Mikrobiyom Çalışmalarının Tasarımı ve Yürütülmesinde Dikkat Edilmesi Gereken Noktalar

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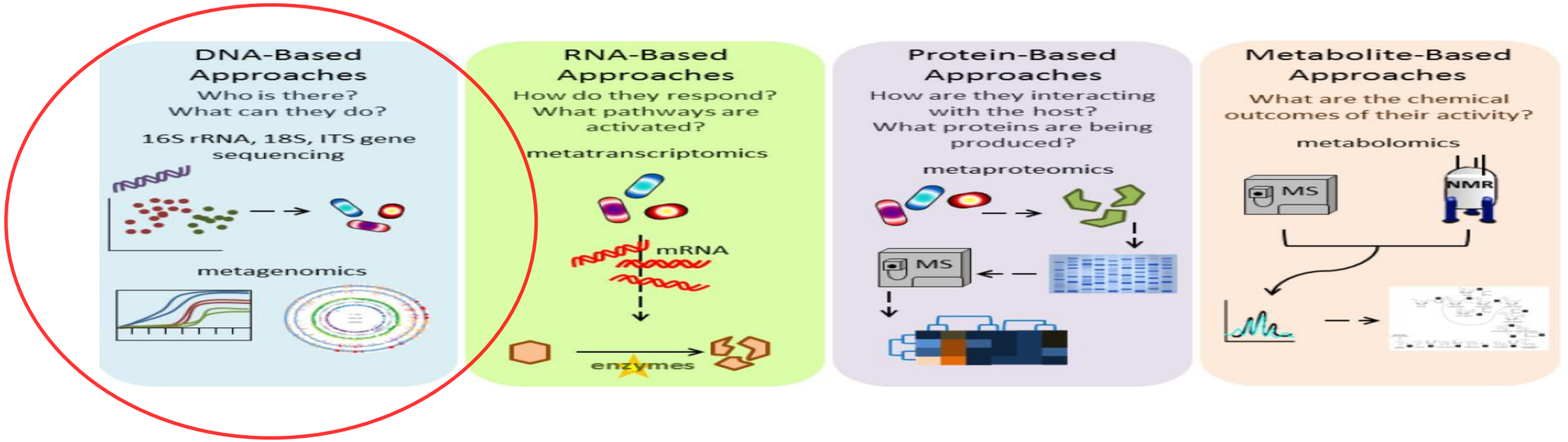
Erciyes Üniversitesi ,Tıp Fakültesi ,Tıbbi Mikrobiyoloji Anabilim Dalı
Genom ve Kök Hücre Merkezi, Metagenomik Birimi, Kayseri

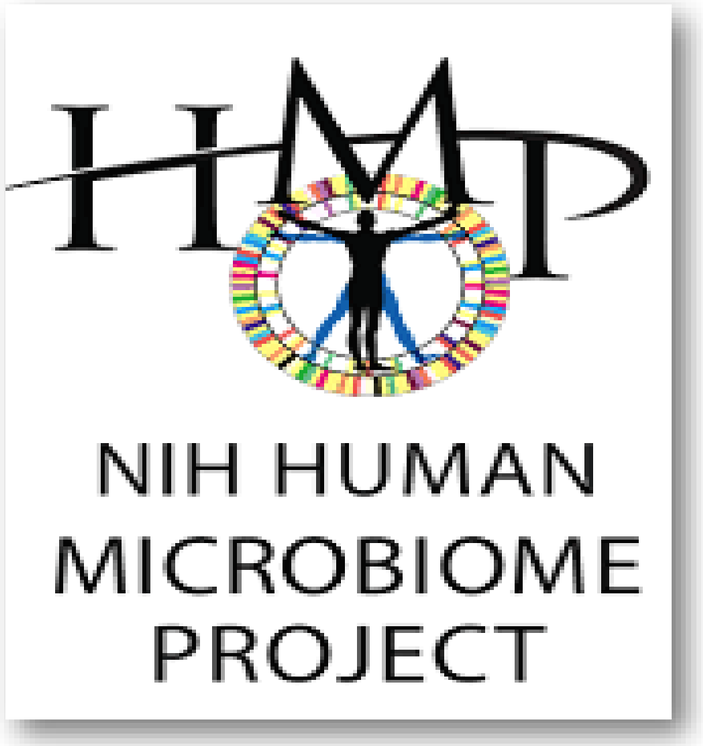
Klimik 2019

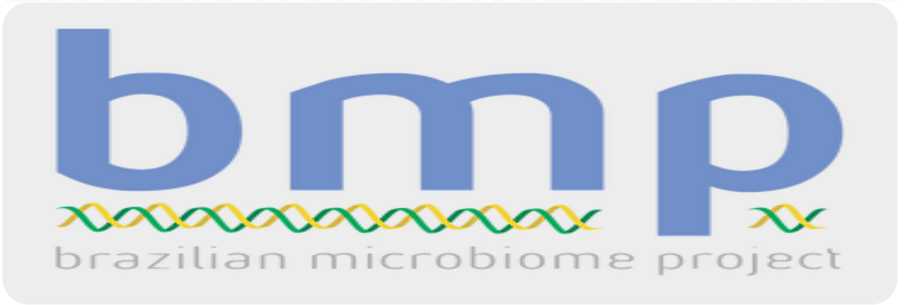
Mikrobiyom Çalışmalarının Kapsamı

Mikrobiyom Çalışmalarının Kapsamı



Metagenom temelli mikrobiyom projeleri





nature

A new genomic blueprint of the human gut microbiota

Alexandre Almeida ✉, Alex L. Mitchell, Miguel Boland, Samuel C. Forster, Gregory B. Gloor, Aleksandra Tarkowska, Trevor D. Lawley & Robert D. Finn ✉

Nature (2019) | Download Citation ⬇

Abstract

The composition of the human gut microbiota is linked to health and disease, but knowledge of individual microbial species is needed to decipher their biological roles. Despite extensive culturing and sequencing efforts, the complete bacterial repertoire of the human gut microbiota remains undefined. Here we identify 1,952 uncultured candidate bacterial species by reconstructing 92,143 metagenome-assembled genomes from 11,850 human gut microbiomes. These uncultured genomes substantially expand the known species repertoire of the collective human gut microbiota, with a 281% increase in phylogenetic diversity. Although the newly identified species are less

nature biotechnology

Resource | OPEN | Published: 04 February 2019

1,520 reference genomes from cultivated human gut bacteria enable functional microbiome analyses

Yuanqiang Zou, Wenbin Xue, [...] Liang Xiao ✉

Nature Biotechnology **37**, 179–185 (2019) | Download Citation ⬇

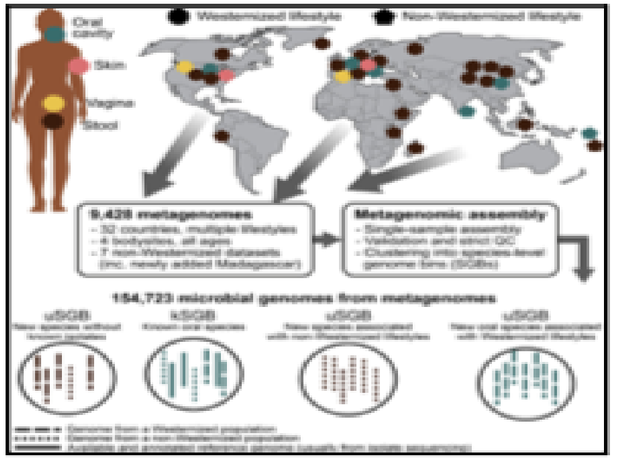
Abstract

Reference genomes are essential for metagenomic analyses and functional characterization of the human gut microbiota. We present the Culturable Genome Reference (CGR), a collection of 1,520 nonredundant, high-quality draft genomes generated from >6,000 bacteria cultivated from fecal samples of healthy humans. Of the 1,520 genomes, which were chosen to cover all major bacterial phyla and

Cell

Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle

Graphical Abstract



Authors

Edoardo Pasolli, Francesco Asnicar, Serena Manara, ..., Christopher Quince, Curtis Huttenhower, Nicola Segata

Correspondence

nicola.segata@unitn.it

In Brief

The human microbiome harbors many unidentified species. By large-scale metagenomic assembly of samples from diverse populations, we uncovered >150,000 microbial genomes that are recapitulated in 4,930 species. Many species (77%) were never described before, increase the mappability of metagenomes, and expand our understanding of global body-wide human microbiomes.

Highlights

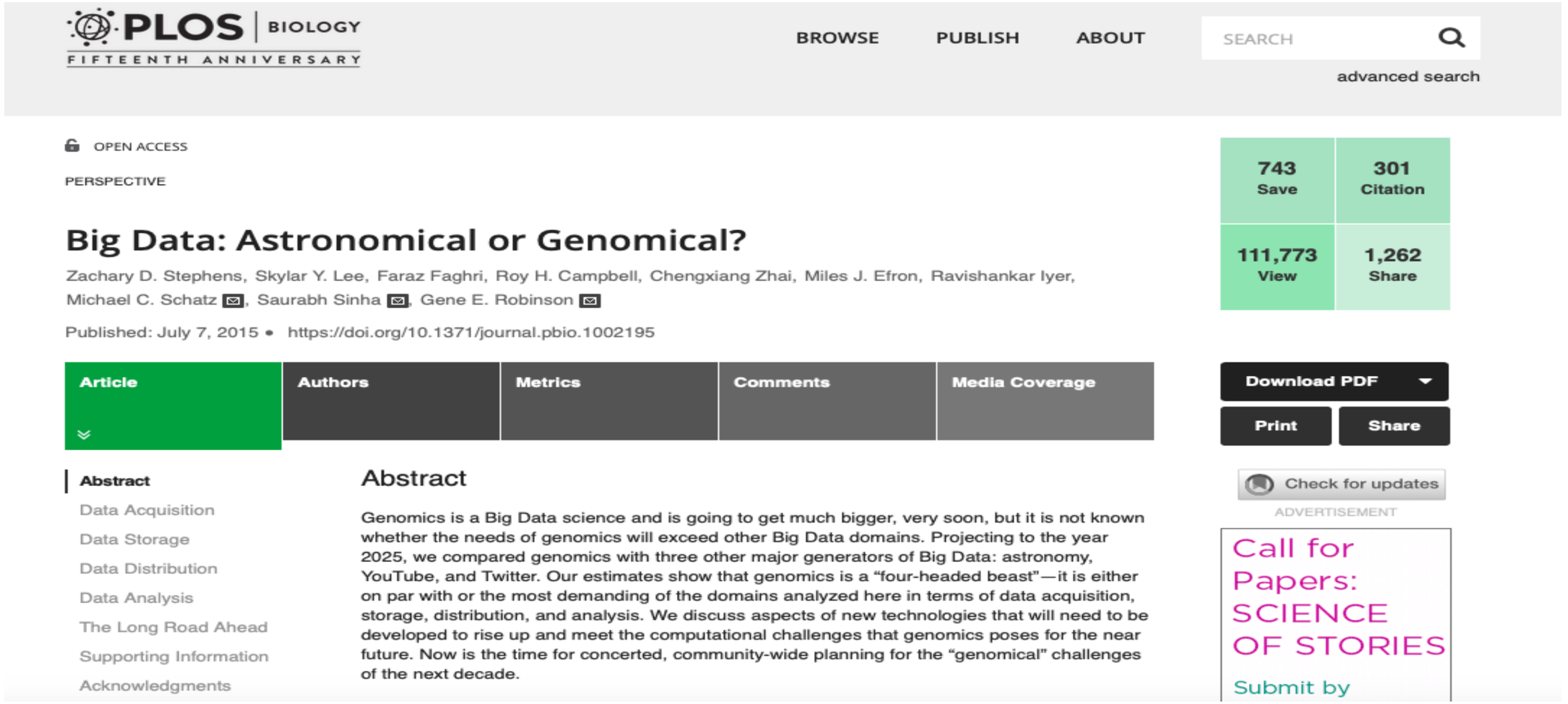
- Large-scale metagenomic assembly uncovered thousands of new human microbiome species
- The new genome resource increases the mappability of gut metagenomes over 87%
- Some of the newly discovered species comprise thousands of reconstructed genomes
- Non-Westernized populations harbor a large fraction of the newly discovered species

