990/Accepted 7 November 1990

A set of oligonucleotide primers capable of initiating enzymatic amplification (polymerase chain reaction) on a phylogenetically and taxonomically wide range of bacteria is described along with methods for their use and examples. One pair of primers is capable of amplifying nearly full-length 16S ribosomal DNA (rDNA) from many bacterial genera; the additional primers are useful for various exceptional sequences. Methods for purification of amplified material, direct sequencing, cloning, sequencing, and transcription are outlined. An obligate intracellular parasite of bovine erythrocytes, *Anaplasma marginale*, is used as an example; its 16S rDNA was amplified, cloned, sequenced, and phylogenetically placed. Anaplasmas are related to the genera *Rickettsia* and *Ehrlichia*. In addition, 16S rDNAs from several species were readily amplified from material found in lyophilized ampoules from the American Type Culture Collection. By use of this method, the phylogenetic study of extremely fastidious or highly pathogenic bacterial species can be carried out without the need to culture them. In theory, any gene segment for which polymerase chain reaction primer design is possible can be derived from a readily obtainable lyophilized bacterial culture.



1990-İnsan Genom Projesinin Başlaması

POLICY FORUM

A New Five-Year Plan for the U.S. Human Genome Project

Francis Collins and David Galas*

The U.S. Human Genome Project is part of an international effort to develop genetic and physical maps and determine the DNA sequence of the human genome and the genomes of several model organisms. Thanks to advances in technology and a tightly focused effort, the project is on track with respect to its initial 5-year goals. Because 3 years have elapsed since these goals were set, and because a much more sophisticated and detailed understanding of what needs to be done and how to do it is now available, the goals have been refined and extended to cover the first 8 years (through September 1998) of the 15-year genome initiative.

In 1990, the Human Genome programs of the National Institutes of Health (NIH) and the Department of Energy (DOE) developed a joint research plan with specific goals for the first 5 years [fiscal year (FY) 1991–95] of the U.S. Human Genome Project (*1*). It has served as a valuable guide for both the research community and the agencies' administrative staff in developing and executing the genome project and assessing its progress for the past 3 years. Great strides have been made toward the achievement of the initial set of goals, particularly with respect to constructing detailed human genetic maps, improving physical maps of the human genome and the genomes of certain model organisms, developing improved technology for DNA sequencing and information handling, and defining the most urgent set of ethical, legal, and social issues associated with the acquisition and use of large amounts of genetic information.

Progress toward achieving the first set of goals for the genome project appears to be on schedule or, in some instances, even ahead of schedule. Furthermore, technological improvements that could not have been anticipated in 1990 have in some areas changed the scope of the project and allowed more ambitious approaches. Earlier this year, it was therefore decided to update and extend the initial goals to address the scope of genome research beyond the completion of the original 5-year plan. A major purpose of revising the plan is to inform and provide a new guide to all participants in the genome project about the project's goals. To obtain the advice needed to develop the extended goals, NIH and DOE held a series of meetings with a large number of scientists and other interested scholars and representatives of the public, including many who previously had not been direct participants in the genome project. Reports of all these meetings are available from the Office of Communications of the National Center for Human Genome Research (NCHGR) and the Human Genome Management Information System of DOE (*2*, *3*). Finally, a group of representative advisors from NIH and DOE drafted a set of new, extended goals for presentation to the National Advisory Council for Human Genome Research of NIH and the Health and Environmental Research Advisory Committee of DOE. These bodies have approved this document as a statement of their advice to the two agencies, and the following represents the goals for FYs 1994–98 (1 October 1993 to 30 September 1998).

General Principles

Several general observations underlie the specific goals (Fig. 1) described here. The first observation is that successful development of new technology for genomic and genetic research has been essential to the achievements of the project to date and will continue to be critical in the future. It was clearly recognized, both in the 1988 National Research Council (NRC) report (*4*) and in the first NIH-DOE plan, that attainment of the ambitious goals originally set for the genome project would require significant technological advances in all areas, such as mapping, sequencing, informatics, and gene identification. As the genome project has proceeded, progress along a broad range of technological fronts has been conspicuous. Among the most notable of these developments have been (i) new types of genetic markers, such as microsatellites, that can be assayed by polymerase chain reaction (PCR); (ii) improved vector systems for cloning large DNA fragments and better experimental strategies and computational methods for assembling those clones into large, overlapping sets (contigs) that compose useful physical maps; (iii) the definition of the sequence tagged site (STS) (*5*) as a common unit of physical mapping; and (iv) improved technology and automation for DNA sequencing. Further substantial improvements in technology are needed in all areas of genome research, especially in DNA sequencing, if the project is to stay on schedule and meet the demanding goals that are being set.

A second general observation concerns an evolution in the levels of biological organization at which genomic research will likely function over the next few years. Initially, attention was focused on the chromosome as the basic unit of genome analysis. Large-scale mapping efforts, in particular, were directed at the construction of chromosome maps. The sophisticated genetic linkage maps now available and the detailed physical maps that are being produced are clear measures of the success of that approach. However, other units of study for the Human Genome Project will also have increasing usefulness in the future. Therefore, further mapping efforts directed at both larger and smaller targets should be encouraged. At one end of the scale, "whole genome" mapping efforts, in which the entire genome is efficiently analyzed, have become feasible with developments in PCR applications and robotics. These approaches generally produce relatively low-resolution maps with current technology. At the other end of the scale, increasing attention needs to be paid to detailed mapping, sequencing, and annotation of regions on the order of one to a few megabases in size. Although small in comparison with the whole genome, a megabase is still large in comparison with the capabilities of conventional molecular genetic analysis. Thus, development of efficient technology for approaching detailed analysis of several-megabase sections of the genome will provide a useful bridge between conventional genetics and genomics, and provide a foundation for innovation from which future methods for analysis of larger regions may arise.

Third, a goal for identifying genes within maps and sequences, implicit in the original plan, has now been made explicit. The progress already made on the original goals, combined with promising new approaches to gene identification, allow this element of genome analysis to be given greater visibility. This increased emphasis on gene identification will greatly enrich the maps that are produced.

It must also be noted that, as in the original 5-year plan, these goals assume a funding level for the U.S. Human Genome Project of $200 million annually, adjusted for inflation. As the detailed cost analysis for the first 5-year plan was performed in

F. Collins is the director of the National Center for Human Genome Research, National Institutes of Health, Bethesda, MD 20892.
D. Galas was associate director, Office of Health and Environmental Research, Department of Energy, Washington, DC 20585.
* Present address: Darwin Molecular, 2405 Carillon Point, Kirkland, WA 98033.

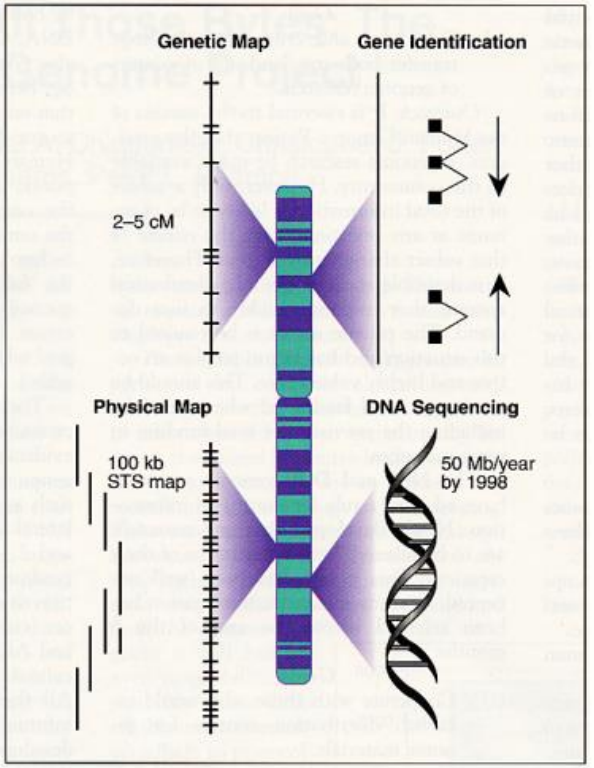


Fig. 1. Graphic overview of the new goals for the human genome. A 2- to 5-cM genetic map is expected to be completed by 1995 and a physical map with STS markers every 100 kb by 1998. Efficient methods for gene identification need to be developed and refined. The DNA sequencing goal of 50 Mb per year by 1998 includes all DNA, both human and model organisms, and assumes an exponential increase in sequencing capacity over time. Other important goals involving model organisms are not shown here, but are described in the text.

1994-NCBI GenBank Kuruldu

NCBI News

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION
National Library of Medicine
National Institutes of Health

Volume 3, Issue 1 February 1994

NCBI Creates New Database, New GenBank Division for STS Data

NCBI is creating a new database and a new GenBank division to facilitate access to the growing body of sequence tagged site (STS) data being generated by genome mapping laboratories. An STS is a short DNA sequence that has a single occurrence in the genome of an organism, thus serving as a physical mapping landmark.

GenBank currently contains about 3,200 STS sequences, most of which come from human sources. Beginning with Release 82.0 (April 1994), STSs will be consolidated into a new division. This reorganization will facilitate cross-comparison of STSs with sequences in other divisions for the purpose of correlating map positions of anonymous sequences with known genes.

In addition, a separate STS database, dbSTS, will be maintained to manage this special class of sequences and provide detailed information about the STS map locations and PCR conditions. Following the model of dbEST,[1] dbSTS will be a dynamic resource. Sequence similarity searches against other divisions of GenBank will be performed on a frequent periodic basis, and the annotations in the STS database will be updated automatically. Methods are under development to enable queries of dbSTS by chromosomal and subchromosomal location. Access will initially be by an e-mail server.

1995- İlk Bakteriyel Tüm Genomun Dizilenmesi

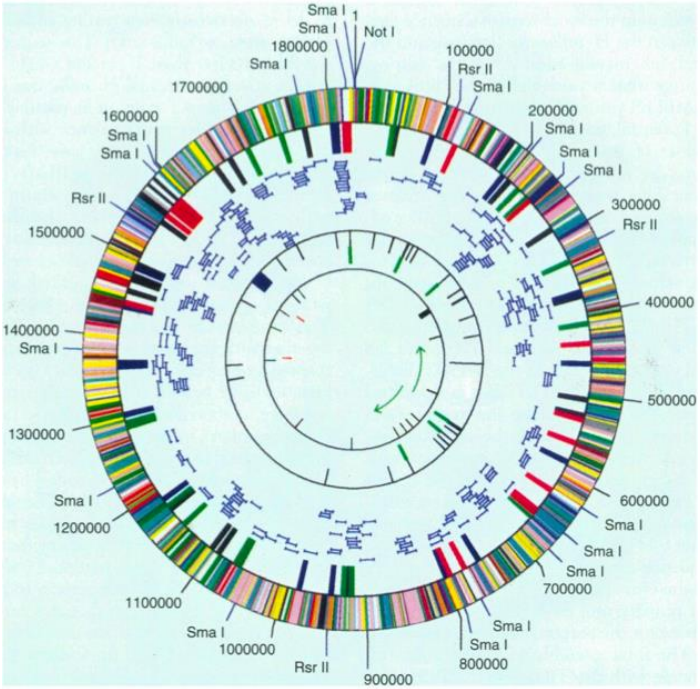
- *Haemophilus influenzae* ve *Mycoplasma genitalium*'un "tüm genom" dizilemesinin yapıldığı duyurulmuştur.

RESEARCH ARTICLE

Whole-Genome Random Sequencing and Assembly of *Haemophilus influenzae* Rd

Robert D. Fleischmann, Mark D. Adams, Owen White, Rebecca A. Clayton, Ewen F. Kirkness, Anthony R. Kerlavage, Carol J. Bult, Jean-Francois Tomb, Brian A. Dougherty, Joseph M. Merrick, Keith McKenney, Granger Sutton, Will FitzHugh, Chris Fields,* Jeannine D. Gocayne, John Scott, Robert Shirley, Li-Ing Liu, Anna Glodek, Jenny M. Kelley, Janice F. Weidman, Cheryl A. Phillips, Tracy Spriggs, Eva Hedblom, Matthew D. Cotton, Teresa R. Utterback, Michael C. Hanna, David T. Nguyen, Deborah M. Saudek, Rhonda C. Brandon, Leah D. Fine, Janice L. Fritchman, Joyce L. Fuhrmann, N. S. M. Geoghagen, Cheryl L. Gnehm, Lisa A. McDonald, Keith V. Small, Claire M. Fraser, Hamilton O. Smith, J. Craig Venter†

An approach for genome analysis based on sequencing and assembly of unselected pieces of DNA from the whole chromosome has been applied to obtain the complete nucleotide sequence (1,830,137 base pairs) of the genome from the bacterium *Haemophilus influenzae* Rd. This approach eliminates the need for initial mapping efforts and is therefore applicable to the vast array of microbial species for which genome maps are unavailable. The *H. influenzae* Rd genome sequence (Genome Sequence DataBase accession number L42023) represents the only complete genome sequence from a free-living organism.



1995- İlk Bakteriyel Tüm Genomun Dizilenmesi

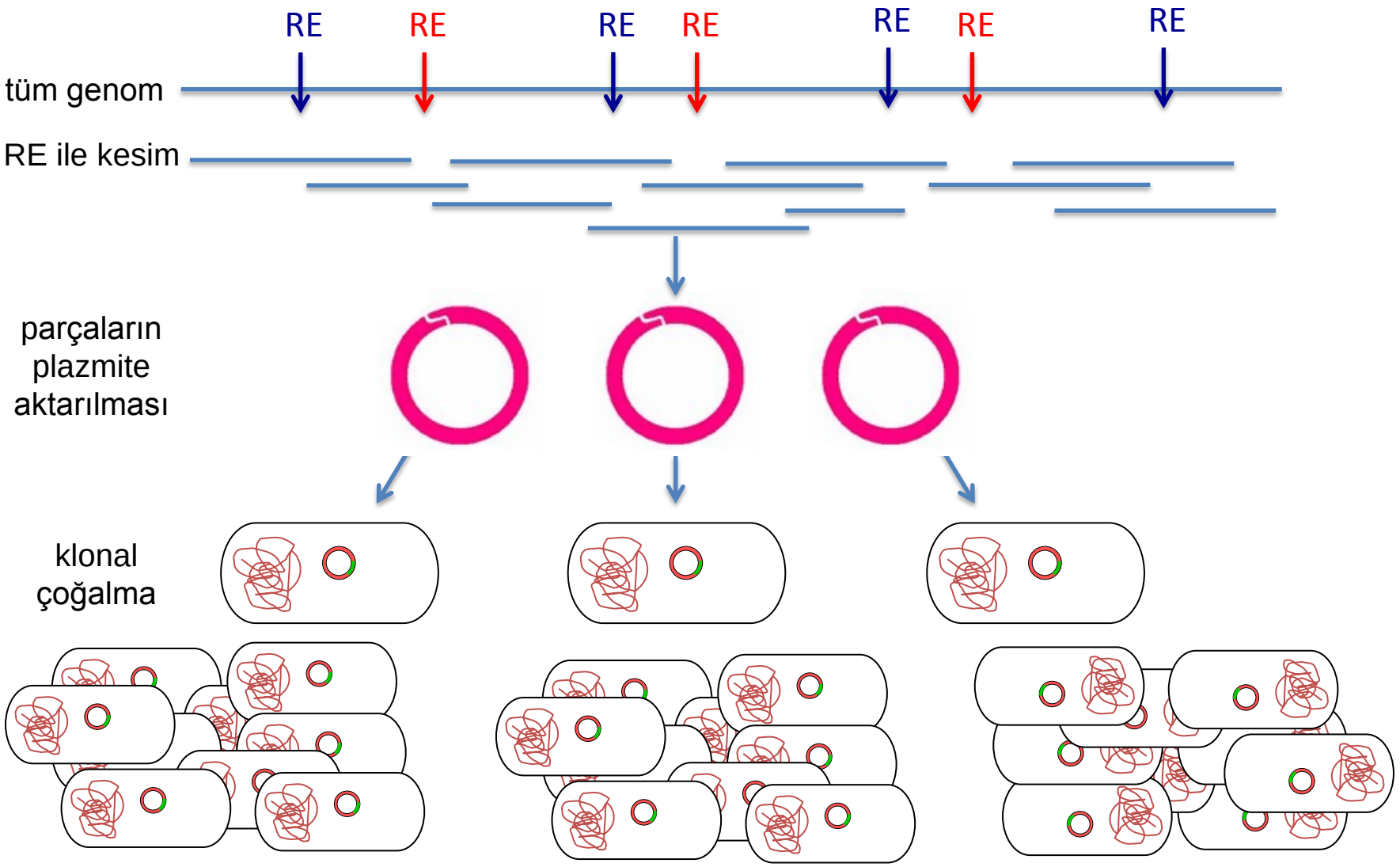
- Bu çalışmada; yeni nesil dizileme sistemlerine de temel oluşturacak "shotgun dizileme" yaklaşımı ortaya konmuştur.

