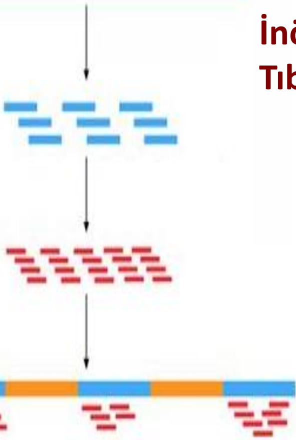
Konak ve Patojen İlişkisinde Genom, Metagenom ve Transkriptomik İnceleme



Barış Otlu
İnönü Üniversitesi Tıp Fakültesi
Tıbbi Mikrobiyoloji Anabilim Dalı

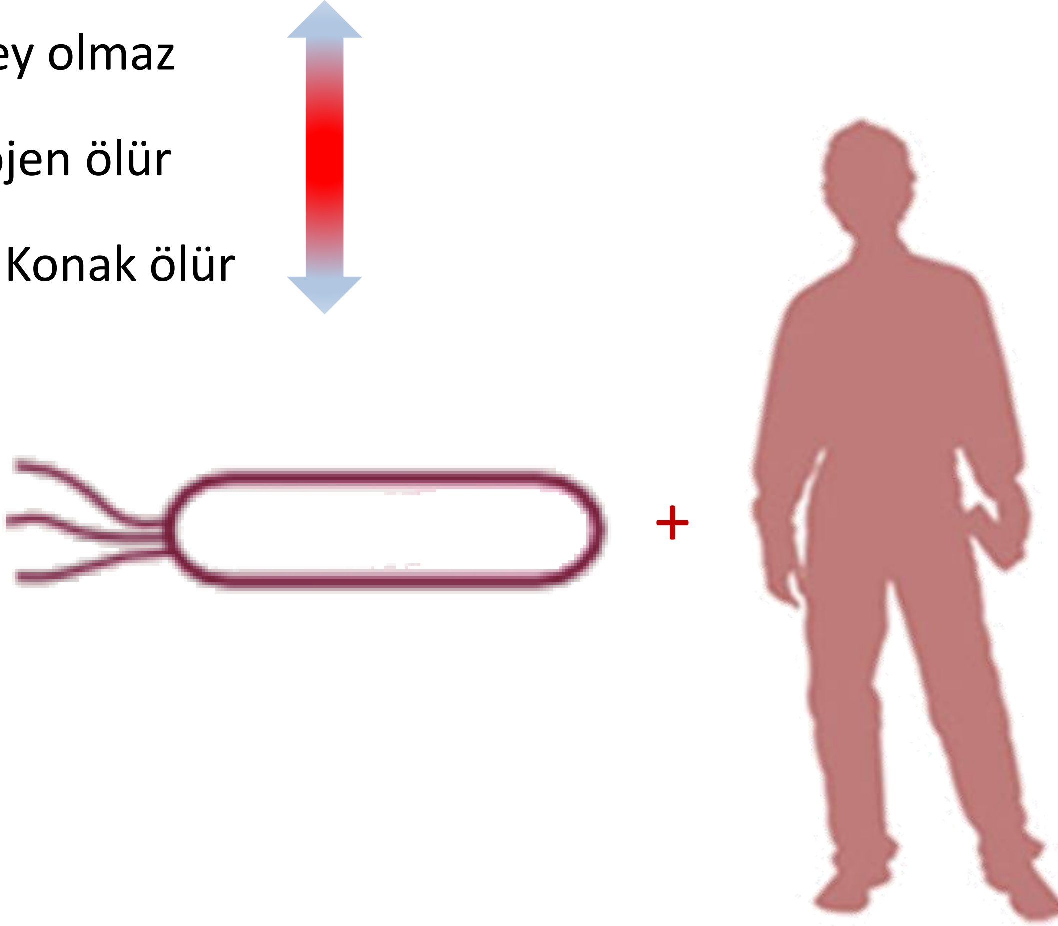
Konak-Patojen İlişkisi- Biyolojik Yolaklar - "Big Data"

- Özetle

Michal (Ed.), Biochemical Pathways, John Wiley and Sons, New York, 1999.

Konak-Patojen İlişkisi

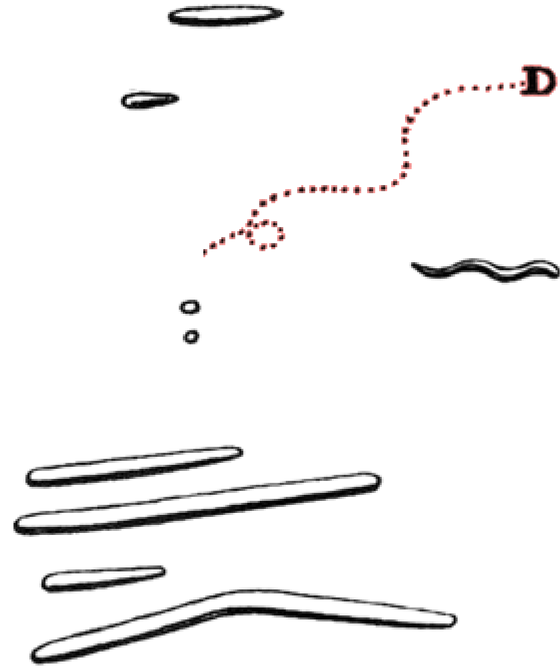
- Hiç bir şey olmaz
  - Patojen ölür
    - Konak ölür



Mikroorganizma Sonrası; 0. zaman

- Mikroorganizmaların ilk kez görülmesi

1680

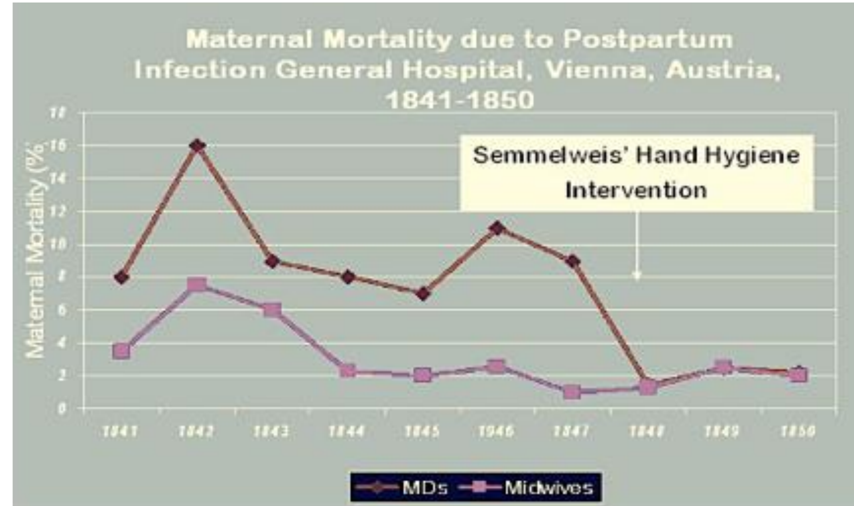


Mikroorganizma Sonrası; 170. yıl

- Ignaz Semmelweis'ın

  1841-1850 yılları arasında Avusturya ve Viyana'da iki farklı doğum hastanesindeki gözlemleri

1850

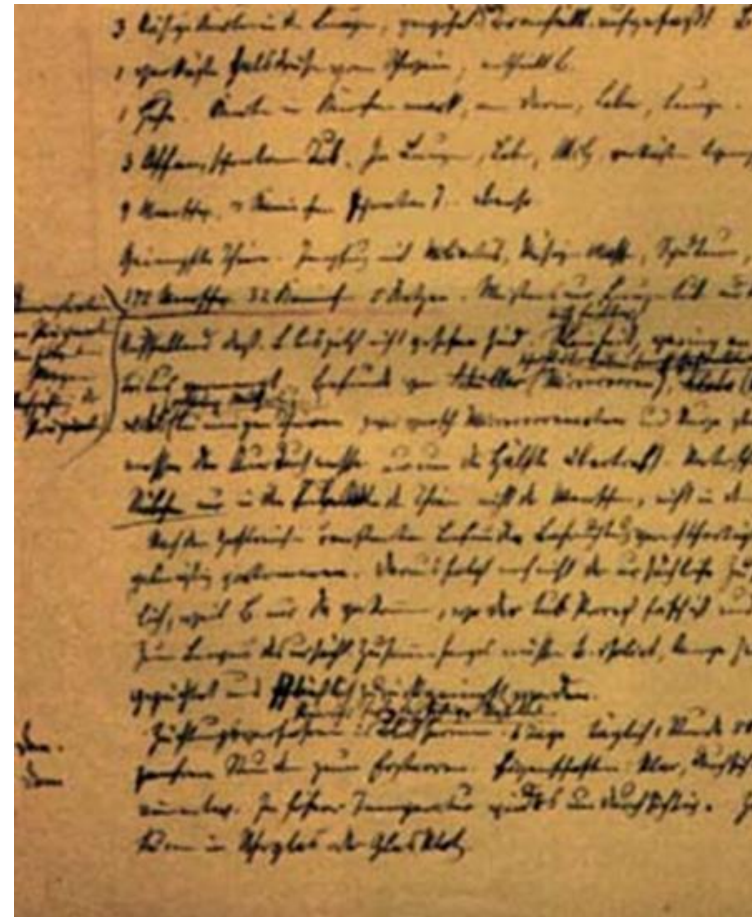


Mikroorganizma Sonrası; 210. yıl

- Robert Koch ve Friedrich Loeffler; hastalık ile bir mikroorganizma arasındaki nedensel ilişkiyi kriterize eden Koch postülatın

1890

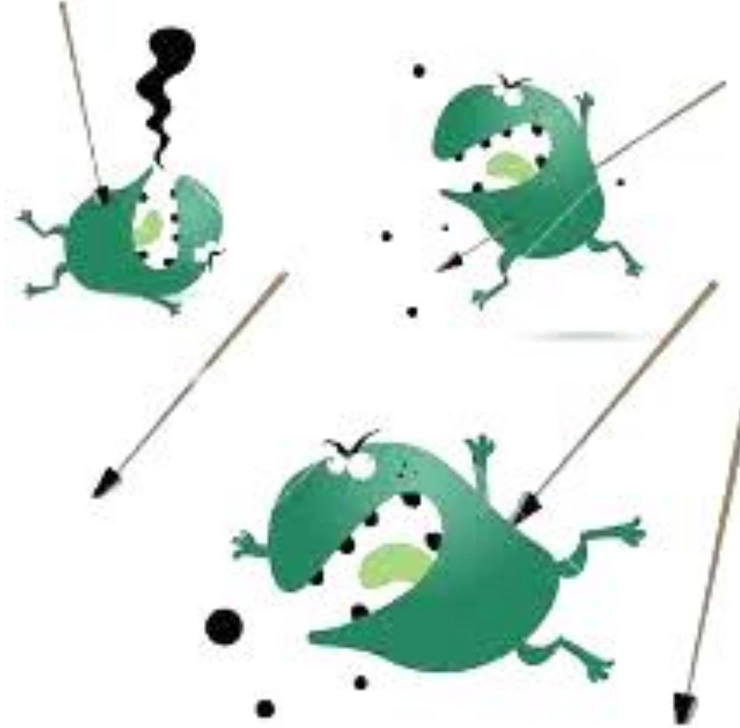
Koch Postülatı



Mikroorganizma Sonrası; 210. yıl

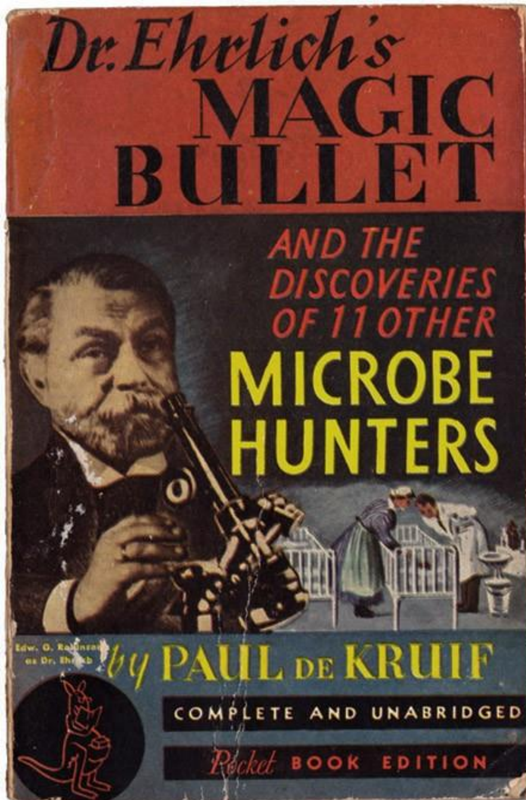
- Bu tarihten sonra mikroorganizmalar ve insanoğlu arasındaki savaş başlamış ve "mikrobu bul, mikrobu yok et" sloganı yıllar boyu sürecek mücadelenin ana fikri olmuştur.

" mikrobu bul, mikrobu yok et"



Mikroorganizma Sonrası;

- Poul de Kruif tarafından 1924 yılında yazılan "Microbe Hunters" kitabında, mikroplarla savaşan cesur ve korkusuz "ölüm savaşçıları" olarak anlatılmaktadır.



Spallanzani

Koch

Paul Ehrlich

Pasteur

Metchnikoff

Mikroorganizma Sonrası; 244. yıl

- Paul Ehrlich'in geliştirdiği “sihirli mermi” kavramı sonucunu vermiş ve bir arsenik türevi olan arsphenamine (salvarsan) ile mikrop katliamına başlanmıştır.

Mikroorganizma Sonrası; Bugün 336. yıl

- Salvarsandan bugünkü modern sefalosporinlere kadar geçen süreç göz önüne alındığında, “mikrobu bul, mikrobu yok et” felsefesinin çok da başarılı olmadığı ve yeni yaklaşımlara ihtiyaç olduğu bir gerçektir.

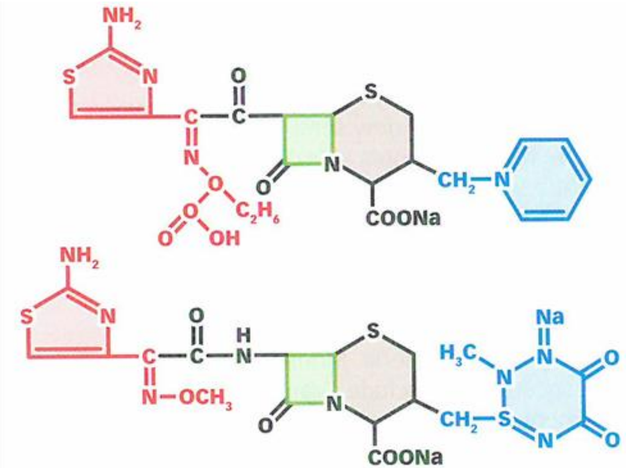
Das neue Ehrlich'sche
Syphilis-Heilmittel
bringen wir Mitte Dezember 1910 unter der geschützten Marke

„SALVARSAN“

in den Handel.

Wegen Bezuges von „Salvarsan“ bitten wir die Herren Apotheker, sich ausschliesslich an die Grossdrogen-Handlungen wenden zu wollen.

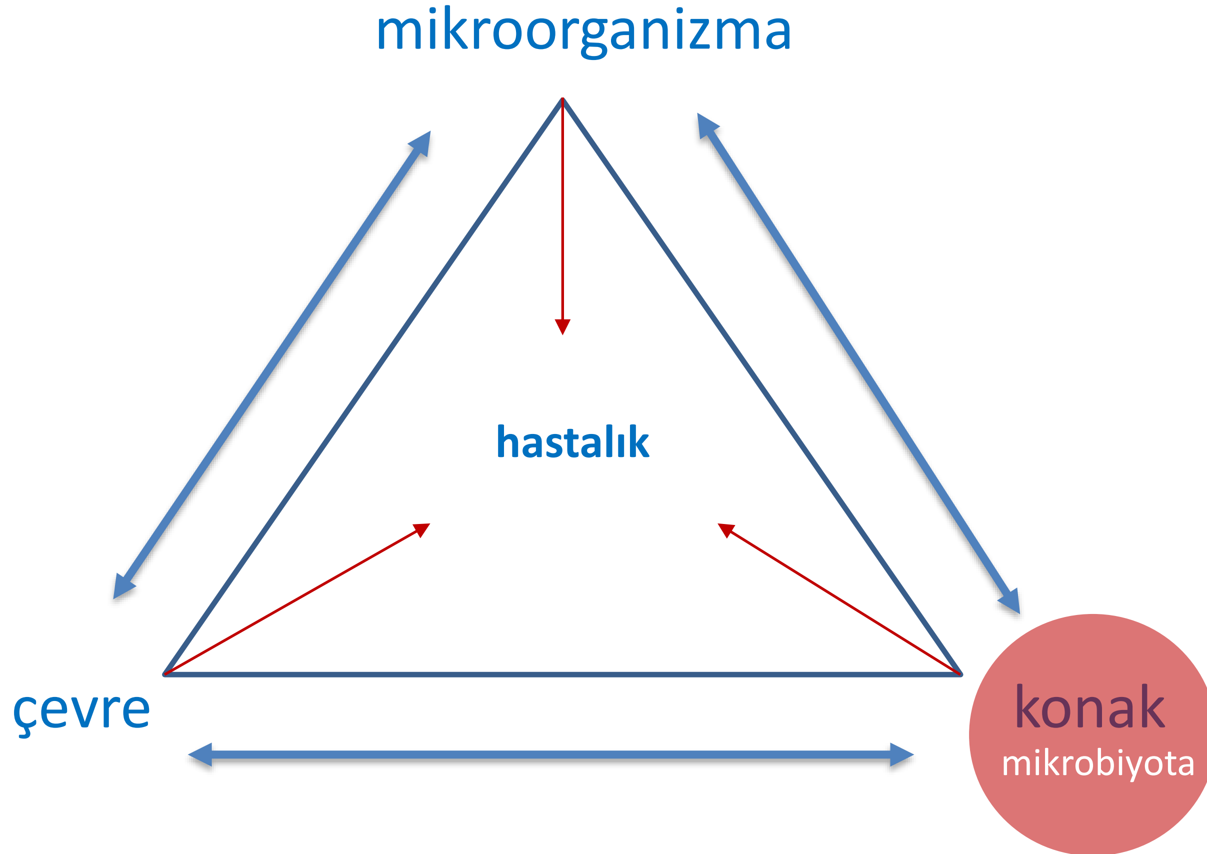
Farbwerke vorm. Meister Lucius & Brüning,
Hoechst a. M.