Pasolli et al., 2019, Cell 176, 649–662
January 24, 2019
https://doi.org/10.1016/j.cell.2019.01.001

CellPress


BIG DATA

Astronomik mi? Genomik mi?



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PERSPECTIVE

Big Data: Astronomical or Genomical?

Zachary D. Stephens, Skylar Y. Lee, Faraz Faghri, Roy H. Campbell, Chengxiang Zhai, Miles J. Efron, Ravishankar Iyer, Michael C. Schatz, Saurabh Sinha, Gene E. Robinson

Published: July 7, 2015 • https://doi.org/10.1371/journal.pbio.1002195

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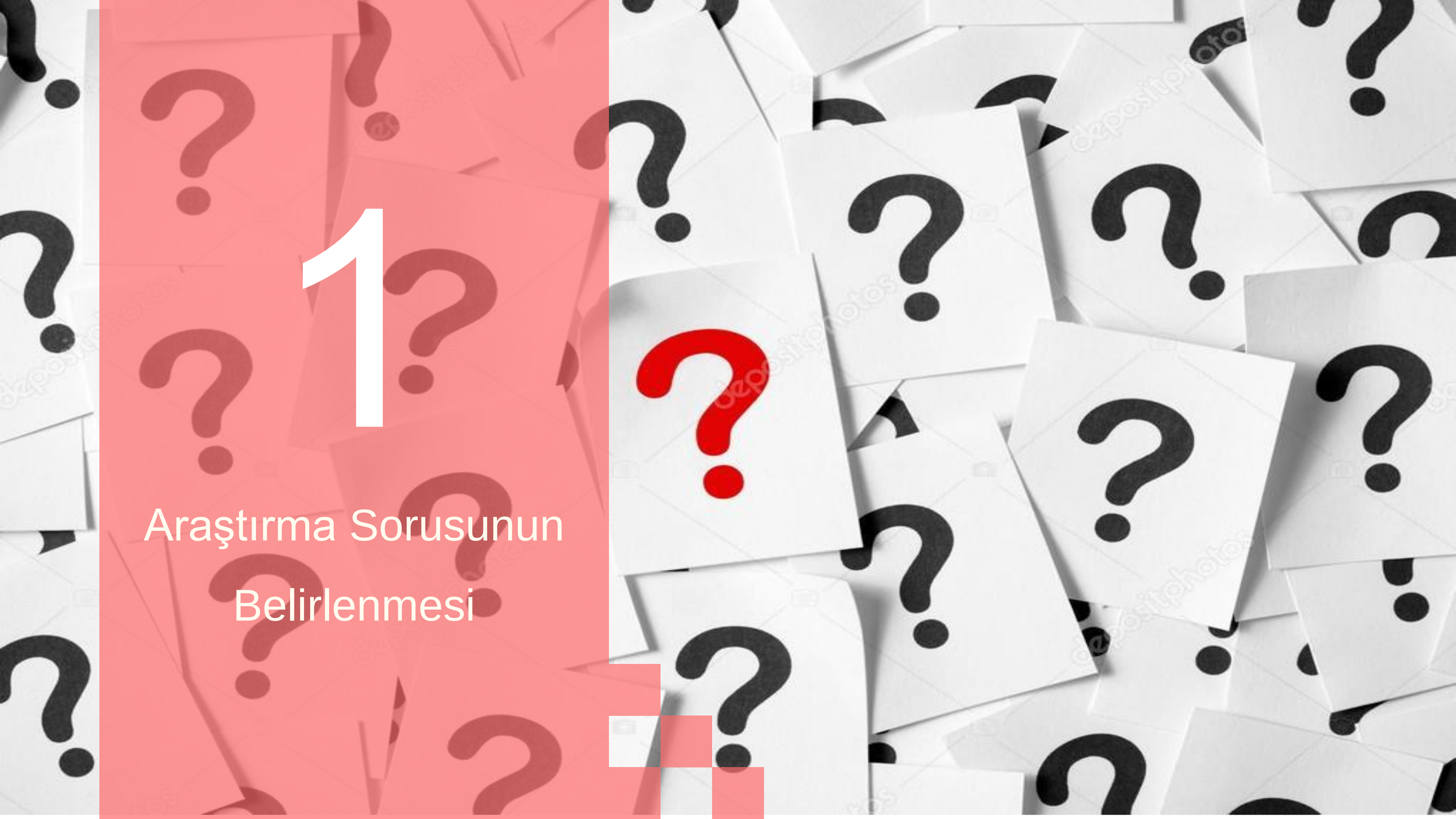
Abstract
Data Acquisition
Data Storage
Data Distribution
Data Analysis
The Long Road Ahead
Supporting Information
Acknowledgments

Abstract

Genomics is a Big Data science and is going to get much bigger, very soon, but it is not known whether the needs of genomics will exceed other Big Data domains. Projecting to the year 2025, we compared genomics with three other major generators of Big Data: astronomy, YouTube, and Twitter. Our estimates show that genomics is a "four-headed beast"—it is either on par with or the most demanding of the domains analyzed here in terms of data acquisition, storage, distribution, and analysis. We discuss aspects of new technologies that will need to be developed to rise up and meet the computational challenges that genomics poses for the near future. Now is the time for concerted, community-wide planning for the "genomical" challenges of the next decade.

Check for updates



1

Araştırma Sorusunun Belirlenmesi

İki Büyük Hata!

Bir bakalım!

Ortada hiç bir hipotez yok!

Obsesif olarak bir hipoteze takılma

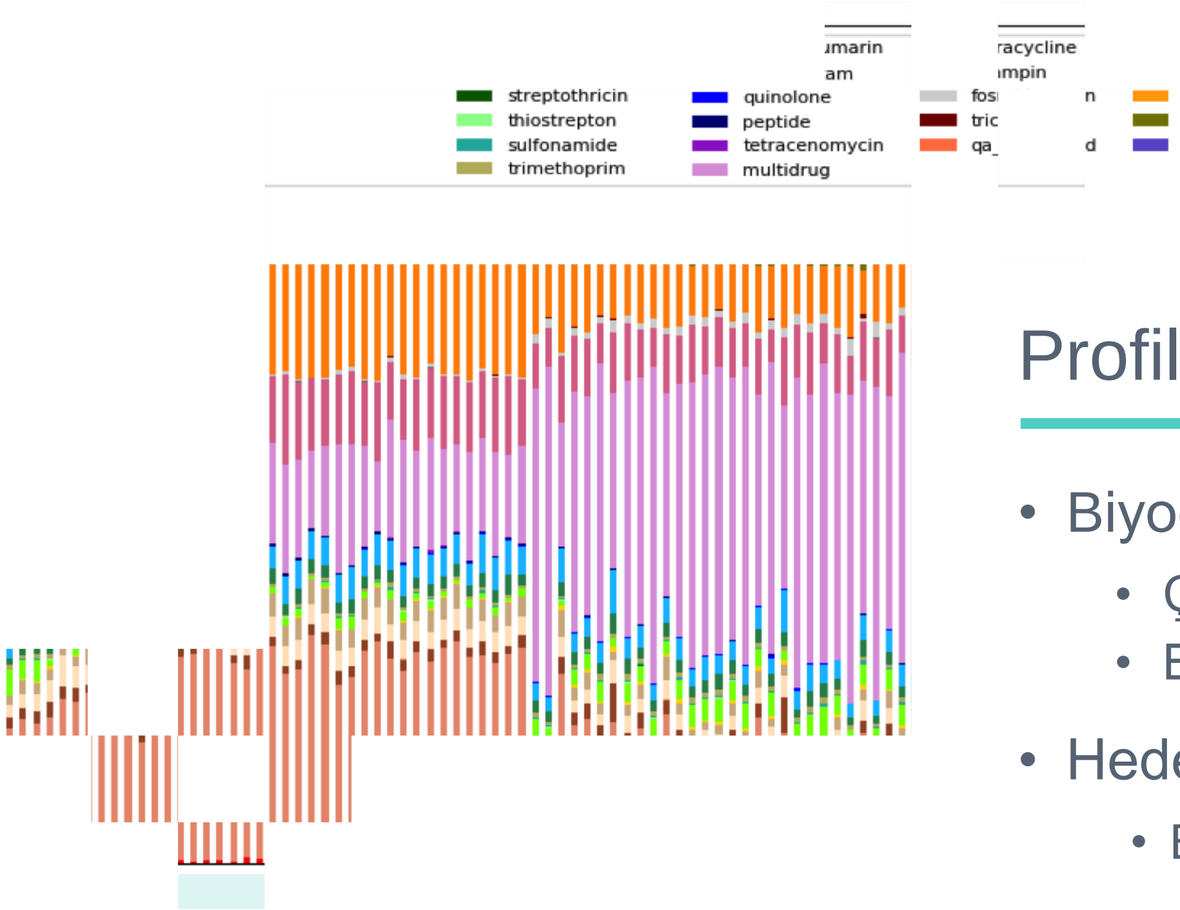
Sadece bir türe ya da ürüne bakmak!

