

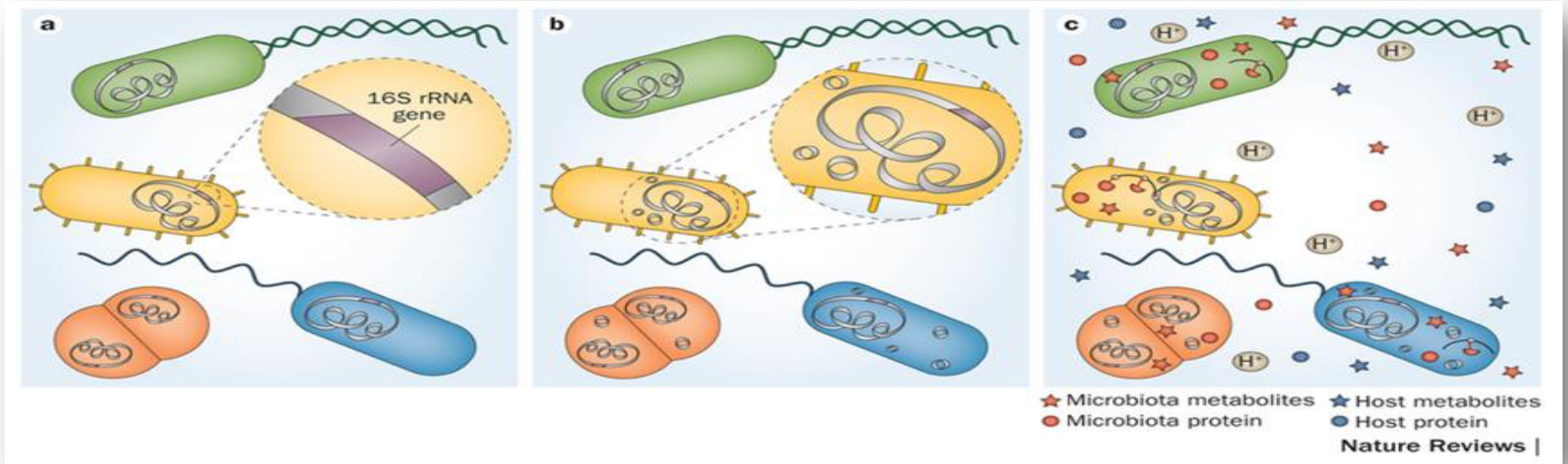
Klinik Metagenomiğin Vaadleri ve Problemleri



Aycan Gundogdu, Ph.D.

*Erciyes Üniversitesi ,Tıp Fakültesi ,Tıbbi Mikrobiyoloji Anabilim Dalı
Genom ve Kök Hücre Merkezi, Metagenomik Birimi, Kayseri*

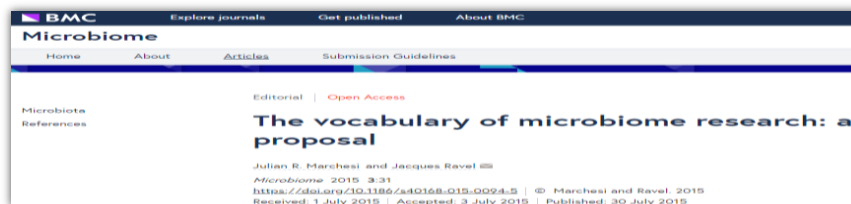
Terminoloji



a. Mikrobiyota: Tanımlanmış bir habitattaki **mikroorganizma** topluluğu

b. Metagenom: Tanımlanmış bir habitattaki **genomların** toplamı

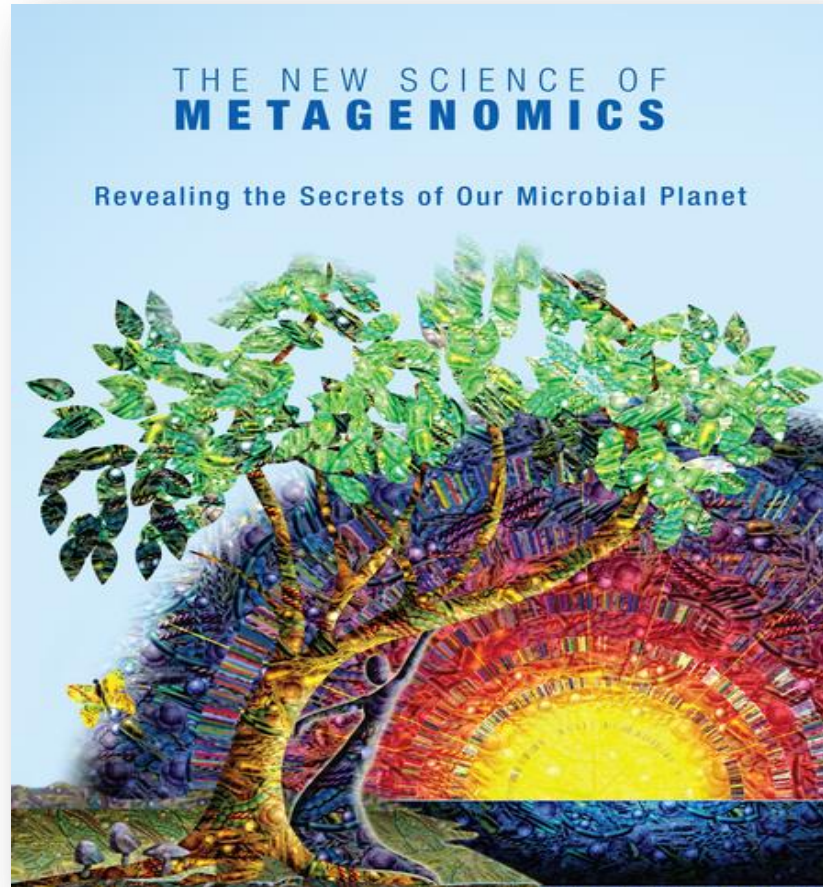
c. Mikrobiyom: Tanımlanmış bir habitattaki tüm **mikroorganizmalar**, **genomları**, **genom ürünleri** ve **çevre etkileri**.