Genome sequencing. The strategy for a shotgun approach to whole genome sequencing is outlined in Table 1. The theory follows from the Lander and Waterman (*14*) application of the equation for the Poisson distribution. The probability that a base is not sequenced is $P_o = e^{-m}$, where m is the sequence coverage.

ACTGCTGTCGAATCGGCCTTTAAGGCTGACTGGTAGCCCGTGAGAGATTTGA

1995- İlk Bakteriyel Tüm Genomun Dizilenmesi

- Her bir DNA parçasının ayrı ayrı dizilenebilmesi için izole edilebilmesi gereklidir.



1995- İlk Bakteriyel Tüm Genomun Dizilenmesi

- Yine bu çalışmada;

  yeni nesil dizileme sistemlerine de temel oluşturacak basamaklar belirlenmiştir.

library construction
DNA'nın parçalara ayrılarak
kütüphanenin oluşturulması

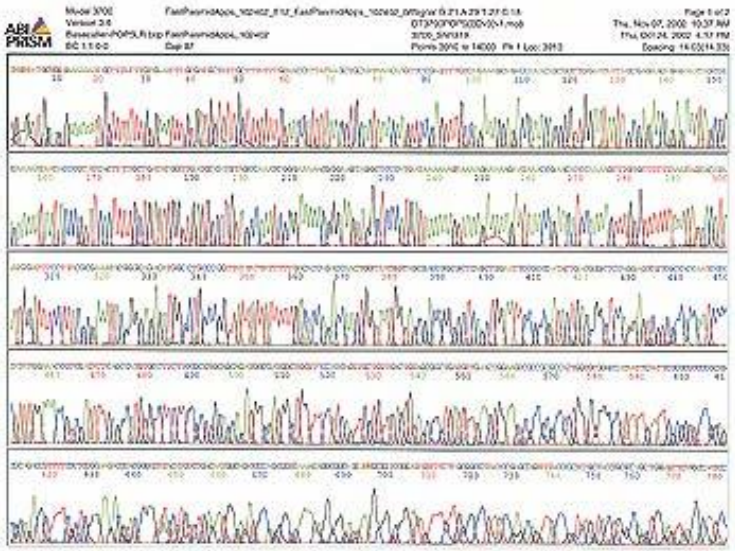
high-throughput sequencing
DNA parçalarının ayrı ayrı
yüksek hacimli dizilenmesi

assembly
dizilerin bir araya getirilmesi

1998-Celera

- İnsan genom projesine, Craig Venter'in kurduğu Celera adlı özel teşebbüs dahil olmuştur.
- Geliştirilen en kritik sistem 96 örneği 3 saat içinde çalışabilen *ABI Prisim 3700* sistemidir.

3 ay içerisinde 20 Gb genom dizileme



1998-Metagenom Terimi

Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products

Jo Handelsman[1], Michelle R Rondon[1], Sean F Brady[2], Jon Clardy[2] and Robert M Goodman[1]

Cultured soil microorganisms have provided a rich source of natural-product chemistry. Because only a tiny fraction of soil microbes from soil are readily cultured, soil might be the greatest untapped resource for novel chemistry. The concept of cloning the metagenome to access the collective genomes and the biosynthetic machinery of soil microflora is explored here.

Addresses: [1]Department of Plant Pathology, University of Wisconsin–Madison, 1630 Linden Drive, Madison, WI 53706, USA. [2]Department of Chemistry and Chemical Biology, Baker Laboratory, Cornell University, Ithaca, NY 14853, USA.

Chemistry & Biology October 1998, **5**:R245–249
http://biomednet.com/elecref/10745521005R0245

Correspondence: Jo Handelsman
E-mail: joh@plantpath.wisc.edu

© Current Biology Ltd ISSN 1074-5521

A new frontier of science is emerging that unites biology and chemistry — the exploration of natural products from previously uncultured soil microorganisms. The approach involves directly accessing the genomes of soil organisms that cannot be, or have not been, cultured by isolating their DNA, cloning it into culturable organisms and screening the resultant clones for the production of new chemicals. The excitement surrounding this new field lies in the vast diversity of unknown soil microflora and the chemical rich-

Despite being familiar and useful, soil is also one of the least understood habitats on earth. The last 25 years of research have revealed that culturing is an excellent method to learn a lot about a tiny proportion of the microorganisms on earth [2–7]. Many lines of evidence show that fewer than 0.1% of the microorganisms in soil are readily cultured using current techniques [8–10]. And, most impressively, the other 99.9% of soil microflora is emerging as a world of stunning, novel genetic diversity. New groups of bacteria have been identified in soil that appear to diverge so deeply from the cultured bacteria that they could represent new phyla, or even new kingdoms of life [11–13]. Groups of *Archaea* related to those found thus far only in the open ocean are soil inhabitants around the world [14,15]. Estimates are that a gram of soil might contain 1,000–10,000 species of unknown prokaryotes [8]. There is likely to be further diversity within species, which current phylogenetic analysis cannot resolve. Because microbes, generally, have great genetic diversity — soil carries the highest populations of microbes of any habitat [16] —and microbes cultured from soil have revealed tremendous chemical virtuosity and utility, the vast majority of as yet unknown microbes could well be a far greater source of new molecular structures than any habitat on earth. Tapping into this source should be a great, joint adventure for biologists and chemists.

Figure 1

Morphological diversity typical of microorganisms cultured from soil on a broad spectrum medium, tryptic soy agar.

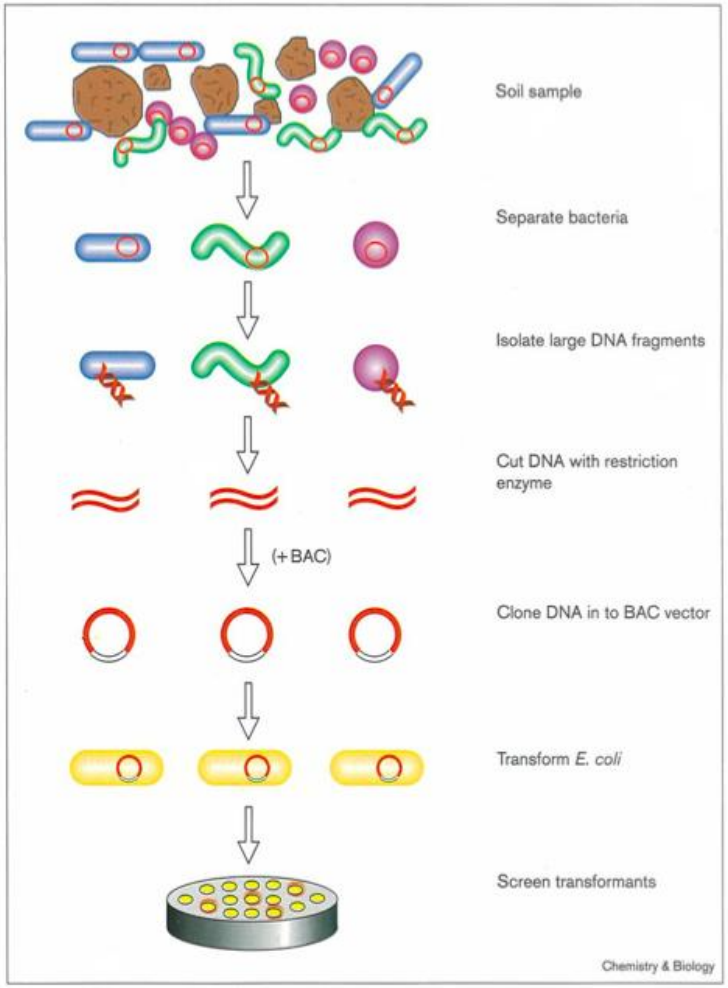
1998-Metagenom Terime

Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products

Jo Handelsman[1], Michelle R Rondon[1], Sean F Brady[2], Jon Clardy[2] and Robert M Goodman[1]

Figure 3

Cloning the metagenome is our process for isolating new pathways for the synthesis of bioactive molecules from noncultured soil microorganisms. DNA is extracted directly from soil, using gentle methods to preserve high-molecular-weight DNA. The DNA is cut using a restriction enzyme and cloned into a bacterial artificial chromosome (BAC), a vector which can carry large fragments of DNA in *E. coli*. The BAC clones are then screened for biological activity and for the production of novel natural products.



2001-İnsan Genom Projesinin Tamamlanması

2001-İnsan Genom Projesinin Tamamlanması

- Projenin tamamlanması;

  postgenomik alanın başlangıcını oluşturmuş.
- Yaklaşık 3 milyar dolar harcanmış ve 11 yıl süre geçmiştir

- Yöntemler;

  - yoğun emek gerektiren,

  - zaman alıcı ve oldukça maliyetli.
- İnsan genom projesi dizileme teknolojileri ile ilgili yeni yaklaşımlara ihtiyaç olduğunun fark edilmesine ve birçok yeni fikrin doğmasına da neden olmuştur.

new generation

young and free

Yeni Nesil Dizileme Sistemlerinin Doğuşu

- Bu dizileme sistemleriyle;

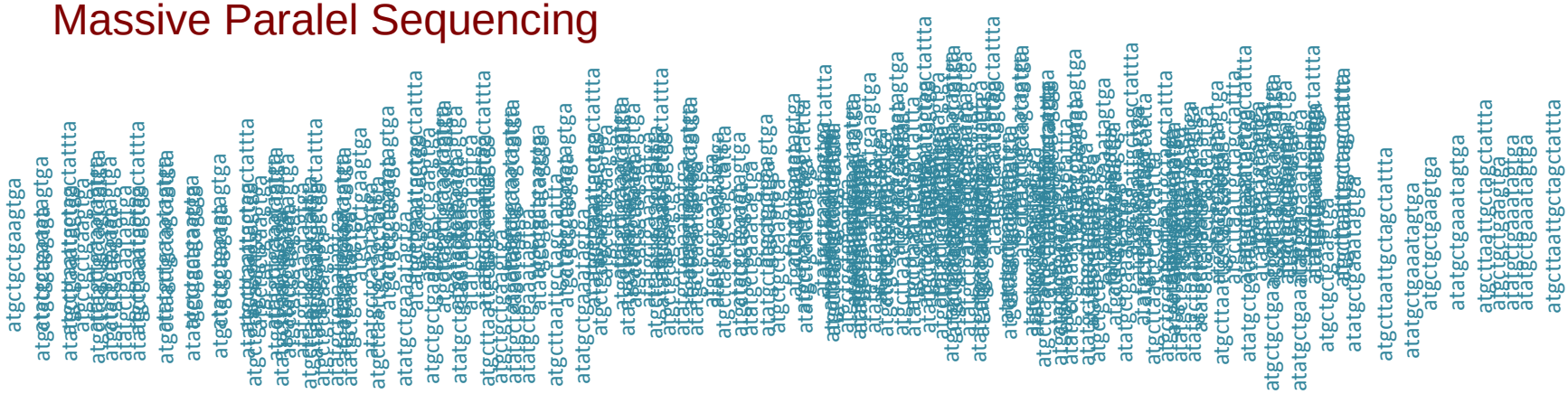
  yüksek doğrulukta ve hızda dizileme yapabilmekte ve

  genom dizi bilinmeyen bir canlının genom dizisinin ortaya çıkarılabilmektedir.

  (De Novo dizileme).

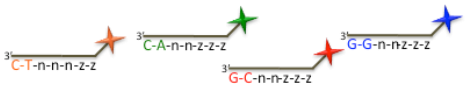
- Bu sistemlerin ortak özeliği;
  - Aynı anda milyonlarca kısa dizilemenin yapılmasıdır.

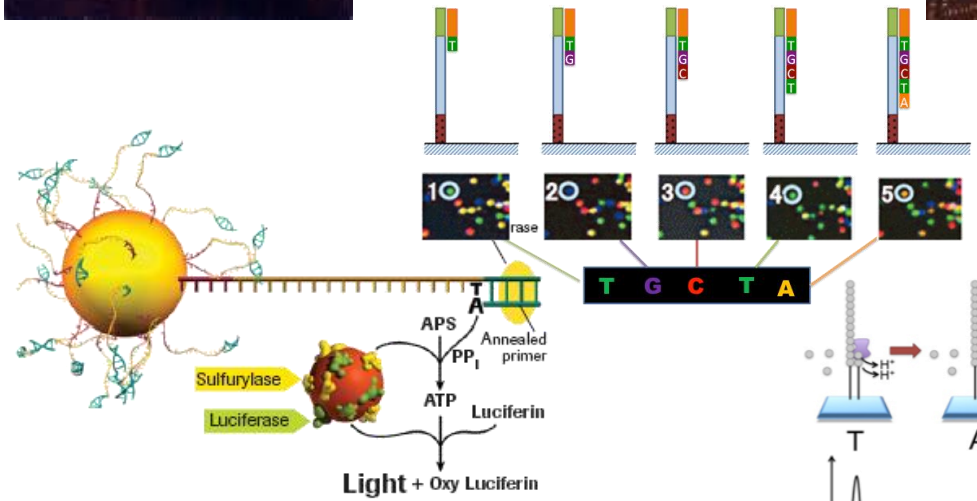
    Massive Paralel Sequencing

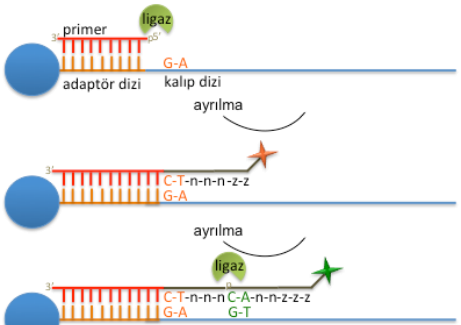


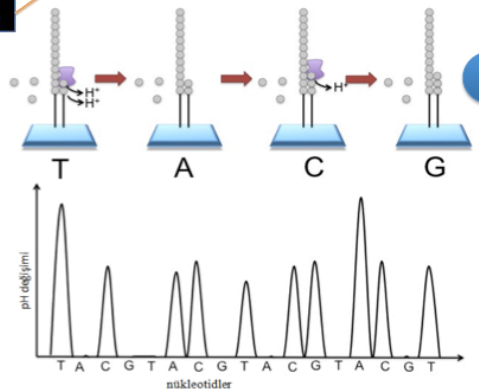
  - Bioinformatik analizlere ihtiyaç duyulması

Yeni Nesil Dizileme Sistemlerinin Doğuşu









454 Life Science *Roche Applied Science*

- Pirodizileme ile “sentez yoluyla dizileme” prensibine dayalıdır.