Araştırma sorusu

En temel soru

Farklı koşullar altında belli sayıda, belli örneklerde mikrobiyom elemanlarında anlamlı değişiklikler var mı?

Araştırma sorusu

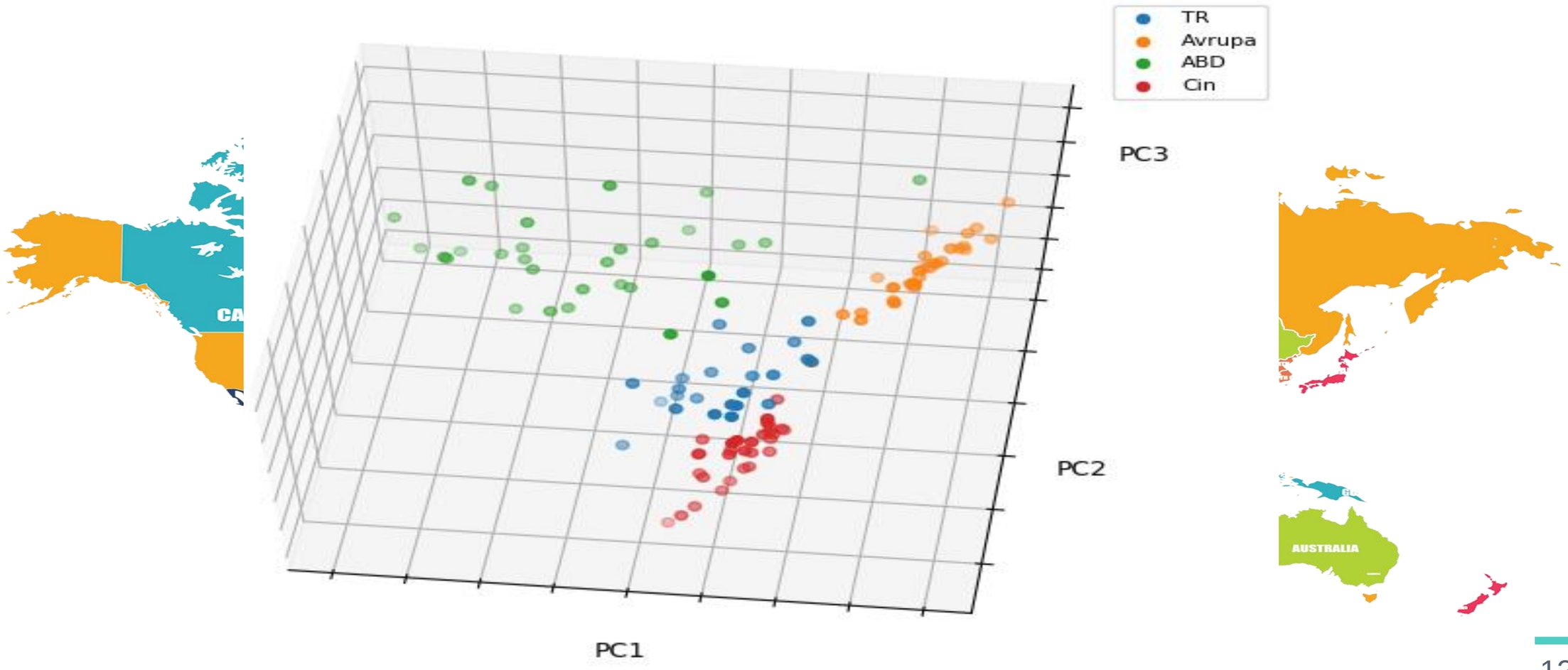


Profilleme

- Biyoçeşitlilik profillemesi
  - Çeşitlilik (alfa-beta diversity)
  - Bağıl bolluk (relative abundance)
- Hedeflenmiş molekül profillemesi
  - Belirli gen ve/veya yolaklar

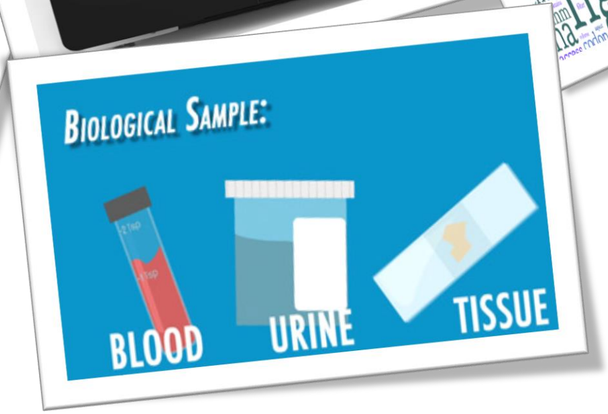
Ülkeler bazında direnç geni çeşitliliği

Çalışma kapsamında > 1 Tbp DNA dizisi *in silico* analiz edildi



2

Deney Tasarımı



Deney Tasarımı

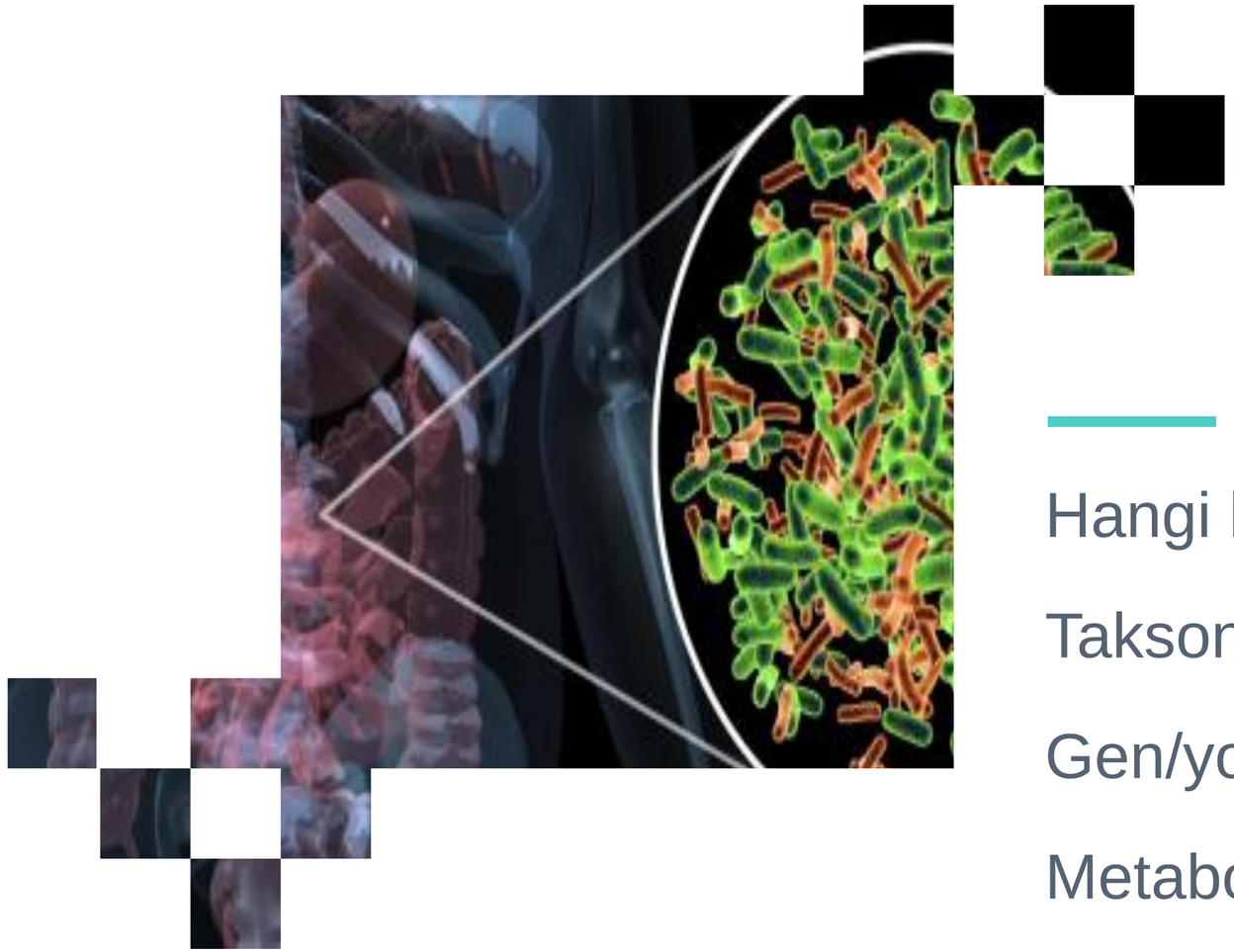
Takım Çalışması!

Multidisipliner çalışma!

PRECISION MEDICINE TEAM FOR INTERPRETATION OF RESULTS

Perhaps the greatest challenge for the practicing infectious disease clinician is interpretation of the results generated by the sequencing laboratory. Due to the complexity of results generated from mNGS, some institutions have implemented precision medicine teams. These teams consist of representatives from medical microbiology, computational biology, infectious diseases, and other clinician groups who can discuss the results and provide interpretation of the mNGS results prior to reporting. This approach ensures that the most clinically relevant data are reported. Additionally, in the authors' experi-

Deney Tasarımı



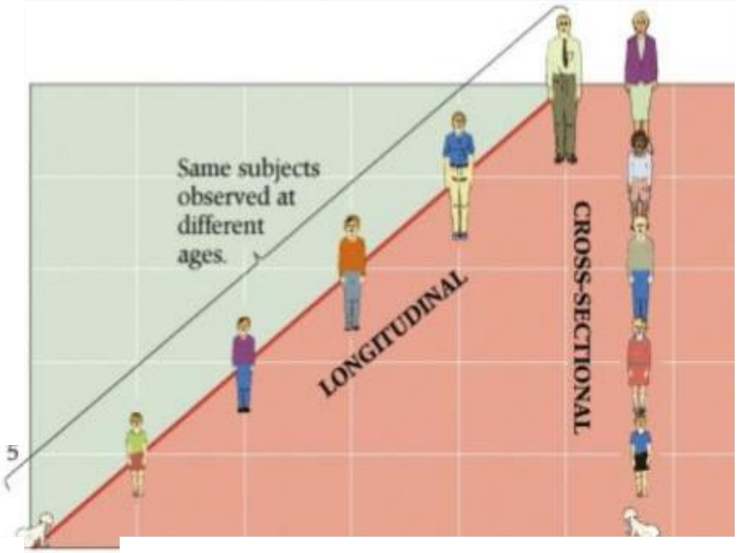
Hangi klinik numune(ler)?

Taksonomik çeşitlilik?

Gen/yolak?

Metabolitler?