Metagenomik

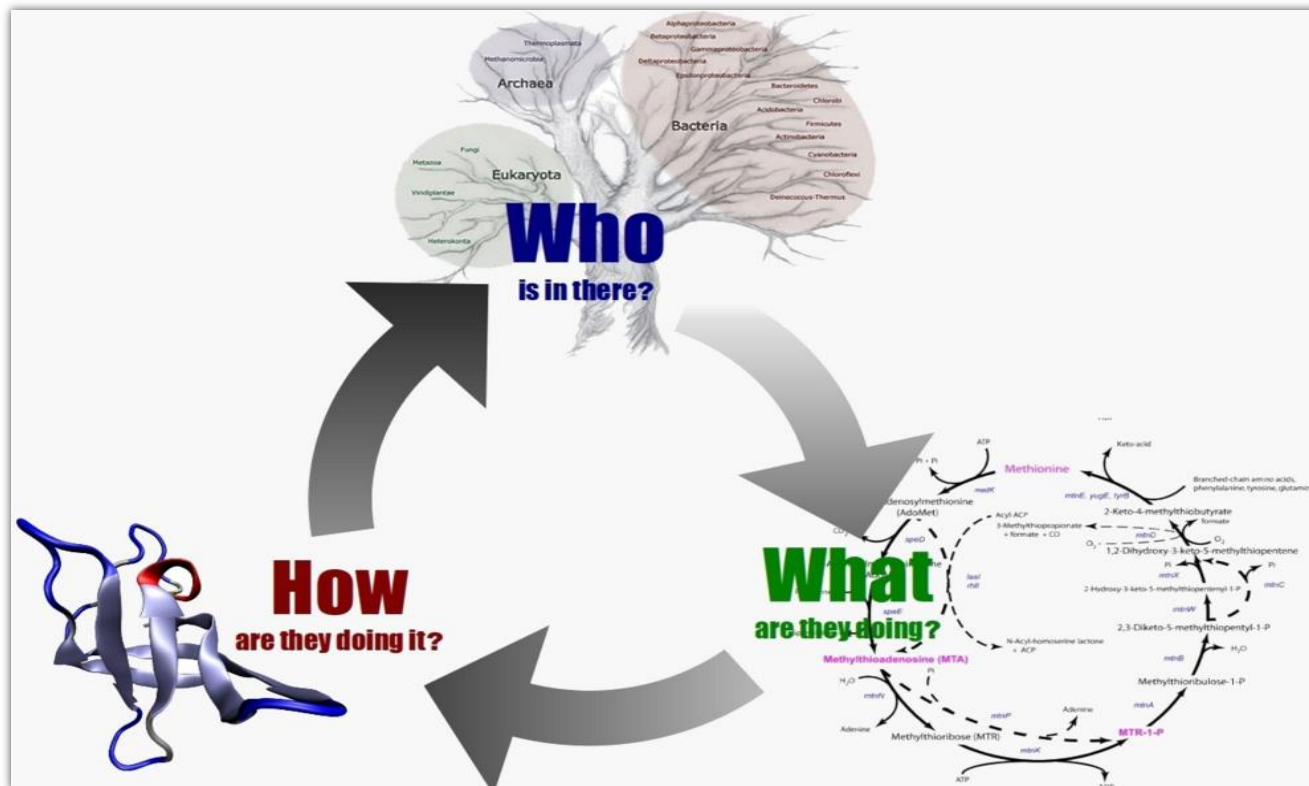
“Mikroskoptan sonraki en büyük buluş”

(National Academy of Sciences)



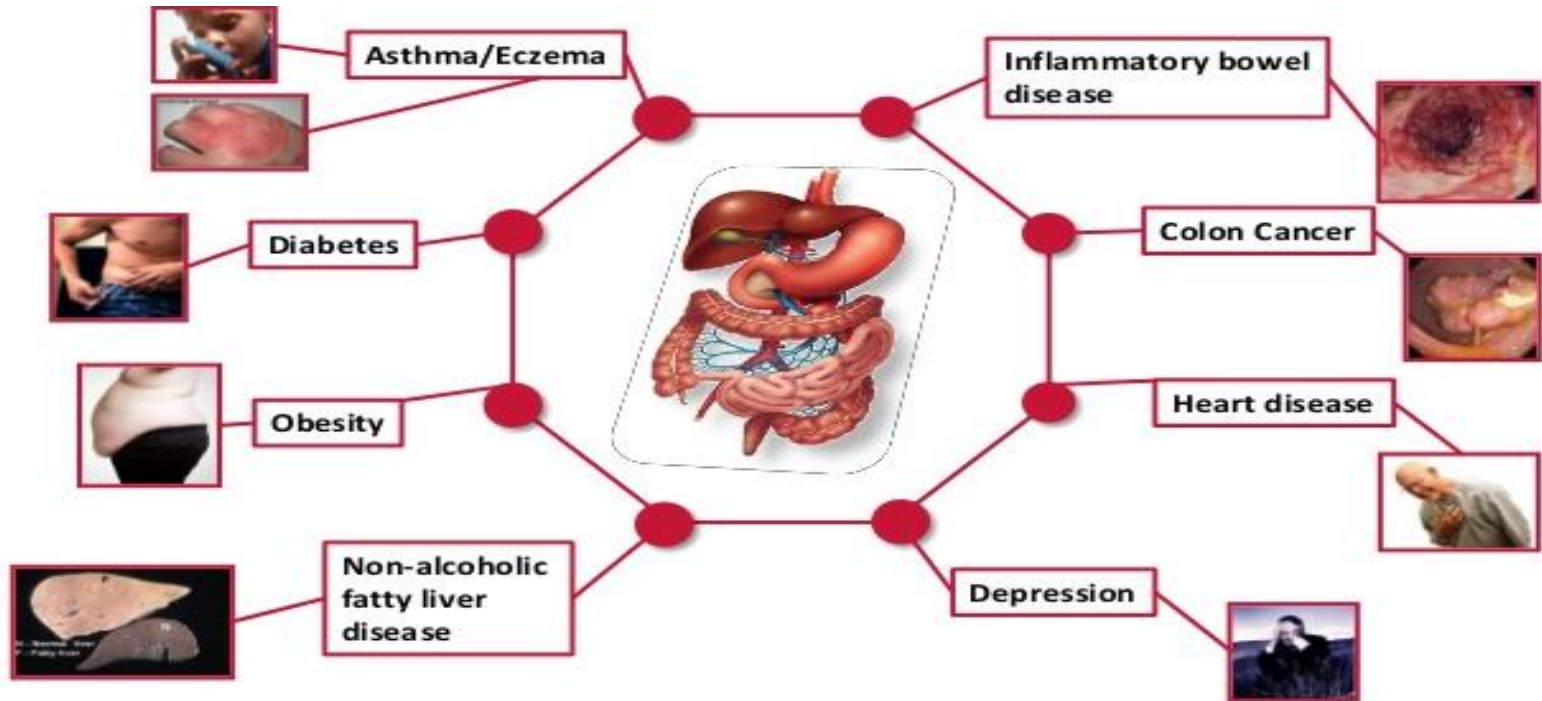
Metagenomik

- ❑ Bir ortamdaki/tanımlanmış habitattaki tüm genetik materyalin **yeni nesil dizileme** ile sekanslanarak **biyoinformatik** yöntemler ile analiz edilmesi



Klinik Metagenomik

□ Mikrobiyal yükü bulunabilecek her türlü klinik örneğin hastalık-sağlık yönünden metagenom boyutunda sekanslanması ve **analizi**



- ❑ Bağırsak Mikrobiyomundan Potansiyel Siroz Biyobelirteçlerinin Keşfi
 - ❑ Veri madenciliği=> biyobelirteç adayı 22 gen! (Patent Baş. No:2017/21166)

- ❑ Alzheimer hastalığı ile intestinal mikrobiyomun potansiyel ilişkisinin metagenomik analizlerle değerlendirilmesi
 - ❑ Alzheimer hastalığı ile ilişkili mikrobiyom elemanları tanımlandı

- ❑ Madde kullanım bozukluklarında bağırsak mikrobiyom disbiyozu ve detoksifikasyonun etkilerinin yeni nesil dizileme ile ortaya çıkarılması
 - ❑ Detoksifikasyon sonrası bağırsak mikrobiyomunda kısmi düzelme!