Kütüphanenin hazırlanması

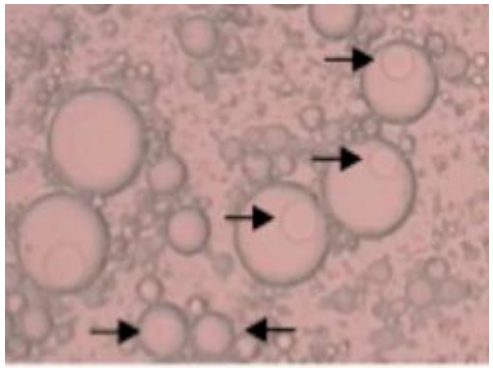
Genomik DNA ‘nın RE ile parçalara ayrılması

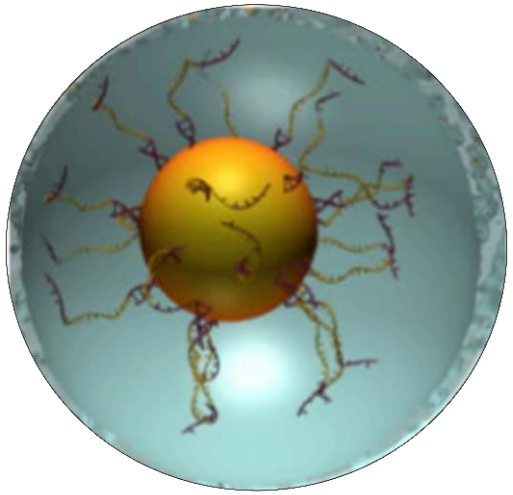
adaptör moleküllerin eklenmesi ve denatürasyon

dizileme primerinin bağlanacağı adaptör

PCR primerinin bağlanacağı adaptör

DNA parçalarının yakalanması ve zenginleştirme





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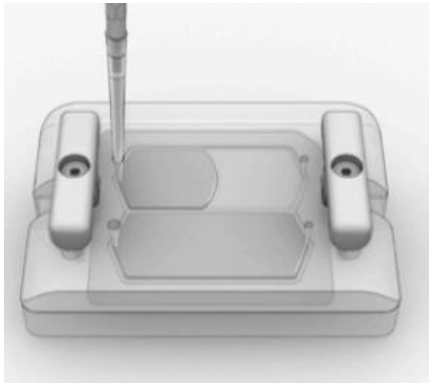
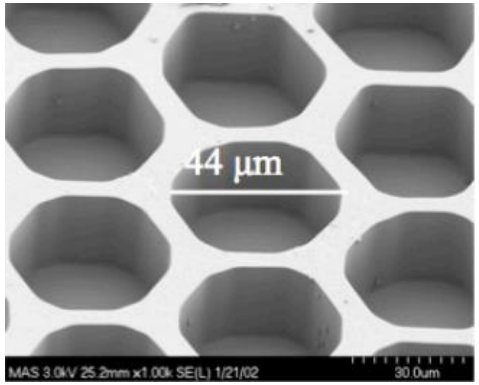
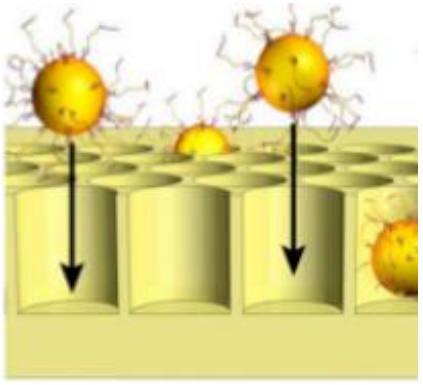
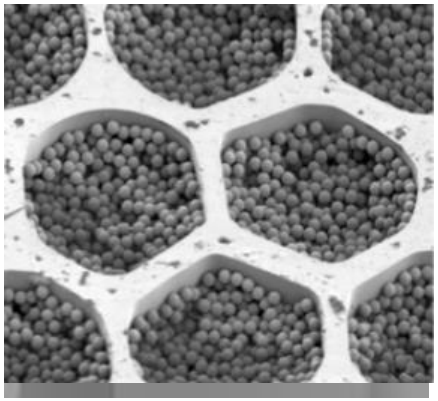
Boncuklar santrifüj ile plak tabanına çöktürülür

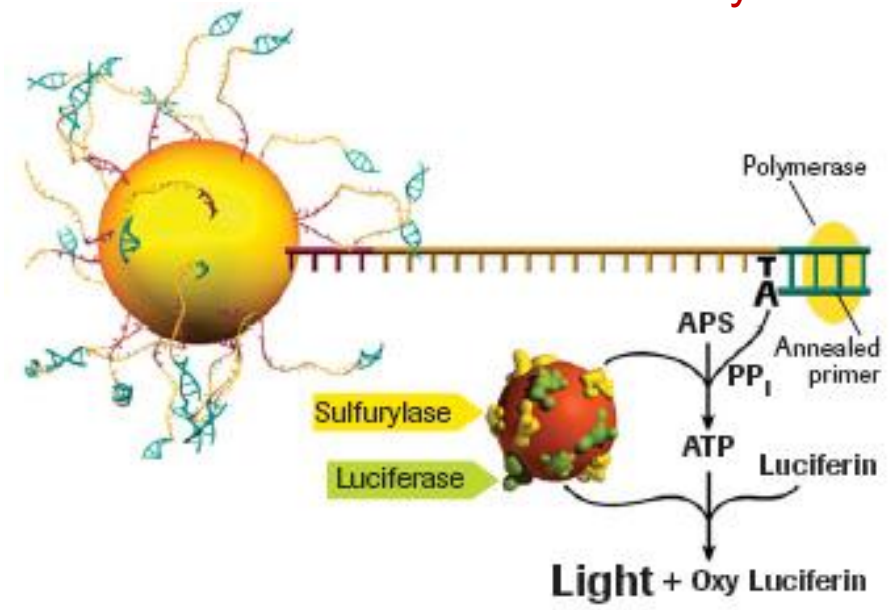


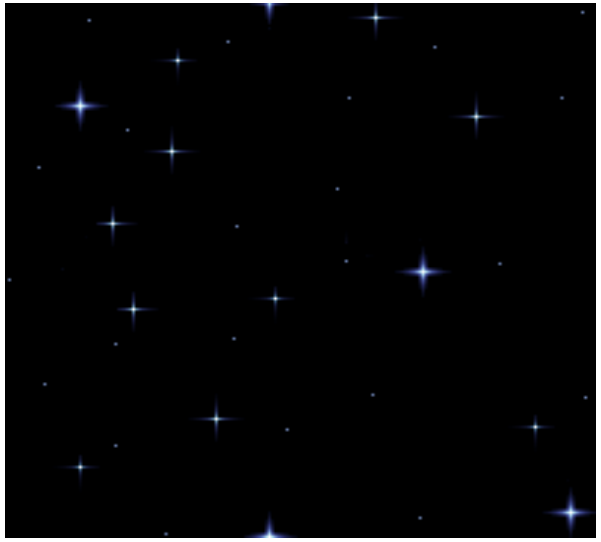






Pirodizileme reaksiyonu ve CCD kamera ile ışımanın tespiti





A

T

G

C

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- Her okumada 200 baz ya da her 12 saatlik çalışma başına toplamda 20 megabaz olacak şekilde 100.000 baz çiftinden fazla okuma yapabilmektedir.
- Bu sistemin 2007'de tanıtımının yapılmasından sonra cihazda şaşırtıcı yenilikler yapılmıştır.

Genome Sequencer FLX Titanium *Roche Applied Science*

- Bu yeniliklerden bir tanesi;

  kuyucuk sayısı arttırılmış pikotitre plaklarıyla 500 baza kadar okuma yapılabilmesidir.

- Bu sistem 8 saatlik tek okumada 500 milyon baz dizileyebilmektedir.

Genome Sequencer FLX Titanium *Roche Applied Science*

OPEN ACCESS Freely available online

PLOS ONE

Plaque Bacterial Microbiome Diversity in Children Younger than 30 Months with or without Caries Prior to Eruption of Second Primary Molars

He Xu[1], Wenjing Hao[1], Qiong Zhou[1], Wenhong Wang[2], Zhongkui Xia[3,4], Chuan Liu[3], Xiaochi Chen[5], Man Qin[1]*, Feng Chen[6]*

1 Department of Pediatric Dentistry, School of Stomatology, Peking University, Beijing, China, **2** Department of Pediatric Dentistry, Maternal and Child Care Service Centre of Haidian District, Beijing, China, **3** BGI, Shenzhen, Guangdong, China, **4** Center of Bioinformatics and Key Laboratory for Neuroinformation of Ministry of Education, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, Sichuan, China, **5** Department of Oral Microorganism, School of Stomatology, Peking University, Beijing, China, **6** Central Laboratory, School of Stomatology, Peking University, Beijing, China

Abstract

Objective: Our primary objective is to phylogenetically characterize the supragingival plaque bacterial microbiome of children prior to eruption of second primary molars by pyrosequencing method for studying etiology of early childhood caries.

Methods: Supragingival plaque samples were collected from 10 caries children and 9 caries-free children. Plaque DNA was extracted, used to generate DNA amplicons of the V1–V3 hypervariable region of the bacterial *16S rRNA* gene, and subjected to 454-pyrosequencing.

Results: On average, over 22,000 sequences per sample were generated. High bacterial diversity was noted in the plaque of children with caries [170 operational taxonomical units (OTU) at 3% divergence] and caries-free children (201 OTU at 3% divergence) with no significant difference. A total of 8 phyla, 15 classes, 21 orders, 30 families, 41 genera and 99 species were represented. In addition, five predominant phyla (*Firmicute, Fusobacteria, Proteobacteria, Bacteroidetes* and *Actinobacteria*) and seven genera (*Leptotrichia, Streptococcus, Actinomyces, Prevotella, Porphyromonas, Neisseria, and Veillonella*) constituted a majority of contents of the total microbiota, independent of the presence or absence of caries. Principal Component Analysis (PCA) presented that caries-related genera included *Streptococcus* and *Veillonella*; while *Leptotrichia, Selenomonas, Fusobacterium, Capnocytophaga* and *Porphyromonas* were more related to the caries-free samples. *Neisseria* and *Prevotella* presented approximately in between. In both groups, the degree of shared organism lineages (as defined by species-level OTUs) among individual supragingival plaque microbiomes was minimal.

Conclusion: Our study represented for the first time using pyrosequencing to elucidate and monitor supragingival plaque bacterial diversity at such young age with second primary molar unerrupted. Distinctions were revealed between caries and caries-free microbiomes in terms of microbial community structure. We observed differences in abundance for several microbial groups between the caries and caries-free host populations, which were consistent with the ecological plaque hypothesis. Our approach and findings could be extended to correlating microbiomic changes after occlusion establishment and caries treatment.

Genome Sequencer FLX Titanium *Roche Applied Science*

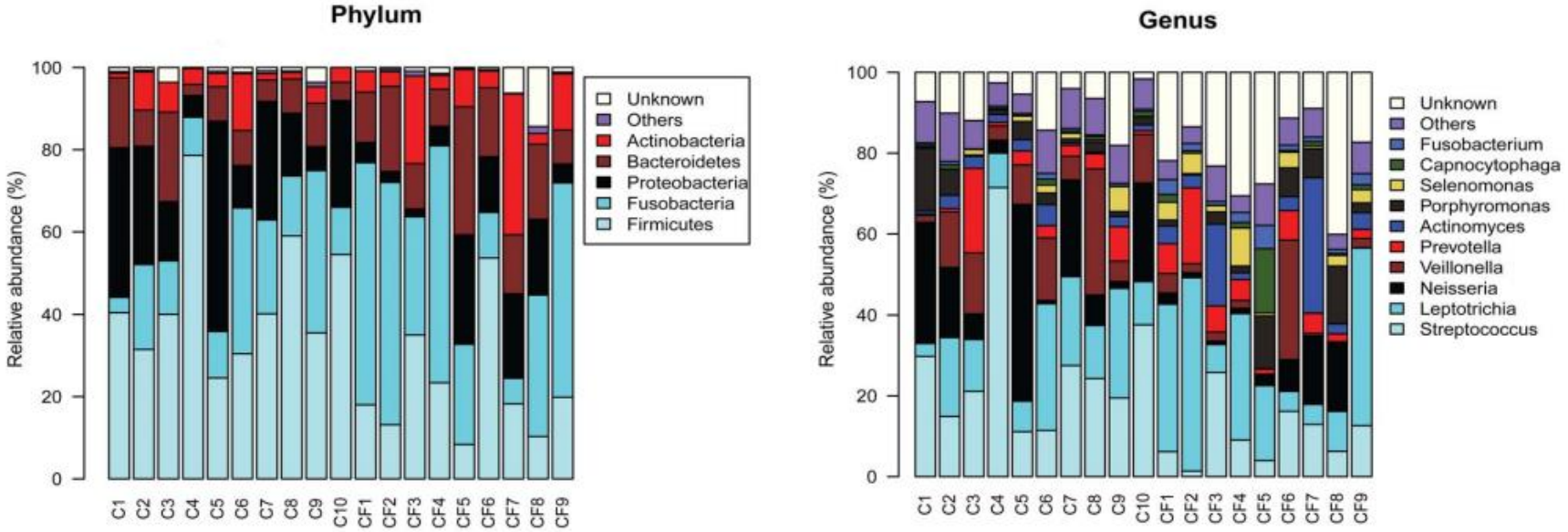
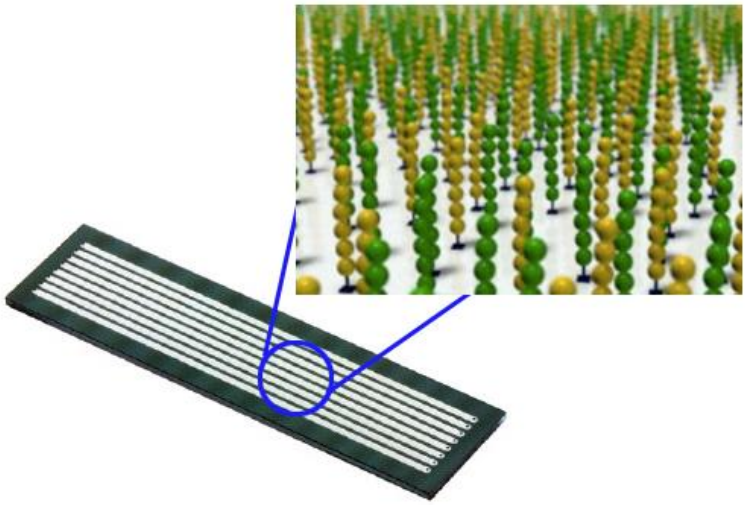


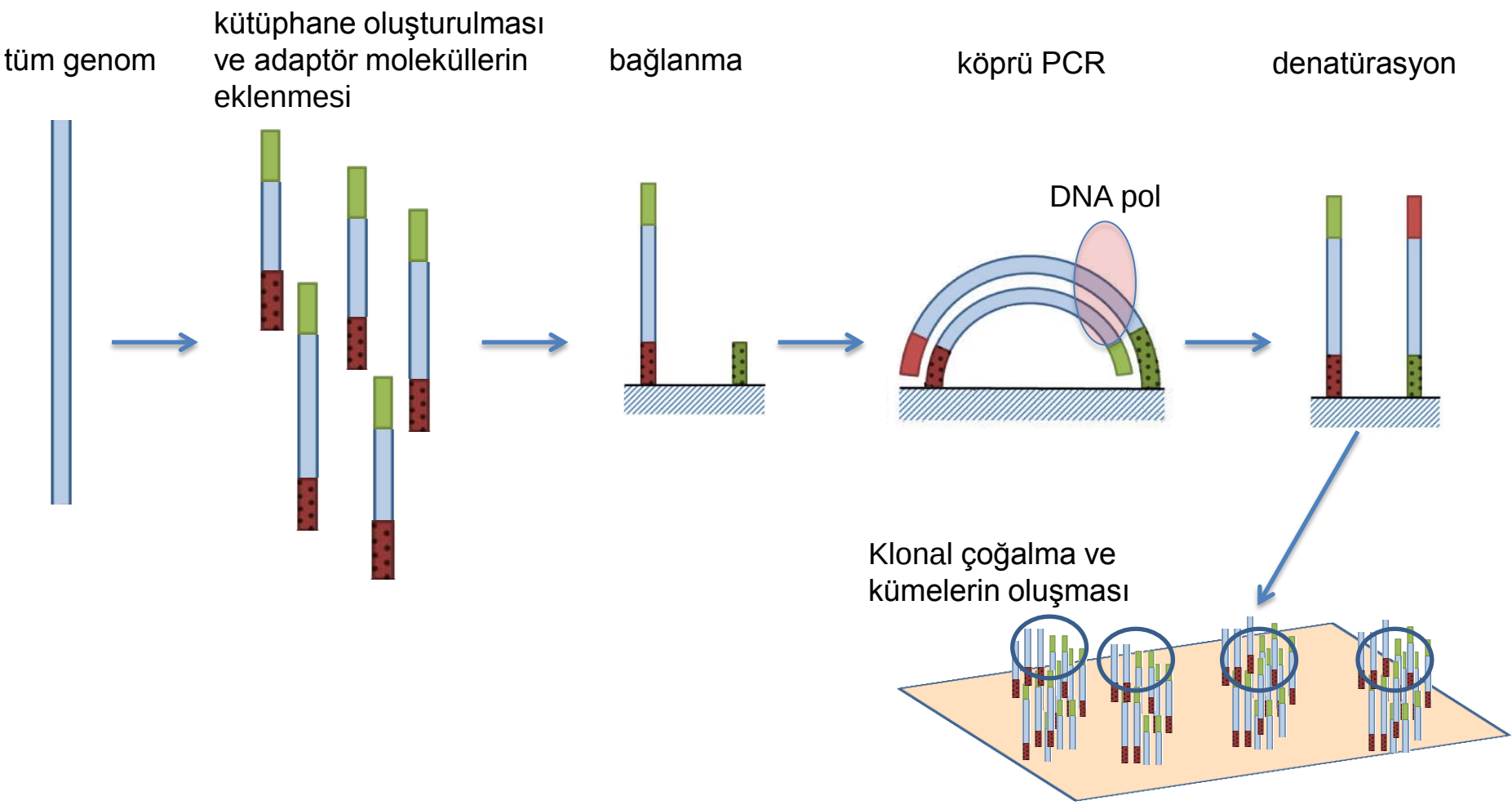
Figure 2. (a) Abundance and prevalence of the different bacterial phyla in 19 plaque samples (b) Abundance and prevalence of the different bacterial genera in 19 plaque samples.
doi:10.1371/journal.pone.0089269.g002

Genome Analyzer *Solexa-illumina*

- Geliştirilen ikinci yeni nesil dizileme cihazıdır ve diğerinden farklı bir yöntem kullanılır.
- Tüm reaksiyon mikroskop slaytına benzer katı bir fazda gerçekleştirilir.