Deney Tasarımı



Boylamsal / Kesitsel

Longitudinal study

vs

Cross-sectional study

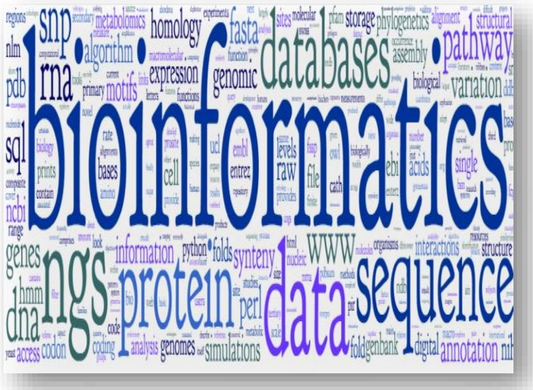
Deney Tasarımı

Araştırma sorusu

Fizibilite

Bütçe

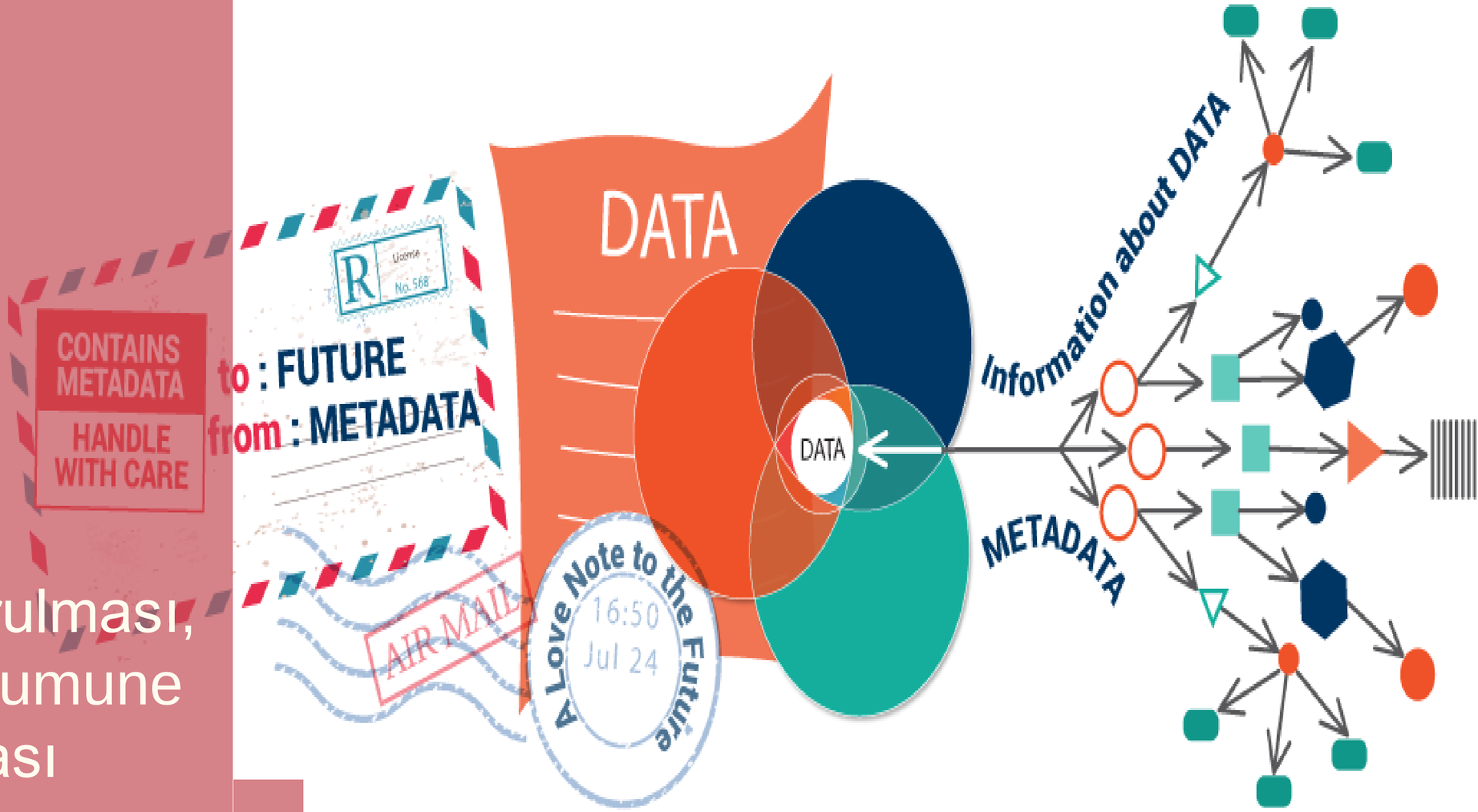
Altyapı



Biyoinformatik

3

Kohort Oluşturulması, Metaveri ve Numune Toplanması



Kohort oluşturulması

Dışlama/İçleme Kriterleri

- Hastalık/sağlık
  - hastalık altın standartla belirlenmiş olmalı
  - evrelenmiş olmalı
- Dışlama kriterleri çok iyi belirlenmiş olmalı

Kohort Büyüklüğü

Fizibiliteye göre mümkün olan en yüksek sayı!

Metaveri

Mümkün olduğunca detaylı ve her birey için standart olmalı!

Metaveri

MICROBIOME

Population-level analysis of gut microbiome variation

Gwen Falony,[1,2]* Marie Joossens,[1,2,3]* Sara Vieira-Silva,[1,2]* Jun Wang,[1,2]* Youssef Darzi,[1,2,3] Karoline Faust,[1,2,3] Alexander Kurilshikov,[4,5] Marc Jan Bonder,[6] Mireia Valles-Colomer,[1,2] Doris Vandeputte,[1,2,3] Raul Y. Tito,[1,2,3] Samuel Chaffron,[1,2,3] Leen Rymenans,[1,2,3] Chloë Verspecht,[1,2] Lise De Sutter,[1,2,3] Gipsi Lima-Mendez,[1,2] Kevin D'hoe,[1,2,3] Karl Jonckheere,[2,3] Daniel Homola,[2,3]† Roberto Garcia,[2,3] Ettje F. Tigchelaar,[6,7] Linda Eeckhaudt,[2,3] Jingyuan Fu,[6,8] Liesbet Henckaerts,[1,9] Alexandra Zhernakova,[6,7] Cisca Wijmenga,[6] Jeroen Raes[1,2,3]‡

Fecal microbiome variation in the average, healthy population has remained under-investigated. Here, we analyzed two independent, extensively phenotyped cohorts: the Belgian Flemish Gut Flora Project (FGFP; discovery cohort; *N* = 1106) and the Dutch LifeLines-DEEP study (LLDeep; replication; *N* = 1135). Integration with global data sets (*N* combined = 3948) revealed a 14-genera core microbiota, but the 664 identified genera still underexplore total gut diversity. Sixty-nine clinical and questionnaire-based covariates were found associated to microbiota compositional variation with a 92% replication rate. Stool consistency showed the largest effect size, whereas medication explained largest total variance and interacted with other covariate-microbiota associations. Early-life events such as birth mode were not reflected in adult microbiota composition. Finally, we found that proposed disease marker genera associated to host covariates, urging inclusion of the latter in study design.

Sequencing-based assessment of microbial communities in human fecal material has linked alterations in gut microbiota composition to disease, as well as chronically suboptimal health and well-being (*1*–*3*). The discovery of these associations has stimulated the search for specific microbiome-based assumed imminent translation of microbiome monitoring into diagnostic and clinical practice. One such hurdle is the lack of knowledge about the impact of host and environmental factors on microbiota variation within an average, healthy population. Such information is essential for robust disease marker identification in clinical

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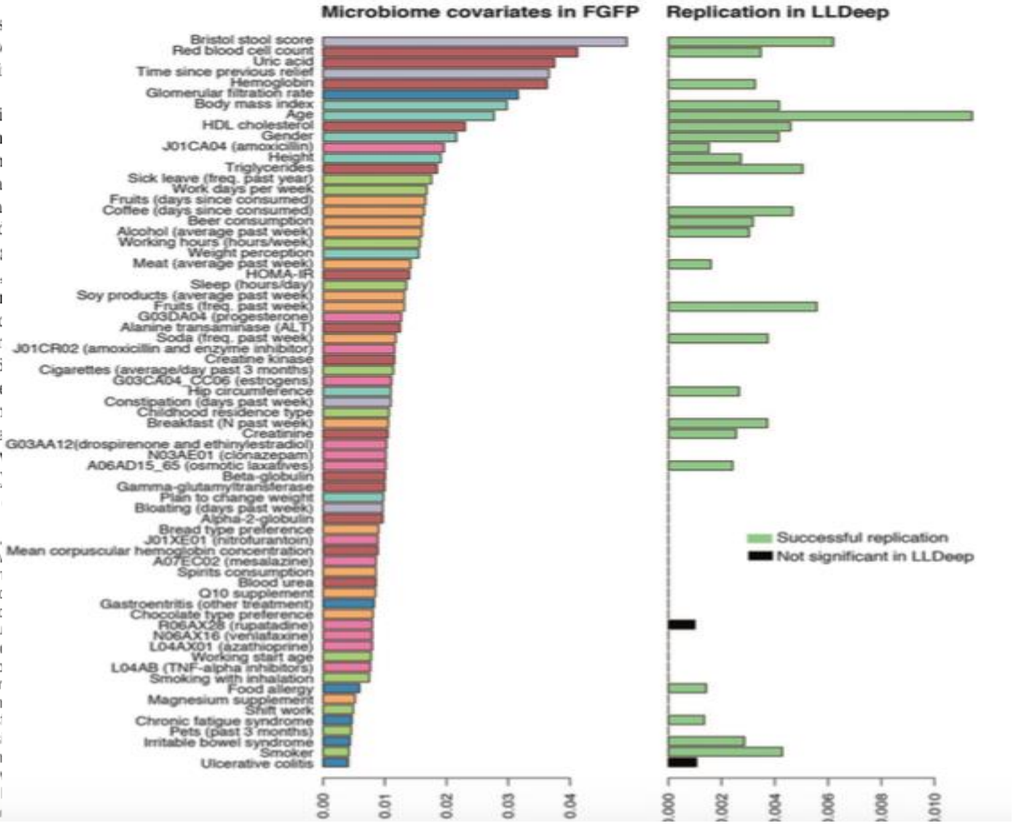

Klinik Numunelerin Toplanması

Steril olmayan numuneler

- Dışkı
- BAL
- Dil/yanak sürüntüsü
- Deri sürüntüsü vb.

“Steril” vücut sıvıları ve dokular

- Kan
- İdrar
- Bos
- Tümör dokusu vb.