Mikroorganizma Sonrası

- Bu yeni yaklaşımların odağında,

  bir mikroorganizmanın hastalık yapması için konağın da ve hatta çevrenin de önemli olduğu bilgisi vardır.



Sistem Biyolojisi

- Geleneksel biyolojik yaklaşımlar;
  daha az değişkeni incelemeye imkan sağlarken sistem biyolojisi, çok sayıda gene, proteine, hücreye, biyolojik ağlara ve bunların etkileşimlerinin çoklu veya tekli sistemlerde incelemesine odaklanmıştır.

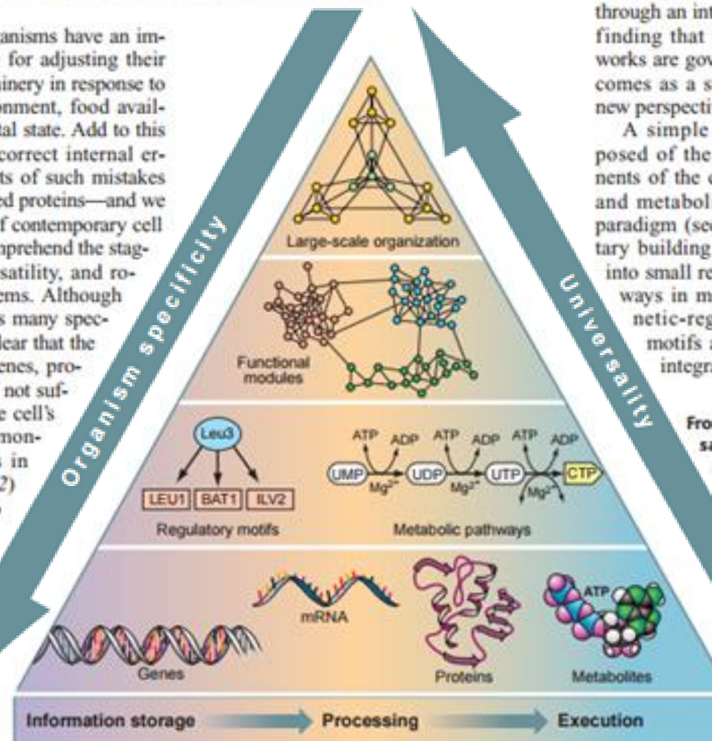
PERSPECTIVES: SYSTEMS BIOLOGY

Life's Complexity Pyramid

Zoltán N. Oltvai and Albert-László Barabási

Cells and microorganisms have an impressive capacity for adjusting their intracellular machinery in response to changes in their environment, food availability, and developmental state. Add to this an amazing ability to correct internal errors—battling the effects of such mistakes as mutations or misfolded proteins—and we arrive at a major issue of contemporary cell biology: our need to comprehend the staggering complexity, versatility, and robustness of living systems. Although molecular biology offers many spectacular successes, it is clear that the detailed inventory of genes, proteins, and metabolites is not sufficient to understand the cell's complexity (*1*). As demonstrated by two papers in this issue—Lee *et al.* (*2*) on page 799 and Milo *et al.* (*3*) on page 824—viewing the cell as a network of genes and proteins offers a viable strategy for addressing the complexity of living systems.

According to the



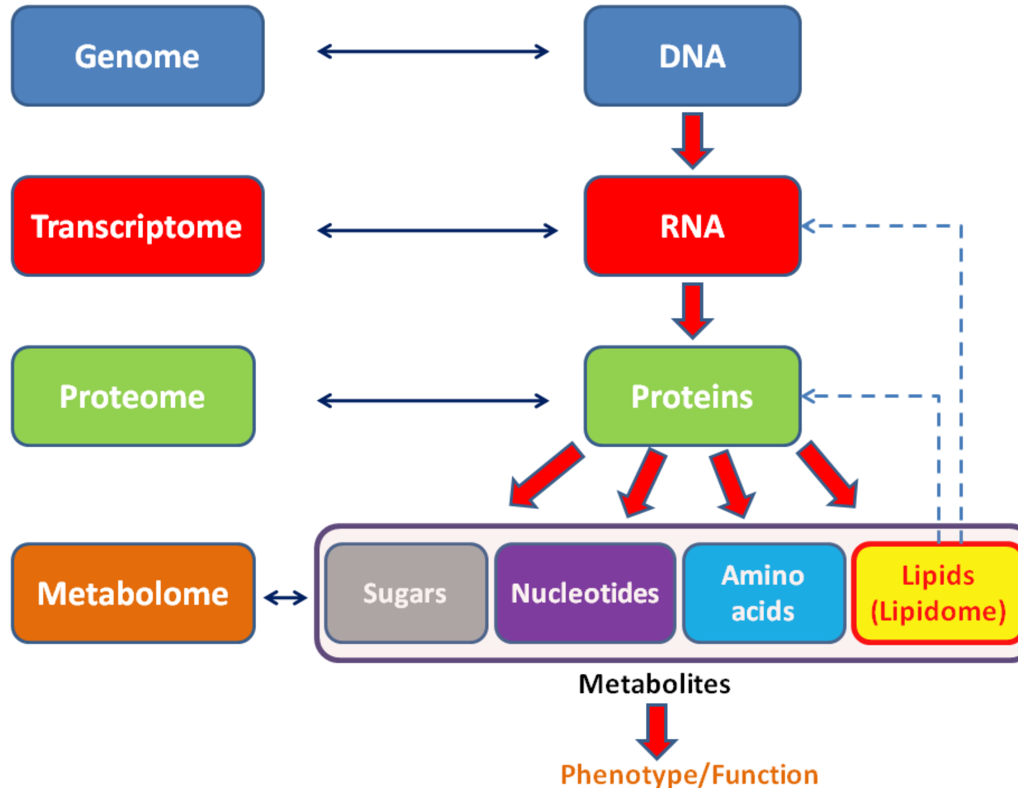
within large networks (*6, 7*). There is clear evidence for the existence of such cellular networks: For example, the proteome organizes itself into a protein interaction network and metabolites are interconverted through an intricate metabolic web (*7*). The finding that the structures of these networks are governed by the same principles comes as a surprise, however, offering a new perspective on cellular organization.

A simple complexity pyramid composed of the various molecular components of the cell—genes, RNAs, proteins, and metabolites—summarizes this new paradigm (see the figure). These elementary building blocks organize themselves into small recurrent patterns, called pathways in metabolism and motifs in genetic-regulatory networks. In turn, motifs and pathways are seamlessly integrated to form functional mod-

From the particular to the universal. The bottom of the pyramid shows the traditional representation of the cell's functional organization: genome, transcriptome, proteome, and metabolome (level 1). There is remarkable integration of the various layers both at the regulatory and the structural level. Insights into the logic of cellular organization can be achieved when we view

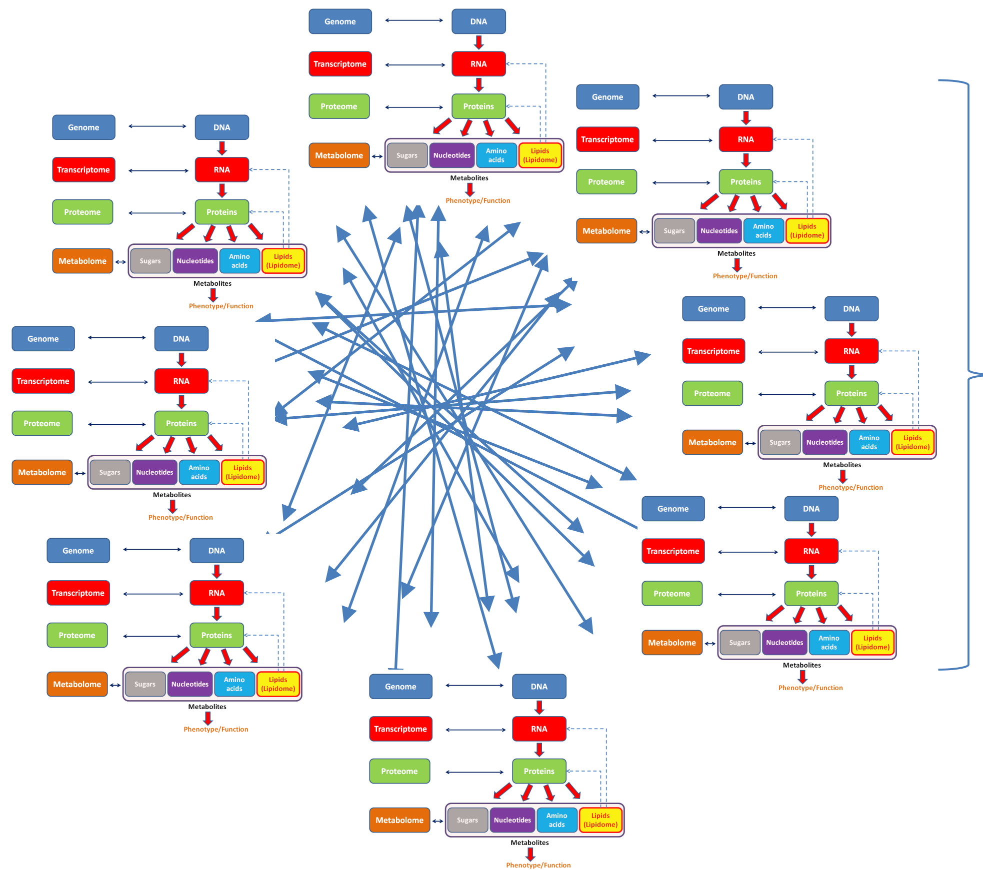
Sistem Biyolojisi ve OMIC

- Gelişen teknoloji ile birlikte genomik, transkriptomik, proteomik, metabolomik alanlarındaki gelişmeler, konak-patojen sistemlerindeki ilişkilerin araştırılmasını imkanlı hale getirmiştir.



Sistem Biyolojisi ve OMIC

- Gelişen teknoloji ile birlikte
  genomik, transkriptomik, proteomik, metabolomik alanlarındaki gelişmeler, konak-patojen sistemlerindeki ilişkilerin araştırılmasını imkanlı hale getirmiştir.



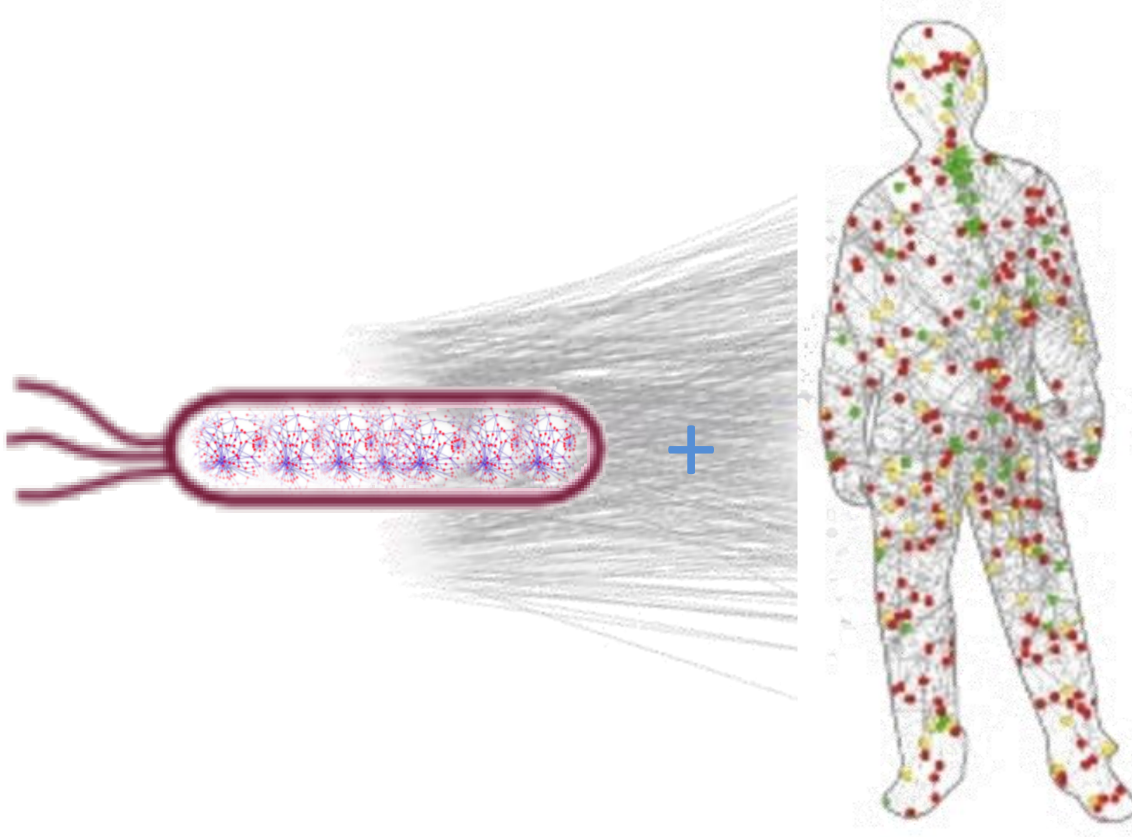
Metagenomik

Metatrankriptomik

Metaproteomik

Sistem Biyolojisi

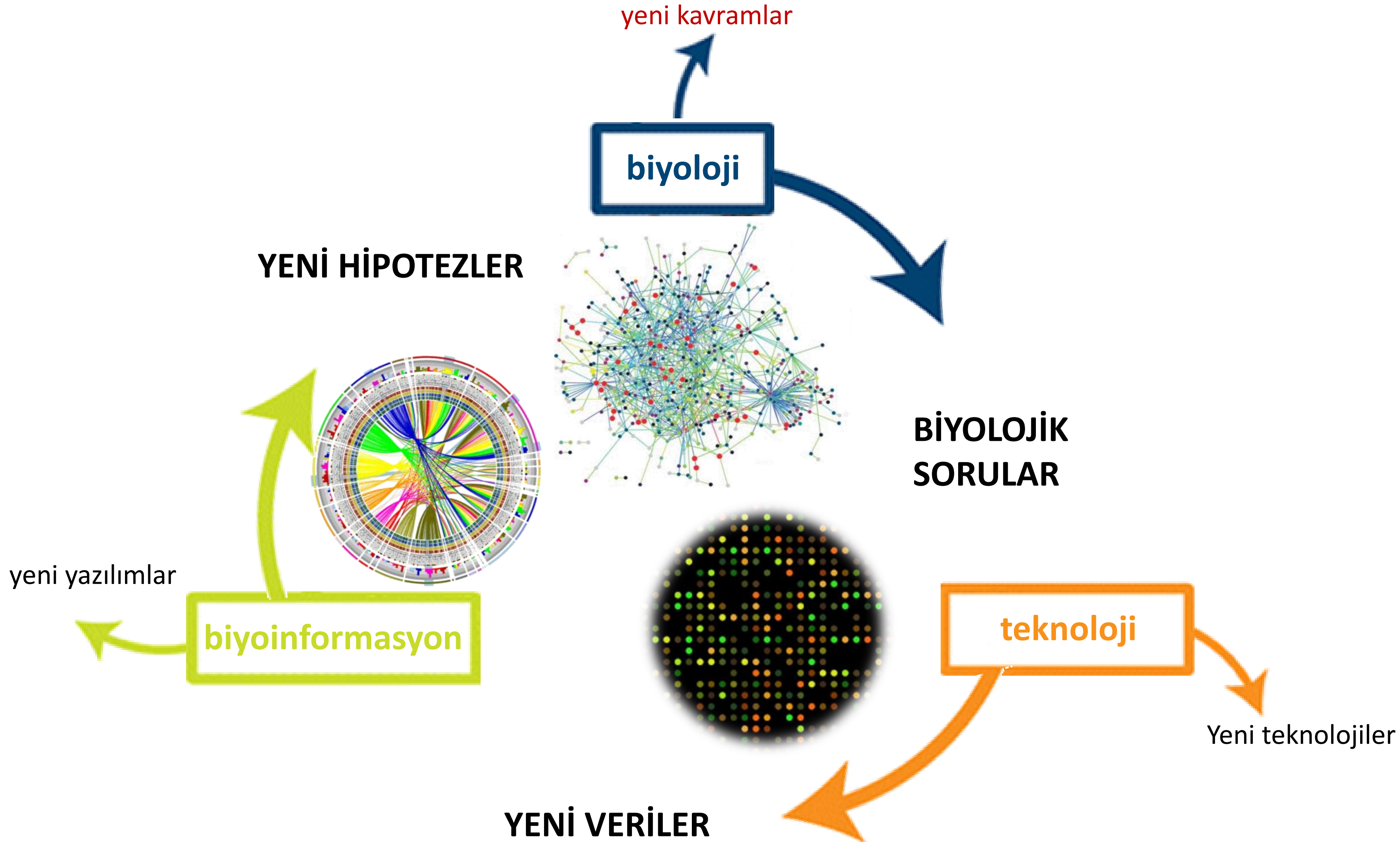
- Patojen mikroorganizmalar hayatta kalabilmek ve çoğalabilmek için
  konak hücre savunma mekanizmalarını yıkmaya çalışırken,
  konak hücre de bazı gen ekspresyon değişiklikleri gerçekleştirerek bu istilaya cevap verirler.



Konak-patojen ilişkilerinde yöntemler

- Bu oldukça karmaşık ve çok boyutlu dinamiklerin ortaya konması; yeni mikrobiyal virülans faktörlerinin ve bunlara karşı gelişen konak yanıtı aydınlatılabilecektir.
- Tüm bu veriler bir arada değerlendirildiğinde, yeni tanı ve tedavi yaklaşımlarının geliştirilmesini sağlayacaktır.