ERÜ Tıp Fakültesi – Genkök Metagenomik & Biyoinformatik Birimi

❑ Akciğer kanseri olan hastalarda bronko-alveolar lavaj mikrobiyom disbiyozunun değerlendirilmesi

❑ Kanserde disbiyozu uğramış BAL mikrobiyomu

❑ Akciğer kanseri olan hastalarda dil mikrobiyom disbiyozunun araştırılması ve hastalığın teşhisine yönelik potansiyelin değerlendirilmesi

❑ %98 oranında ayırım gücü

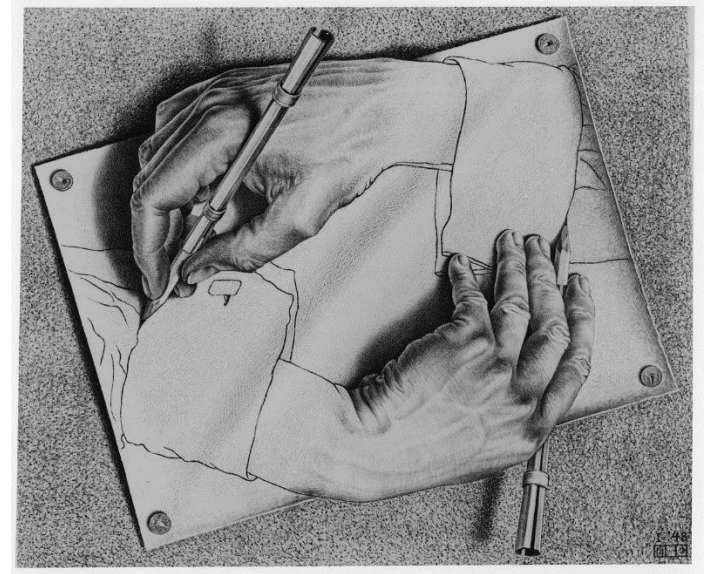
❑ Ortopedik Protez Enfeksiyon Mikrobiyotasının Metagenomik Yöntemlerle *de novo* İncelenmesi

❑ Nanogözenek DNA Dizilemesi İle Hızlı Patojen Tanıma Yapabilen Algoritmaların Geliştirilmesi

Etiyopatoloji faktörü mü? V Komplikasyon mu?

Patogeneizde rol oynuyor Δ Komplikasyon Δ Prognoza katkı

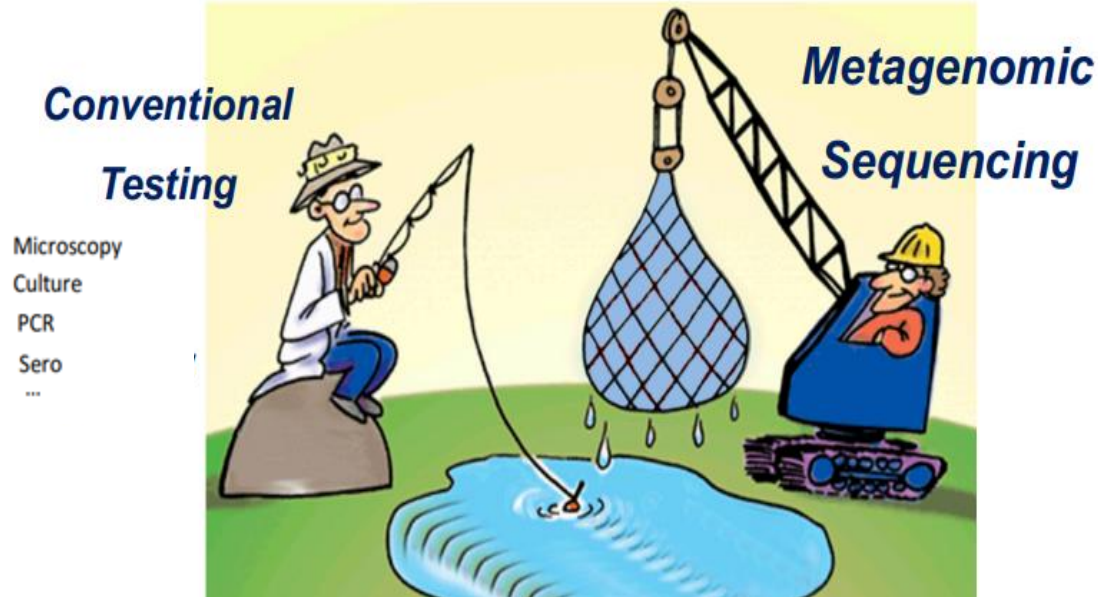
Which came first?



M.C. Escher, *Drawing Hands*, 1948

Enfeksiyon Hastalıklarında Klinik Metagenomik

- ❑ Teşhis ve tedaviye yönelik biyobelirteç/biyomolekül keşfi
- ❑ Konvansiyonel yöntemler ile tespit edilemeyen patojenlerin tespiti
- ❑ Erken/Hızlı patojen tanıma

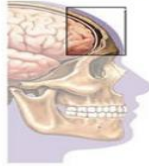


Kültür negatifse!

❑ Konvansiyonel yöntemler ile tespit edilemeyen (yeni?) patojenler

Critical need for better Diagnostic tests

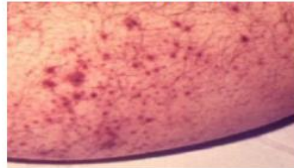
Meningitis/ Encephalitis:
60-80% unknown cause



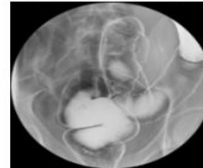
Pneumonia:
15-25% unknown cause



Hemorrhagic Fever:
20% unknown cause



Diarrheal Disease:
50% unknown cause



Kültür negatifse => Klinik Metagenomik

Metagenomic sequencing complements routine diagnostics in identifying viral pathogens in lung transplant recipients with unknown etiology of respiratory infection

Dagmara W. Lewandowska , Peter W. Schreiber , Macé M. Schuurmans, Bettina Ruehe, Osvaldo Zagordi, Cornelia Bayard, Michael Greiner, Fabienne D. Geissberger, Riccarda Capaul, Andrea Zbinden, Jürg Böni, Christian Benden, Nicolas J. Mueller , Alexandra Trkola , Michael Huber  

Published: May 23, 2017 • <https://doi.org/10.1371/journal.pone.0177340>

Table 1. Continued

Case(s)	mNGS Results and Specimen Type
A retrospective series of 16 cases of infectious keratitis. All specimens were FFPE tissues.	Sequencing generated 20 million to 46 billion reads per sample. On average, 9 of the reads were classified as human.