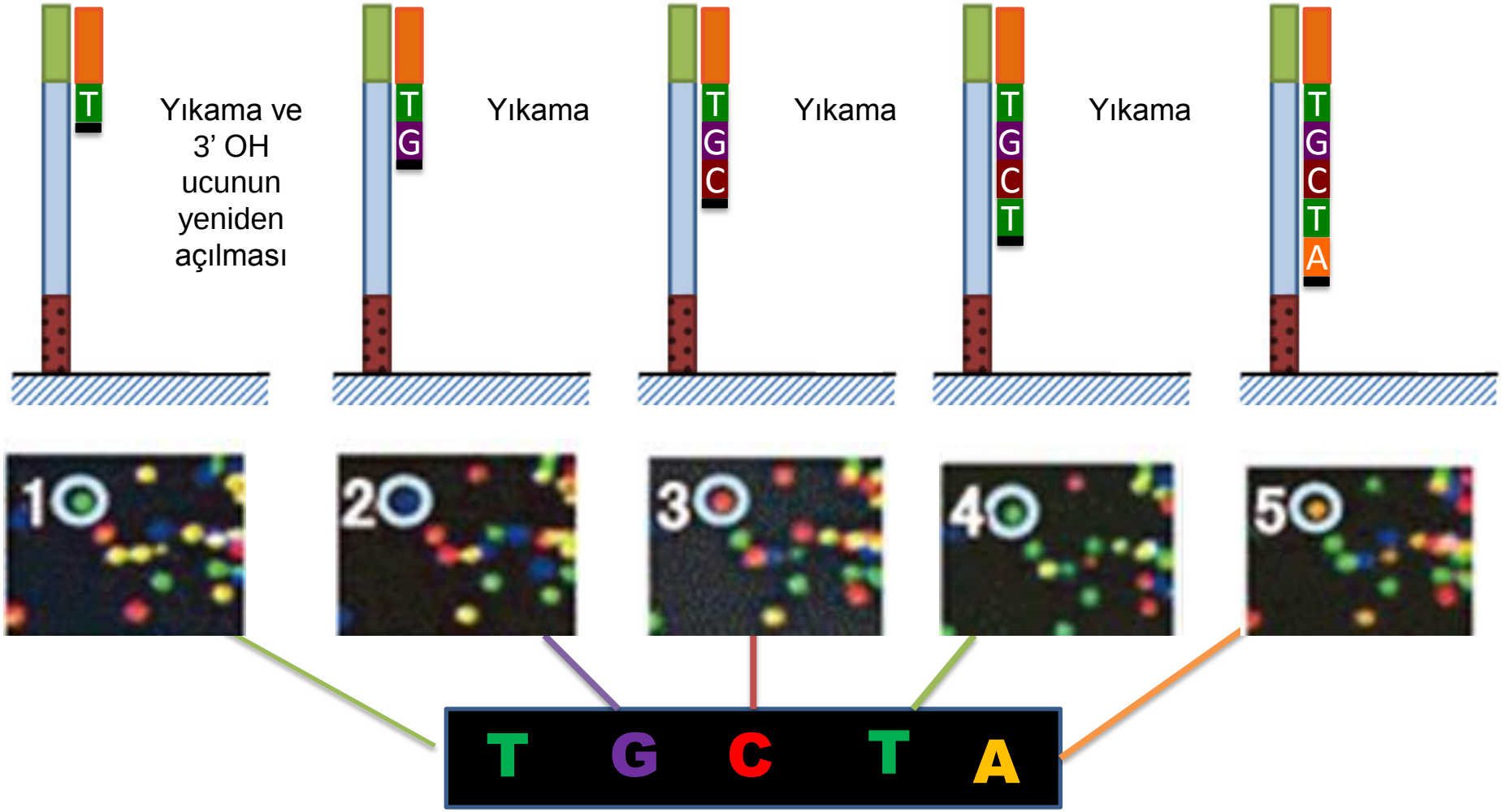


Genome Analyzer *illumina*



Genome Analyzer *illumina*

- Nükleotidlerin 3’ OH uçları kimyasal olarak kapatılmış olduğundan zincir sonlanmasına neden olur.



HiSeq 2000 *illumina*

- Tek yükleme ile iki bireye ait genomu kişi başı 10.000 dolarlık bir maliyetle dizileyebilmektedir.

Sequencing by Synthesis

Cluster Generation

Kütüphane hazırlanması
1-14 gün

↓

Zenginleştirme
5 saat

↓

Sentez ile dizileme
2-10 gün

↓

Verilerin analizi
2 gün

HiSeq 2000 *illumina*

Lazarevic *et al. Journal of Clinical Bioinformatics* 2012, **2**:4
http://www.jclinbioinformatics.com/content/2/1/4

JCBi JOURNAL OF CLINICAL BIOINFORMATICS

RESEARCH Open Access

Analysis of the salivary microbiome using culture-independent techniques

Vladimir Lazarevic[1*], Katrine Whiteson[1], Nadia Gaïa[1], Yann Gizard[1], David Hernandez[1], Laurent Farinelli[2], Magne Østerås[2], Patrice François[1] and Jacques Schrenzel[1]

Abstract

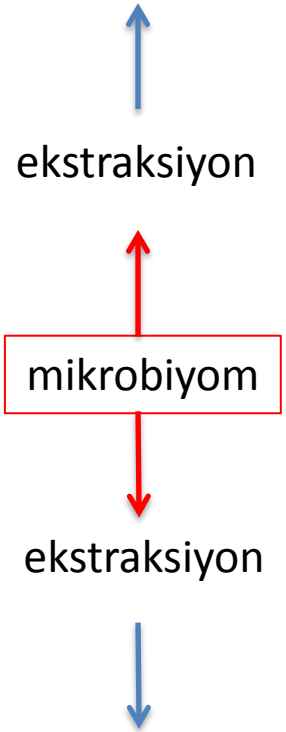
Background: The salivary microbiota is a potential diagnostic indicator of several diseases. Culture-independent techniques are required to study the salivary microbial community since many of its members have not been cultivated.

Methods: We explored the bacterial community composition in the saliva sample using metagenomic whole genome shotgun (WGS) sequencing, the extraction of 16S rRNA gene fragments from metagenomic sequences (16S-WGS) and high-throughput sequencing of PCR-amplified bacterial 16S rDNA gene (16S-HTS) regions V1 and V3.

Results: The hierarchical clustering of data based on the relative abundance of bacterial genera revealed that distances between 16S-HTS datasets for V1 and V3 regions were greater than those obtained for the same V region with different numbers of PCR cycles. Datasets generated by 16S-HTS and 16S-WGS were even more distant. Finally, comparison of WGS and 16S-based datasets revealed the highest dissimilarity.
The analysis of the 16S-HTS, WGS and 16S-WGS datasets revealed 206, 56 and 39 bacterial genera, respectively, 124 of which have not been previously identified in salivary microbiomes. A large fraction of DNA extracted from saliva corresponded to human DNA. Based on sequence similarity search against completely sequenced genomes, bacterial and viral sequences represented 0.73% and 0.0036% of the salivary metagenome, respectively. Several sequence reads were identified as parts of the human herpesvirus 7.

Conclusions: Analysis of the salivary metagenome may have implications in diagnostics e.g. in detection of microorganisms and viruses without designing specific tests for each pathogen.

16S Yüksek hacimli dizileme



Tüm genom dizleme
(metagenomik dizileme)

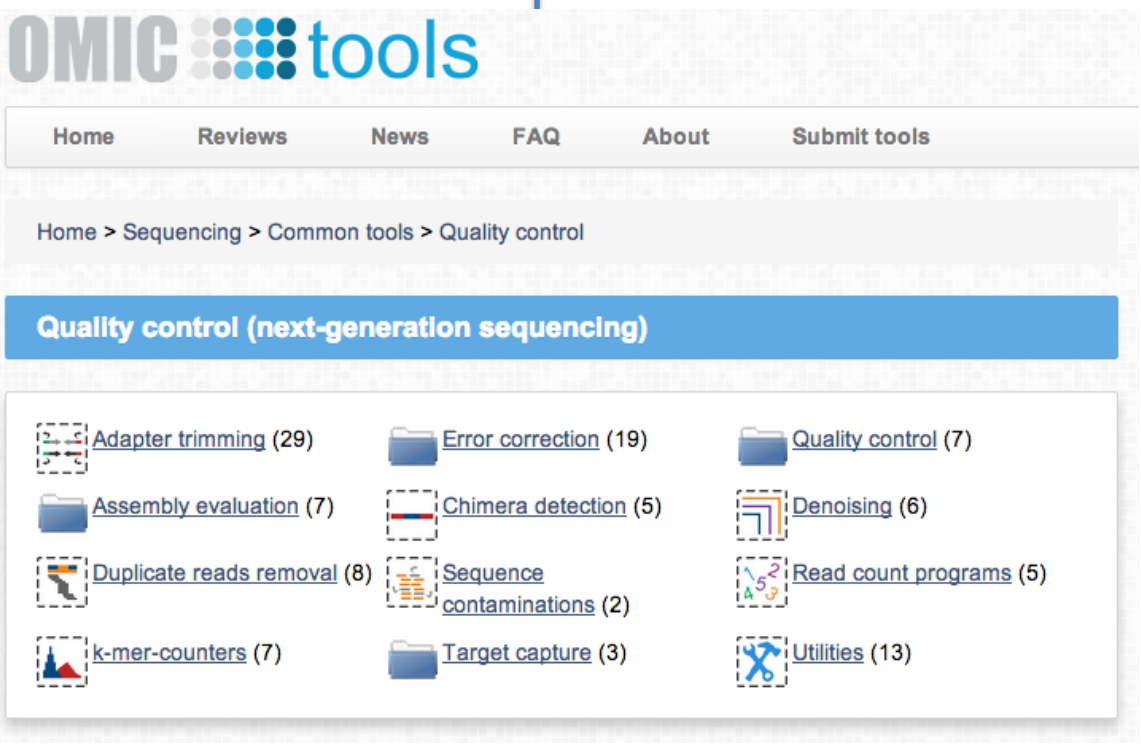
HiSeq 2000 *illumina*

Table 1 Description of the 4 sequence datasets and 14 subsets

	Dataset						
	16S-HTS, 20 cycles		16S-HTS, 25 cycles		16S-HTS, 30 cycles		WGS
Generated reads	16,345,598		10,732,108		6,150,466		14'724,838
Randomly chosen read pairs	1,200,000		1,200,000		1,200,000		1,000,000
16S rDNA region subset	V1	V3	V1	V3	V1	V3	
Filtered forward reads	592,322	566,310	576,701	564,774	546,710	569,459	1,000,000
Filtered reverse reads	532,945	530,504	534,229	523,548	548,009	495,531	1,000,000

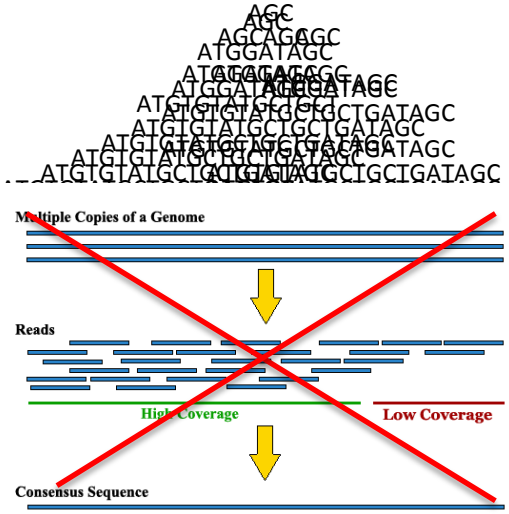
36 tür

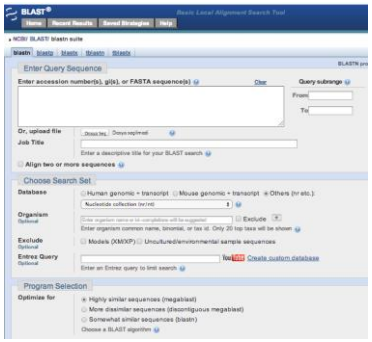
Neden daha az ?

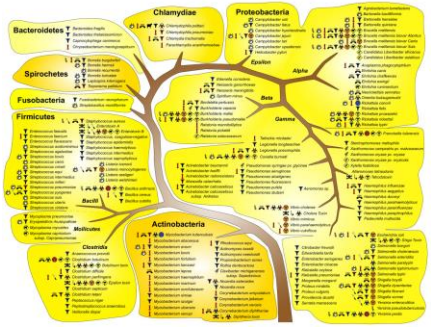


HiSeq 2000 *illumina*

- Mikrobiyota çalışmalarında “tüm genom dizileme” yaklaşımında okunan dizilerin birleştirilme (assemble) şansı şu an için çok zor.