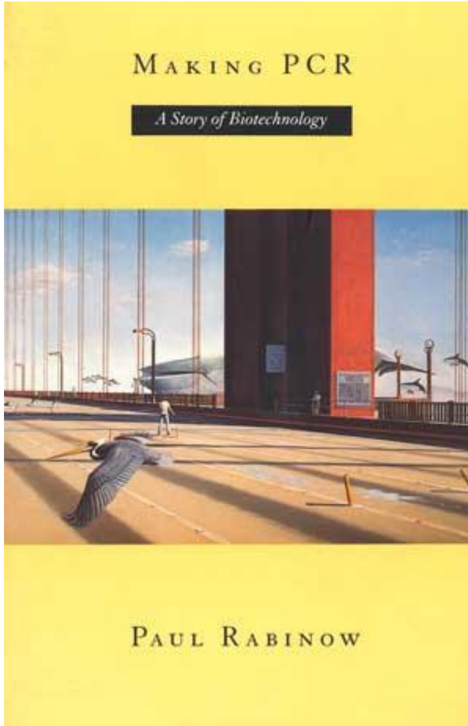
Sistem Biyolojisi



www.systemsbiology.org

Polymerase chain reaction (PCR)

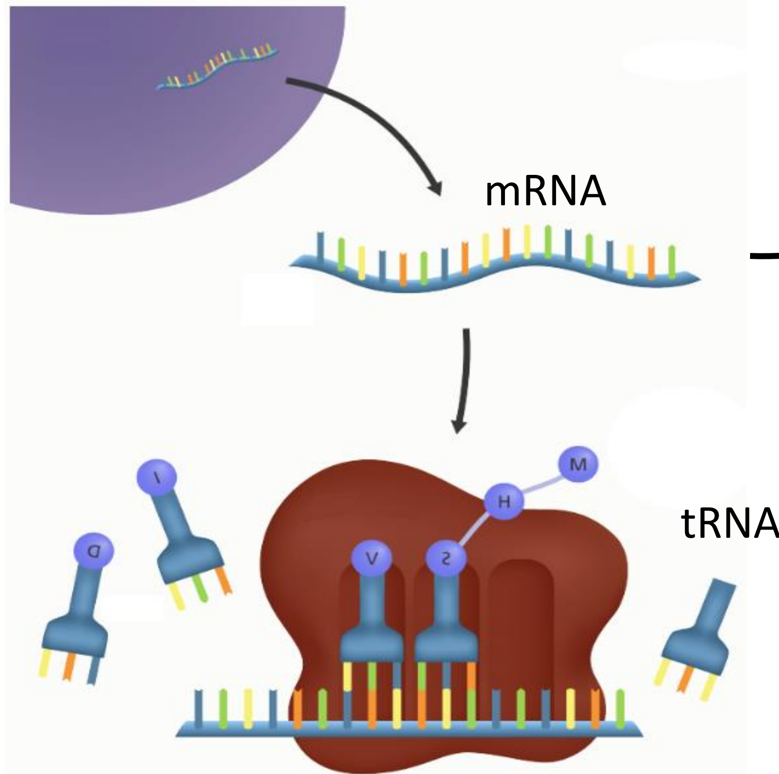
- Polimeraz zincirleme tepkimesinin 1983 yılında tanıtılması, biyolojinin her alanında olduğu gibi moleküler biyoloji alanında da yeni bir çağın başlangıcına neden olmuş ve genomdan bilgi akışında büyük bir ivme yaşanmıştır.



- *PCR*
- *Multiplex PCR*
- *Nested PCR*
- *Seminetested PCR*
- *Broad Range PCR*
- *Hot Start PCR*
- *Touchdown PCR*
- *Reverse Transcription PCR*

Revers transcriptase-PCR

- Ters transkriptaz-PZT yönteminin geliştirilmesiyle RNA’yı cDNA’ya çevirmek mümkün olmuş ve transkiripsiyonel seviyede incelemeler olanaklı hale gelmiştir.



mRNA up-regülasyon↑

mRNA down-regülasyon↓

www.yourgenome.org’dan uyarlanmıştır.

Revers transcriptase-PCR

- Deneysel fare modelinde yapılan bir çalışmada;
  Burkholderia pseudomallei enfeksiyonu sırasında konağın gösterdiği sitokin yanıtının, enfeksiyonun kronik / akut ilerleyişe etki edip etmediği araştırılmıştır.

ELSEVIER

Acta Tropica 74 (2000) 229–234

ACTA TROPICA

www.elsevier.com/locate/actatropica

Proinflammatory cytokine mRNA responses in experimental *Burkholderia pseudomallei* infection in mice

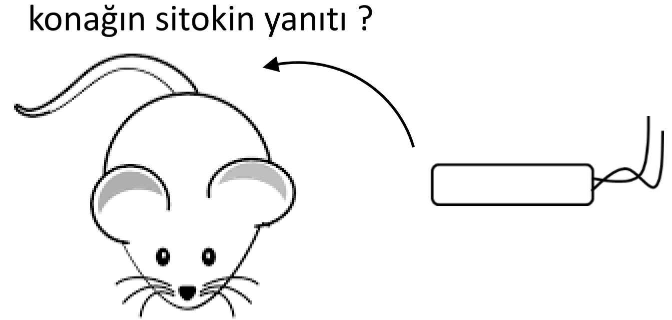
Glen C. Ulett [a,*], Natkunam Ketheesan [b], Robert G. Hirst [a]

[a] *Department of Microbiology and Immunology, James Cook University, Townsville, Queensland 4811, Australia*
[b] *Department of Medicine, North Queensland Clinical School, University of Queensland, Queensland 4810, Australia*

Abstract

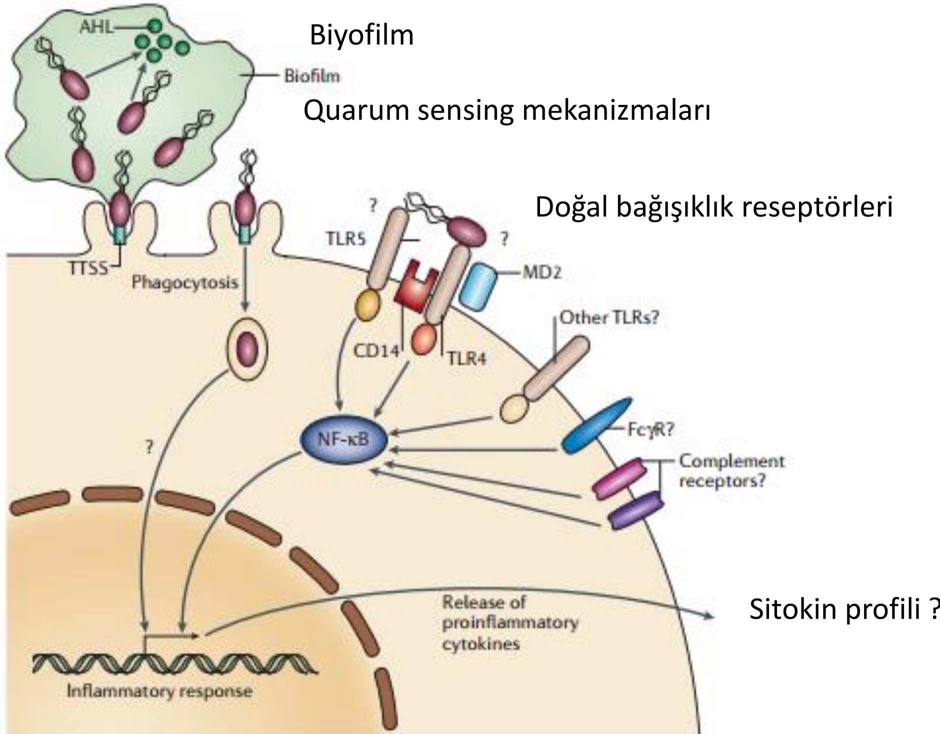
Melioidosis is a potentially fatal disease of both human and animals caused by the bacterium *Burkholderia pseudomallei*. Disease is endemic in tropical and subtropical regions of Southeast Asia and Northern Australia. The pathogenesis of melioidosis is poorly understood. In particular, the host responses that occur following infection, and the specific host-pathogen interactions that result in the development of either acute or chronic infection are unclear. Using an established murine model, we investigated early proinflammatory cytokine responses believed to be critical in the development of acute and chronic *B. pseudomallei* infection. Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) was used to assess levels of mRNA for tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6) in the liver of mice following infection. We demonstrate that the level of mRNA for these cytokines increase moderately in chronic infection in C57BL/6 mice. However, in acute infection in BALB/c mice, mRNA responses for these cytokines were shown to be comparatively greater. These results demonstrate that early proinflammatory cytokine responses are important in the immunopathogenesis of melioidosis. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Burkholderia pseudomallei*; Melioidosis; Cytokine; C57BL/6; BALB/c; mRNA



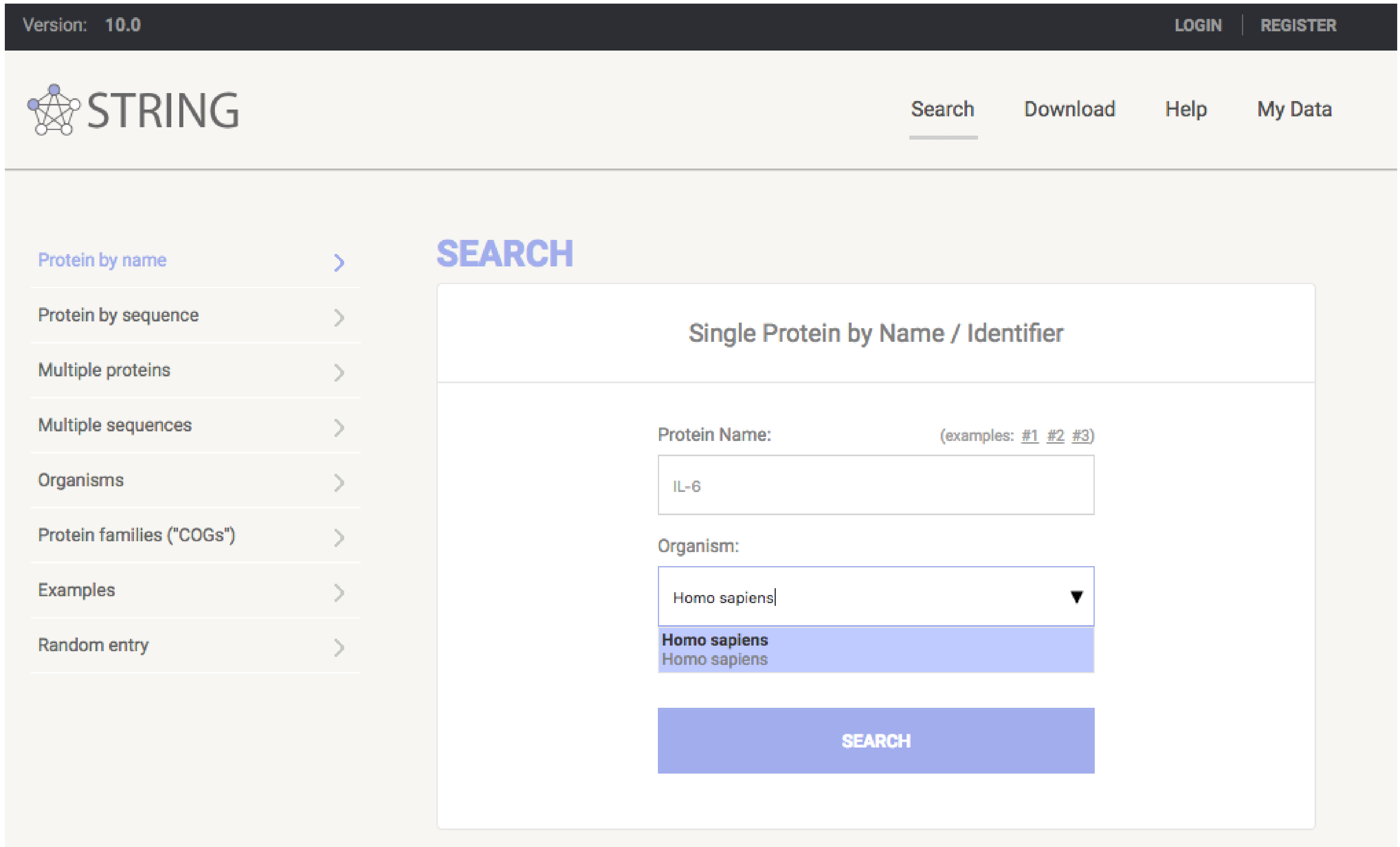
Revers transcriptase-PCR

- Bu amaçla;
  tümör nekroz faktör-α (TNF-α), interlökin-1β (IL-1β) ve interlökin-6 (IL-6) mRNA düzeyleri araştırılmış ve genlerin ekspresyon düzeylerinin artmasının enfeksiyonun kronikleşmesine neden olduğu tespit edilmiştir.



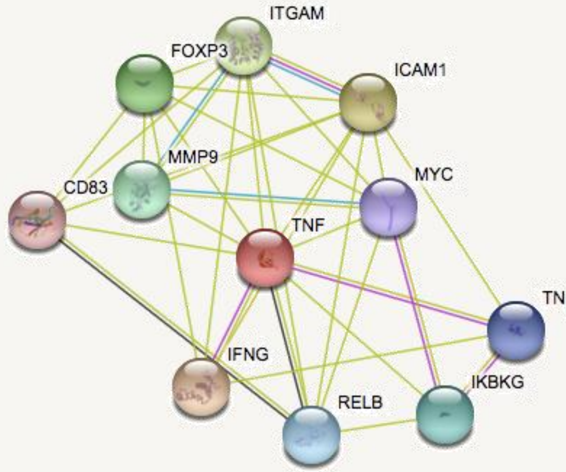
Revers transcriptase-PCR

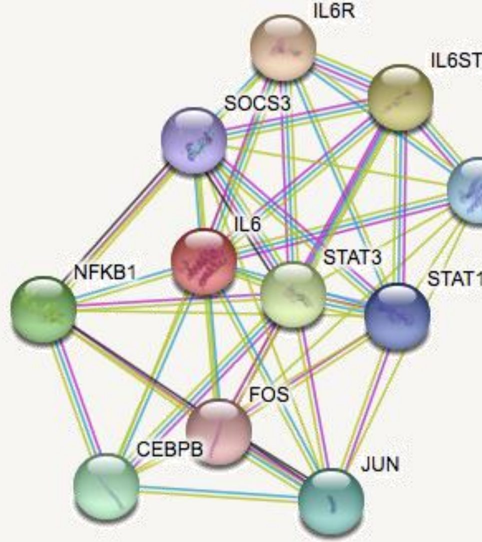
- Sistem biyoloji veri tabanı; TNF-α, IL-1β, IL-6

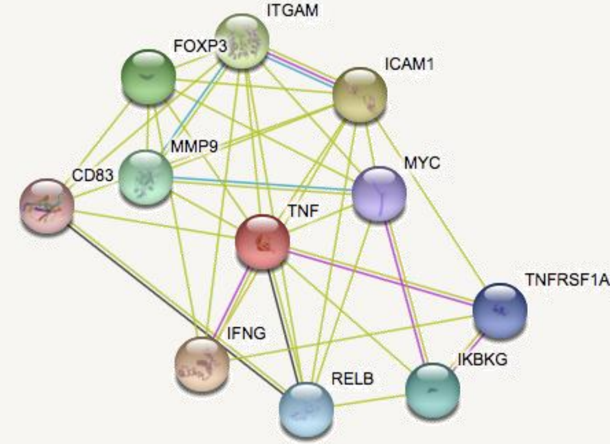


Revers transcriptase-PCR

- Belirlenen üç genin , diğer genlerle/proteinlerle birçok kategoride olabilecek etkileşimleri.

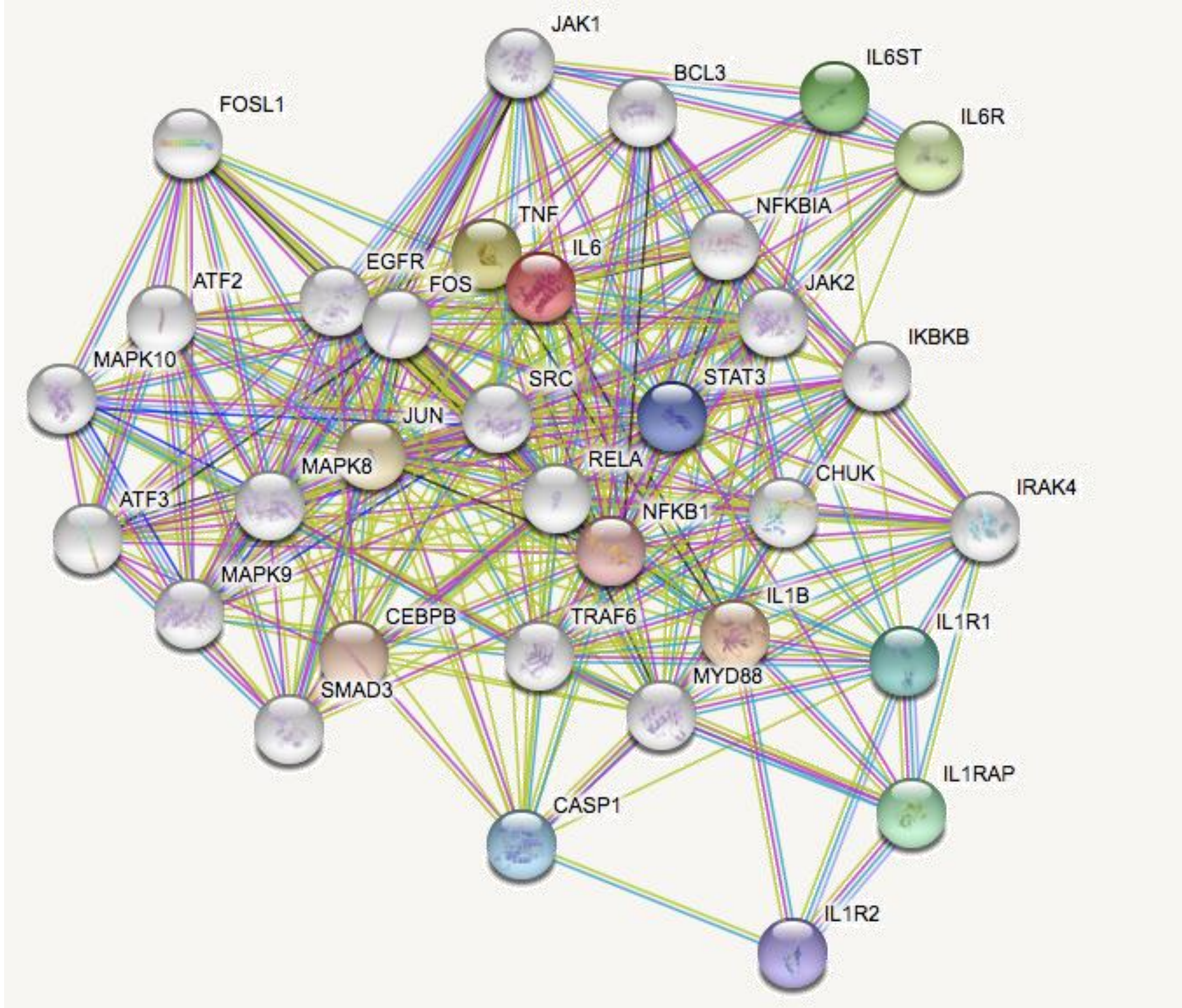






Revers transcriptase-PCR

- Belirlenen üç genin birlikte diğer genlerle birçok kategoride olabilecek etkileşimleri.