OPEN ACCESS Freely available online

Identification of a Novel Human Papillomavirus by Metagenomic Analysis of Samples from Patients with Febrile Respiratory Illness

Dutilh³, Yan Wei Lim^{1,2}, Bradley S. Schneider², Travis Taylor², David Metzgar^{4,5}, Christopher A. Myers⁴, Patrick J. Blair⁴, Bahador Nosrat^{1,5}, Rohwer^{1,5}

¹ University, San Diego, California, United States of America, ² Global Viral Forecasting (now known as Metablot), San Francisco, California, United States of America, ³ Centre for Molecular and Biomolecular Informatics/Nijmegen Centre for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands, ⁴ Naval Medical Research Center, U.S. Navy, San Diego, California, United States of America, ⁵ Center for Microbial Sciences, San Diego State University, San Diego, California, United States of America

med.	Abiotrophia defectiva endocarditis	Patient treated with antibiotics for 4 weeks and discharged.	[49]
al	Novel rhabdovirus: Bas-Congo virus	First 2 patients died, third survived after treatment with fluids, blood transfusion and empiric antibiotics. No subsequent cases identified.	[50]
s.	Confirmation of known viral infections in 6-hour turnaround time.	Clinical outcomes not provided.	[41]
			[42]
			[43]

A Novel Prosthetic Joint Infection Pathogen, *Mycoplasma salivarium*, Identified by Metagenomic Shotgun Sequencing

Matthew Thoendel, Patricio Jeraldo, Kerryl E. Greenwood-Quaintance, Nicholas Chia, Matthew P. Abdel, James M. Steckelberg, Douglas R. Osmon, Robin Patel  Author Notes

Clinical Infectious Diseases, Volume 65, Issue 2, 15 July 2017, Pages 332–335, <https://doi.org/10.1093/cid/cix296>

Published: 01 April 2017 

April 10, 2013

A Culture-Independent Sequence-Based Metagenomics Approach to the Investigation of an Outbreak of Shiga-Toxigenic *Escherichia coli* O104:H4

Nicholas J. Loman, MBBS, PhD; Chrystala Constantinidou, PhD; Martin Christner, MD; [et al](#)

Article Information

JAMA. 2013;309(14):1502-1510. doi:10.1001/jama.2013.3231

Stool samples positive (n = 22) or negative (n = 5) for *Clostridioides* (previously *Clostridium*) *difficile* were tested by mNGS.

Abbreviations: AST, antimicrobial susceptibility testing; BAL, bronchoalveolar lavage; ESB, extended-spectrum β -lactamase; FFPE, formalin-fixed, paraffin-embedded; LRTI, lower respiratory tract infection; mNGS, metagenomic next-generation sequencing; RSV, respiratory syncytial virus; RT-PCR, reverse-transcription polymerase chain reaction.

Kültür negatifse => Klinik Metagenomik

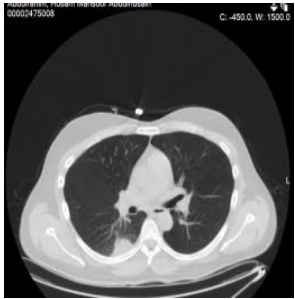
➤ Ortopedik Protez Enfeksiyon Mikrobiyotasının Metagenomik Yöntemlerle *de novo* Incelenmesi (Tübitak-116S290)

	Doku	Protez
Protez Enfeksiyonu		
1. hasta	Rothia spp. Streptococcus spp. Cutibacterium acnes C. acnes faji Tanımlanmamış mantar	Cutibacterium acnes C. acnes faji
2. hasta	Cutibacterium acnes C. acnes faji	Cutibacterium acnes C. acnes faji
Aseptik Gevşeme		
1. hasta	Veillonella spp.	Staphylococcus spp. Corynebacterium spp.
2. hasta	Cutibacterium acnes Veillonella spp.	Cutibacterium acnes
3. hasta	Cutibacterium acnes C. acnes faji	Cutibacterium acnes C. acnes faji

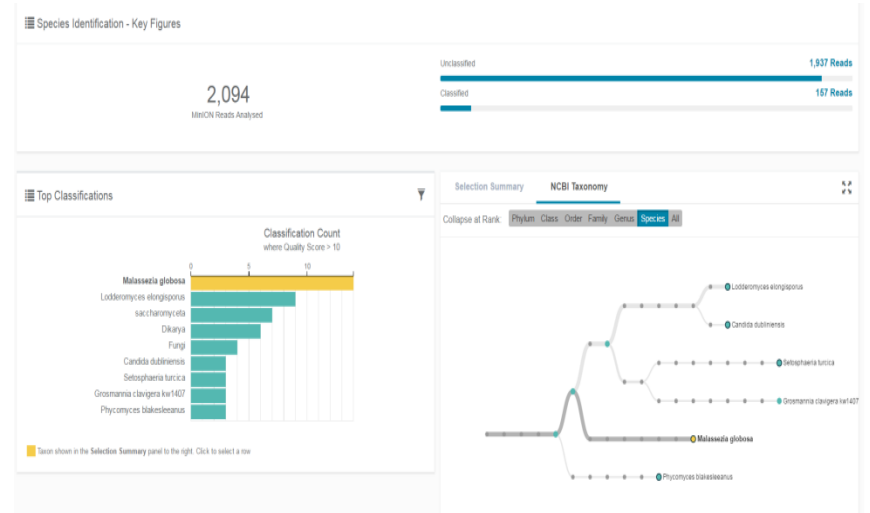
Kültür negatifse => Klinik Metagenomik

➤ Nanogözenek DNA Dizilemesi İle Hızlı Patojen Tanıma Yapabilen Algoritmaların Geliştirilmesi (Tübitak-116S083)