Aynı kişiden farklı zamanlarda alınan numuneler?

HHS Public Access
Author manuscript
Nat Microbiol. Author manuscript; available in PMC 2018 June 25.

Published in final edited form as:
Nat Microbiol. 2018 March ; 3(3): 347–355. doi:10.1038/s41564-017-0096-0.

Stability of the human faecal microbiome in a cohort of adult men

Raaj S. Mehta[1,2], **Galeb S. Abu-Ali**[3,4], **David A. Drew**[1,2], **Jason Lloyd-Price**[3,4], **Ayshwarya Subramanian**[3,4], **Paul Lochhead**[1,2], **Amit D. Joshi**[1,2], **Kerry L. Ivey**[5,6], **Hamed Khalili**[1,2], **Gordon T. Brown**[1,2], **Casey DuLong**[3], **Mingyang Song**[1,2], **Long H. Nguyen**[1,2], **Himel Mallick**[3,4], **Eric B. Rimm**[5,7], **Jacques Izard**[8], **Curtis Huttenhower**[3,4,*], and **Andrew T. Chan**[1,2,7,*]

[1]Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

[2]Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

[3]Biostatistics Department, Harvard T. H. Chan School of Public Health, Boston, MA, USA

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[5]Department of Nutrition, Harvard T. H. Chan School of Public Health, Boston, MA, USA

[6]South Australian Health and Medical Research Institute, Infection and Immunity Theme, School of Medicine, Flinders University, Adelaide, Australia

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[8]University of Nebraska, Lincoln, NE, USA

Abstract

Characterizing the stability of the gut microbiome is important to exploit it as a therapeutic target and diagnostic biomarker. We metagenomically and metatranscriptomically sequenced the faecal microbiomes of 308 participants in the Health Professionals Follow-Up Study. Participants provided four stool samples—one pair collected 24–72 h apart and a second pair ~6 months later. Within-person taxonomic and functional variation was consistently lower than between-person variation over time. In contrast, metatranscriptomic profiles were comparably variable within and

between subjects due to higher within-subject longitudinal variation. Metagenomic instability accounted for ~74% of corresponding metatranscriptomic instability. The rest was probably attributable to sources such as regulation. Among the pathways that were differentially regulated, most were consistently over- or under-transcribed at each time point. Together, these results suggest that a single measurement of the faecal microbiome can provide long-term information regarding organismal composition and functional potential, but repeated or short-term measures may be necessary for dynamic features identified by metatranscriptomics.

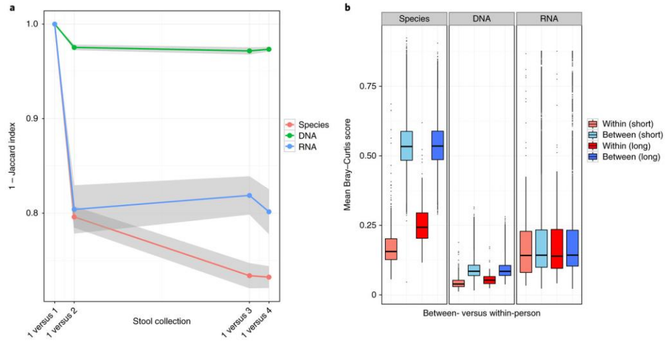


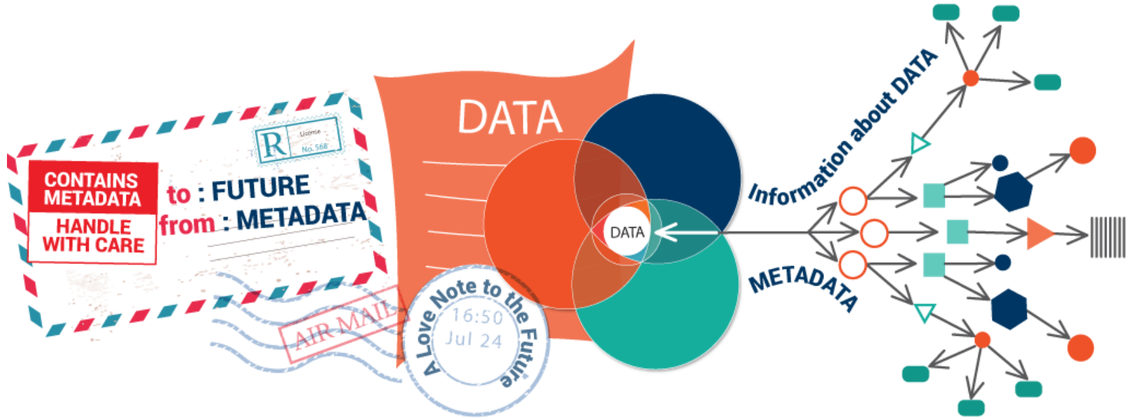
Fig. 2. Inter-individual differences in organismal composition and functional potential appear to be preserved, unlike the more variable metatranscriptomes

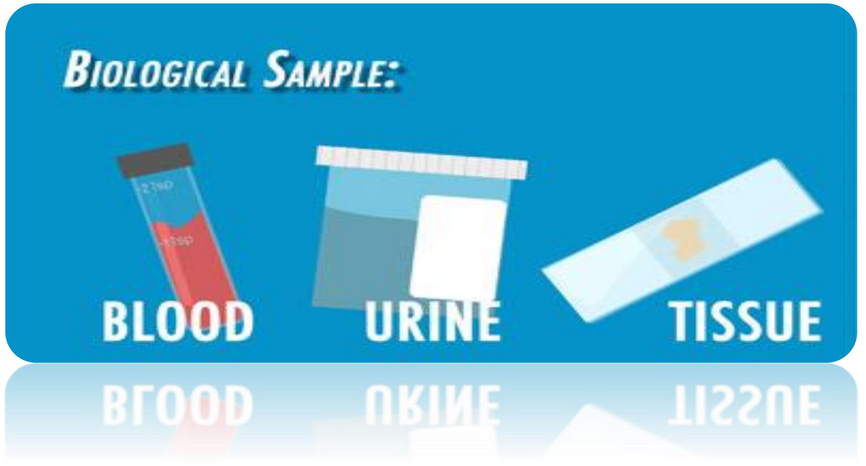
a, 1 – Jaccard Index (fraction of shared features) between all possible pairwise combinations of the first faecal sample with the other three samples collected from each individual (n = 308). 95% confidence intervals are shown in grey. **b**, Bray–Curtis β-diversity scores within and between subjects for short- (24–72 h; n = 308 individuals) and intermediate-term intervals (6 months; n = 160 individuals). Here, species represents taxonomic profile abundances, DNA represents metagenomic functional profiles, and RNA represents metatranscriptomes. Boxplot whiskers include observations within 1.5 interquartile range of the upper and lower quartiles.