Revers transcriptase-PCR

- Epstein–Barr virus (EBV) ve nazofarengeal baş-boyun kanserleri

OPEN ACCESS Freely available online

A Global View of the Oncogenic Landscape in Nasopharyngeal Carcinoma: An Integrated Analysis at the Genetic and Expression Levels

Chunfang Hu[1], Wenbin Wei[1], Xiaoyi Chen[1,2], Ciaran B. Woodman[1], Yunhong Yao[1,2], John M. Nicholls[3], Irène Joab[4], Sim K. Sihota[1], Jian-Yong Shao[5], K. Dalia Derkaoui[6], Aicha Amari[7], Stephanie L. Maloney[1], Andrew I. Bell[1], Paul G. Murray[1], Christopher W. Dawson[1], Lawrence S. Young[1], John R. Arrand[1]*

1 School of Cancer Sciences, University of Birmingham, Birmingham, United Kingdom, **2** Department of Pathology, Guangdong Medical College, Zhanjiang, Guangdong, China, **3** Department of Pathology, University of Hong Kong, Hong Kong, China, **4** UMR542 Inserm-Université Paris Sud, Villejuif, France, **5** Department of Molecular Diagnostics, Sun Yat-Sen University Cancer Centre, Guangzhou, China, **6** Laboratoire de Biologie du Développement et de la Différenciation, Faculté des Sciences, Université d'Oran, Oran, Algeria, **7** ORL Centre Hospitalier et Universitaire, Oran, Algeria

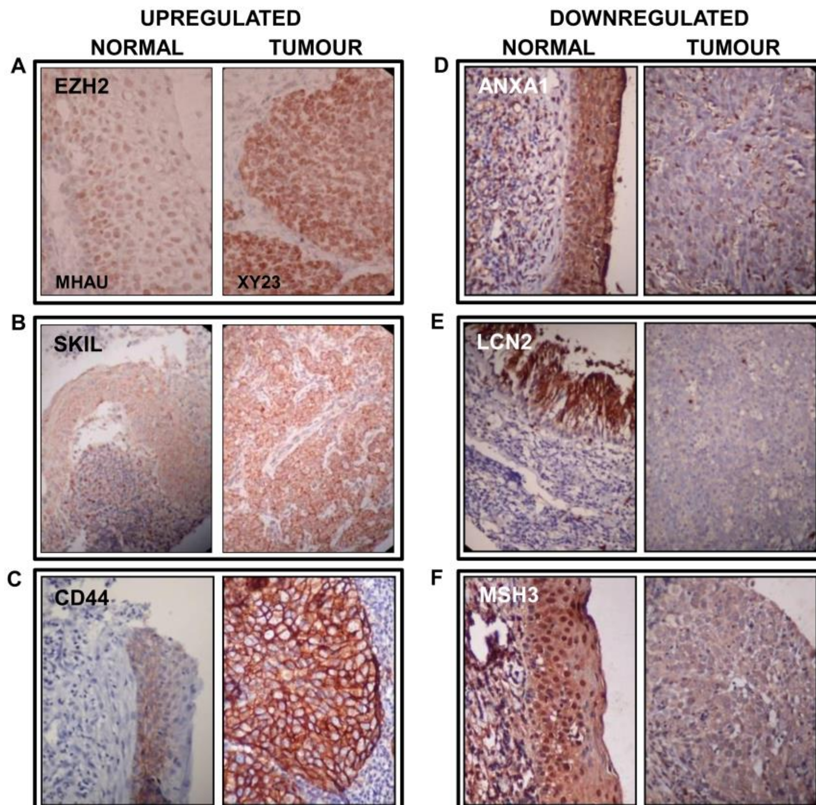
Abstract

Previous studies have reported that the tumour cells of nasopharyngeal carcinoma (NPC) exhibit recurrent chromosome abnormalities. These genetic changes are broadly assumed to lead to changes in gene expression which are important for the pathogenesis of this tumour. However, this assumption has yet to be formally tested at a global level. Therefore a genome wide analysis of chromosome copy number and gene expression was performed in tumour cells micro-dissected from the same NPC biopsies. Cellular tumour suppressor and tumour-promoting genes (TSG, TPG) and Epstein-Barr Virus (EBV)-encoded oncogenes were examined. The EBV-encoded genome maintenance protein EBNA1, along with the putative oncogenes LMP1, LMP2 and BARF1 were expressed in the majority of NPCs that were analysed. Significant downregulation of expression in an average of 76 cellular TSGs per tumour was found, whilst a per-tumour average of 88 significantly upregulated, TPGs occurred. The expression of around 60% of putative TPGs and TSGs was both up-and down-regulated in different types of cancer, suggesting that the simplistic classification of genes as TSGs or TPGs may not be entirely appropriate and that the concept of context-dependent onco-suppressors may be more extensive than previously recognised. No significant enrichment of TPGs within regions of frequent genomic gain was seen but TSGs were significantly enriched within regions of frequent genomic loss. It is suggested that loss of the FHIT gene may be a driver of NPC tumourigenesis. Notwithstanding the association of TSGs with regions of genomic loss, on a gene by gene basis and excepting homozygous deletions and high-level amplification, there is very little correlation between chromosomal copy number aberrations and expression levels of TSGs and TPGs in NPC.

Revers transcriptase-PCR

- Bu çalışmada;

  EBV ile enfekte olan ve olmayan kişilerde tümör supresör (TSG) ve tümör promotör genlerin (TPG) gen ekspresyonları incelenmiş.

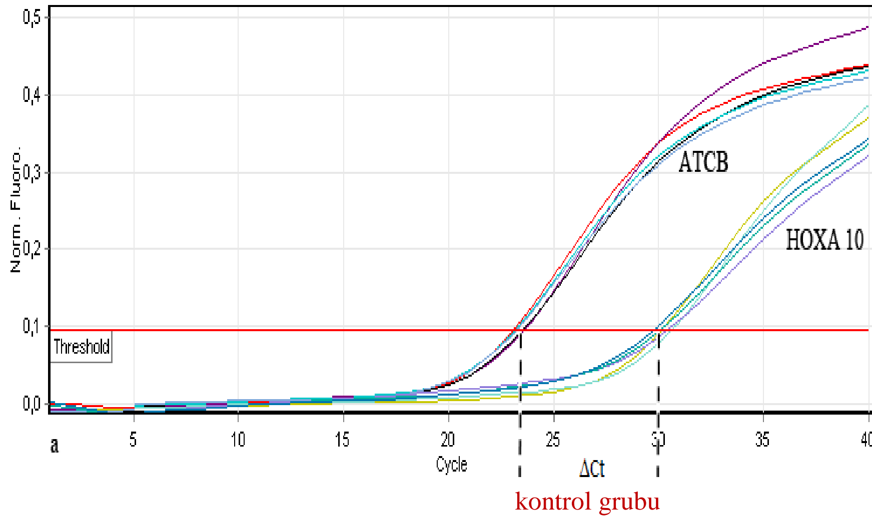


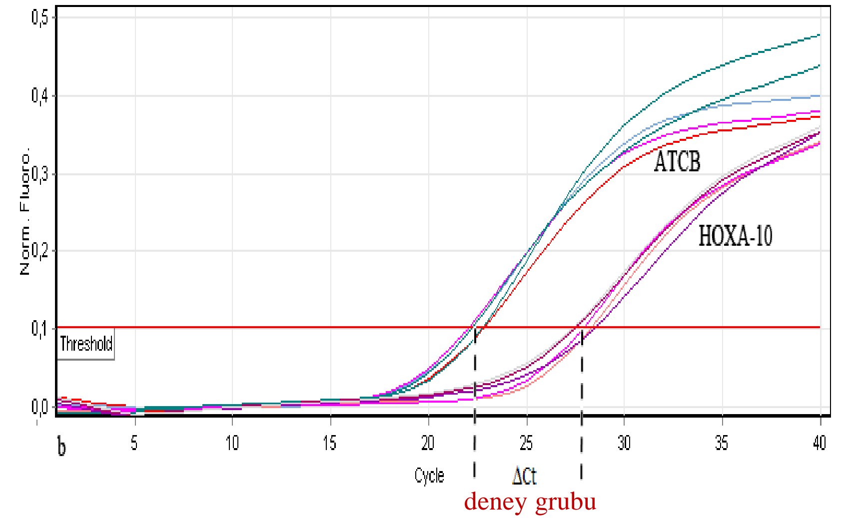
EBNA, BARF, LMP-1, LMP-2

Expression of EBV genes and EBV genome status of samples used in this study. The expression of the EBV-encoded genes BARF1, LMP1, LMP2 and EBNA1 was determined by RT-PCR using the primers listed in Table S8. Products were separated by agarose gel electrophoresis and visualised under U.V. light after staining with ethidium bromide. Cellular GAPDH expression was used as a positive control. The primers used in the detection of LMP1 transcripts flank the 33 base pair repeat region. Thus the variation in size of product is due to the different numbers of repeats in the LMP1 coding sequence in the various viral genomes. EBV gene expression in samples YH7 and YH8 was determined in separate experiments (not shown). EBV genome status was determined by PCR as described in the Methods. Samples HK4 and C666-1 were examined separately

Real time-PCR

- Bunlardan “bağıl kantitasyon” yöntemi bir hücredeki transkripsiyon düzeylerinin belirlenebilmesi için kullanılmaktadır.





Kontrol Grubu: ΔCt = Ct (hedef gen) - Ct (ATCB)

Deney Grubu: ΔCt = Ct (hedef gen) - Ct (ATCB)

ΔΔCt = ΔCt (deney grubu) - ΔCt (kontrol grubu)

Ratio = $2^{-\Delta\Delta CT}$

Real time-PCR

- Pumbwe ve arkadaşlarının yaptığı çalışmada;
  Bacteroides fragilis enfeksiyonunda safra tuzlarının rolü araştırılmıştır.

Microbial Pathogenesis 43 (2007) 78–87

www.elsevier.com/locate/micpath

Bile salts enhance bacterial co-aggregation, bacterial-intestinal epithelial cell adhesion, biofilm formation and antimicrobial resistance of *Bacteroides fragilis*

Lilian Pumbwe[a,b,*], Christopher A. Skilbeck[a], Viviane Nakano[a,b], Mario J. Avila-Campos[c], Roxane M.F. Piazza[d], Hannah M. Wexler[a,b]

[a] *Greater Los Angeles Veterans Administration Healthcare Systems, CA, USA*
[b] *Department of Medicine, University of California, Los Angeles, CA, USA*
[c] *Laboratorio de Anaerobios, Departamento de Microbiologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, SP, Brazil*
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Accepted 12 April 2007
Available online 20 April 2007

Abstract

Bacteroides fragilis is the most common anaerobic bacterium isolated from human intestinal tract infections. Before *B. fragilis* interacts with the intestinal epithelial cells, it is exposed to bile salts at physiological concentrations of 0.1–1.3%. The aim of this study was to determine how pre-treatment with bile salts affected *B. fragilis* cells and their interaction with intestinal epithelial cells. *B. fragilis* NCTC9343 was treated with conjugated bile salts (BSC) or non-conjugated bile salts (BSM). Cellular ultrastructure was assessed by electron microscopy, gene expression was quantified by comparative quantitative real-time RT-PCR. Adhesion to the HT-29 human intestinal cell line and to PVC microtitre plates (biofilm formation) was determined. Exposure to 0.15% BSC or BSM resulted in overproduction of fimbria-like appendages and outer membrane vesicles, and increased expression of genes encoding RND-type efflux pumps and the major outer membrane protein, OmpA. Bile salt-treated bacteria had increased resistance to structurally unrelated antimicrobial agents and showed a significant increase in bacterial co-aggregation, adhesion to intestinal epithelial cells and biofilm formation. These data suggest that bile salts could enhance intestinal colonization by *B. fragilis* via several mechanisms, and could therefore be significant to host–pathogen interactions.
Published by Elsevier Ltd.

Keywords: Colonization; Membrane; Signaling; Stress; Tolerance

Real time-PCR

- Bu amaçla;

  safra tuzları ile karşılaşmış ve karşılaşmamış *B. fragilis* suşlarının HT29 barsak hücre kültür hattı üzerindeki etkileri gen ekspresyon düzeyinde RT-PCR ile incelenmiştir.

5.3. DNA procedures

Total cellular chromosomal DNA was isolated with the DNeasy tissue kit, according to the manufacturers' instructions (Qiagen; Valencia, CA). BSH genes were detected by PCR with the following primers: BF1508-forward, 5′-TAG AAG GTG GTA CGC ATC-3′ and BF1508-reverse, 5′-GCT GTC AGA CCT TCA CGA-3′; BF3386-forward, 5′-CCG TCG TAA TGA TAC TCG-3′ and BF3586-reverse, 5′-CAT CAG TGA TAG CCA TGT GC-3′. PCR conditions used for both genes included an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min.

5.4. RNA procedures

Total cellular RNA was isolated from logarithmic growth phase cells (OD_{600} 0.4) with the RNeasy-RNA Protect® kit (Qiagen, Valencia, CA).

Gene expression was quantified by real-time comparative quantitative RT-PCR with the Quantitect® SYBR® Green one-step RT-PCR kit (Qiagen, Valencia, CA) on the SmartCycler® (Cepheid, Sunnyvale, CA). Final amounts of 250 ng of RNA were converted to cDNA and reactions were normalized with 16SrRNA. Primers used for *bmeB* efflux pump genes have been described previously [22]. Primers used for cholylglycine hydrolase genes were as shown above. Primers used for *ompA* were *ompA*-forward, 5′-GGA TAT GAC GGT GTT GCC AG-3′ and *ompA*-reverse, 5′-TAG CAG CAG CCA TGT CAT TC-3′. Expression was quantified by the $\Delta\Delta C_t$ approximation method [22]. Experiments were repeated five times. Data were analyzed by Student's *t*-test and a value of $P \leqslant 0.05$ was considered significant. A $\geqslant$2-fold difference in expression was considered significant.

Real-time PCR

- Safra tuzları ile önceden muamele edilen suşların;

  fimbria, dış membran proteini (ompA), dış membran vezikülleri ve dışa atım pompa geni ekspresyon düzeylerinin arttığı görülmüştür.

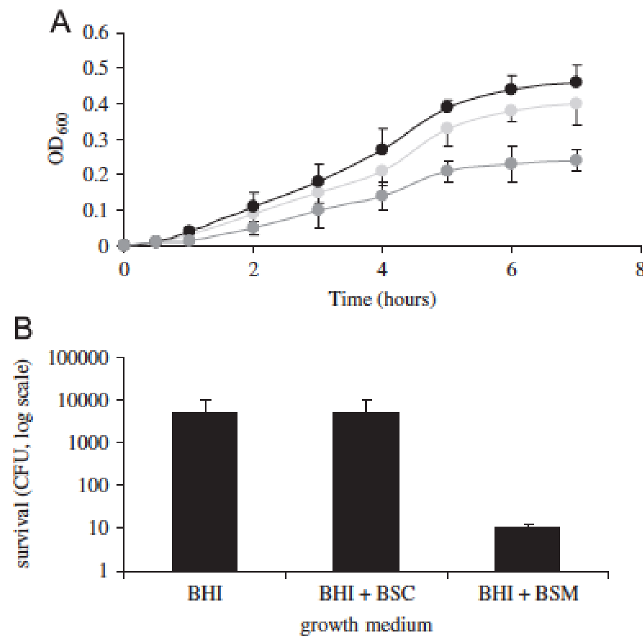


Fig. 1. (A) Growth rates of *B. fragilis* NCTC 9343 with or without 0.15% bile salt treatment. Black is untreated, light gray is treated with 0.15% bile salt conjugate (BSC) and dark gray is treated with 0.15% bile salt mixture (BSM), (B) survival after 12 h with or without exposure to 5% BSC or 5% BSM. An average of five data sets was performed for each experiment.