Hasta: T-ALL, Nörtopenik ateş,
İnvaziv fungal enfeksiyon şüphesi



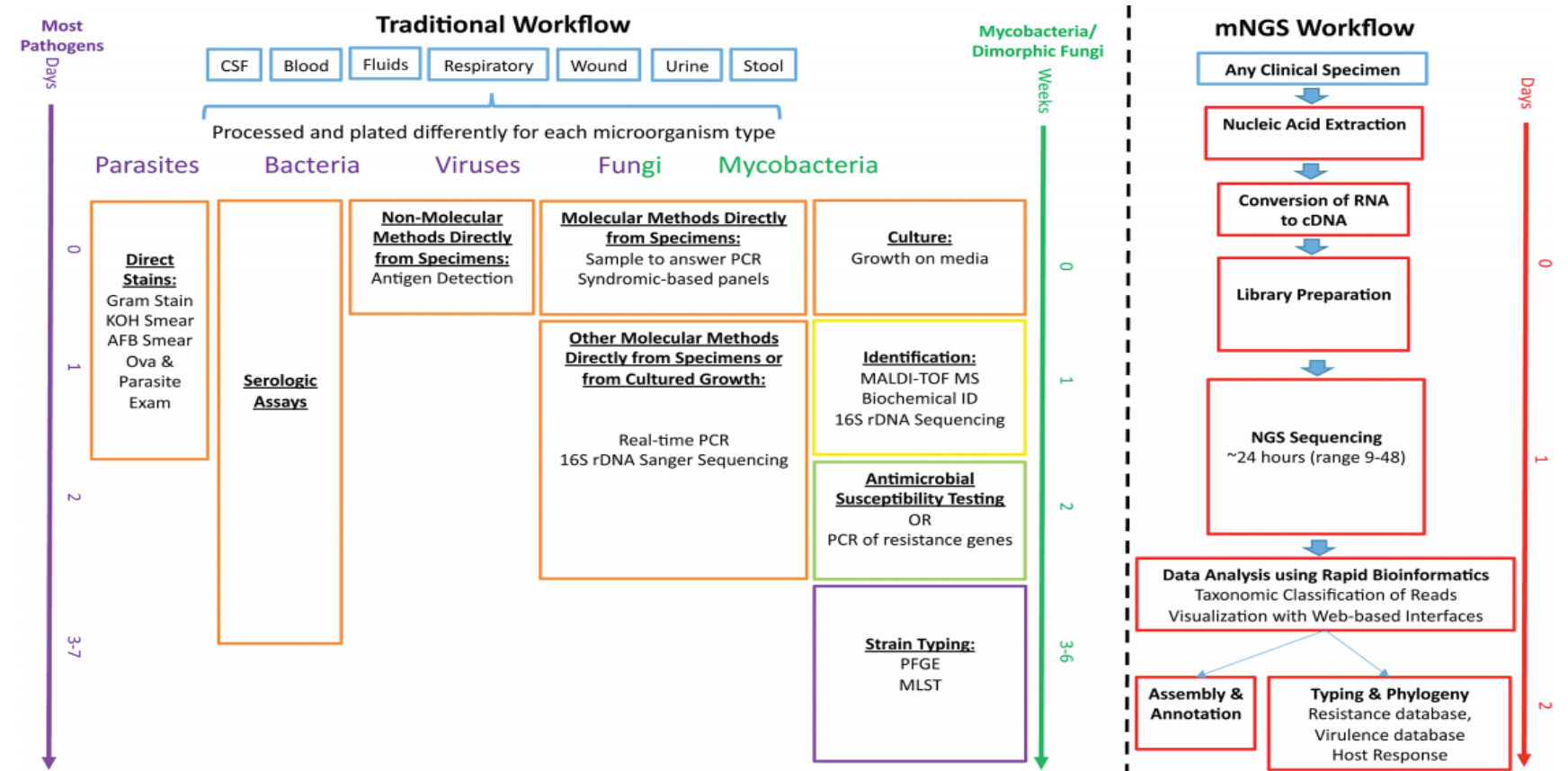
Vorikonazol
9. gün



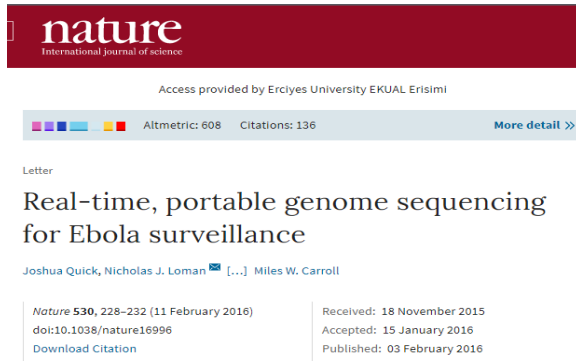
***Malassezia globosa*,**
Lodderomyces elongisporis



Rutin iş akışı vs Metagenomik iş akışı



Erken Teşhis – Hızlı patojen tanıma



Identification of bacterial pathogens and antimicrobial resistance directly from clinical urines by nanopore-based metagenomic sequencing

K. Schmidt, S. Mwaigwisya, L. C. Crossman, M. Doumith, D. Munroe, C. Pires, A. M. Khan, N. Woodford, N. J. Saunders, J. Wain ... [Show more](#)

Journal of Antimicrobial Chemotherapy, Volume 72, Issue 1, 1 January 2017, Pages 104–114, <https://doi.org/10.1093/jac/dkw397>

Published: 25 September 2016 [Article history](#)

Grumaz et al. *Genome Medicine* (2016) 8:73
DOI 10.1186/s13073-016-0326-8

Genome Medicine

RESEARCH

Open Access

Next-generation sequencing diagnostics of bacteremia in septic patients

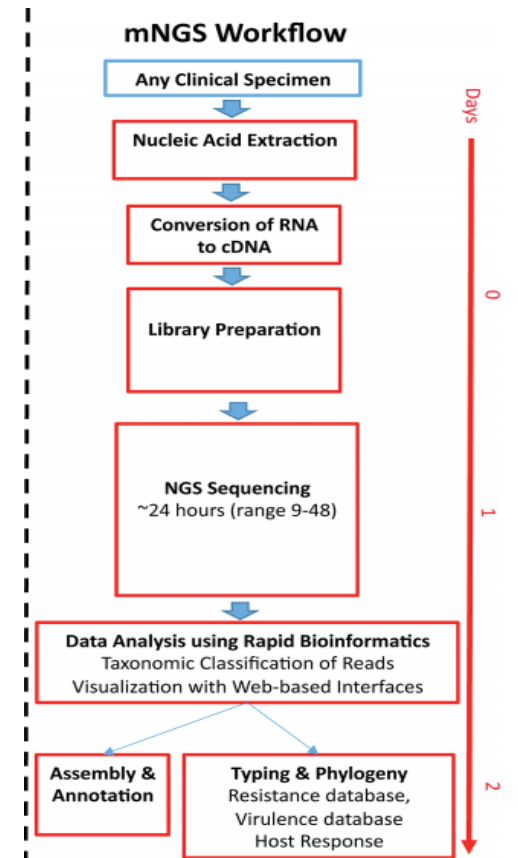
Silke Grumaz^{1†}, Philip Stevens^{2,4†}, Christian Grumaz¹, Sebastian O. Decker³, Markus A. Weigand³, Stefan Hofer³, Thorsten Brenner³, Arndt von Haeseler^{4,5} and Kai Sohn^{1,2*}

THE NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing

Michael R. Wilson, M.D., Samia N. Naccache, Ph.D., Erik Samayoa, B.S., C.L.S., Mark Biagtan, M.D., Hiba Bashir, M.D., Guixia Yu, B.S., Shahriar M. Salamat, M.D., Ph.D., Sneha Somasekar, B.S., Scot Federman, B.A., Steve Miller, M.D., Ph.D., Robert Sokolic, M.D., Elizabeth Garabedian, R.N., M.S.L.S., Fabio Candotti, M.D., Rebecca H. Buckley, M.D., Kurt D. Reed, M.D., Teresa L. Meyer, R.N., M.S., Christine M. Seroogy, M.D., Renee Galloway, M.P.H., Sheryl L. Henderson, M.D., Ph.D., James E. Gern, M.D., Joseph L. DeRisi, Ph.D., and Charles Y. Chiu, M.D., Ph.D.



Hızlı patojen tanıma-ECCMID 2017

Rapid pathogen detection in ambiguous infections using
real-time nanopore sequencing technology



Aycan Gundogdu, Ph.D.
Department of Microbiology and Clinical Microbiology,
Faculty of Medicine, Erciyes University, Turkey



Hızlı patojen tanıma- “A proof-of-concept study”

❑ 1. hasta -peritonal tüberküloz kliniği

❑ Abdominal ağrı, ateş, kilo kaybı, peritonda kalınlık artışı, PPD=18, BAL ve periton mai kültür negatif (ENZ, PZR; BACTEC)

❑ 2. hasta- 9 ay önce bruselloz tanısı almış

❑ Kan kültür & Serolojik tanı negatif (1/20, 1/40)

❑ 1. hasta için; Sekansın 20. dakikasında ilk *Mycobacterium tuberculosis*,

❑ 2. hasta için 30. dakikada ilk *Brucella melitensis* okuması

❑ 50 dakikada güven eşiği aşıldı

❑ Numune elimize ulaştıktan sonra 6 saat içinde etkenler tespit edildi!



Enfeksiyonda => Teşhis/tedavi odaklı biyobelirteç

- ❑ Serbest dolaşan RNA' lar (**miRNA**, piwiRNA, lncRNA, ciRNA, sirRNA)
- ❑ miRNA ifade imzaları patojene spesifik
- ❑ Aşı adayı!