4

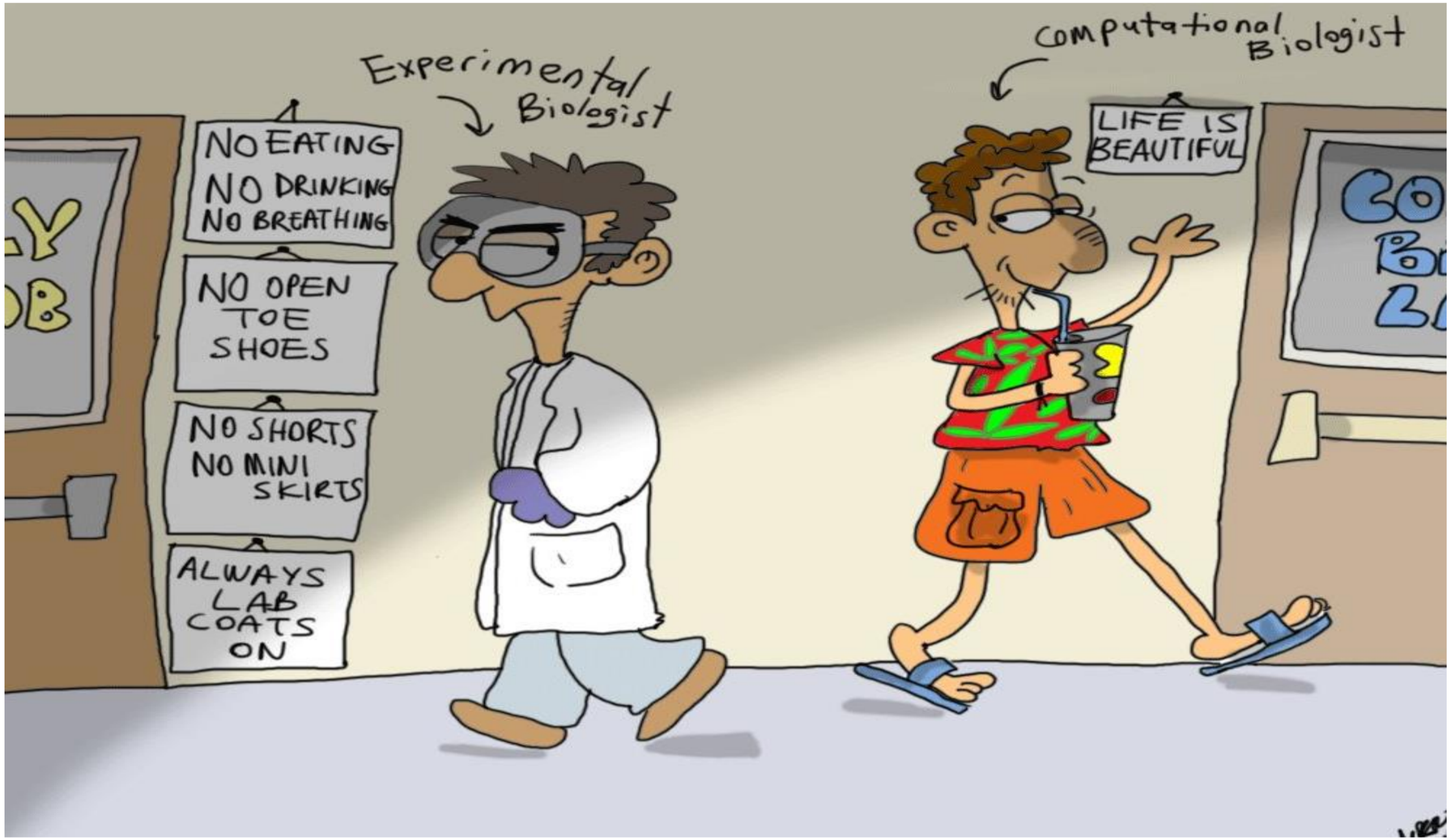
Islak ve Kuru Laboratuvar Süreçleri

Mikrobiyom Çalışmaları

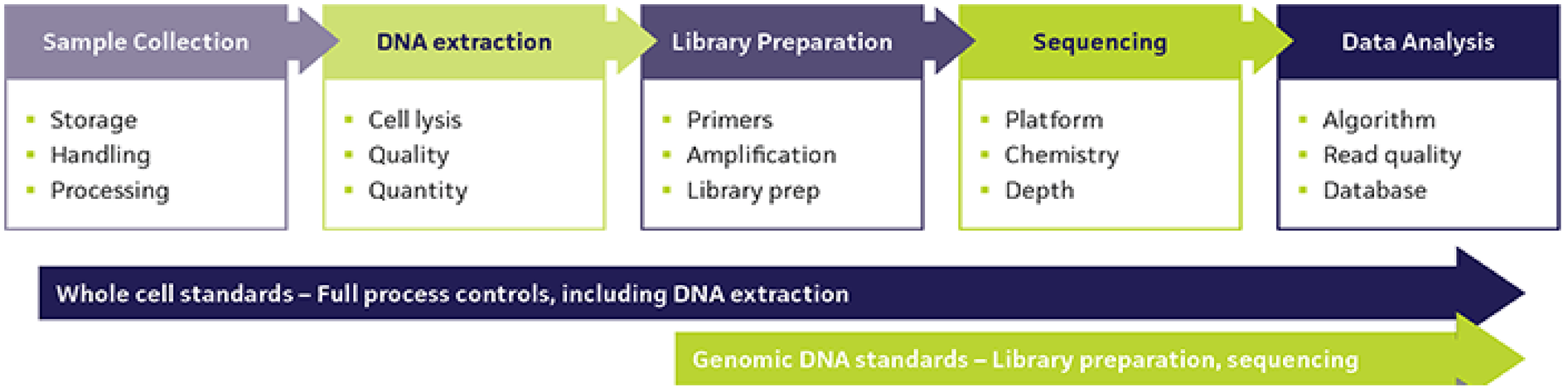




Laboratuvar Süreçleri



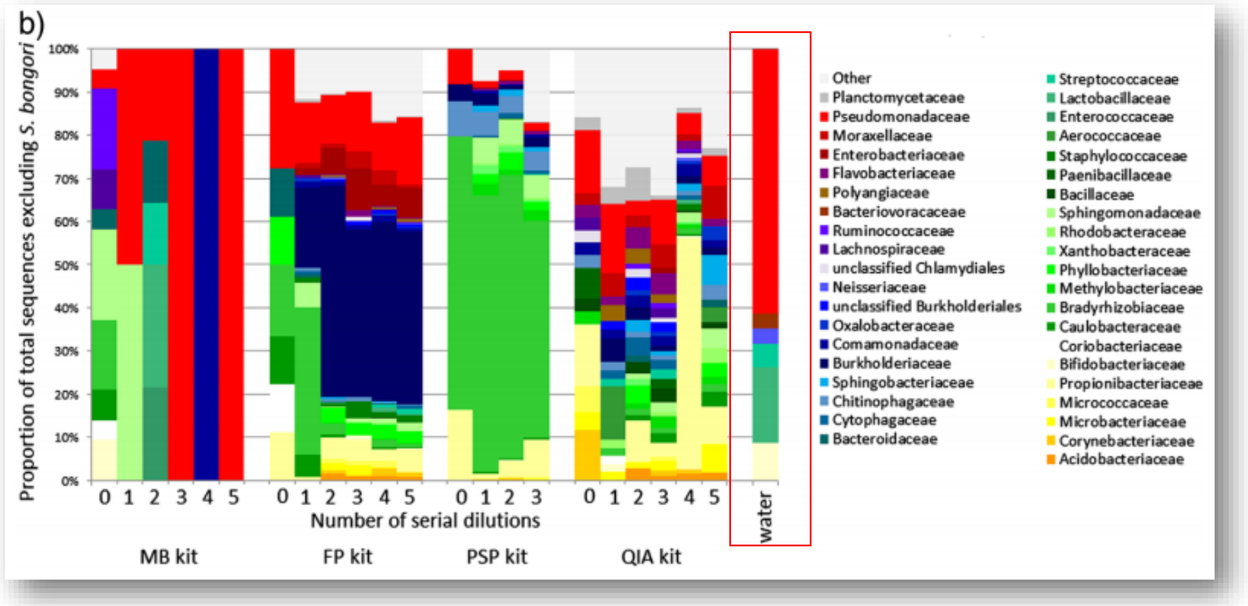
Laboratuvar Süreçleri



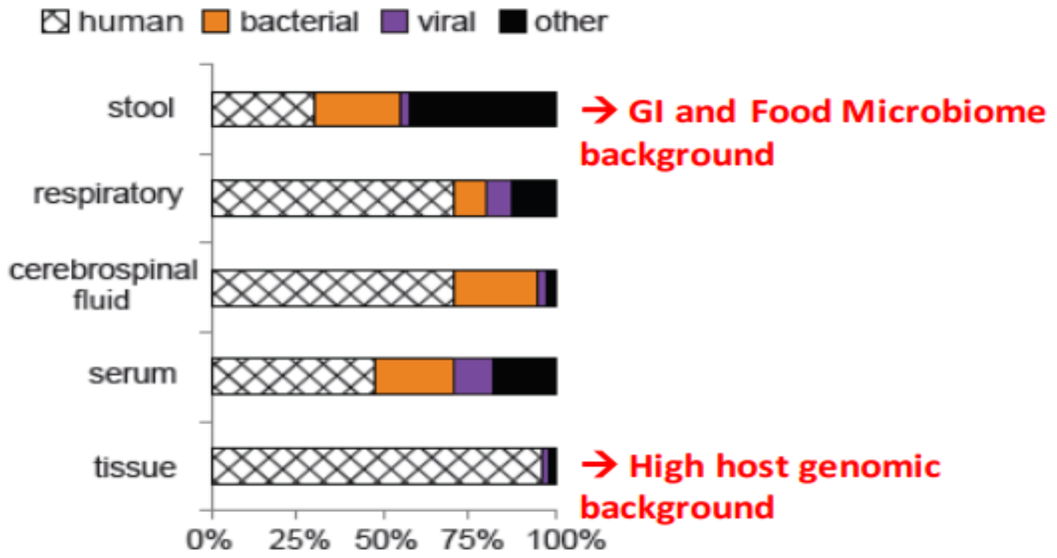
- ❑Klinik örneklerden uygun kalite ve nicelikte dsDNA izolasyonu
- ❑Kütüphane oluşturma
- ❑Yeni nesil sistemlerinde dizileme
- ❑Araştırma sorusuna uygun biyoinformatik analizler

dsDNA izolasyonu

- Kit ile izole ederim
  - mi acaba?