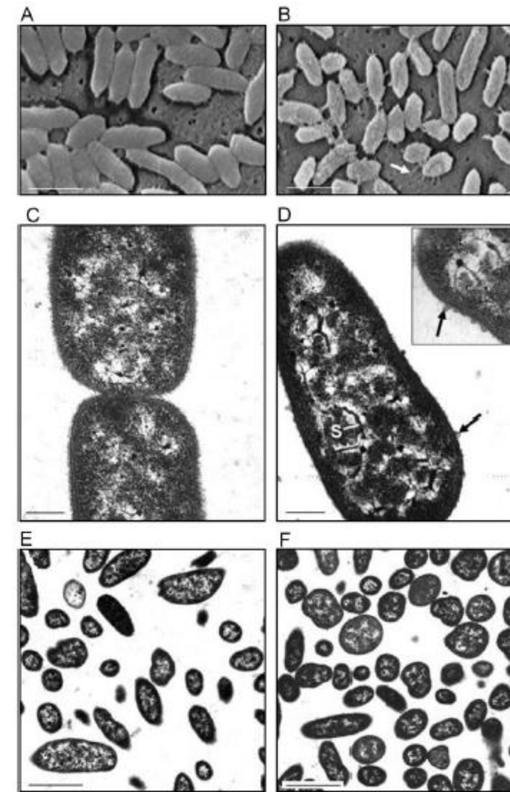


Fig. 2. Morphology of untreated vs. bile salt-treated NCTC9343. (A) SEM of 0.15% bile salt conjugate (BSC)-treated cells. Scale bar = 2.0 μm. (B) SEM of 0.15% bile salt mixture (BSM)-treated cells. The white arrow indicates the fimbria-like appendages on the surface of the 0.15% BSM-treated cells. Scale bar = 2.0 μm. (C, E) TEM of 0.15% BSC-treated cells. Scale bar on C = 0.2 μm, scale bar on E = 1.0 μm. (D, F) TEM of 0.15% BSM-treated cells. The black arrows indicate the circular surface structures (outer membrane vesicles) on 0.15% BSM-treated cells and S indicates the electron dense regions formed by the nucleic acids and ribosomes in the 0.15% BSM-treated cells. Scale bar on *D* = 0.2 μm, scale bar on *F* = 1.0 μm.

Real time-PCR

- Bununla birlikte;

  intestinal epitel hücrelerine adezyonu ve biyofilm üretiminin arttığı tespit edilmiş.

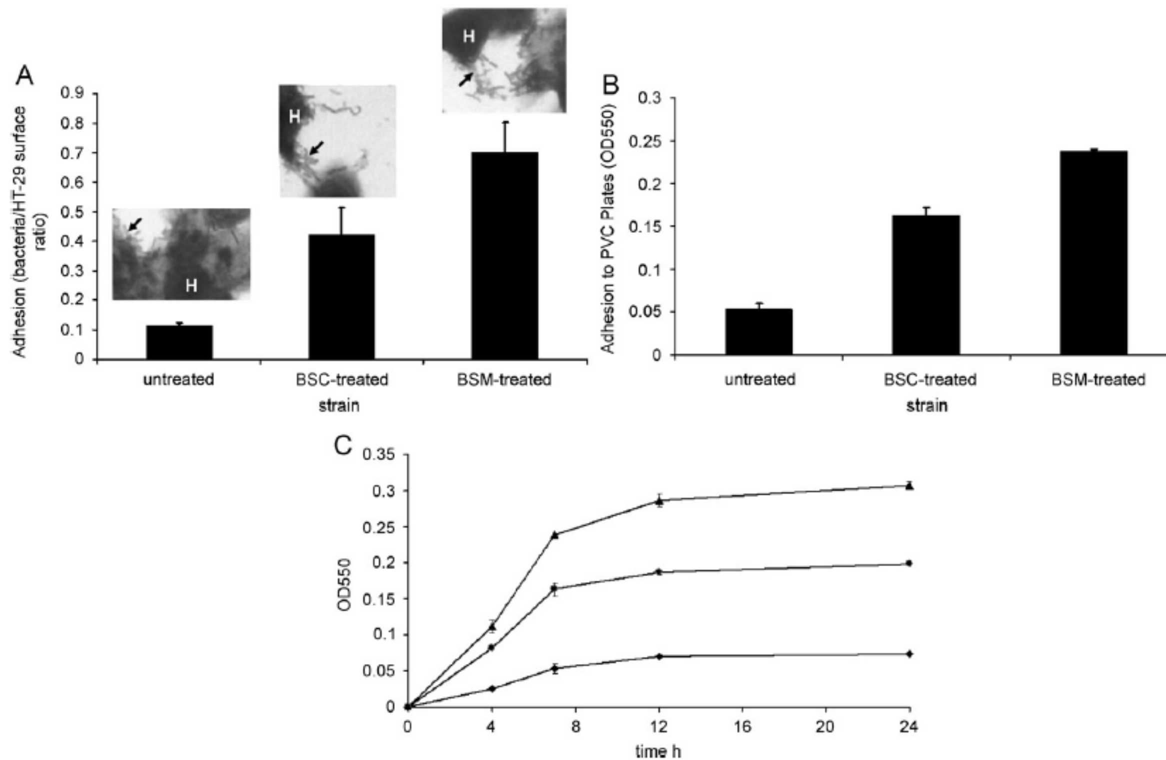
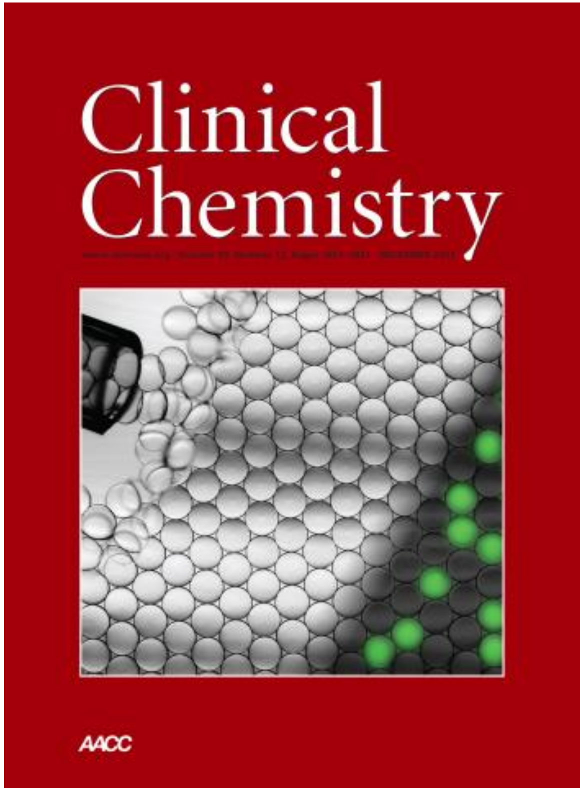


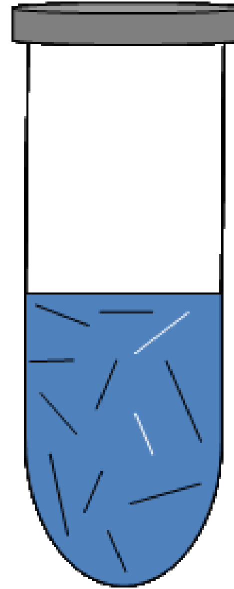
Fig. 3. Bacterial adhesion to HT-29 human intestinal cells (A) and biofilm formation (B). Compared with the untreated, bacteria treated with bile salt conjugate (BSC) or bile salt mixture (BSM) had increased adhesion to HT-29 cells and enhanced biofilm formation. HT-29 adhesion data were represented ratios of the HT-29 cell surface covered by bacteria ± standard deviation. Untreated bacteria adhered in chains whereas BSC or BSM-treated bacteria adhered in clusters (A-inserts). Arrows indicate bacterial cells; H = HT-29 intestinal epithelial cells. Biofilm formation was measured as the mean OD_{550} of bacteria bound to PVC microtitre plates ± standard deviation from five experiments. Biofilm formation kinetics (C) showed that untreated cells formed significantly less biofilms than bile salt-treated cells. Diamonds = untreated; circles = BSC-treated; triangles = BSM-treated. Data are expressed as OD_{550} ± standard deviation. Error bars were small and sometimes not visible.

Dijital PCR

- Başlangıçtaki hedef miktarının hesaplanması için denen yöntemlerden biri de 1990 yıllardan beri araştırılan “limit dilüsyon” ve Poisson dağılım analizidir.

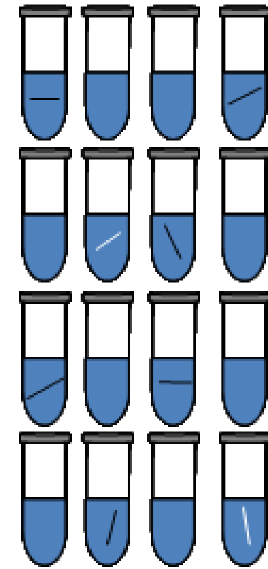


Geleneksel PCR



porsiyonlama

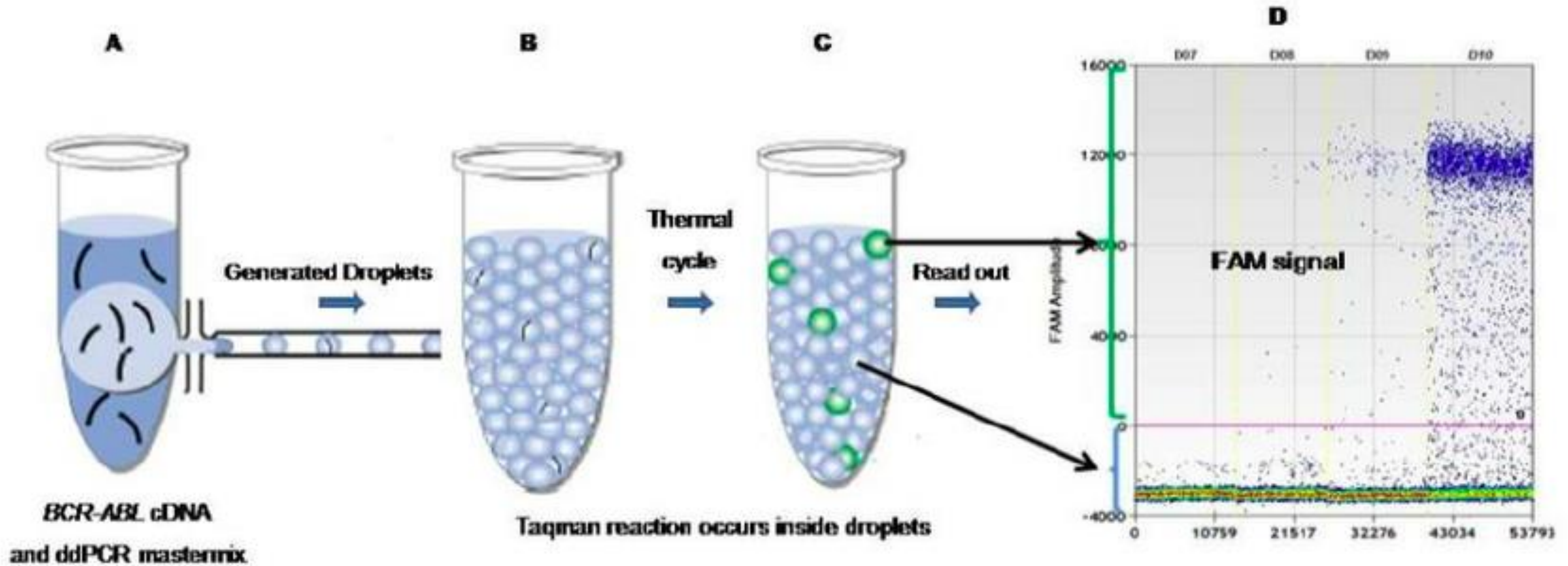
Dijital PCR



Dijital PCR

- Dijital PZT'nin en büyük avantajı "mutlak kantitasyon" için standart eğrilerine ihtiyaç duymaması ve "bağıl kantitasyonu" (gen ekspresyonları için) geleneksel PZT yöntemlerinden daha doğru hesaplamasıdır.

    - Emülsiyon PCR, Damlacık PCR



Dijital PCR

- Stauber Rački ve arkadaşların dPZT kullanarak yaptıkları çalışmada, gastrointestinal sistem enflamasyonu olan çocukların dışkılarında, ince barsak enfeksiyonu ile ilişkili olabilecek 18 farklı transkript düzeyi araştırılmıştır.

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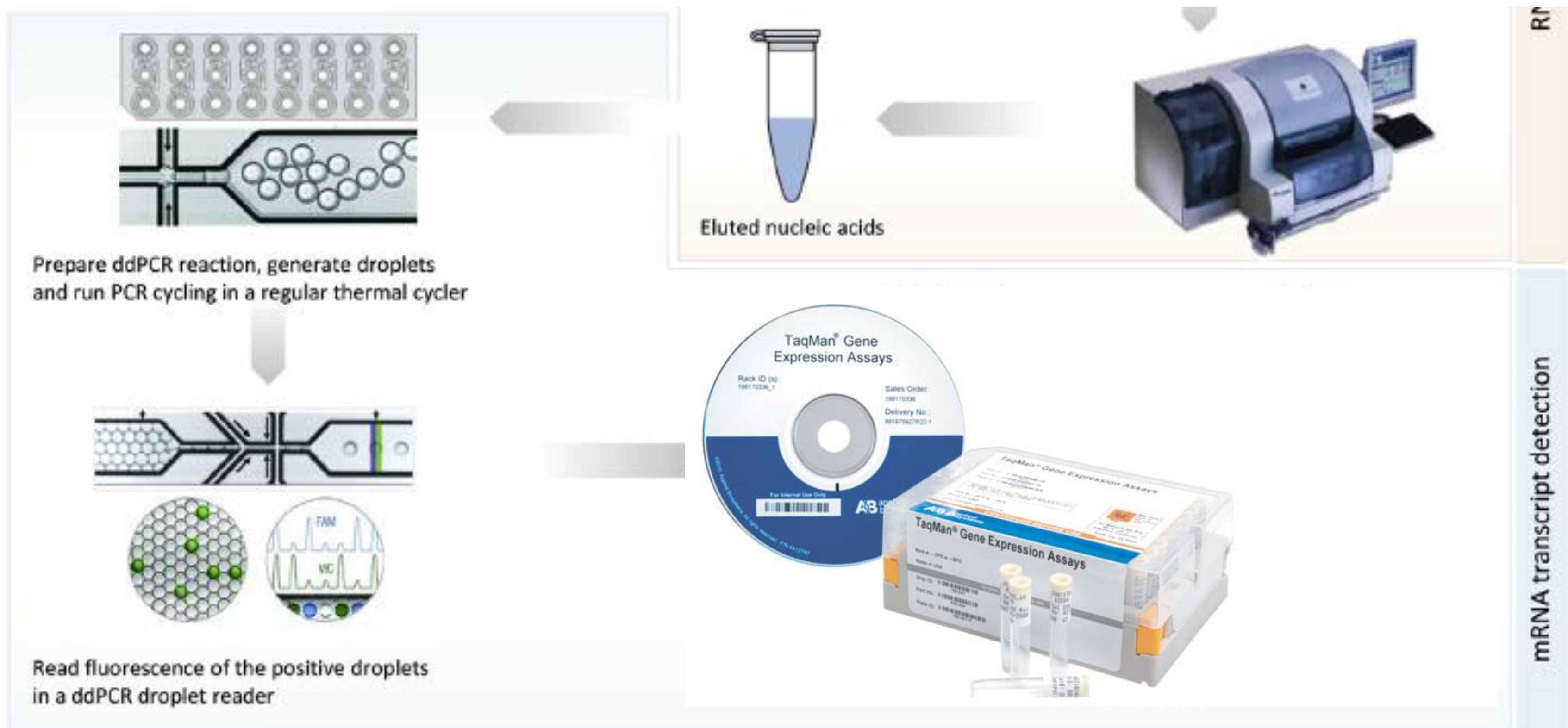


Fig. 1. Methodology used to isolate and detect fecal host transcripts.

Dijital PCR

- Çalışmanın sonucunda;

  konağın enflamatuar transkript miktarlarının tespit edilmesi ile gastrointestinal sistem hastalıklarının tespitinde özgül bir mRNA'nın bir biyobelirteç olarak kullanılabileceği belirtilmektedir.

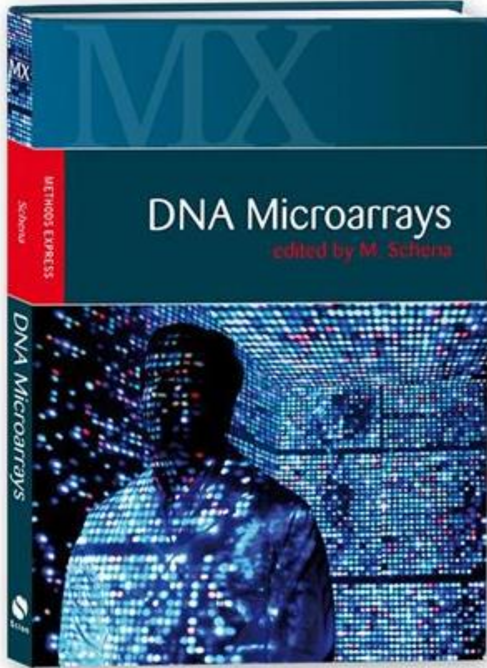
Table 1
Summary of 18 transcripts correlated with L:M.