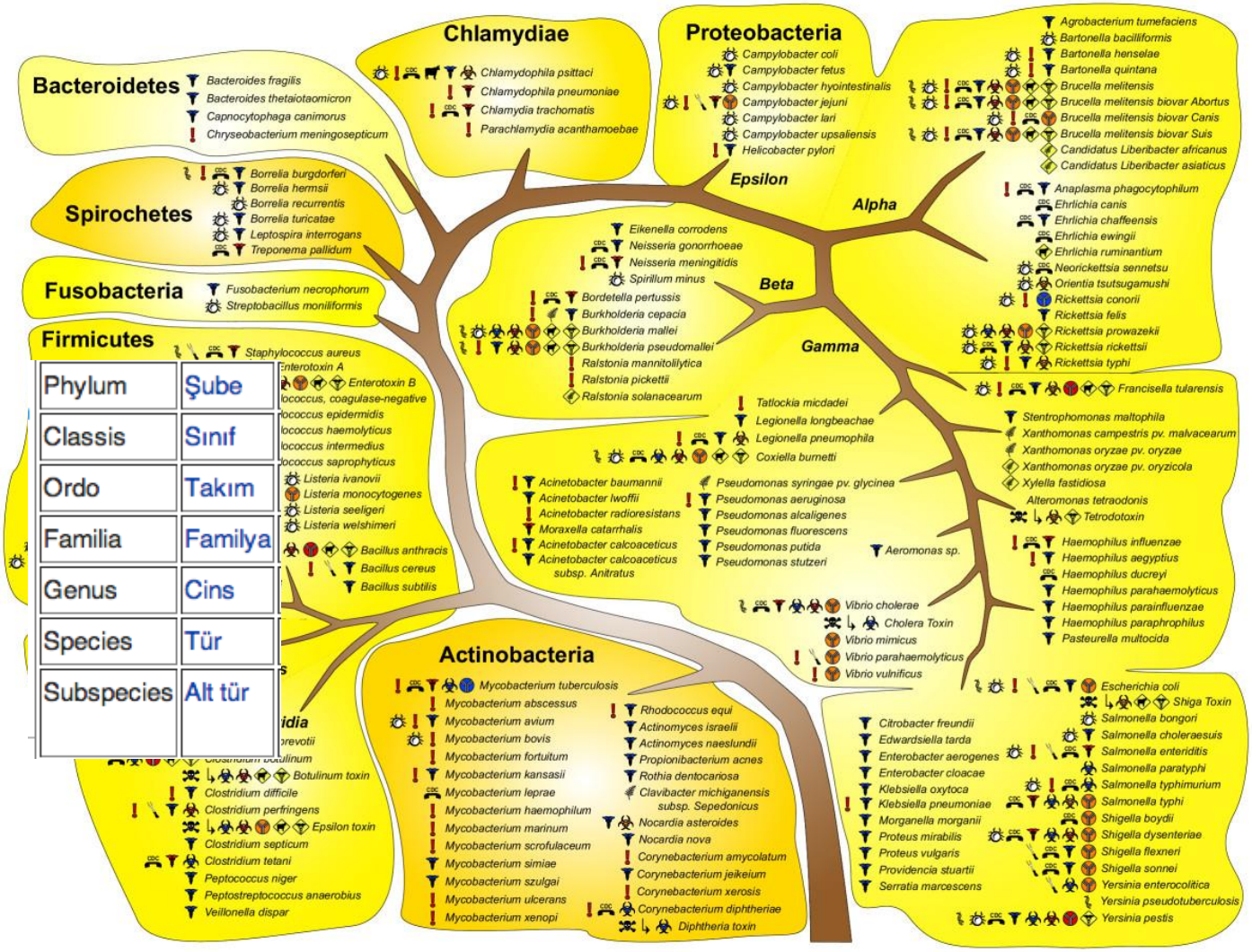




http://www.biomedcentral.com/1471-2180/5/19/figure/F2?highres=y

HiSeq 2000 *illumina*

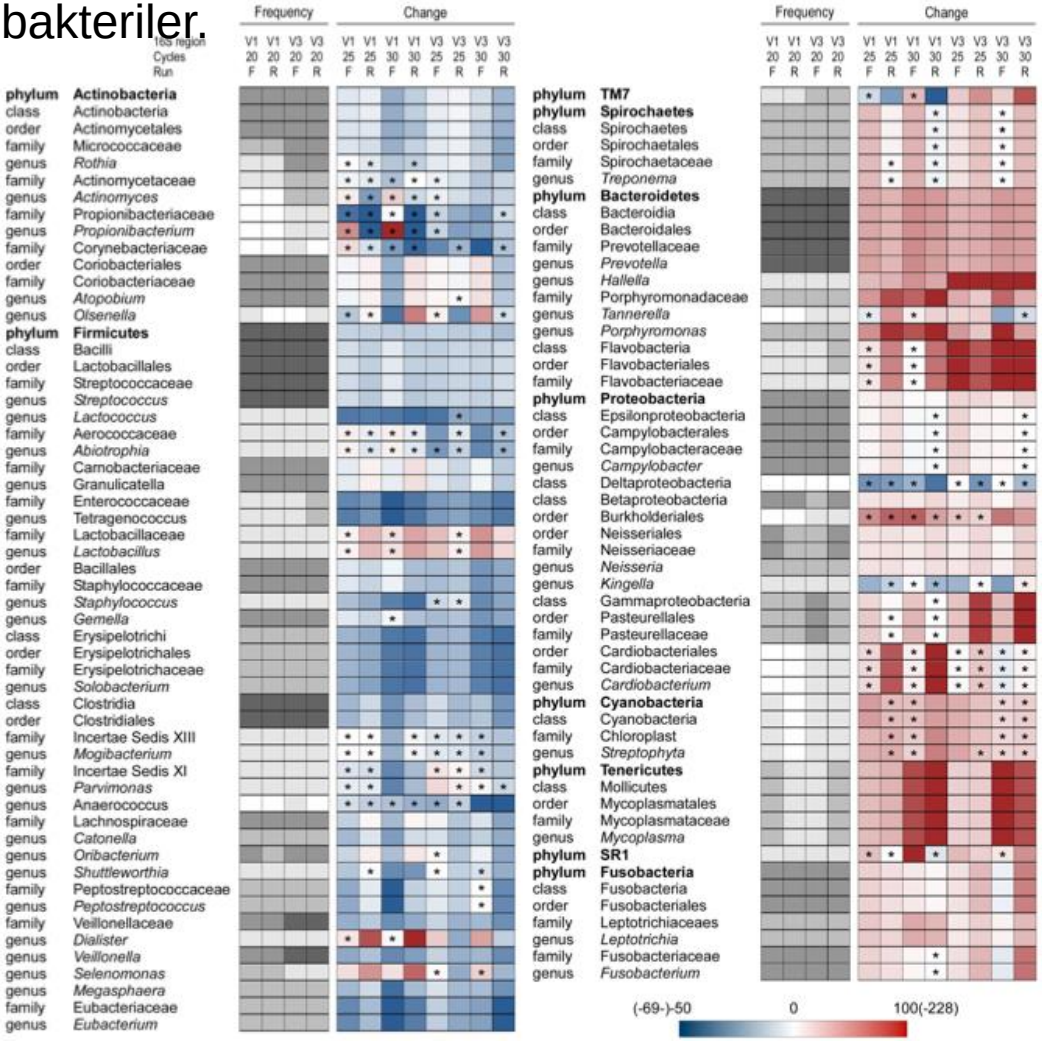
ATTTGCGCTGTTAGATTTATTTGCATACTTATTAATGATTTA



http://www.biomedcentral.com/1471-2180/5/19/figure/F2?highres=y

HiSeq 2000 *illumina*

- Bu çalışmada 16S “yüksek hacimli dizileme” yaklaşımı ile tespit edilebilen bakteriler.



HiSeq 2000 *illumina*

- Virüsler ve fajlar sadece "tüm genom dizileme" yaklaşımı ile tespit edilebilir.

Table 4 Detection of viral sequences in the WGS dataset using BLASTN

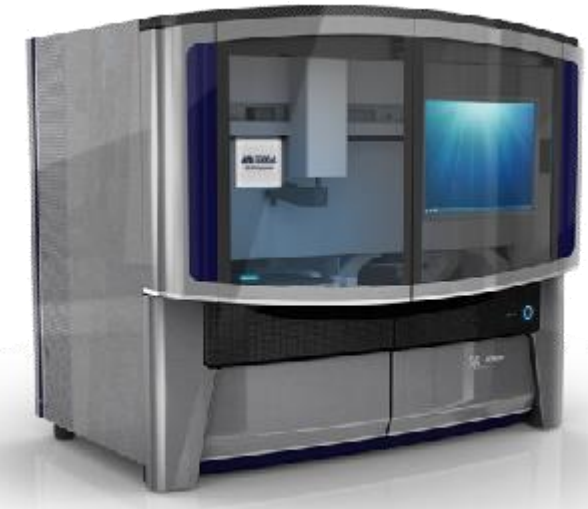
	Number of hits [a]	
	F	R
Virus		
Human herpesvirus 7	17	15
Porcine endogenous retrovirus E	1	-
Paramecium bursaria chlorella virus-1 FR483	1	1
Phage		
Enterobacteria phage lambda	12	11
Enterobacteria phage phiX174	6	7
Streptococcus phage SM1	1	-

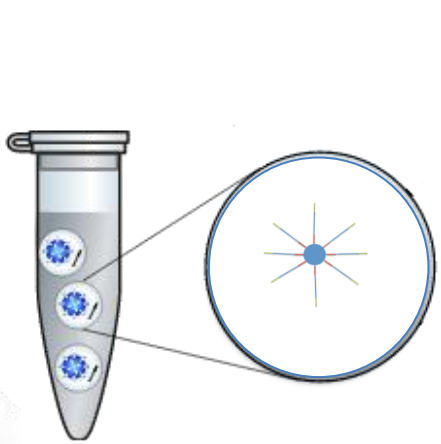
F, forward run; R, reverse run.

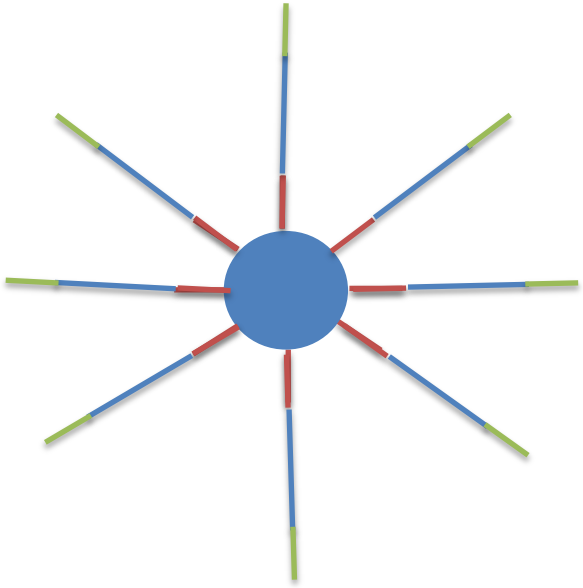
[a] The following BLASTN parameters were used: wordsize = 16, e ≤ 10^{-30}, sequence identity ≥ 94%, alignment length ≥ 90 nt.

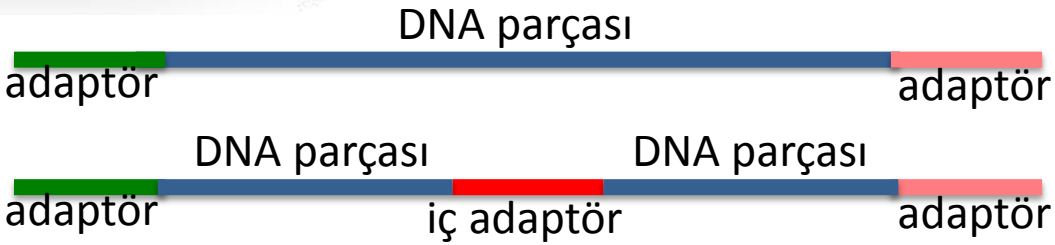
SOLID *Applied Biosytem*

- Örneğin hazırlık aşamaları, hedefin klonal (küme) olarak çoğaltılması ve emülsiyon PCR basamakları 454 sistemine oldukça benzerlik göstermektedir.



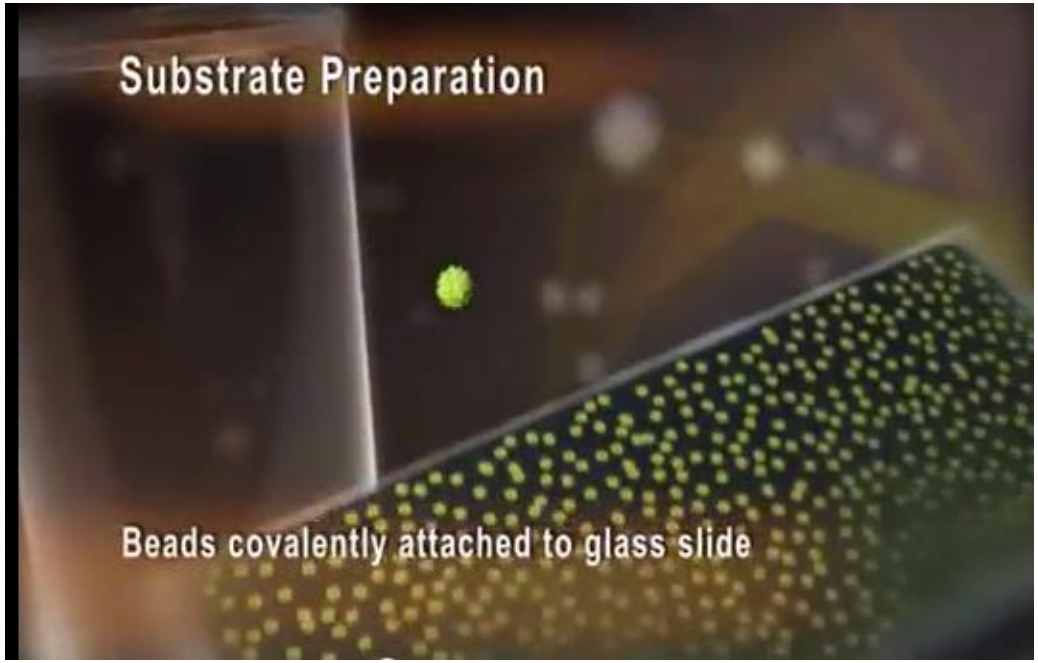




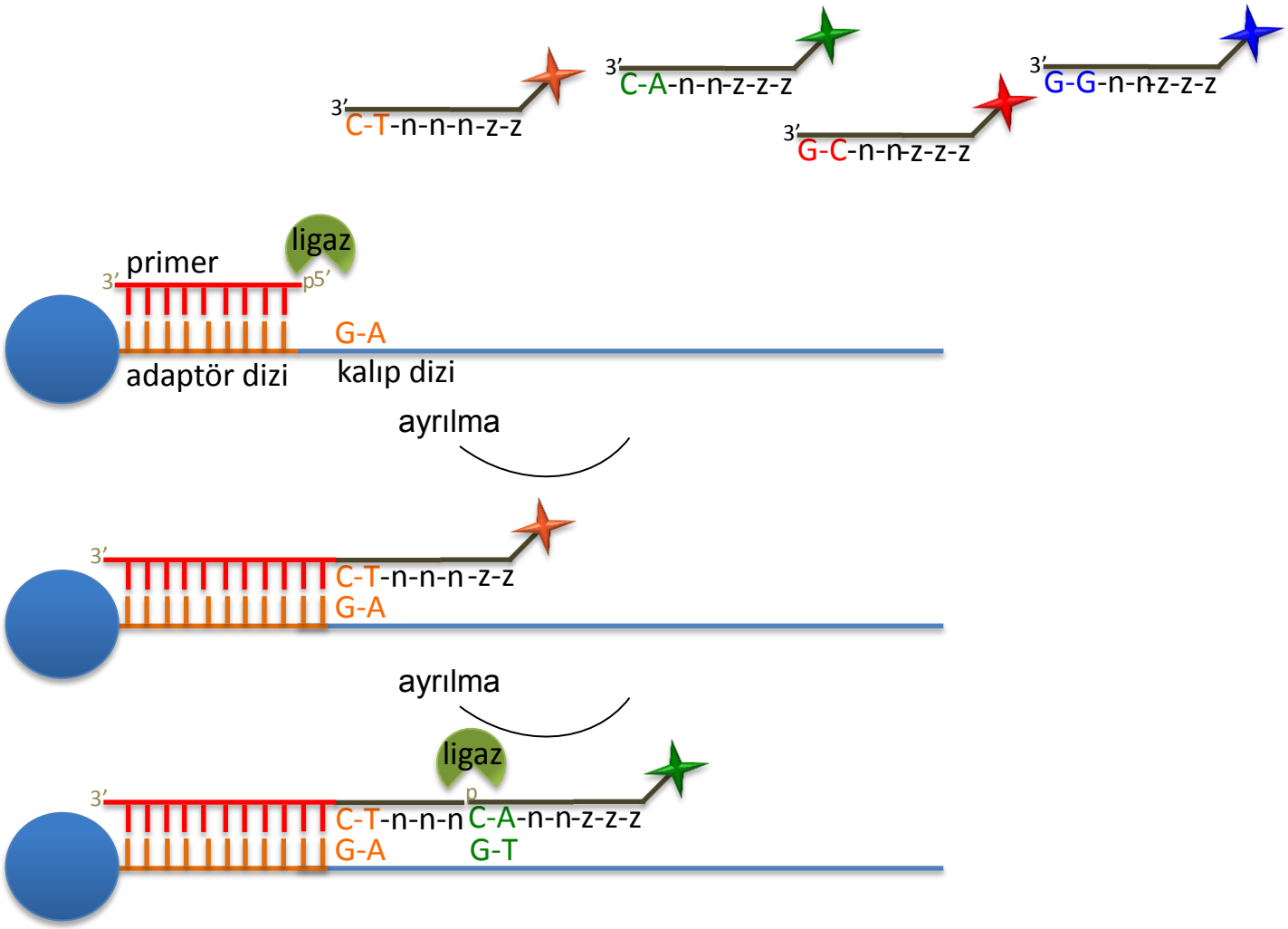


SOLID *Applied Biosytem*

- Bu dizileme yönteminde diğerlerinden farklı olarak, çoğalmış DNA, boncuklardan saflaştırılarak slayt yüzeyine kovalent olarak bağlanır.