Circulating MicroRNAs: Potential and Emerging Biomarkers for Diagnosis of Human Infectious Diseases

Parmila Verma^{1†}, Rajan K. Pandey^{1†}, Priyanka Prajapati² and Vijay K. Prajapati^{1*}

¹ Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Ajmer, India, ² Arya Kanya College, Meerut, India

miRNA-181 in *Helicobacter pylori* infection. The diagnosis of infection with the help of a biomarker is a non-invasive tool that has shown to have a key role in early diagnosis of infection. The discovery of circulating miRNA in the blood of infected patients has the potential to become a powerful non-invasive biomarker in coming future.

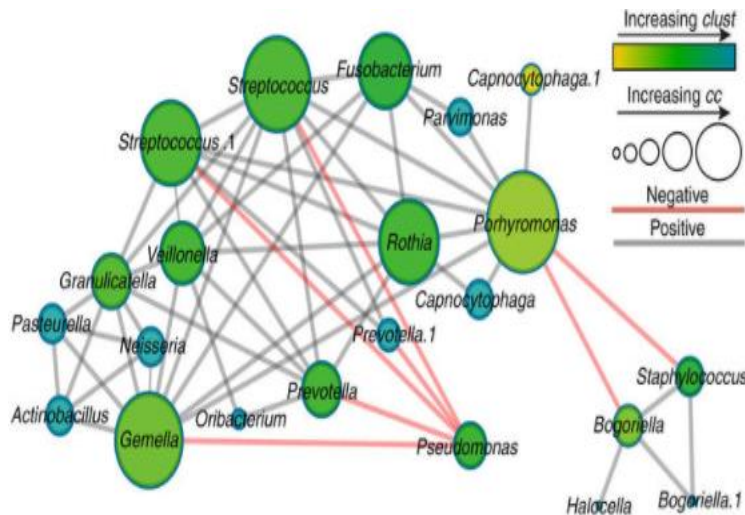
Reviewed by:
Yves Renaudineau,
University of Western Brittany, France
Paras Jain,
Albert Einstein College of Medicine,
USA

*Correspondence:
vijay.pra@csr.org

immunodeficiency virus (HIV); miRNA-122, miRNA-21, and miRNA-34a in hepatitis; miRNA-361-5p and miRNA-29c in tuberculosis; miRNA-16 and miRNA-451 in malaria and miRNA-181 in *Helicobacter pylori* infection. The diagnosis of infection with the help of a biomarker is a non-invasive tool that has shown to have a key role in early diagnosis of infection. The discovery of circulating miRNA in the blood of infected patients has the potential to become a powerful non-invasive biomarker in coming future.

Enfeksiyonda => Teşhis/tedavi odaklı biyobelirteç

- ❑ Serbest dolaşan RNA' lar (miRNA, piwiRNA, lncRNA, ciRNA, sirRNA)
- ❑ Mikrobiyom içerisindeki ekolojik ilişkiler => tedavi
 - ❑ Mikrobiyom elamanları arasındaki ekolojik dengeyi anlama
 - ❑ Disbiyozu engelleyecek şekilde mikrobiyomu modüle etme!

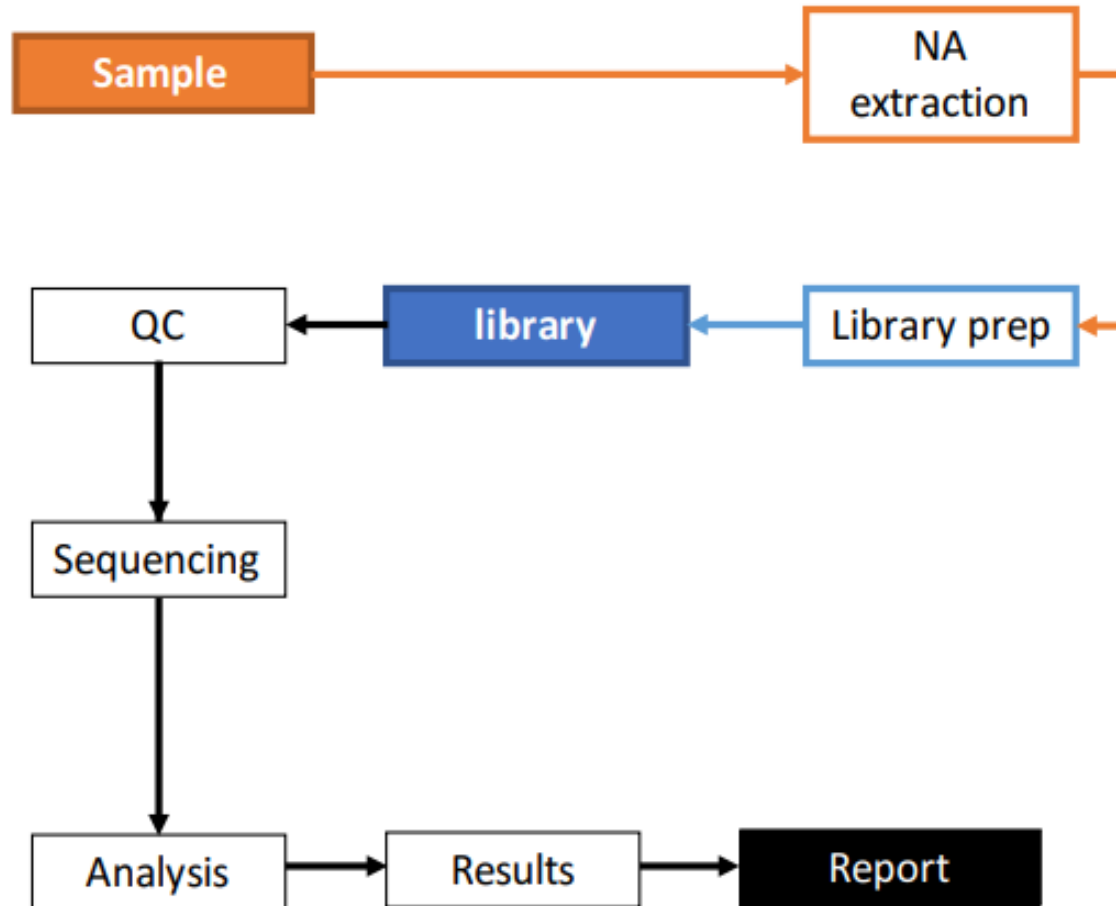


Suppression of *Clostridium difficile* in the Gastrointestinal Tracts of Germfree Mice Inoculated with a Murine Isolate from the Family *Lachnospiraceae*

Angela E. Reeves,^a Mark J. Koenigsnecht,^b Ingrid L. Bergin,^c and Vincent B. Young^{a,b}

Department of Microbiology & Immunology,^a Department of Internal Medicine/Division of Infectious Diseases,^b and Unit for Laboratory Animal Medicine,^c The University of Michigan, Ann Arbor, Michigan, USA