Gene symbol	Gene function	Transcript Concentration[1] Median (25th, 75th percentiles)	Spearman's r with L:M	*P* value of r
ACP1	Acts on tyrosine phosphorylated proteins, low-MW aryl phosphates and natural and synthetic acyl phosphates. Isoform 3 does not possess phosphatase activity	0.007 (0.005, 0.011)	−0.104	0.043
AQP9	Forms a channel with a broad specificity. Mediates passage of non-charged solutes including carbamides, polyols, purines, and pyrimidines, whereas amino acids, cyclic sugars, and ions are excluded	0.065 (0.019, 0.150)	0.299	0.01
BIRC3	Regulates caspases and apoptosis, modulates inflammatory signaling and immunity, mitogenic kinase signaling and cell proliferation, as well as cell invasion. Acts as an E3 ubiquitin-protein ligase	0.119 (0.075, 0.192)	−0.125	0.005
CD53	Mediates signal transduction promoting cell development. Complexes with integrins. Mutations in this gene result in immunodeficiency	0.071 (0.031, 0.172)	0.316	0.006
CDX1	Caudal type homeobox 1. Plays a role in the terminal differentiation of the intestine	0.026 (0.016, 0.042)	−0.157	<0.001
DECR1	Auxiliary enzyme of beta-oxidation. It participates in the metabolism of unsaturated fatty enoyl-CoA esters. Catalyzes the NADP-dependent reduction of 2,4-dienoyl-CoA to yield trans-3-enoyl-CoA	0.035 (0.022, 0.047)	0.220	0.043
DEFA6	Has antimicrobial activity against Gram-negative and Gram-positive bacteria. Protects cells against infection with HIV-1	0.038 (0.015, 0.096)	0.138	0.009
HLADRA	Binds peptides derived from antigens that access the endocytic route of antigen presenting cells and presents them on the cell surface for recognition by the CD4 T-cells	0.176 (0.103, 0.279)	−0.136	0.002
IFI30	Lysosomal thiol reductase that reduces protein disulfide bonds. Facilitates the complete unfolding of proteins destined for lysosomal degradation. Plays an important role in antigen processing	0.168 (0.081, 0.288)	0.264	0.024
LYZ	Lysozyme has primarily a bacteriolytic function; those in tissues and body fluids are associated with the monocyte-macrophage system and enhance the activity of immunoagents	0.083 (0.040, 0.137)	0.297	0.011
MUC12	Mucin 12. Codes for key protein in mucous layer. Involved in epithelial cell protection, adhesion modulation, signaling and epithelial cell growth regulation. Stimulated by inflammatory cytokines	0.321 (0.162, 0.541)	−0.271	0.021
PIK3AP1	Signaling adapter that contributes to B-cell development. Links Toll-like receptor signaling to PI3K activation, preventing excessive inflammatory cytokine production. Activates natural killer cells	0.048 (0.022, 0.121)	0.249	0.029
REG1A	Acts as an inhibitor of spontaneous calcium carbonate precipitation. Associated with intestinal, brain and pancreas regeneration	0.040 (0.018, 0.103)	0.188	<0.001
REG3A	Bactericidal C-type lectin which acts exclusively against Gram-positive bacteria and mediates bacterial killing by binding to surface-exposed carbohydrate moieties of peptidoglycan	0.049 (0.019, 0.086)	0.262	0.011
S100A8	Calprotectin, a calcium- and zinc-binding protein which plays a prominent role in the regulation of inflammatory processes and immune response. Induces neutrophil chemotaxis and adhesion	0.386 (0.154, 1.169)	0.096	0.025
SELL	Cell surface adhesion protein. Promotes initial tethering and rolling of leukocytes in endothelia	0.009 (0.003, 0.038)	0.295	0.008
SI	Sucrase isomaltase. A disaccharidase that plays an important role in carbohydrate digestion. Isomaltase activity is specific for α-1,4- and α-1,6-oligosaccharides	0.017 (0.008, 0.036)	−0.100	0.006
TNF	Cytokine that binds to TNFRSF1A/TNFR1. Secreted by macrophages, potent pyrogen, promotes cell death. Induces IL-12 production in dendritic cells	0.004 (0.002, 0.008)	−0.153	<0.001

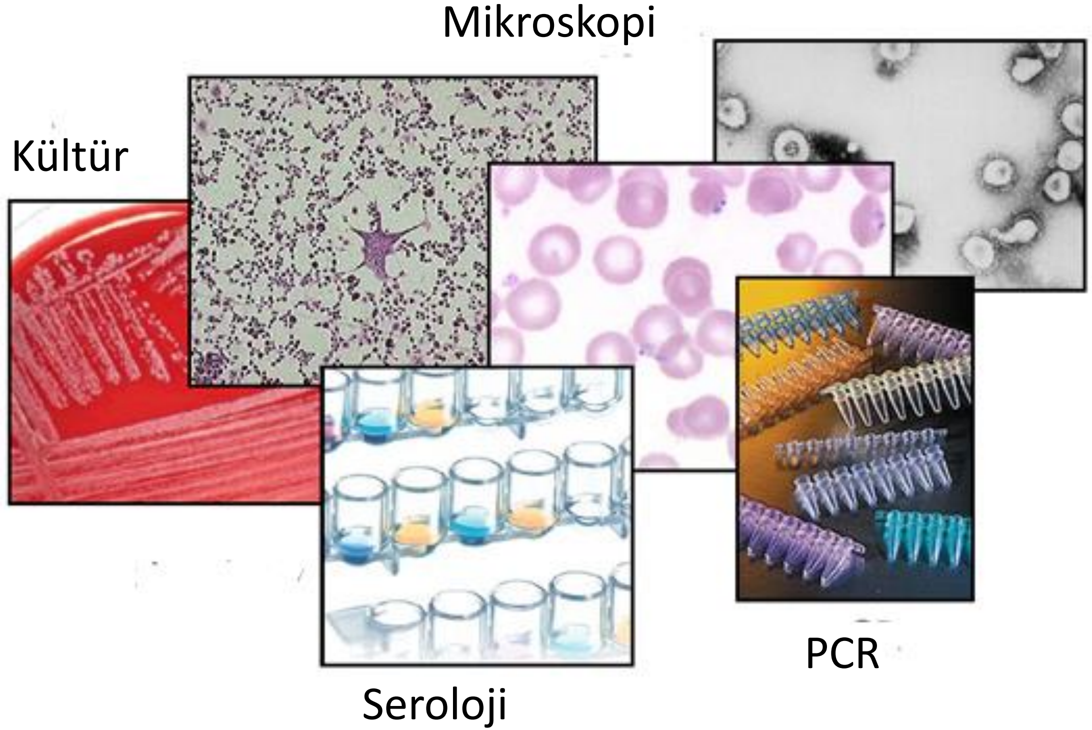
[1] Expressed as copies/copy GAPDH.

Microarray temelli yöntemler

- Konak-patojen etkileşimlerinin neden olduğu gen ekspresyon profillerinin belirlenmesinde en sık kullanılan yöntemlerden biridir.

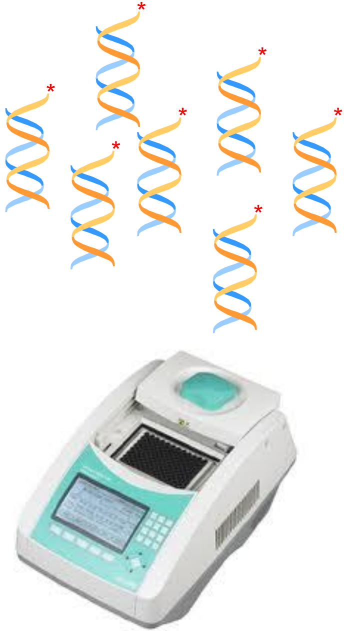


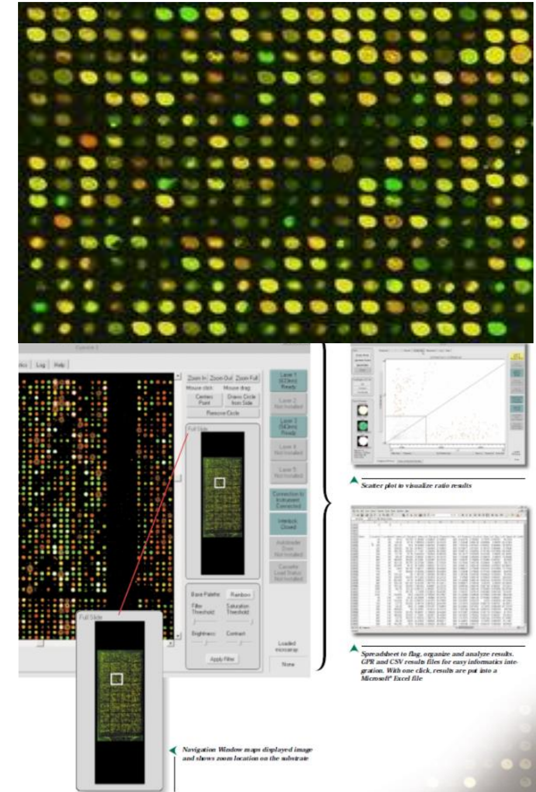
Geleneksel Mikrobiyolojik Tanı Yöntemleri



Microarray temelli yöntemler

- PCR ile elde işaretlenmiş cDNA/cRNA amplikonları çok sayıda farklı oligonükleotid prob içeren katı yüzeylerde, kendisine uyan probla hibridize olması temeline dayanmaktadır.





Microarray temelli yöntemler

- Hartmann ve arkadaşlarının yaptığı çalışmada, 1918 ve 2009 pandemilerine neden olan influenza virüsleri iki mevsimsel virüs suşuna karşı insan dentritik hücrelerinin gösterdiği transkripsiyonel yanıt incelenmiştir.

Human Dendritic Cell Response Signatures Distinguish 1918, Pandemic, and Seasonal H1N1 Influenza Viruses

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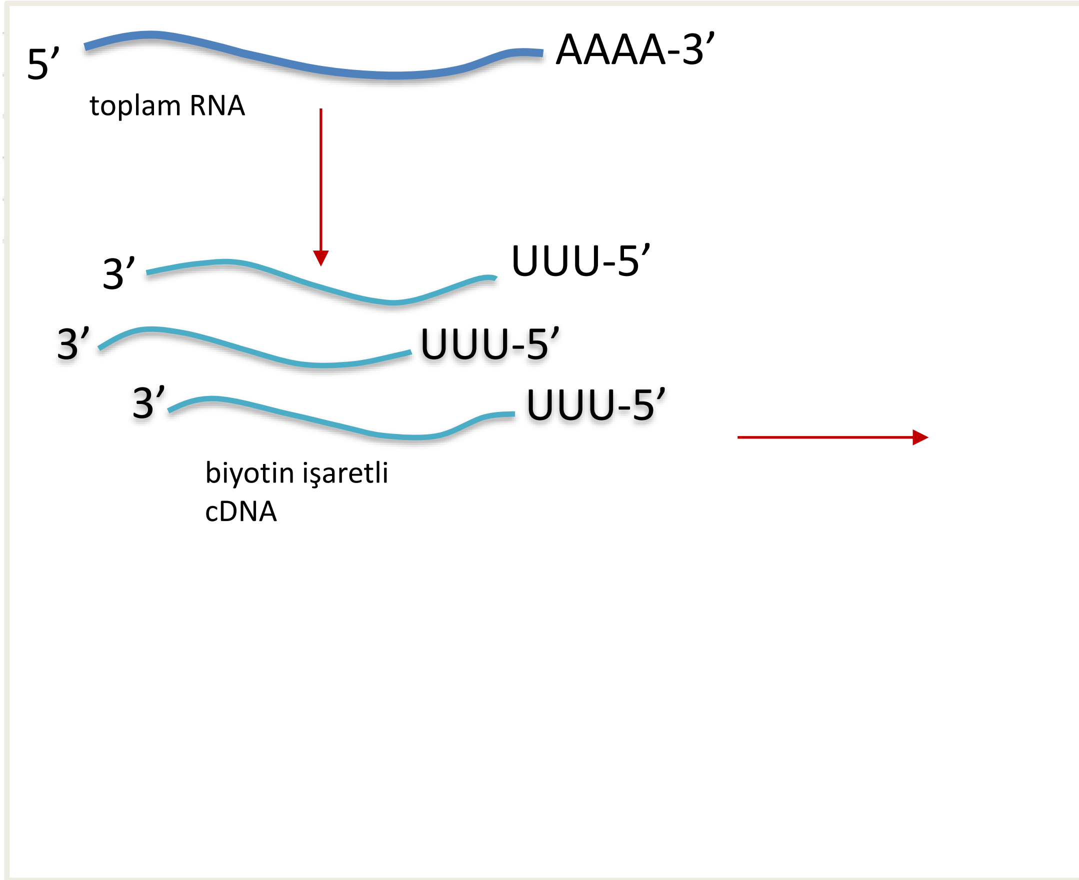
Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, New York, USA[a]; Center for Translational Systems Biology, Icahn School of Medicine at Mount Sinai, New York, New York, USA[b]; Department of Pathology, Yale School of Medicine, New Haven, Connecticut, USA[c]; Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York, USA[d]; Interdepartmental Program in Computational Biology and Bioinformatics, Yale University, New Haven, Connecticut, USA[e]; Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland[f]; Global Health & Emerging Pathogens Institute at Icahn School of Medicine, New York, New York, USA[g]; Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA[h]

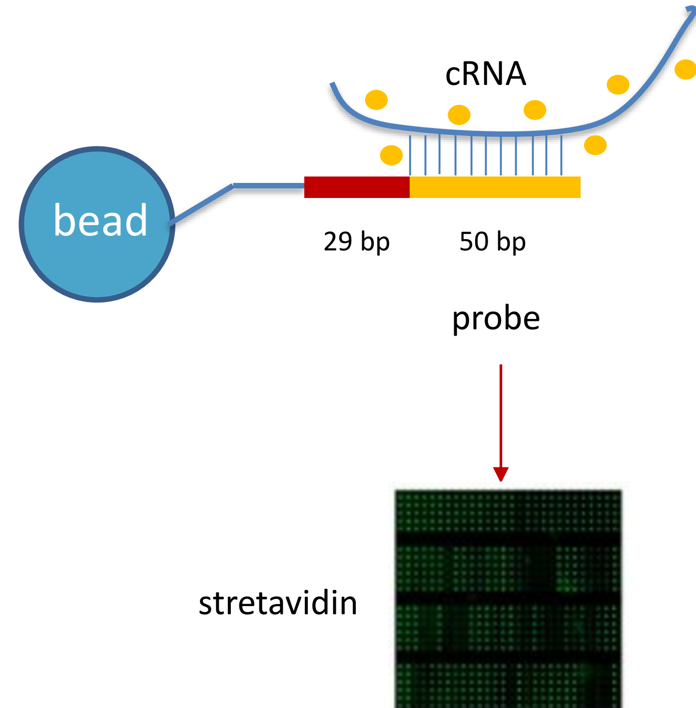
ABSTRACT

Influenza viruses continue to present global threats to human health. Antigenic drift and shift, genetic reassortment, and cross-species transmission generate new strains with differences in epidemiology and clinical severity. We compared the temporal transcriptional responses of human dendritic cells (DC) to infection with two pandemic (A/Brevig Mission/1/1918, A/California/4/2009) and two seasonal (A/New Caledonia/20/1999, A/Texas/36/1991) H1N1 influenza viruses. Strain-specific response differences included stronger activation of NF-κB following infection with A/New Caledonia/20/1999 and a unique cluster of genes expressed following infection with A/Brevig Mission/1/1918. A common antiviral program showing strain-specific timing was identified in the early DC response and found to correspond with reported transcript changes in blood during symptomatic human influenza virus infection. Comparison of the global responses to the seasonal and pandemic strains showed that a dramatic divergence occurred after 4 h, with only the seasonal strains inducing widespread mRNA loss.

Microarray temelli yöntemler

- Bu çalışmada Human HT-12 v4 Expression BeadChip (Illumina, San Diego, CA)





Microarray temelli yöntemler

- Dört influenza suşu içinde 58 gen ekspresyonu ortak.

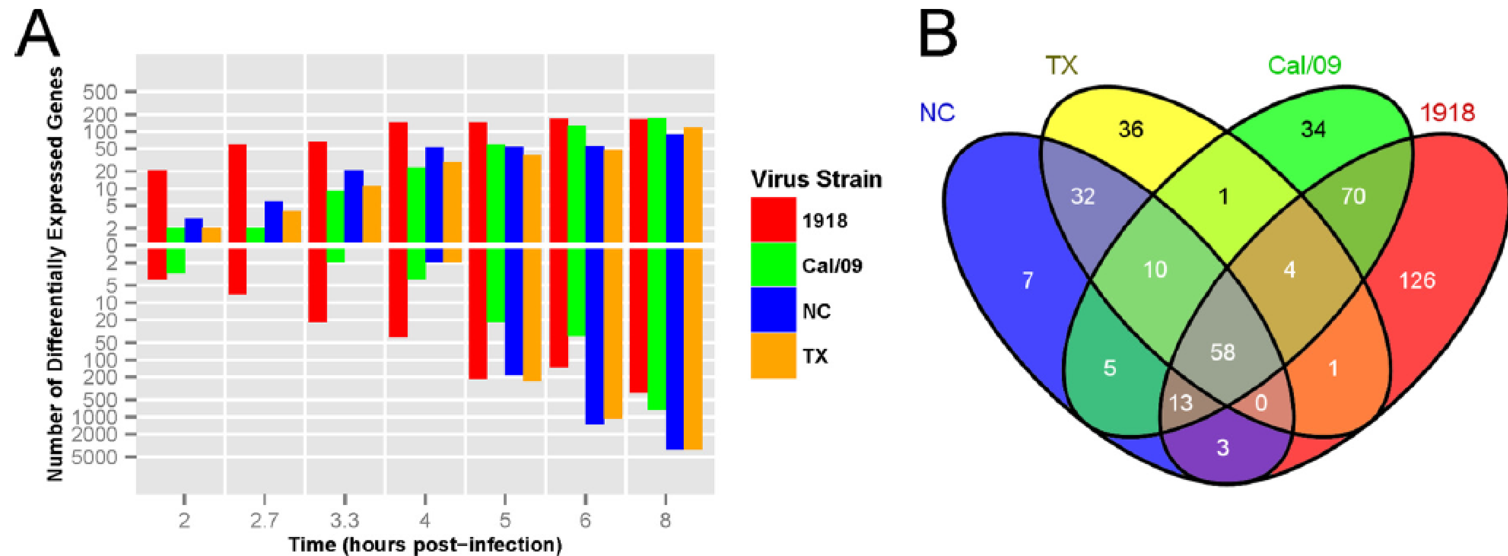


FIG 2 The transcriptional response to infection with seasonal and pandemic influenza strains share a core. Differentially expressed genes were identified by comparing their levels of expression at each time point with those of the time-matched control genes (absolute fold change, ≥2; FDR < 0.05). The numbers of up- or downregulated genes in DC infected with the 1918 (red), Cal/09 (green), NC (blue), and TX (orange) strains at each time point are indicated. (B) A common core of 58 genes was identified by analyzing the overlap among genes that were upregulated at any time point for each strain (a complete list of genes is provided in Table SA2 in the supplemental material).