SOLID *Applied Biosytem*



SOLID *Applied Biosytem*

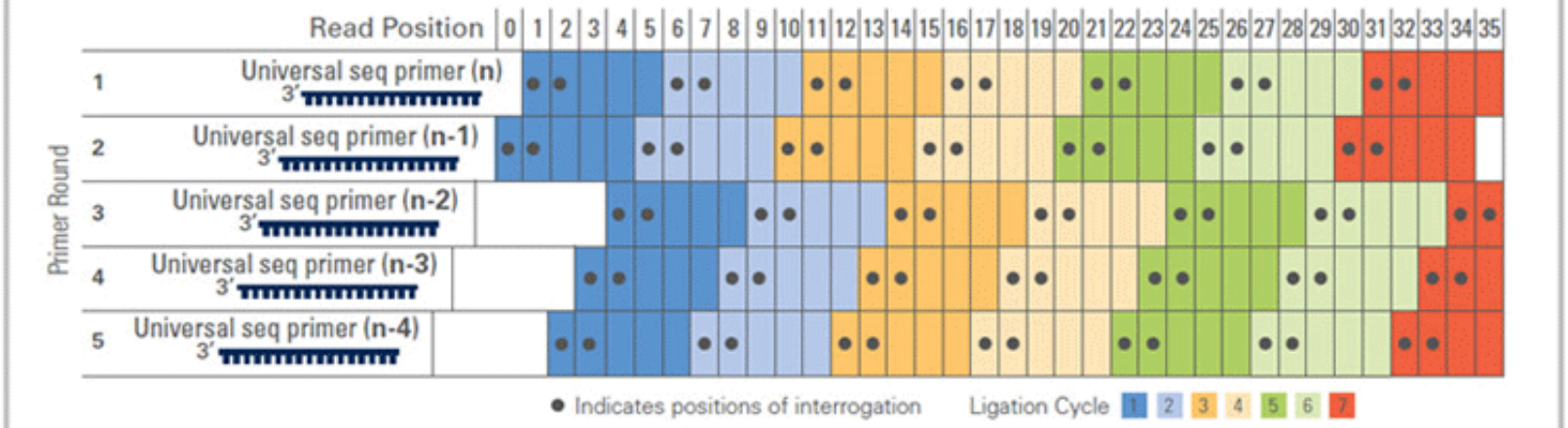
- Bu teknolojinin en farklı yanı

  ise prob bağlanma işleminin iki oligonükleotid tarafından kontrol edilmesidir.

- Bu metot sentezleyerek dizileme yaklaşımına göre belirleyici şekilde

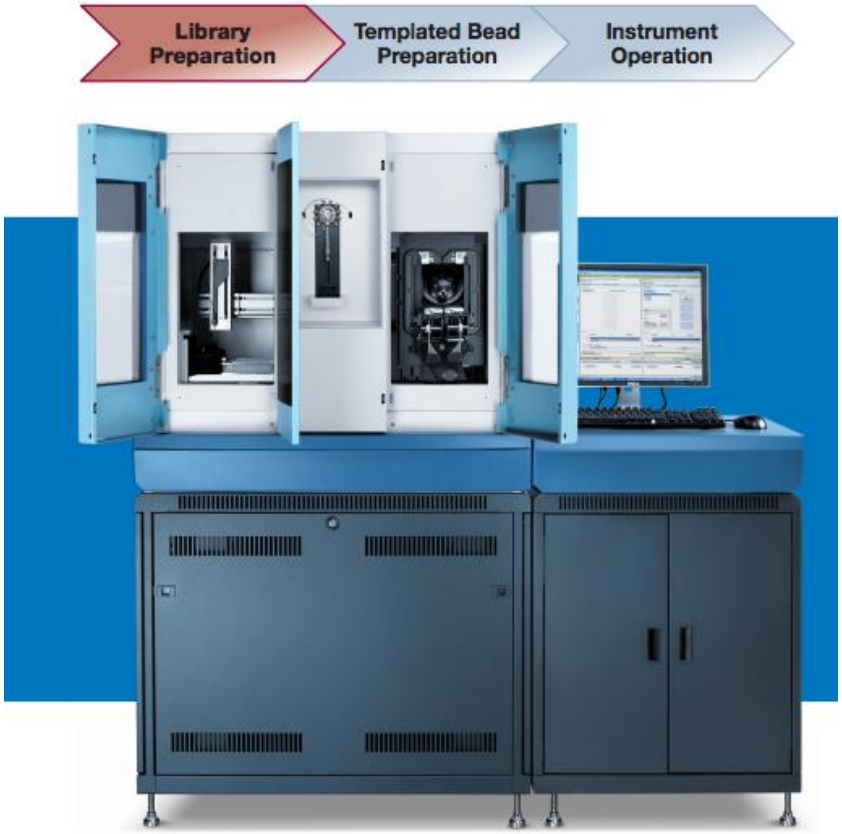
  yüksek özgüllük ve yüksek doğruluk göstermektedir.

DUAL INTERROGATION OF EACH BASE



SOLID *Applied Biosytem*

- *SOLID* cihazı geliştirilerek her okumadaki baz sayısı artırılmış ve daha kolay iş akışı sağlanmıştır.
- Tek slayt üzerinde okuma uzunluğu artırılarak 10 günde 60 gigabazdan fazla ve dizileme yapabilmektedir.

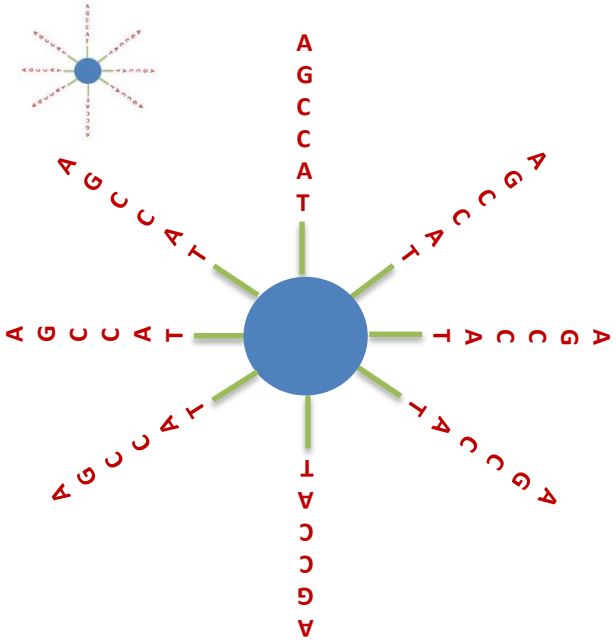


SOLID *Applied Biosytem*

- 2010 yılında yeni geliştirilen SOLID 4hq cihazı 14 günde 300 gigabaz okuma yapabilmektedir ve tek genom maliyeti yaklaşık 3000 dolardır.

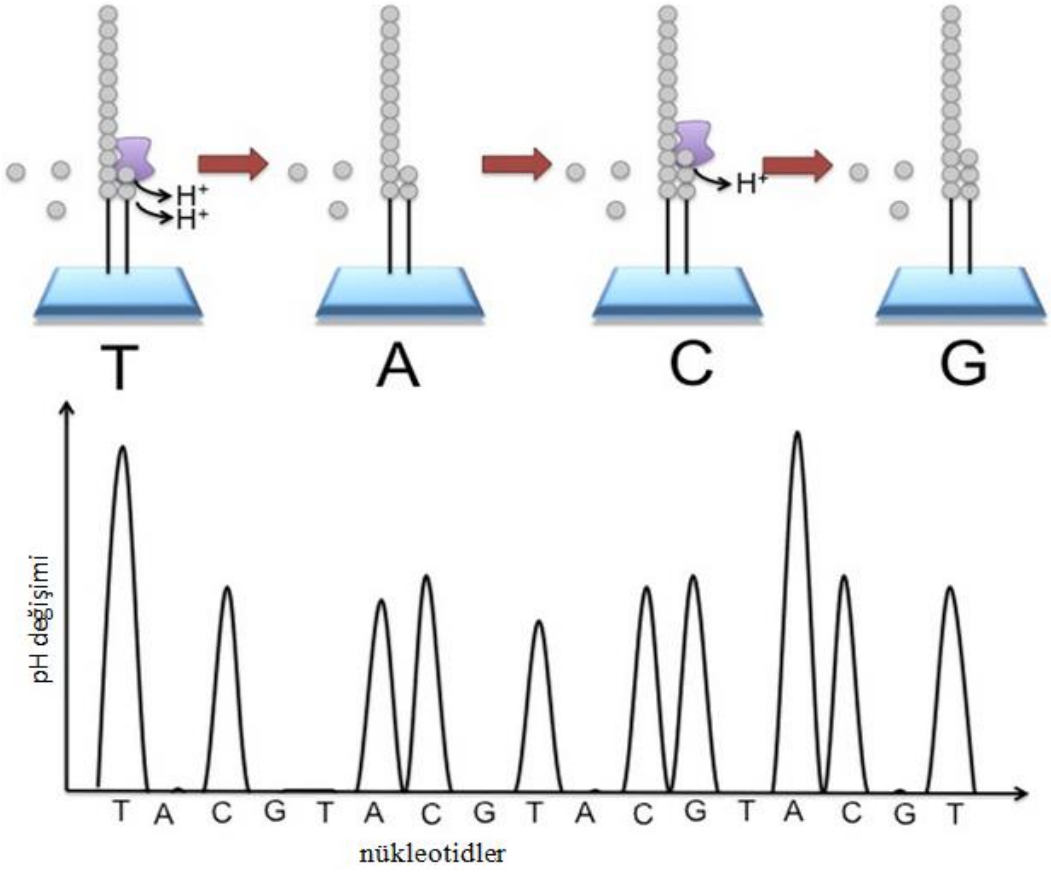
İon Torrent

- Herhangi bir optik okuma veya floresan ışımanın yer almadığı bir sistem olan bu sistem 2010 yılında tanıtılmış diğerlerinden oldukça farklı bir sistemdir.



İon Torrent

- İyon yarı iletken dizilemesine dayalı bu dizileme yöntemi, DNA'nın polimerizasyonu sırasında açığa çıkan hidrojen iyonlarının tespitine dayalıdır.



İon Torrent

- Cihazın en önemli bileşeni kimyasal değişimleri tespit etmek amacıyla kullanılan yarı iletken çiplerdir.

- Cihazın İyon Proton II çipine sahip modeli, 660 milyon çipten oluşmakta ve tüm insan genomunun dizilenmesi için kullanılabilmektedir.

İon Torrent

OPEN ACCESS Freely available online

PLOS ONE

Meconium Microbiome Analysis Identifies Bacteria Correlated with Premature Birth

Alexandria N. Ardissone[1], Diomel M. de la Cruz[2], Austin G. Davis-Richardson[1], Kevin T. Rechcigl[1], Nan Li[2], Jennifer C. Drew[1], Roberto Murgas-Torrazza[2], Renu Sharma[3], Mark L. Hudak[3], Eric W. Triplett[1], Josef Neu[2]*

1 Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, United States of America, **2** Department of Pediatrics, University of Florida, Gainesville, Florida, United States of America, **3** Department of Pediatrics, University of Florida, Jacksonville, Florida, United States of America

Abstract

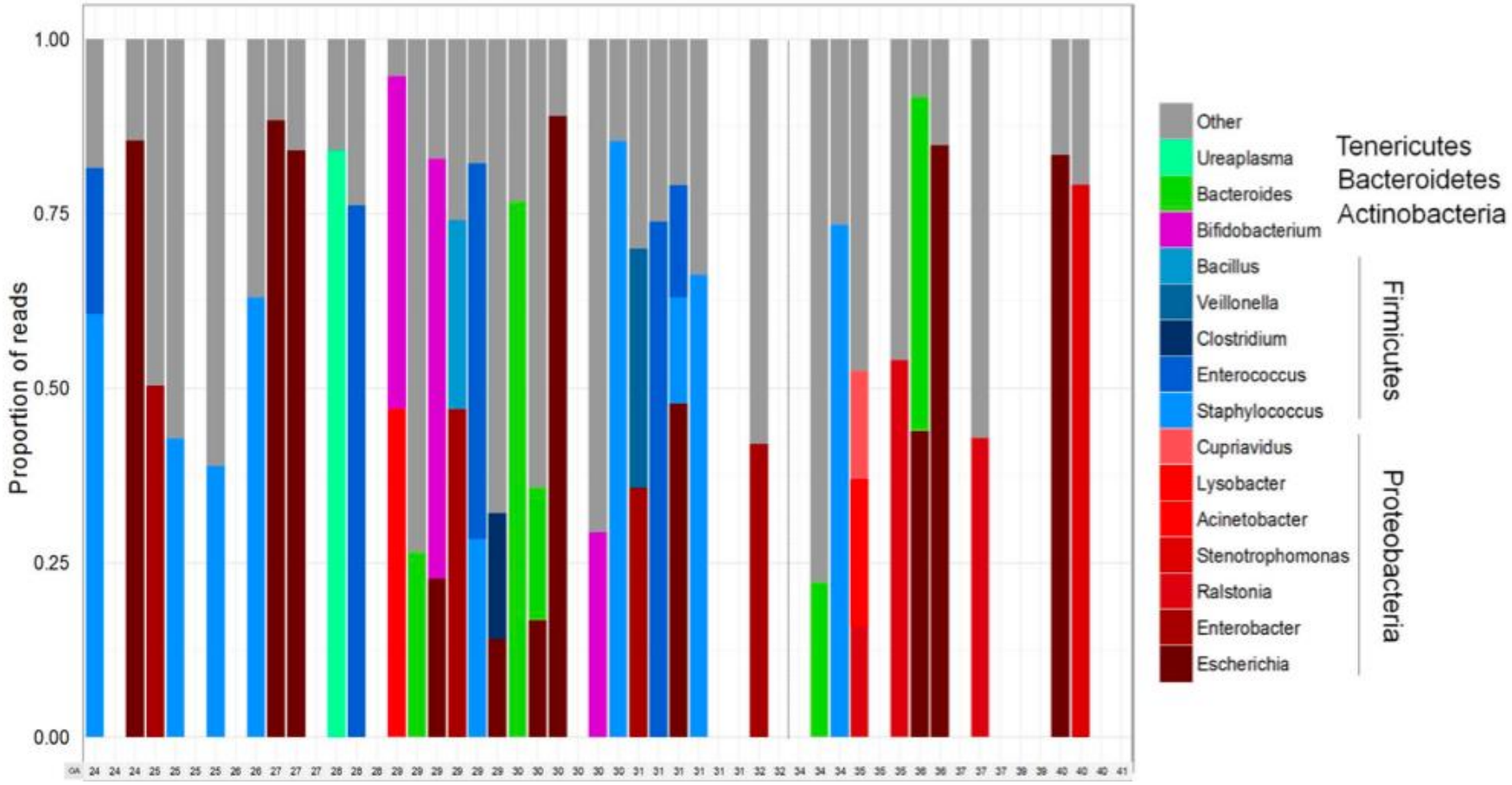
Background: Preterm birth is the second leading cause of death in children under the age of five years worldwide, but the etiology of many cases remains enigmatic. The dogma that the fetus resides in a sterile environment is being challenged by recent findings and the question has arisen whether microbes that colonize the fetus may be related to preterm birth. It has been posited that meconium reflects the in-utero microbial environment. In this study, correlations between fetal intestinal bacteria from meconium and gestational age were examined in order to suggest underlying mechanisms that may contribute to preterm birth.

Methods: Meconium from 52 infants ranging in gestational age from 23 to 41 weeks was collected, the DNA extracted, and 16S rRNA analysis performed. Resulting taxa of microbes were correlated to clinical variables and also compared to previous studies of amniotic fluid and other human microbiome niches.

Findings: Increased detection of bacterial 16S rRNA in meconium of infants of <33 weeks gestational age was observed. Approximately 61·1% of reads sequenced were classified to genera that have been reported in amniotic fluid. Gestational age had the largest influence on microbial community structure ($R = 0{\cdot}161$; $p = 0{\cdot}029$), while mode of delivery (C-section versus vaginal delivery) had an effect as well ($R = 0{\cdot}100$; $p = 0{\cdot}044$). *Enterobacter*, *Enterococcus*, *Lactobacillus*, *Photorhabdus*, and *Tannerella*, were negatively correlated with gestational age and have been reported to incite inflammatory responses, suggesting a causative role in premature birth.

Interpretation: This provides the first evidence to support the hypothesis that the fetal intestinal microbiome derived from swallowed amniotic fluid may be involved in the inflammatory response that leads to premature birth.