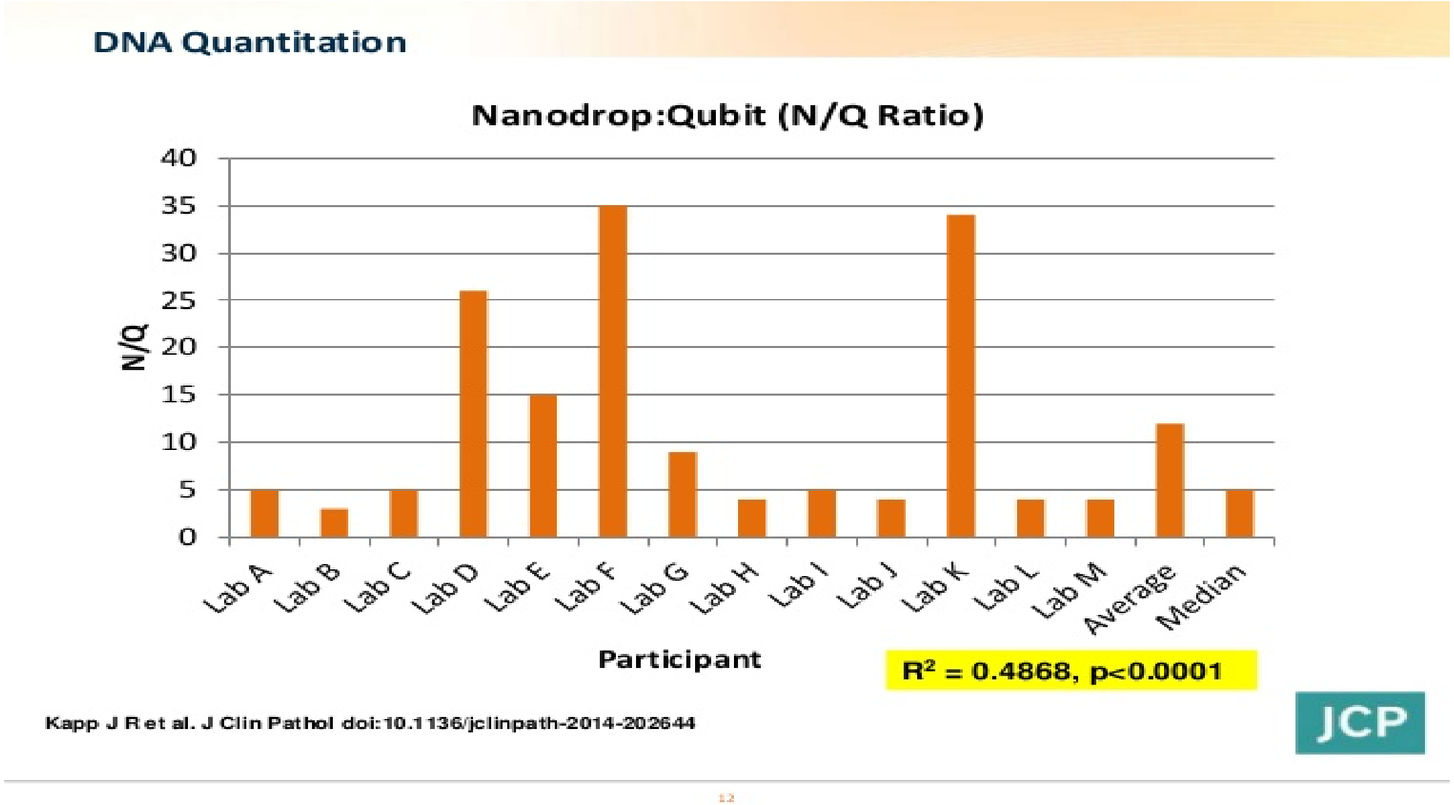
Batch Effects



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

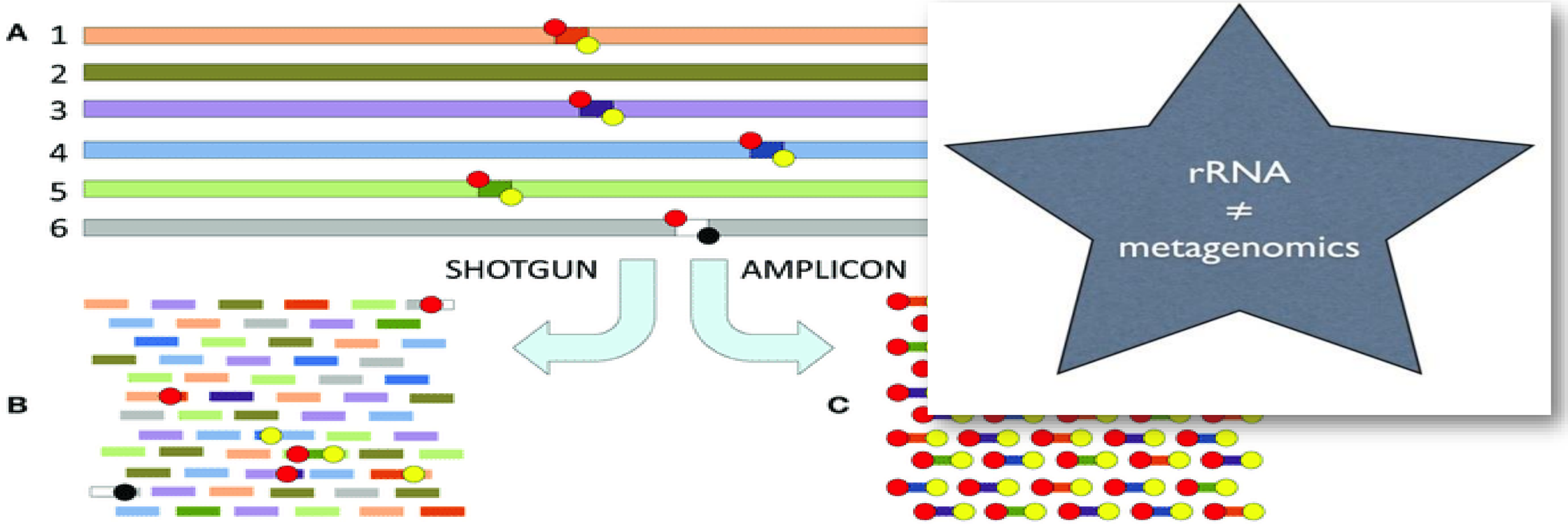
dsDNA kalite ve ölçümü

- Nanodrop ile ölçerim
  - mi acaba?



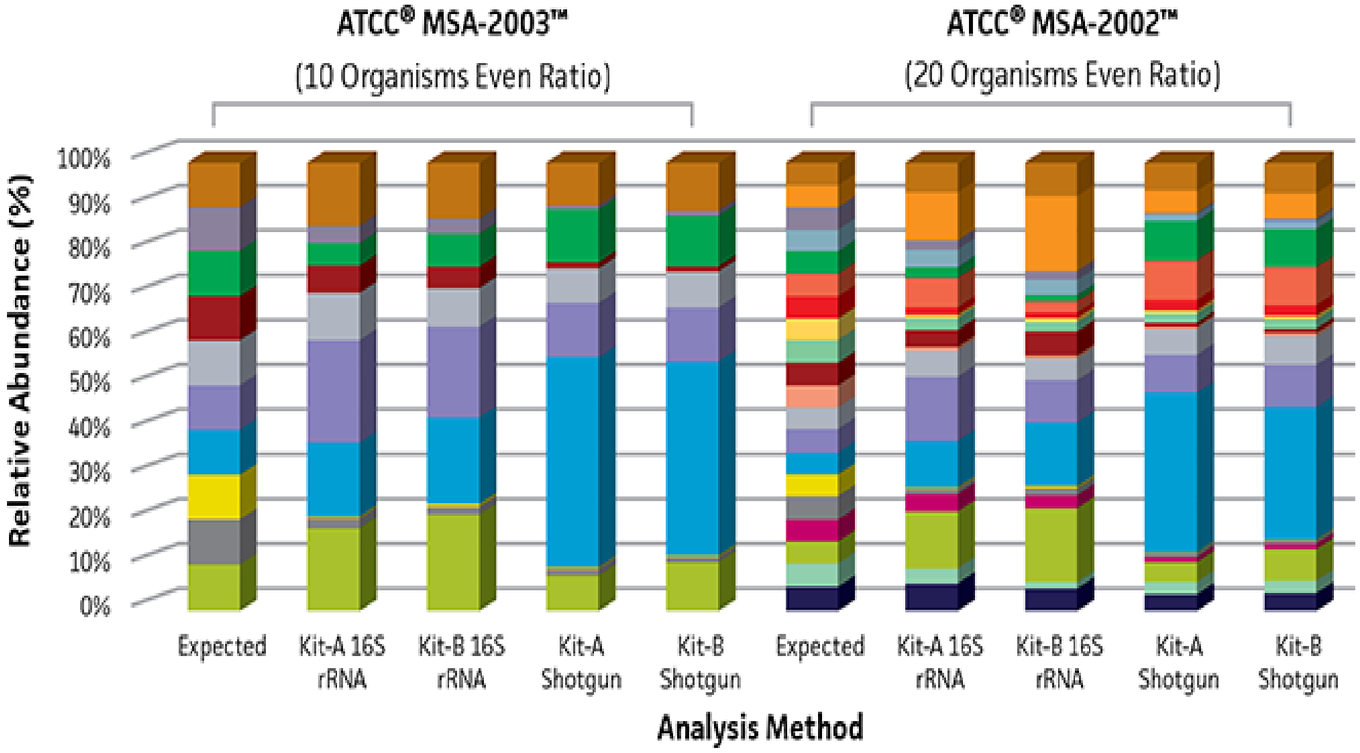
Kütüphane hazırlığı

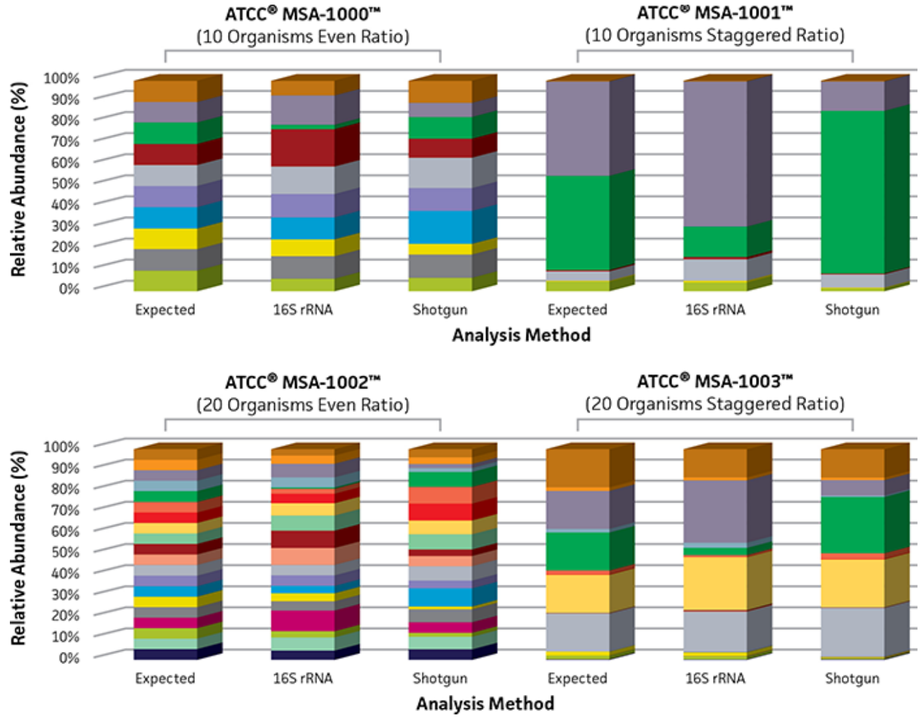
Metagenom dizileme VS amplikon dizleme



Kütüphane hazırlığı

- Pozitif/negatif kontroller!

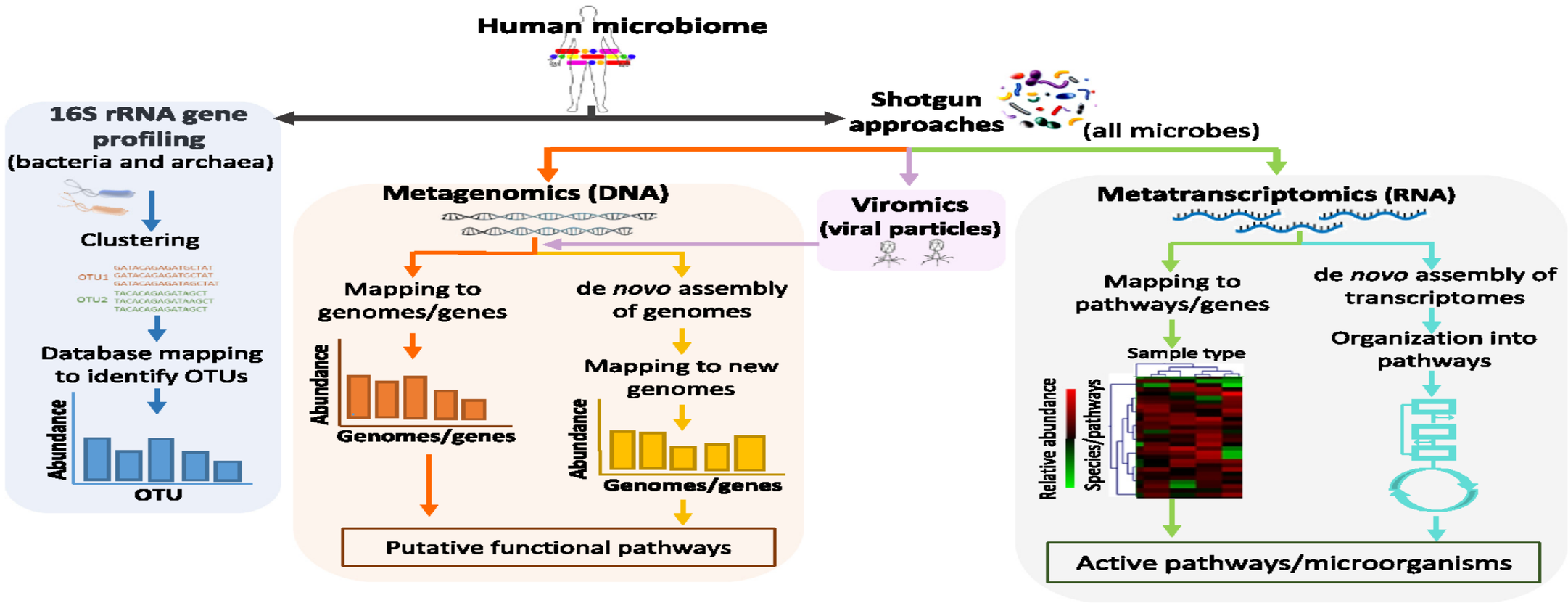




Yeni nesil dizileme teknolojileri

- Roche 454 (2004) - Pirosekanslama
- Illumina (2006) – Sentezleme ile dizileme
- Ion Torrent (2011)- Elektronik iyon değişimi dizilemesi
- PacBio (2011) – Tek molekül, gerçek zamanlı dizileme
- Oxford MinION (2015)- Nanogözenek dizilemesi