Microarray temelli yöntemler

- Çalışmanın en çarpıcı ve şaşırtıcı bulgularından biri pandemik ve mevsimsel virüsler tarafından oluşturulan enfeksiyon sırasında, mevsimsel virüs suşları tarafından gelişen enfeksiyonda yaygın mRNA kaybının gösterilmiş olmasıdır.

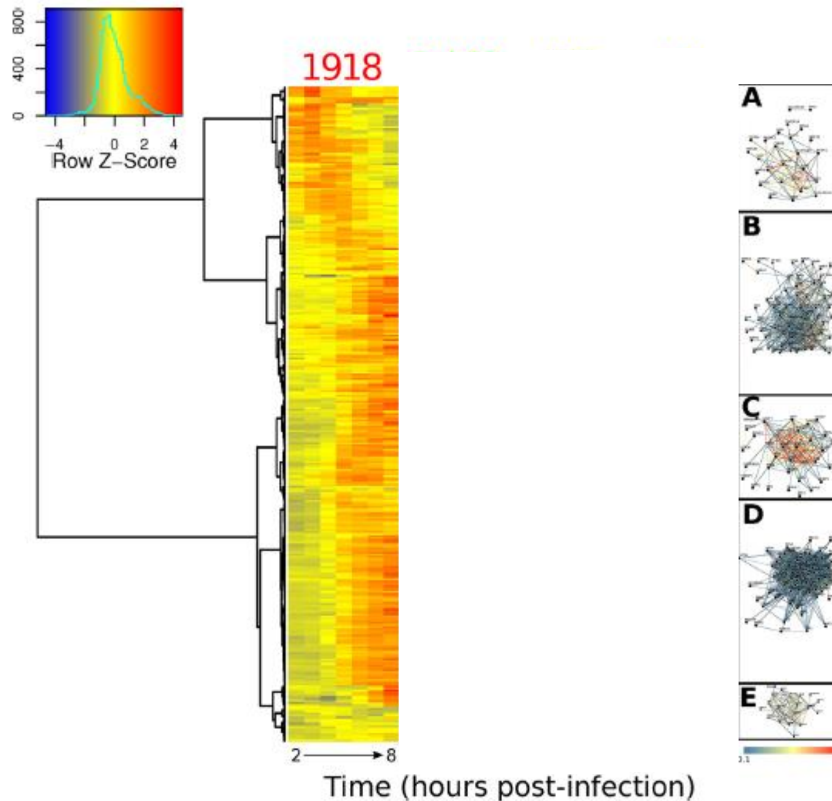


FIG 3 Temporal clustering identifies five distinct gene expression patterns. Hierarchical clustering of the expression profiles for upregulated genes (fold change, ≥ 2; FDR < 0.05 with respect to the time-matched controls) identified five groups (A to E). Temporal profiles for individual genes are depicted in the heatmap by row-normalized fold changes from profiles after allantoic fluid stimulation, while the rightmost set of plots shows the average activity of each cluster, estimated by QuSAGE, following infection by pandemic strains (solid lines indicate 1918 [red] and Cal/09 [green]) and seasonal strains (dashed lines indicate NC [blue] and TX [orange]). Immunospecific functional interactions between the genes in each cluster were predicted using ImmuNet (networks adjacent to the heatmap). See http://tsb.mssm.edu/primeportal/?q=Immunet_interactive_networks.

Microarray temelli yöntemler

Cluster E

Symbol	defense response	regulation of transcription	response to virus	immune response	Gene Name
IL29	■	■	■	■	interleukin 29 (interferon, lambda 1)
CXCL11	■			■	chemokine (C-X-C motif) ligand 11
TNFAIP6	■				tumor necrosis factor, alpha-induced protein 6
IL6	■	■	■	■	interleukin 6 (interferon, beta 2)
TNF	■	■	■	■	tumor necrosis factor (TNF superfamily, member 2)
NFKBIZ	■	■			nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
CXCL9	■			■	chemokine (C-X-C motif) ligand 9
TRIM22		■	■	■	tripartite motif-containing 22
ASCL2		■			achaete-scute complex homolog 2 (Drosophila)
BATF3		■			basic leucine zipper transcription factor, ATF-like 3
CCL5	■		■	■	chemokine (C-C motif) ligand 5
IFNB1	■	■	■		interferon, beta 1, fibroblast
IL28A			■		interleukin 28A (interferon, lambda 2)

Microarray temelli yöntemler

- Sitkiewicz ve arkadaşları *Streptococcus agalactiae*'nın (grup B streptekok, GBS) insan amniyotik sıvısına gösterdiği adaptasyon sonucu değişen transkriptomu incelenmiştir.

OPEN ACCESS Freely available online

Transcriptome Adaptation of Group B *Streptococcus* to Growth in Human Amniotic Fluid

Izabela Sitkiewicz[1¤], Nicole M. Green[1], Nina Guo[1], Ann Marie Bongiovanni[2], Steven S. Witkin[2], James M. Musser[1,2*]

1 Center for Molecular and Translational Human Infectious Diseases Research, The Methodist Hospital Research Institute, and Department of Pathology, The Methodist Hospital, Houston, Texas, United States of America, 2 Weill Medical College of Cornell University, New York, New York, United States of America

Abstract

Background: *Streptococcus agalactiae* (group B *Streptococcus*) is a bacterial pathogen that causes severe intrauterine infections leading to fetal morbidity and mortality. The pathogenesis of GBS infection in this environment is poorly understood, in part because we lack a detailed understanding of the adaptation of this pathogen to growth in amniotic fluid. To address this knowledge deficit, we characterized the transcriptome of GBS grown in human amniotic fluid (AF) and compared it with the transcriptome in rich laboratory medium.

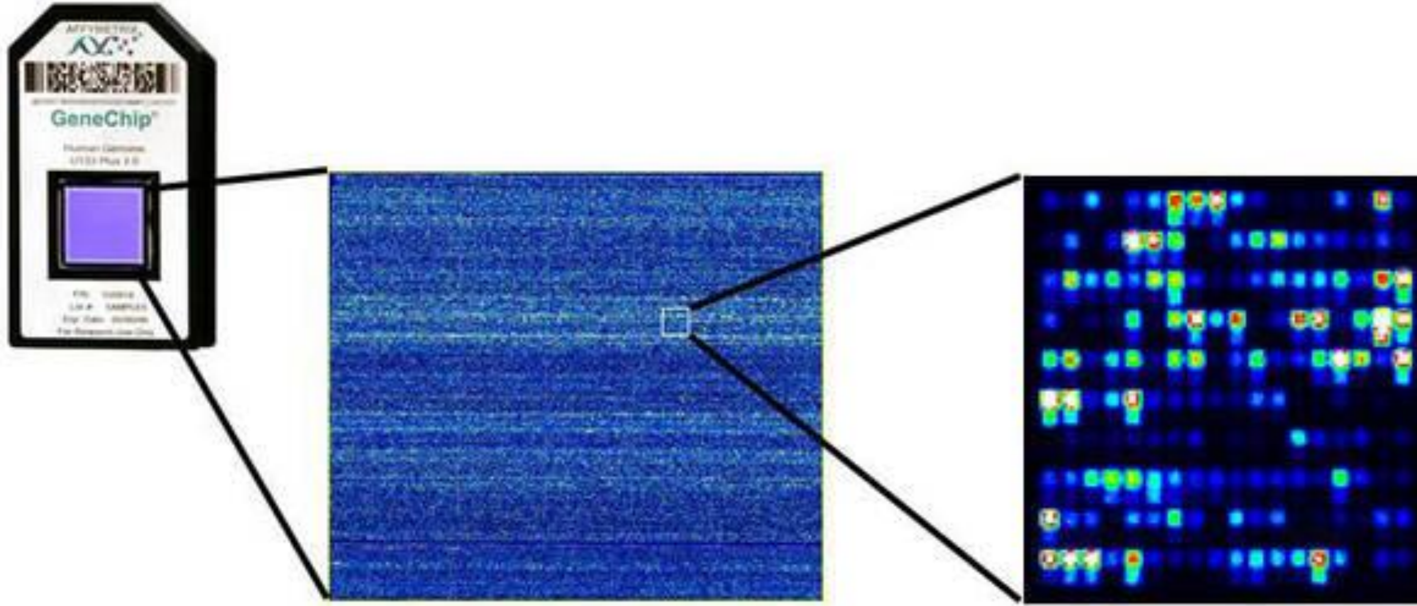
Methods: GBS was grown in Todd Hewitt-yeast extract medium and human AF. Bacteria were collected at mid-logarithmic, late-logarithmic and stationary growth phase. We performed global expression microarray analysis using a custom-made Affymetrix GeneChip. The normalized hybridization values derived from three biological replicates at each growth point were obtained. AF/THY transcript ratios representing greater than a 2-fold change and P-value exceeding 0.05 were considered to be statistically significant.

Principal Findings: We have discovered that GBS significantly remodels its transcriptome in response to exposure to human amniotic fluid. GBS grew rapidly in human AF and did not exhibit a global stress response. The majority of changes in GBS transcripts in AF compared to THY medium were related to genes mediating metabolism of amino acids, carbohydrates, and nucleotides. The majority of the observed changes in transcripts affects genes involved in basic bacterial metabolism and is connected to AF composition and nutritional requirements of the bacterium. Importantly, the response to growth in human AF included significant changes in transcripts of multiple virulence genes such as adhesins, capsule, and hemolysin and IL-8 proteinase what might have consequences for the outcome of host-pathogen interactions.

Conclusions/Significance: Our work provides extensive new information about how the transcriptome of GBS responds to growth in AF, and thus new leads for pathogenesis research.

Microarray temelli yöntemler

- Bu çalışmada transkriptomik inceleme için *Affymetrix chip* (Affymetrix, Santa Clara, CA, USA) kullanılmış. 1.994 probe özel olarak eklenmiş.



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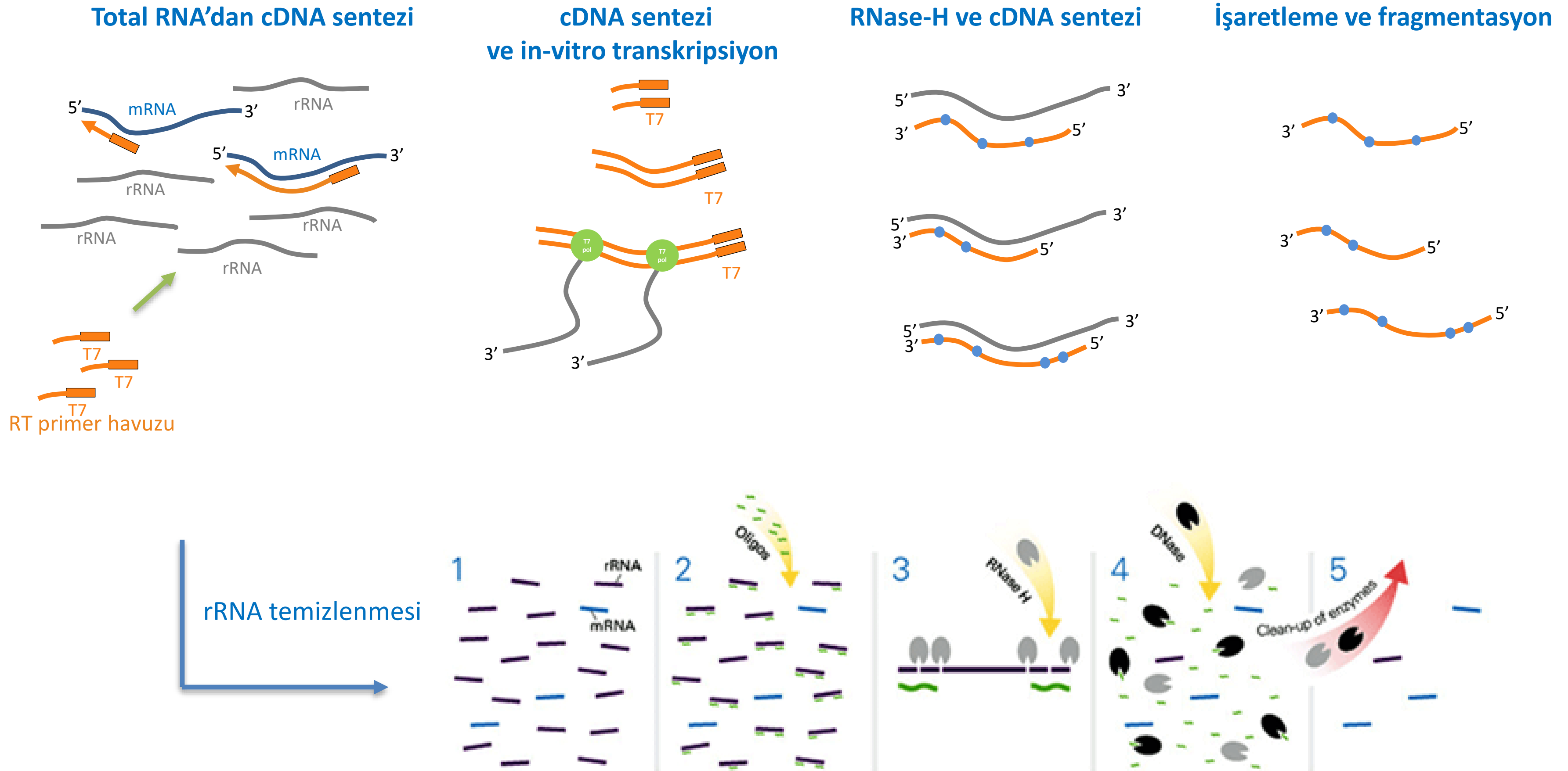

https://www.westburg.eu

Microarray temelli yöntemler

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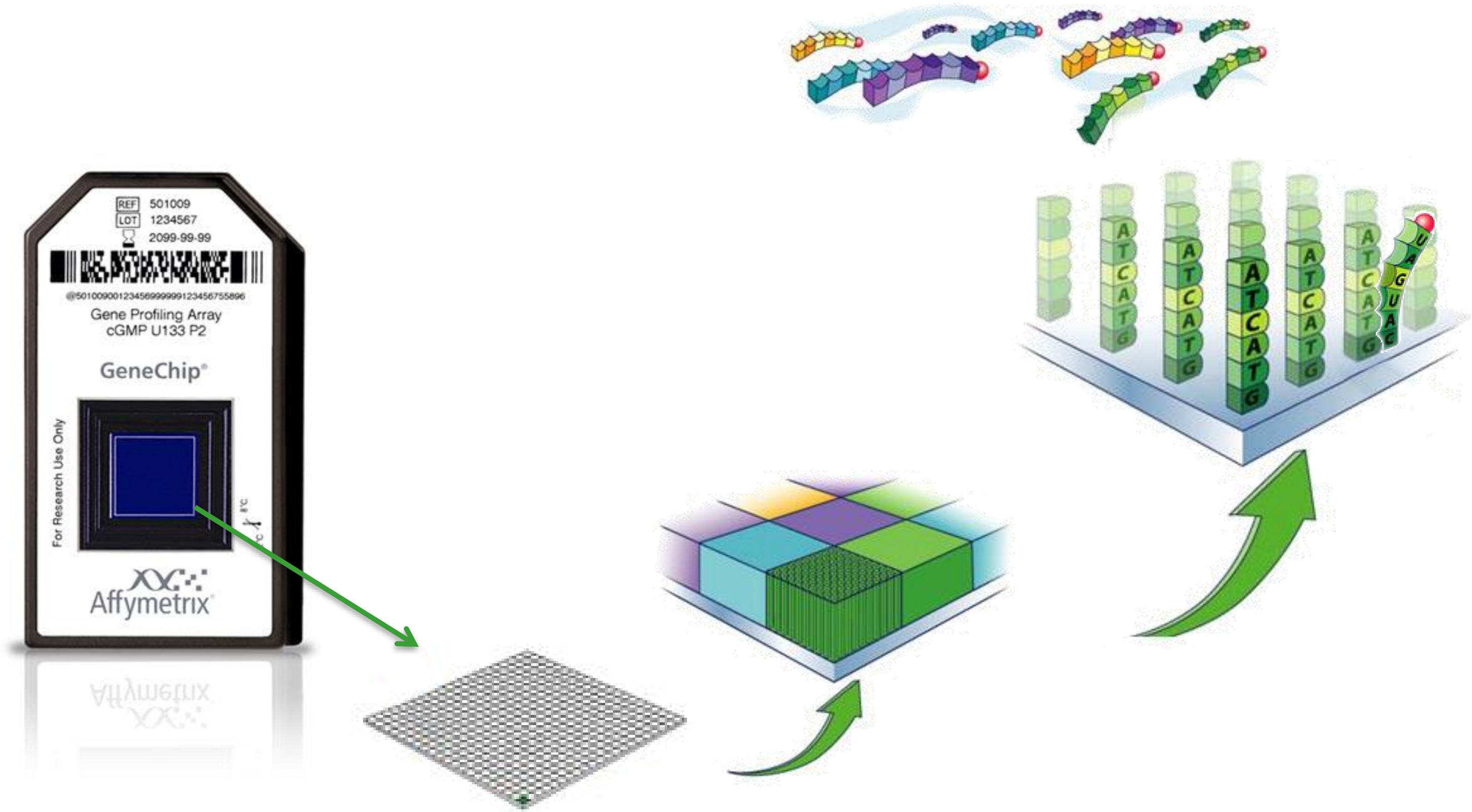