İon Torrent


1.00
0.75
0.50
0.25
0.00
Proportion of reads
GA
Other
Ureaplasma
Bacteroides
Bifidobacterium
Bacillus
Veillonella
Clostridium
Enterococcus
Staphylococcus
Cupriavidus
Lysobacter
Acinetobacter
Stenotrophomonas
Ralstonia
Enterobacter
Escherichia
Tenericutes
Bacteroidetes
Actinobacteria
Firmicutes
Proteobacteria

İon Torrent

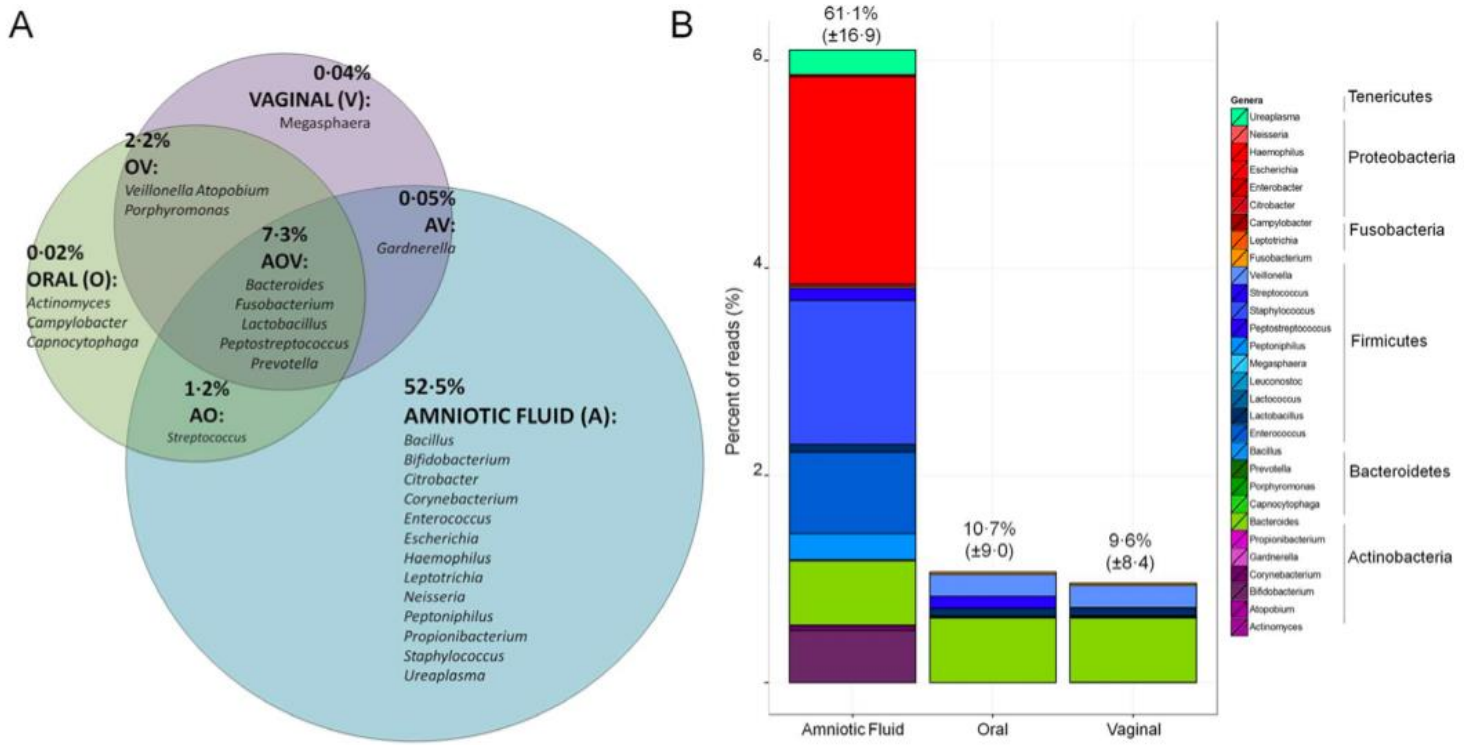
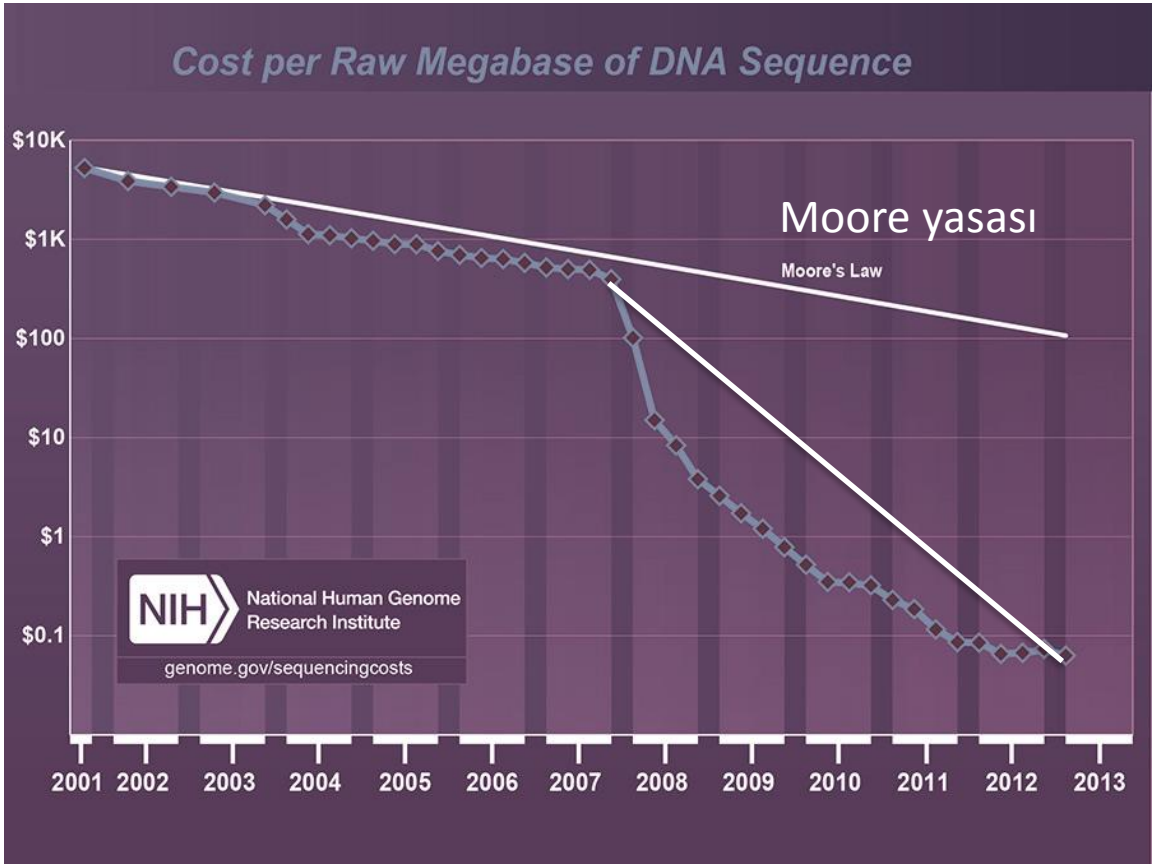


Figure 2. Meconium microbiome is most suggestive of amniotic fluid origin. (A) The average percent relative abundance in meconium samples of this study for genera reported in amniotic fluid, and the oral and vaginal cavities of pregnant women[6,7,27] are displayed by the Venn diagram which distinguishes unique and shared maternal environments of genera. (B) The potential total mean contribution and standard deviation of any particular maternal locale (amniotic fluid[5,6], oral[21], or vaginal[21]), and the phyletic distribution of contributing genera is shown in the stacked bar plot. The color assignment is as follows: Actinobacteria = purple; Bacteroidetes = green; Firmicutes = blue; Fusobacteria = orange; Proteobacteria = red; Tenericutes = aquamarine.

Yeni Nesil Dizileme Cihazları:

İlk yeni nesil dizileme sistemleri,

yüksek doğruluk, hız ve düşük fiyata tahminden daha hızlı eriştiler.



Yöntem iki dezavantaja sahipti.

- PCR'ın pahalı ve zaman alıcı bir işlem olmasıdır.
- Diğeri ise bu aşamada hatalı bazların çoğaltılmasıdır.

İkinci Yeni Nesil Dizileme Cihazları:

Tek molekül dizileme sistemleri

Faster human genome sequencing

Yingrui Li & Jun Wang

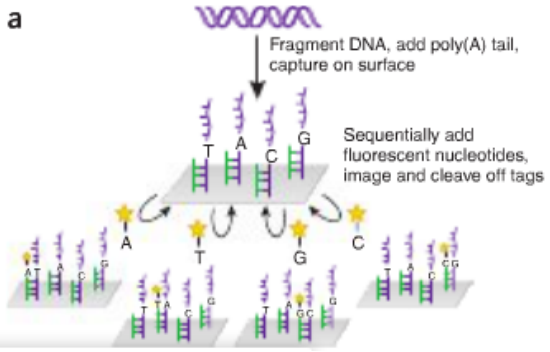
Advances in parallelization allow a human genome to be sequenced using single-molecule technology.

Genome sequencing has reached another milestone: the sequencing of a human genome using single-molecule technology (**Fig. 1a**). This feat, reported by Pushkarev *et al.*[1] in this issue, was achieved by three researchers in a matter of weeks for an estimated reagent cost of $48,000, highlighting the rapid pace of technology development in the sequencing field[2] and bringing us a step closer to the day when genome analysis is routinely applied in personalized medicine.

Pushkarev *et al.*[1] used a new sequencer from Helicos BioSciences, one of a growing number of cutting-edge sequencing instruments that includes Roche 454's GS FLX Titanium[3], Illumina's Genome Analyzer II (ref. 4) and Applied Biosystems' SOLiD 3.0 (ref. 5). All of these machines are based on similar cyclic-array sequencing principles—'sequencing-by-synthesis' or 'sequencing-by-ligation'—but each uses distinct chemistries, leading to different performance characteristics (**Table 1**).

useful, for example, in targeting medicines to patients most likely to benefit.

The single-molecule sequencing technology used by Pushkarev *et al.*[1] does not require amplification, ligation or cloning. This provides a potential advantage that was not specifically mentioned by the authors but is worth noting here: improved sequencing accuracy. Amplification steps required by other technologies introduce errors that result in uneven coverage of the genome (caused in part by variation in local G+C percentage between regions) and spuriously identified SNPs. The authors detected SNPs and copy number



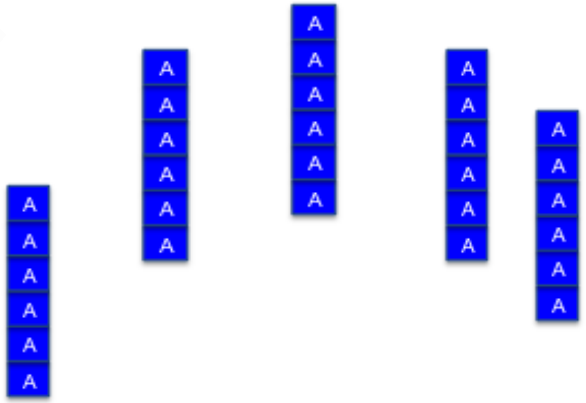
Helicos Genetic Analysis *Helicos BioSciences*

Original research shouldn't start with copies.

Helicos

Helicos Genetic Analysis *Helicos BioSciences*

- Bu sistemde, poly(dA) kuyruğu eklenen DNA parçaları, katı yüzeye bağlanmış oligo(dT) primerleri ile yakalanır.



Helicos Genetic Analysis *BioSciences*

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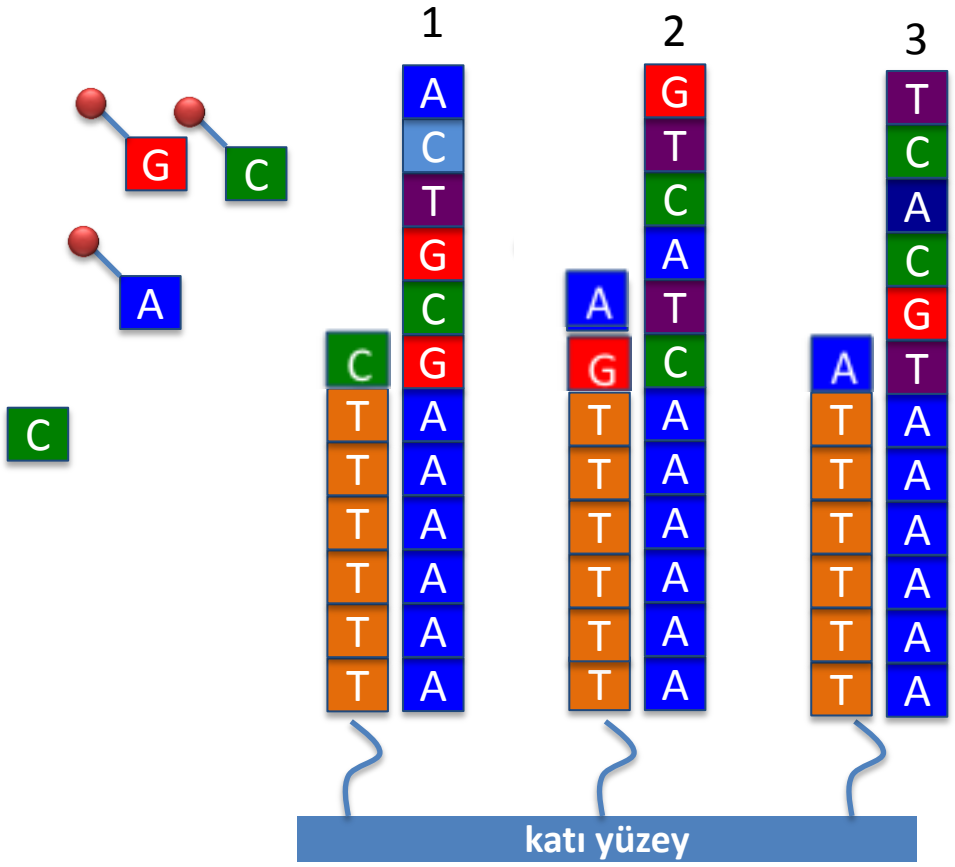
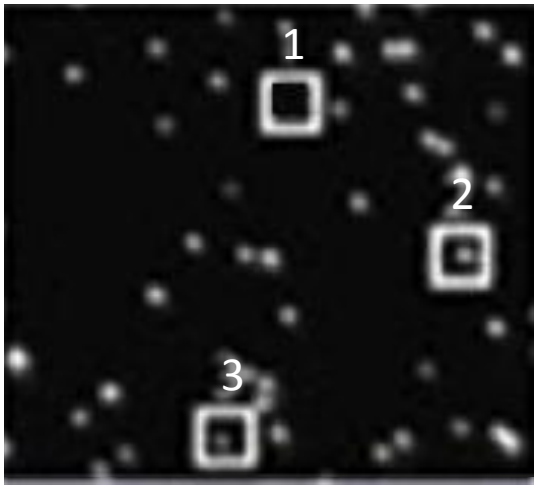




Helicos Genetic Analysis *Helicos BioSciences*

- Cihazın fiyatı 1.3 milyon dolar

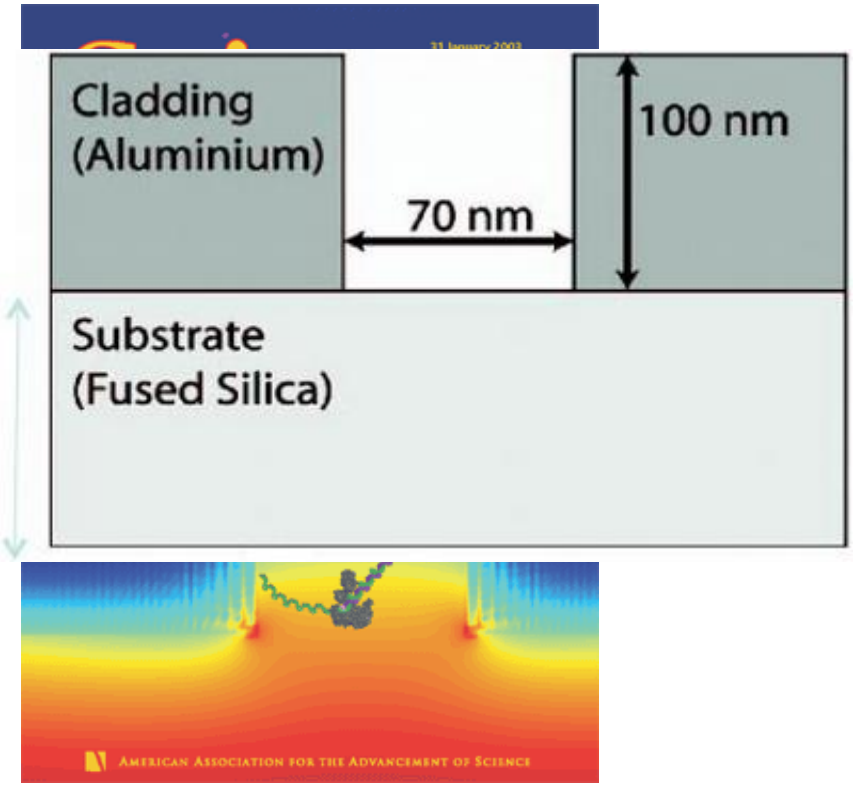
  ve çok fazla hata oranı olması ve çok kısa okumalar yapmasından dolayı popülaritesi sınırlıdır.