Araştırma sorusuna uygun biyoinformatik analizler



Araştırma sorusuna uygun biyoinformatik analizler

World Journal of Gastroenterology

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World J Gastroenterol 2018 December 14; 24(46): 5223-5233

DOI: 10.3748/wjg.v24.i46.5223

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Modulation of faecal metagenome in Crohn's disease: Role of microRNAs as biomarkers

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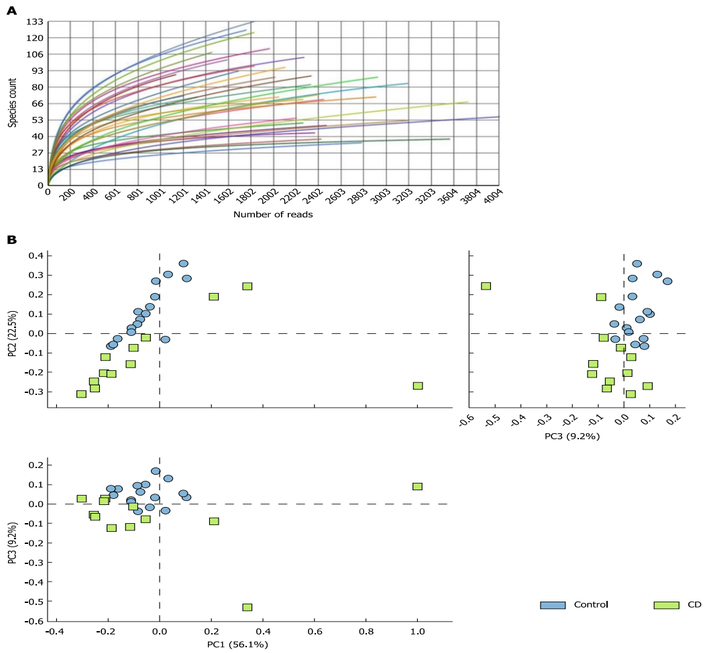

Abstract

BACKGROUND

The gut microbiota plays a key role in the maintenance of intestinal homeostasis and the development and activation of the host immune system. It has been shown that commensal bacterial species can regulate the expression of host genes. 16S rRNA gene sequencing has shown that the microbiota in inflammatory bowel disease (IBD) is abnormal and characterized by reduced diversity. MicroRNAs (miRNAs) have been explored as biomarkers and therapeutic targets, since they are able to regulate specific genes associated with Crohn's disease (CD). In this work, we aim to investigate the composition of gut microbiota of active treatment-naïve adult CD patients, with miRNA profile from gut microbiota.

AIM

To investigate the composition of gut microbiota of active treatment-naïve adult CD patients, with miRNA profile from gut microbiota.

METHODS

Patients attending the outpatient clinics at Valme University Hospital without relevant co-morbidities were matched according to age and gender. Faecal samples of new-onset CD patients, free of treatment, and healthy controls were collected. Faecal samples were homogenized, and DNA was amplified by PCR using primers directed to the 16S bacterial rRNA gene. Pyrosequencing was performed using GS-Junior platform. For sequence analysis, MG-RAST server with the database Ribosomal Project was used. MiRNA profile and their relative abundance were analyzed by quantitative PCR.

RESULTS

Microbial community was characterized using 16S rRNA gene sequencing in 29 samples (n = 13 CD patients, and n = 16 healthy controls). The mean Shannon diversity was higher in the healthy control population compared to CD group (5.5 *vs* 3.7). A reduction in *Firmicutes* and an increase in *Bacteroidetes* were found. *Clostridia* class was also significantly reduced in CD. Principal components analysis showed a grouping pattern, identified in most of the subjects in both groups, showing a marked difference between control and CD groups. A functional metabolic study showed that a lower metabolism of carbohydrates (P = 0.000) was found in CD group, while the metabolism of lipids was increased. In CD patients, three miRNAs were induced in affected mucosa: mir-144 (6.2 ± 1.3 fold), mir-519 (21.8 ± 3.1) and mir-211 (2.3 ± 0.4).

CONCLUSION

Changes in microbial function in active non-treated CD subjects and three miRNAs in affected *vs* non-affected mucosa have been found. miRNAs profile may serve as a biomarker.

Key words: Crohn's disease; Dysbiosis; microRNAs; *Firmicutes*; *Bacteroidetes*

Core tip: In this study, we have found a shift in microbial gut community composition that supports dysbiosis in Crohn's disease (CD) patients. The greatest interest of our work is that we have only included new-onset adult CD patients. We found that active non-treated CD patients had a low *Firmicutes*/*Bacteroidetes* ratio, less biodiversity in the structure of microbial communities and a significantly different pattern on gut microbiota distribution. Three microRNAs (miRNAs) have been found induced in affected mucosa vs non-affected mucosa in CD, indicating that miRNA profile may serve as biomarker for active disease.

Rojas-Feria M, Romero-García T, Fernández Caballero-Rico JÁ, Pastor Ramírez H, Avilés-Recio M, Castro-Fernandez M, Chueca Porcuna N, Romero-Gómez M, García F, Grande L, Del Campo JA. Modulation of faecal metagenome in Crohn's disease: Role of microRNAs as biomarkers. *World J Gastroenterol* 2018; 24(46): 5223-5233

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INTRODUCTION

Up to now, the pathogenesis of inflammatory bowel disease (IBD) has not been clarified. A plausible theory is that IBD develops in genetically susceptible individuals due to the abnormal immune response against luminal antigens and microbiota[1]. The targets of this response are thought to be antigens derived from constituents of the microbiota.

The gut microbiota plays a key role in the maintenance of intestinal homeostasis and the development and activation of the host immune system. The composition of microbiota community evolves during the first years of life, increasing the microbial diversity gradually. During this evolution, the host genetics and the environmental factors can shape the microbiome composition.

On the other hand, it has been shown that commensal bacterial species can regulate the expression of host genes. 16S rRNA gene sequencing has shown that the microbiota in IBD is abnormal and characterized by reduced diversity. The causality between IBD and alterations in microbiota remains incompletely understood but one theory is that altered microbiota composition and function in IBD result in increased immune stimulation or enhanced mucosal permeability.

A strong genetic component has been described in IBD, with the identification of about 200 loci associated with the development of the disease[2]. However, this can only explain a 16%-23% of the heritable of IBD[3-5]. Epigenetic factors can mediate interactions between the environment and the genome and could therefore play a central role in the pathogenesis of IBD and other

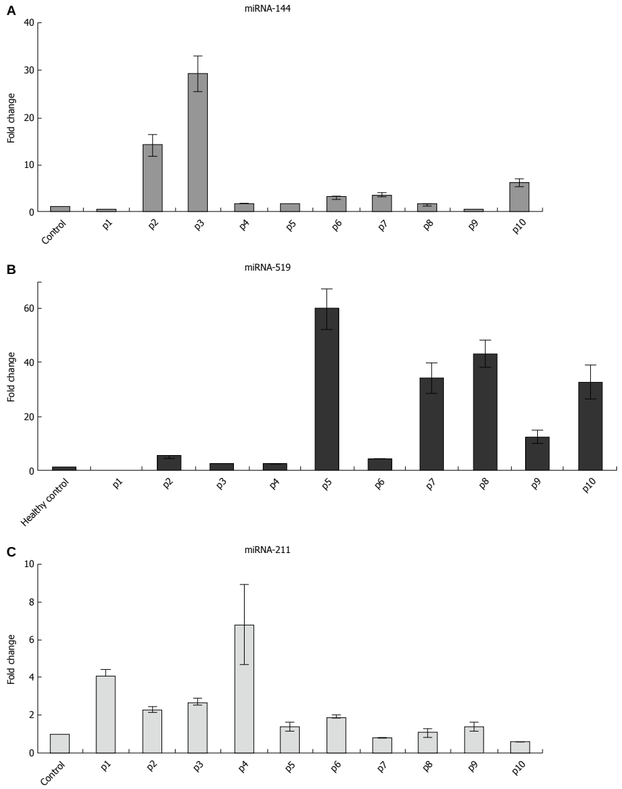
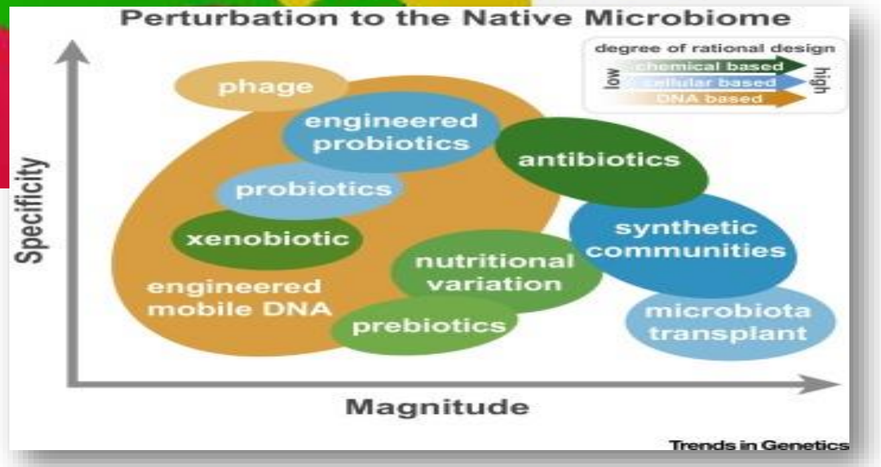


Figure 6 Three microRNAs were found increased in samples from patients with Crohn's disease. Individual microRNA levels in 10 patients with Crohn's disease are represented. miRNA: MicroRNA.


SONRASINDA?
SONRASINDA?

TRANSLATIONAL MICROBIOME



Teşekkürler

GenKok Metagenomics Lab.