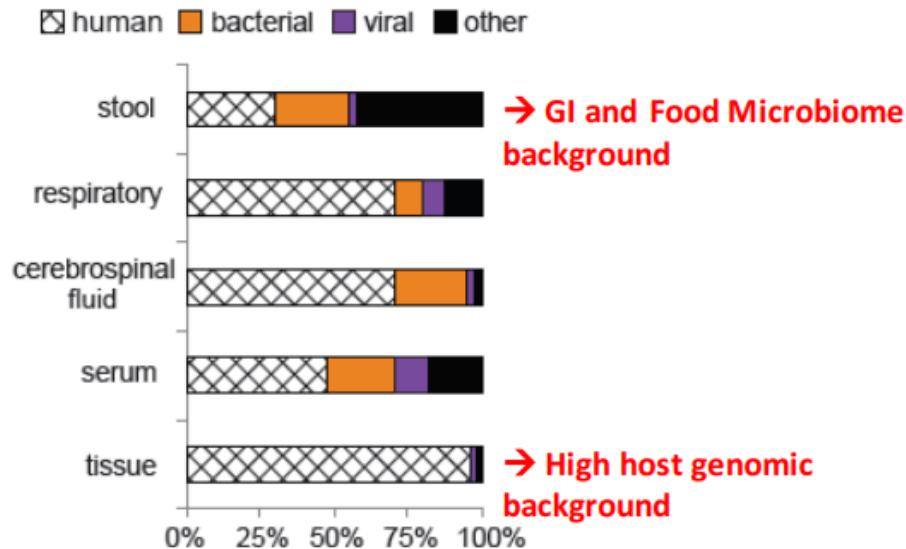
Klinik Metagenomik İş Akışı



Klinik Metagenomiğin Problemleri

❑ Konak DNA'sı!

❑ Steril dokularda toplam okumanın %0.00001– %0.7' si patojene ait

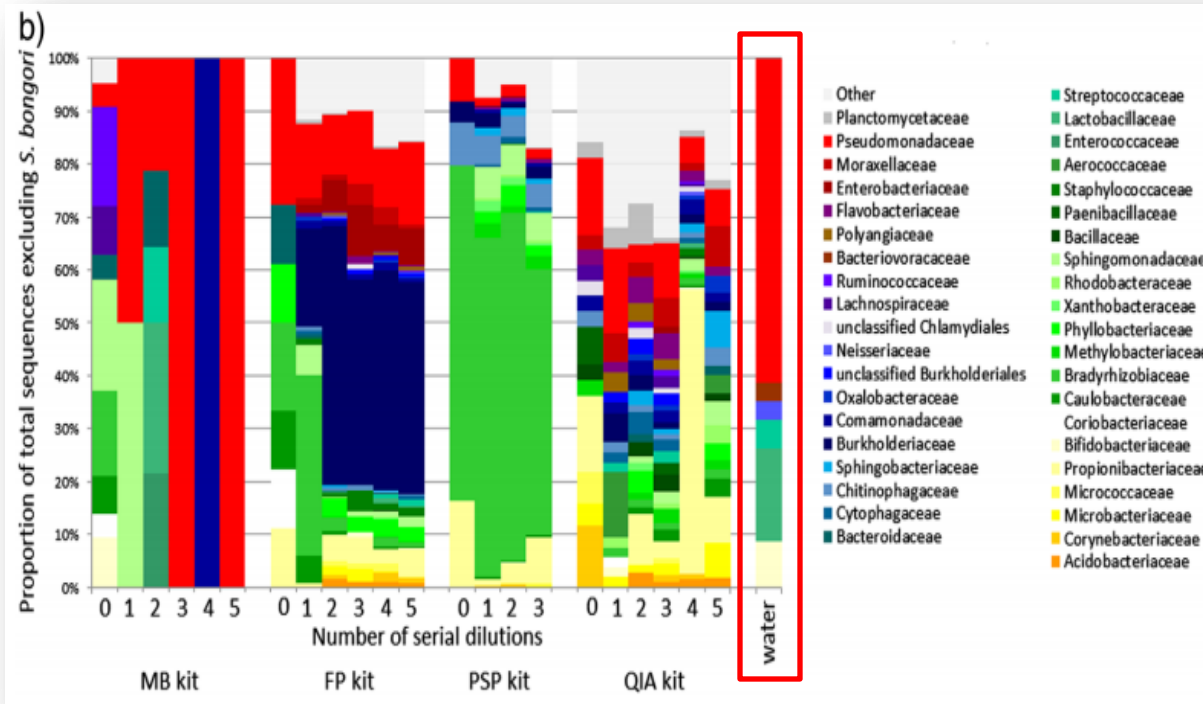


Klinik Metagenomiğin Problemleri

❑ Konak DNA'sı!

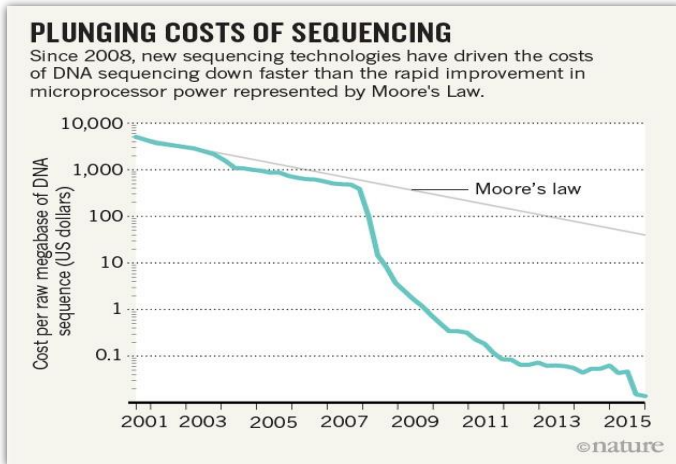
❑ Kontaminasyon!

❑ “Batch Effects”