	Helicos Initial Spec	Leading Next-Gen Instrument*
Per Sample (Channel)	50 per run	8 per run
Sequencing Reagents	$360.00	$430.00
Sample Prep Labor	$13.00	$85.00
Sample Prep Reagents	$8.00	$625.00
Total Aftermarket Costs	$381.00	$1,140
Sample Prep Premium Paid	~6%	265%

Helicos is Faster and Cheaper

Pacific Biosciences

"Sıfır mod dalga alanı" (zero-mode waveguide ZMW) olarak adlandırılan, optik kuyucuk tabanına tek bir DNA polimeraz bağlanmıştır.



Pacific Biosciences

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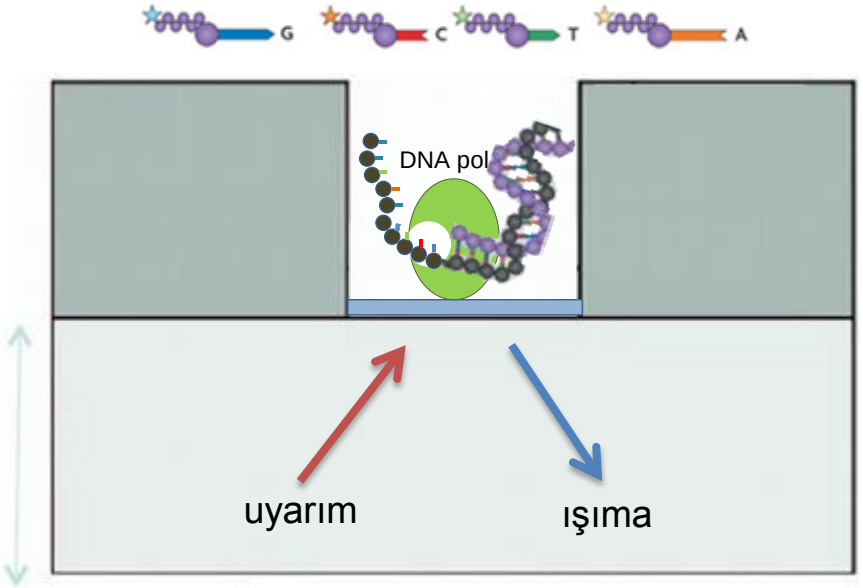
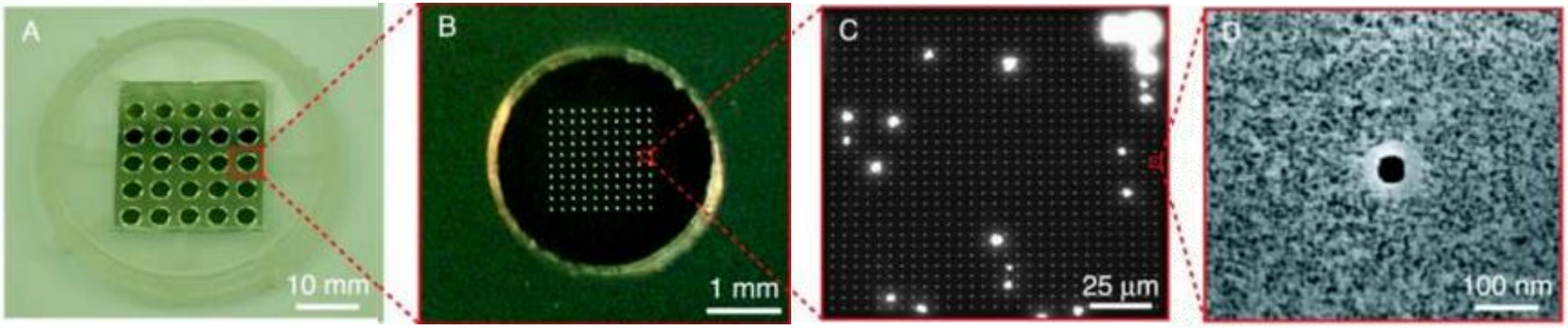




Pacific Biosciences

Fichot and Norman *Microbiome* 2013, **1**:10
http://www.microbiomejournal.com/content/1/1/10

COMMENTARY **Open Access**

Microbial phylogenetic profiling with the Pacific Biosciences sequencing platform

Erin B Fichot and R Sean Norman*

Abstract

High-throughput sequencing of 16S rRNA gene amplicons has revolutionized the capacity and depth of microbial community profiling. Several sequencing platforms are available, but most phylogenetic studies are performed on the 454-pyrosequencing platform because its longer reads can give finer phylogenetic resolution. The Pacific Biosciences (PacBio) sequencing platform is significantly less expensive per run, does not rely on amplification for library generation, and generates reads that are, on average, four times longer than those from 454 (C2 chemistry), but the resulting high error rates appear to preclude its use in phylogenetic profiling. Recently, however, the PacBio platform was used to characterize four electrosynthetic microbiomes to the genus-level for less than USD 1,000 through the use of PacBio's circular consensus sequence technology. Here, we describe in greater detail: 1) the output from successful 16S rRNA gene amplicon profiling with PacBio, 2) how the analysis was contingent upon several alterations to standard bioinformatic quality control workflows, and 3) the advantages and disadvantages of using the PacBio platform for community profiling.

Keywords: PacBio, Pacific Biosciences, Amplicon, 16S rRNA, Circular consensus sequence (ccs), Microbial diversity

Daha da Yeni Nesil Dizileme Sistemleri

nature International weekly journal of science

Nanopore genome sequencer makes its debut

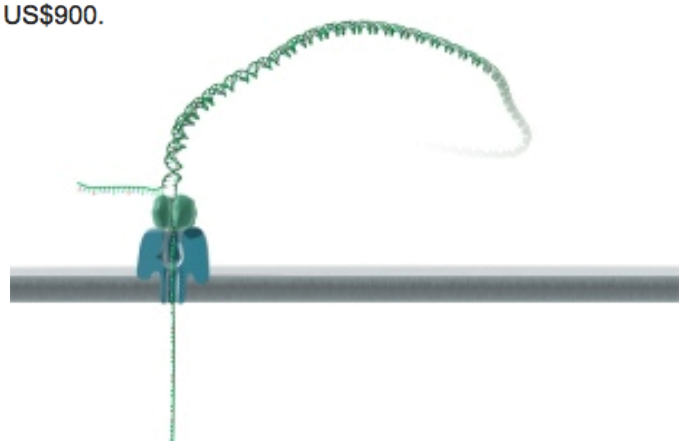
Technique promises it will produce a human genome in 15 minutes.

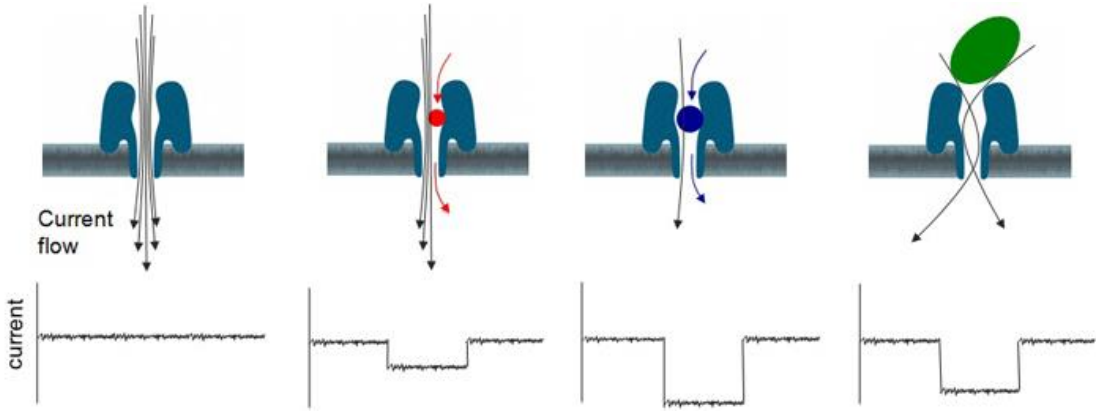
Erika Check Hayden

17 February 2012

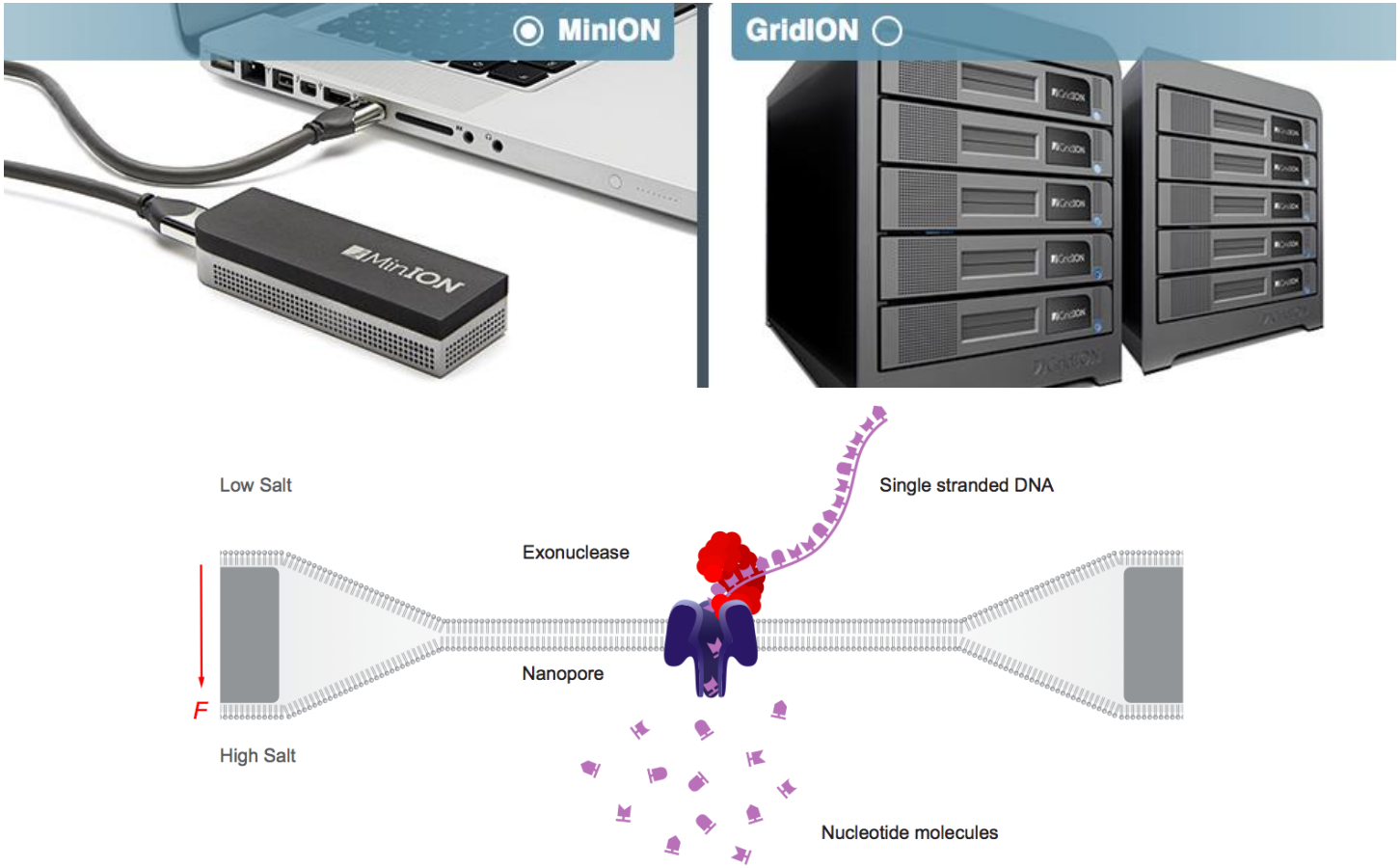
Technology that its parent company says will sequence a hur
opened its first data run to scrutiny today.

Oxford Nanopore Technologies, based in Oxford, UK, reveale
system at the Advances in Genome Biology and Technology
firm expects to start selling its new machine in the second ha
the world's first miniaturized, disposable sequencer — the Mi
US$900.





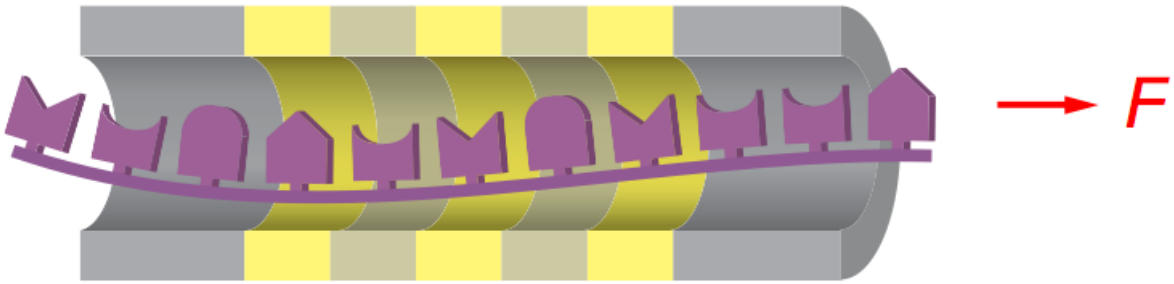
Oxford Nanopore Technology



IBM DNA Transitstor Technology

DNA'nın (sadece tek iplik halde geçebileceği)

"karbon nano tüplerden" (elektron tünelleri) geçerken meydana gelen elektrik akımını ölçülmesi prensibine dayalıdır.

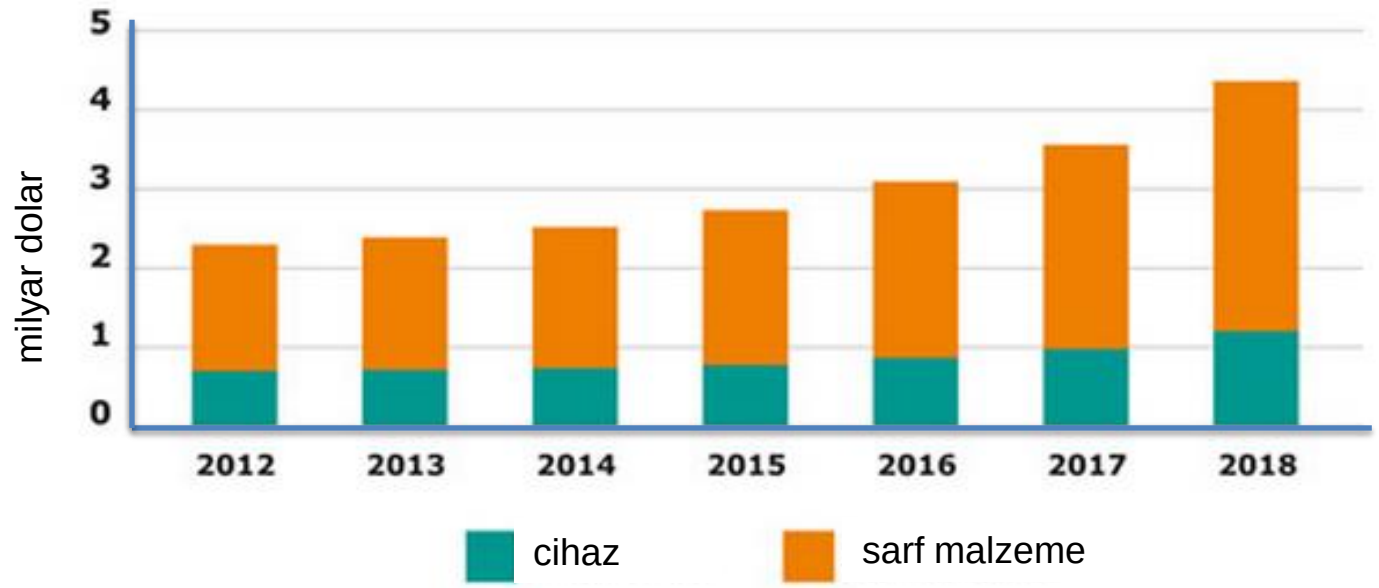


Yeni Nesil Dizileme Sistemleri

Tüm bu üstün özelliklerine rağmen,
bu sistemlerin rutin mikrobiyoloji
laboratuvarlarında yerini alması için henüz
erken dönemlerde olduğumuz söylenebilir.

Yeni Nesil Dizileme Pazarı

- 2018 de tahmin edilen pazar payı 4 milyar dolar.



http://www.electronics.ca/store/next-generation-sequencing.html

Yeni Nesil Dizileme Sistemlerini Kim Üretiyor ?

Bu çalışmalara başlamadan önce,

iyi hipotezler kurup, yeni nesil dizileme cihazlarının ürettiği milyonlarca veriyi nasıl işleyeceğimizi öğrenmek.