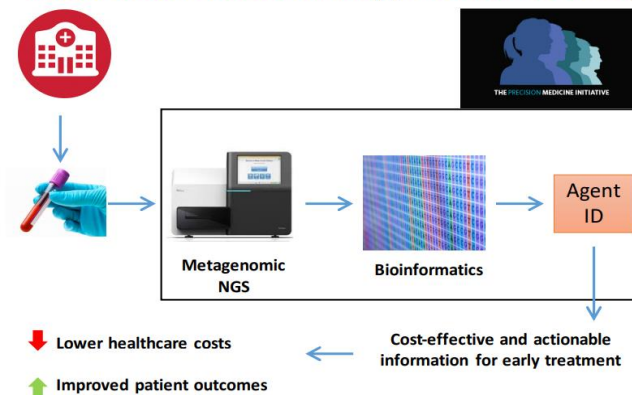


Klinik Metagenomiğin Problemleri

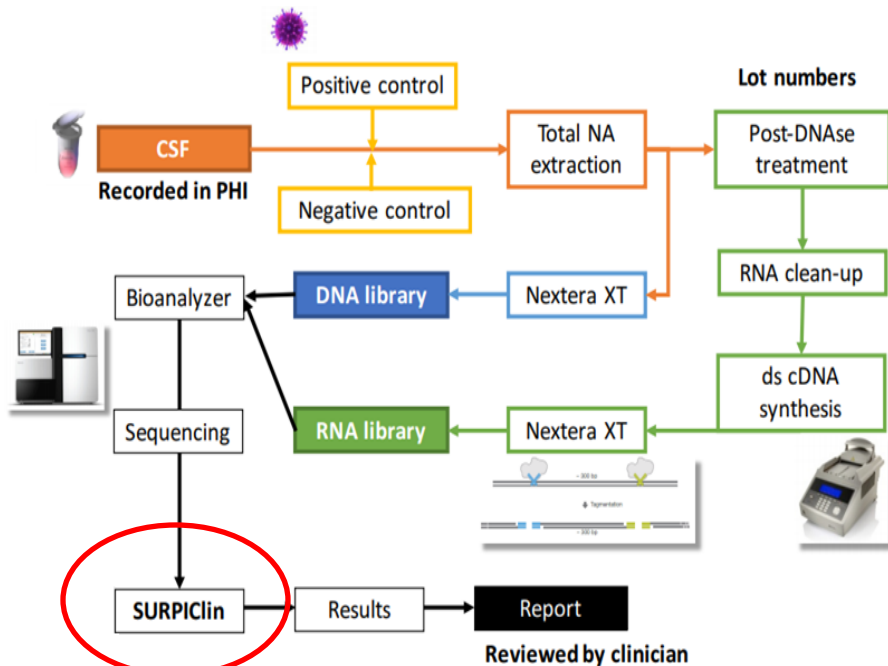
- ❑ Konak DNA' sı!
- ❑ Kontaminasyon!
- ❑ Farklı patojenler için farklı izolasyon gereksinimleri
 - ❑ DNA ? RNA?
- ❑ Etken & Kolonizasyon ayrımı?
- ❑ Maliyet



Accelerating Diagnosis with Next-Gen Testing can Impact Clinical Decision-Making ("Precision Medicine")



Klinik Metagenomiğin Problemleri (mi?)



Clinical Metagenomics for the Diagnosis of Acute Infectious Diseases



Timeline – Clinical CSF mNGS assay in full speed



- High yield cases chosen by PM consult services
- 6 prospective CSF samples sequenced each week
- Results reported in 56h
- Cost-benefit and clinical outcomes analysis after 1 year, 300 samples

Steril doku paradoksu!

❑Doğal flora elamanı? Enfeksiyon?

[Current Bladder Dysfunction Reports](#)

March 2016, Volume 11, [Issue 1](#), pp 18–24

The Bladder Is Not Sterile: History and Current Discoveries on the Urinary Microbiome

Authors

[Authors and affiliations](#)

Krystal Thomas-White, Megan Brady, Alan J. Wolfe, Elizabeth R. Mueller 

[< Previous Article](#)

[December 2016](#) Volume 215, Issue 6, Pages 682–683

[Next Article >](#)

Challenging dogma: the endometrium has a microbiome with functional consequences!

[Linda C. Giudice](#), MD, PhD  

University of California, San Francisco, San Francisco, CA

SCIENTIFIC REPORTS

OPEN

The Microbiome of Aseptically Collected Human Breast Tissue in Benign and Malignant Disease

Received: 14 April 2016
Accepted: 07 July 2016
Published: 03 August 2016

Tina J. Hieken¹, Jun Chen^{2,3}, Tanya L. Hoskin², Marina Walther-Antonio^{3,4}, Stephen Johnson², Sheri Ramaker⁴, Jian Xiao², Derek C. Radisky⁵, Keith L. Knutson⁶, Krishna R. Kalari², Janet Z. Yao³, Larry M. Baddour⁷, Nicholas Chia^{3,4,8} & Amy C. Degnim¹

RESEARCH ARTICLES

The Placenta Harbors a Unique Microbiome

BY KJERSTI AAGAARD, JUN MA, KATHLEEN M. ANTONY, RADHIKA GANU, JOSEPH PETROSINO, JAMES VERSALOVIC

SCIENCE TRANSLATIONAL MEDICINE | 21 MAY 2014 : 237RA65 | 

Metagenomic approaches demonstrate that the human placenta is not sterile but harbors a unique microbiome.

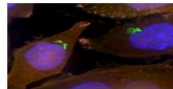
Ravid Straussman Lab

Tumor microenvironment, tumor microbiome and resistance to anti-cancer therapy

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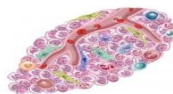
Research



The Tumor Microbiome

Characterizing, visualizing and studying the effects that the bacteria inside tumors might have on different cancer hallmarks.

[Read more >](#)



Tumor Secretome Dependent Precision Anti-Cancer Therapy

Tailoring novel drug combinations to overcome tumor microenvironment mediated innate resistance.

[Read more >](#)

Steril doku paradoksu!

□Doğal flora elamanı? Enfeksiyon?

Lauder et al. *Microbiome* (2016) 4:29
DOI 10.1186/s40168-016-0172-3

Microbiome

RESEARCH Open Access

Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota

Abigail P. Lauder^{1†}, Aoife M. Roche^{1†}, Scott Sherrill-Mix¹, Aubrey Bailey¹, Alice L. Laughlin¹, Kyle Bittinger¹, Rita Leite², Michal A. Elowitz², Samuel Parry^{2*} and Frederic D. Bushman^{1*}

Perez-Muñoz et al. *Microbiome* (2017) 5:48
DOI 10.1186/s40168-017-0268-4

Microbiome

REVIEW Open Access

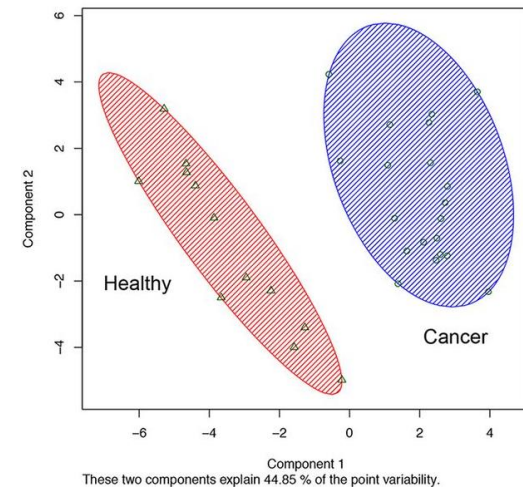
A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: implications for research on the pioneer infant microbiome

Maria Elisa Perez-Muñoz¹, Marie-Claire Arrieta^{2,3}, Amanda E. Ramer-Tait⁴ and Jens Walter^{1,5*}

AMERICAN SOCIETY FOR MICROBIOLOGY Applied and Environmental Microbiology

The Microbiota of Breast Tissue and Its Association with Breast Cancer

Camilla Urbaniak,^{a,b} Gregory B. Gloor,^a Muriel Brackstone,^a Leslie Scott,^a Mark Tangney,^a Gregor Reid^{a,b}
^aLawson Health Research Institute, London, Ontario, Canada; ^bDepartment of Microbiology and Immunology, Western University, London, Ontario, Canada; ^cDepartment of Biochemistry, Western University, London, Ontario, Canada; ^dLondon Regional Cancer Program, London, Ontario, Canada; ^eCork Cancer Research Centre, University College Cork, Cork, Ireland



A photograph of a traditional Chinese garden. A circular window, known as a 'yuanmen', is set into a white wall. Through the window, a lush green weeping willow tree hangs over a stone path that leads towards a small waterfall. The scene is peaceful and natural. The text 'Hastalık ve Saęlıęa Açılan Geçit; Mikrobiyom' is overlaid on the image in white, bold, sans-serif font.

Hastalık ve Saęlıęa Açılan Geçit; Mikrobiyom