Microarray temelli yöntemler

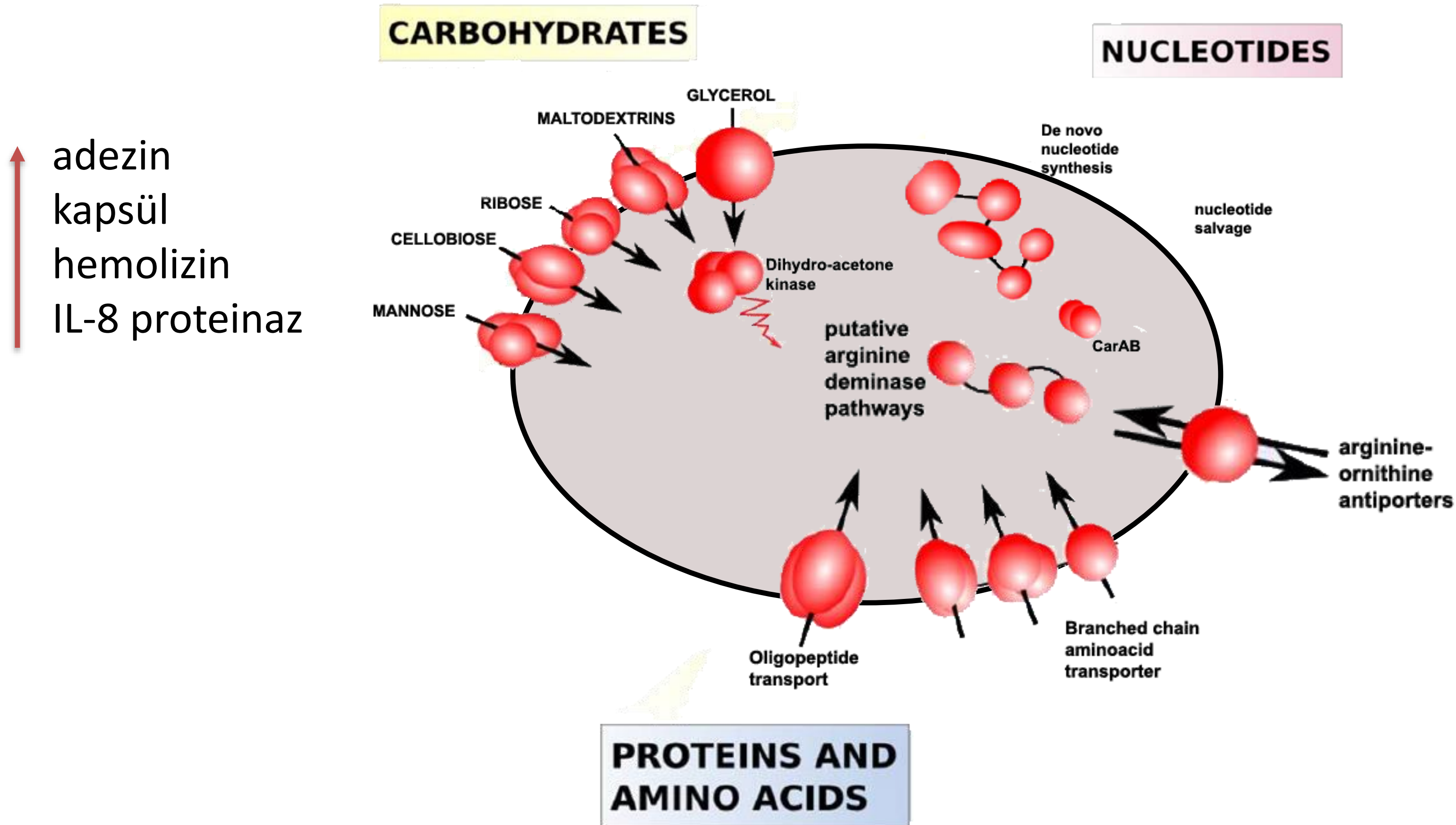
- GBS'ların amniyotik sıvıda herhangi bir stres yanıtı vermeden hızla üredikleri gözlendi.
- *Todd Hewitt-yeast extract* sıvı besiyerinde üreyen bakteriler ile amniyotik sıvıda üretilen bakteriler karşılaştırıldığında;
  - adezin
  - kapsül
  - hemolizin
  - IL-8 proteinaz gibi birçok virülans genin transkripsiyonundaki belirgin değişiklikler gerçekleşmiş.

Microarray temelli yöntemler

- Transkriptomda gözlenen değişikliklerin çoğu;

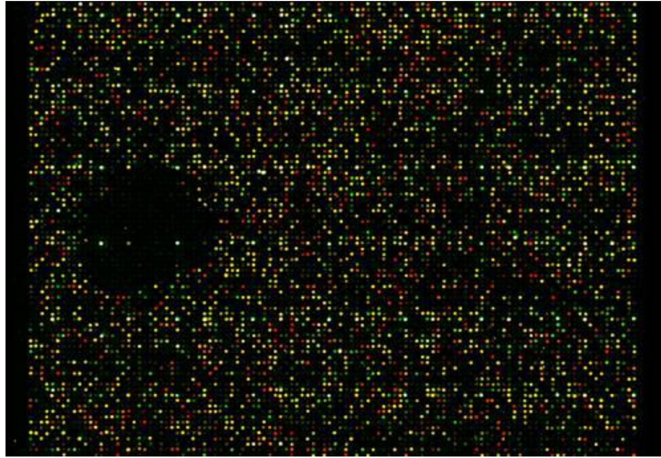
  amniyotik sıvı içeriği ve beslenme gereksinimlerine bağlı olarak temel bakteriyel metabolizmayla ilişkilidir.

↑ adezin
kapsül
hemolizin
IL-8 proteinaz



Microarray temelli yöntemler

- Mikrodizin sistemleri;
  - saptama kapasitesinin belirlenen hedeflerle sınırlı olması (prob bağımlı sistemler)
  - yüksek arka-plan gürültüsü ve çapraz-bağlanma gibi bazı dezavantajlara sahiptir.



Dizi analizi temelli yöntemler

- Nükleik asit dizi analizi yöntemlerinin keşfi,

  biyolojinin her alanında olduğu gibi konak-patojen etkileşimlerinin incelenmesi açısından

  da oldukça önemli sonuçları olmuştur.

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

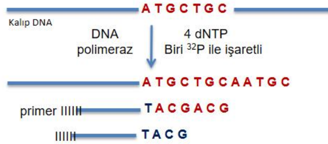
A Rapid Method for Determining Sequences in DNA by Primed Synthesis with DNA Polymerase

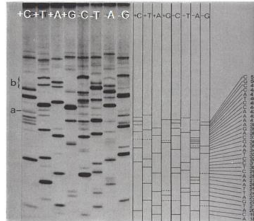
F. Sanger and A. R. Coulson

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.





Tüm Genom Dizileme

Nature Vol. 265 February 24 1977

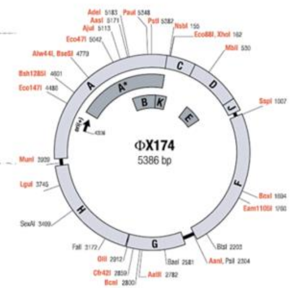
articles

Nucleotide sequence of bacteriophage ΦX174 DNA

F. Sanger, G. M. Air[1], B. G. Barrell, N. L. Brown[1], A. R. Coulson, J. C. Fiddes, C. A. Hutchison III[2], P. M. Slocombe[3] & M. Smith[1]

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.



Dizi analizi temelli yöntemler

- Nükleik asit dizi analizi yöntemlerinin keşfi,

  biyolojinin her alanında olduğu gibi konak-patojen etkileşimlerinin incelenmesi açısından

  da oldukça önemli sonuçları olmuştur.

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.

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

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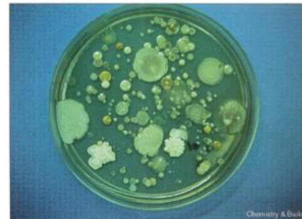
A new frontier of science is emerging that unites biology and chemistry — the exploration of natural products from previously uncultured soil microorganisms. The approach involves directly accessing the genomes of soil organisms that cannot be, or have not been, cultured by isolating their DNA, cloning 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

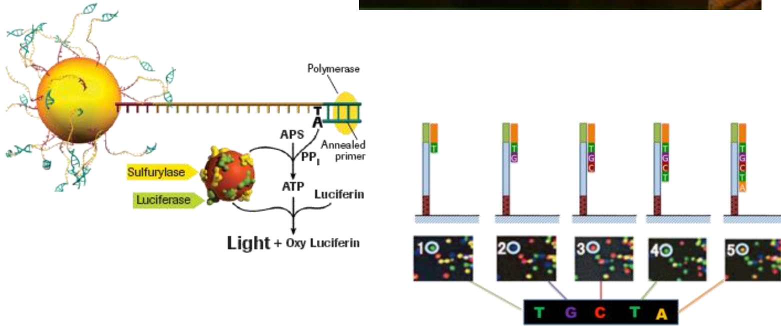


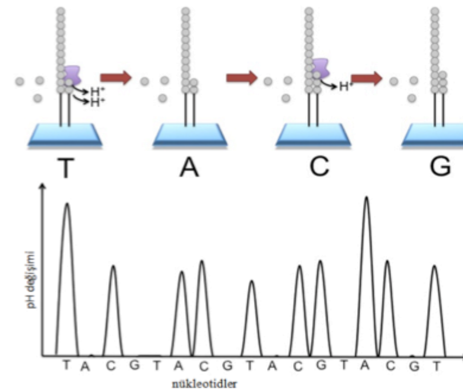
Morphological diversity typical of microorganisms cultured from soil on a broad spectrum medium, tryptic soy agar.

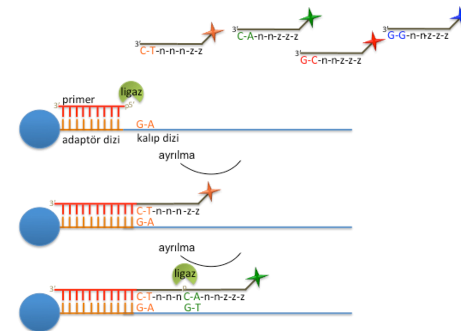
Dizi analizi temelli yöntemler

Yeni Nesil Dizileme Sistemleri

- Düşük masraflı ve daha uzun DNA dizilemesine olan ihtiyaç "yüksek hacimli dizileme" (massive parallel sequencing) teknolojilerinin geliştirilmesine yol açmıştır.



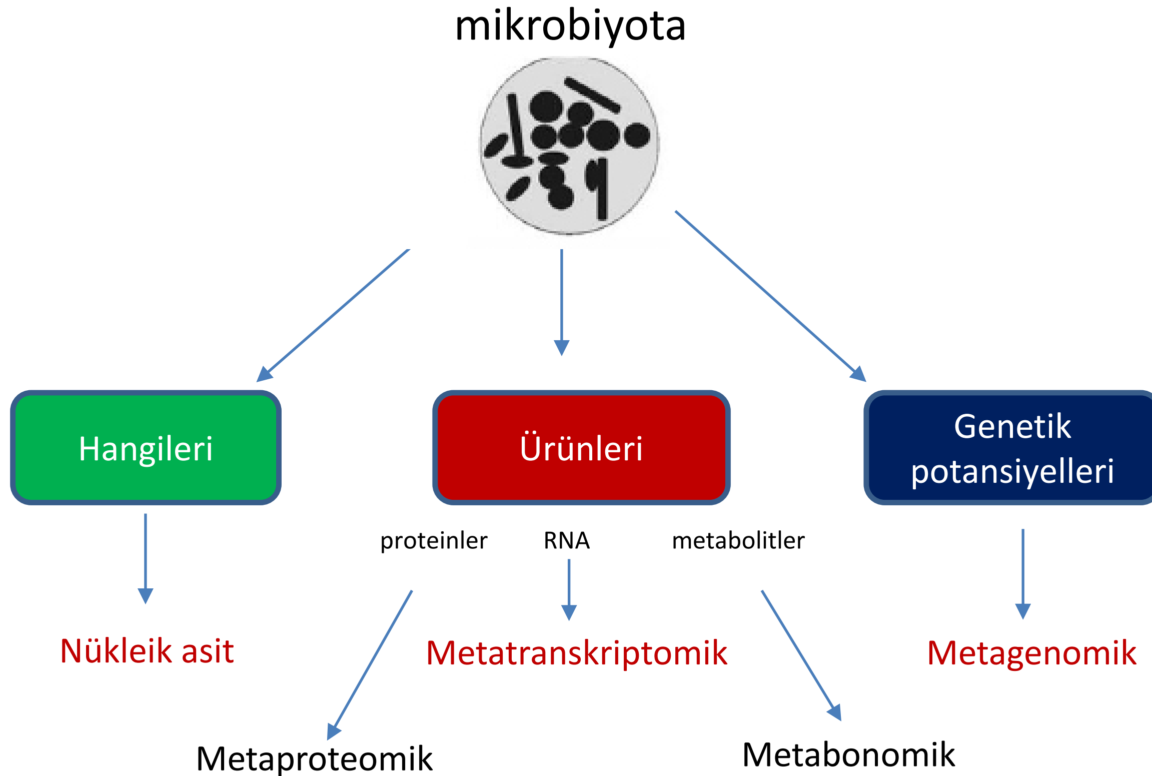




Dizi analizi temelli yöntemler

Yeni Nesil Dizileme Sistemleri

- Yeni nesil dizileme yöntemlerinin keşfi metagenomik ve metatranskriptomik alanlarındaki çalışmalarda büyük bir ivme yaratmıştır.



Reproduced from Gut, Zoetendal EG, M Rajilic-Stojanovic & WM de Vos. Vol 57, issue 11, pages 1605-15, 2008.

Dizi analizi temelli yöntemler

- Yöntemin bir diğer avantajı da;
  - mRNA, ncRNA, (non-coding RNA) ayrımını yapabilmesi
  - RNA uç birleşme bölgelerinin (splice junction) tespit edilebilmesidir.

Advancing RNA-Seq analysis

Brian J Haas & Michael C Zody

New methods for analyzing RNA-Seq data enable *de novo* reconstruction of the transcriptome.

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npg

Sequencing of RNA has long been recognized as an efficient method for gene discovery[1] and remains the gold standard for annotation of both coding and noncoding genes[2]. Compared with earlier methods, massively parallel sequencing of RNA (RNA-Seq)[3] has vastly increased the throughput of RNA sequencing and allowed global measurement of transcript abundance. Two reports in this issue introduce approaches for RNA-Seq analysis that capture genome-wide transcription and splicing in unprecedented detail. Trapnell *et al.*[4] describe a software package, Cufflinks, for simultaneous discovery of transcripts and quantification of expression levels and apply it to study gene expression and splicing during the differentiation of mouse myoblast cells. Taking a similar approach, Guttman *et al.*[5] use software called Scripture to reannotate the transcriptomes of three mouse cell lines, defining complete gene models for hundreds of new large intergenic noncoding RNAs (lincRNAs)[6].

Although transcript sequencing has been possible for nearly 20 years, until recently it required the construction of clone libraries. Projects to determine full-length gene structures for human, mouse and other important models have taken years to complete[7]. With new sequencing technologies, no cloning is needed, allowing direct sequencing of cDNA fragments. In a matter of days and at a small fraction of the cost of earlier projects, one can achieve reasonably complete coverage of a transcriptome[8]. But this approach has been hindered by a substantial challenge:

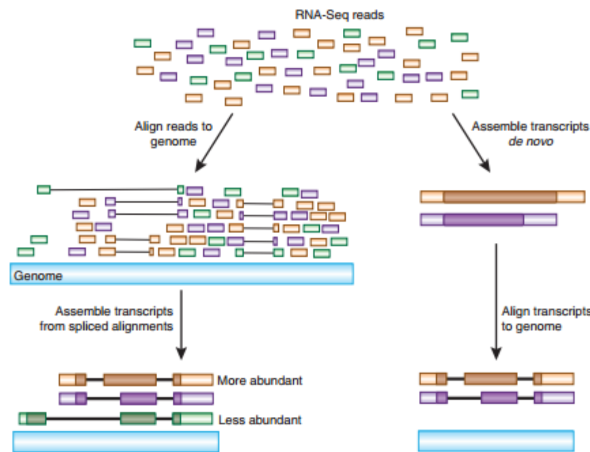


Figure 1 Strategies for reconstructing transcripts from RNA-Seq reads. The 'align-then-assemble' approach (left) taken by Trapnell *et al.*[4] and Guttman *et al.*[5] first aligns short RNA-Seq reads to the genome, accounting for possible splicing events, and then reconstructs transcripts from the spliced alignments. The 'assemble-then-align' approach (right) first assembles transcript sequences *de novo*—that is, directly from the RNA-Seq reads. These transcripts are then splice-aligned to the genome to delineate intron and exon structures and variations between alternatively spliced transcripts. As *de novo* assembly is likely to work only for the most abundant transcripts, the align-then-assemble method should be more sensitive, although this warrants further investigation. RNA-Seq reads are colored according to the transcript isoform from which they were derived. Protein-coding regions of reconstructed transcript isoforms are depicted in dark colors.

Dizi analizi temelli yöntemler

Yeni Nesil Dizileme Sistemleri

- Yeni nesil dizilmenin yığın verileri için geliştirilen biyoinformatik araçlar;

SOFTWARE Open Access

Taxonomer: an interactive metagenomics analysis portal for universal pathogen detection and host mRNA expression profiling

Steven Flygare[1†], Keith Simmon[2†], Chase Miller[1], Yi Qiao[1], Brett Kennedy[1], Tonya Di Sera[1], Erin H. Graf[3], Keith D. Tardif[4], Aurélie Kapusta[1], Shawn Rynearson[1], Chris Stockmann[5], Krista Queen[6], Suxiang Tong[6], Karl V. Voelkerding[3,4], Anne Blaschke[5], Carrie L. Byington[5], Seema Jain[6], Andrew Pavia[5], Krow Ampofo[5], Karen Eilbeck[2,7], Gabor Marth[1,7], Mark Yandell[1,7*] and Robert Schlaberg[3,4*]

Abstract

Background: High-throughput sequencing enables unbiased profiling of microbial communities, universal pathogen detection, and host response to infectious diseases. However, computation times and algorithmic inaccuracies have hindered adoption.

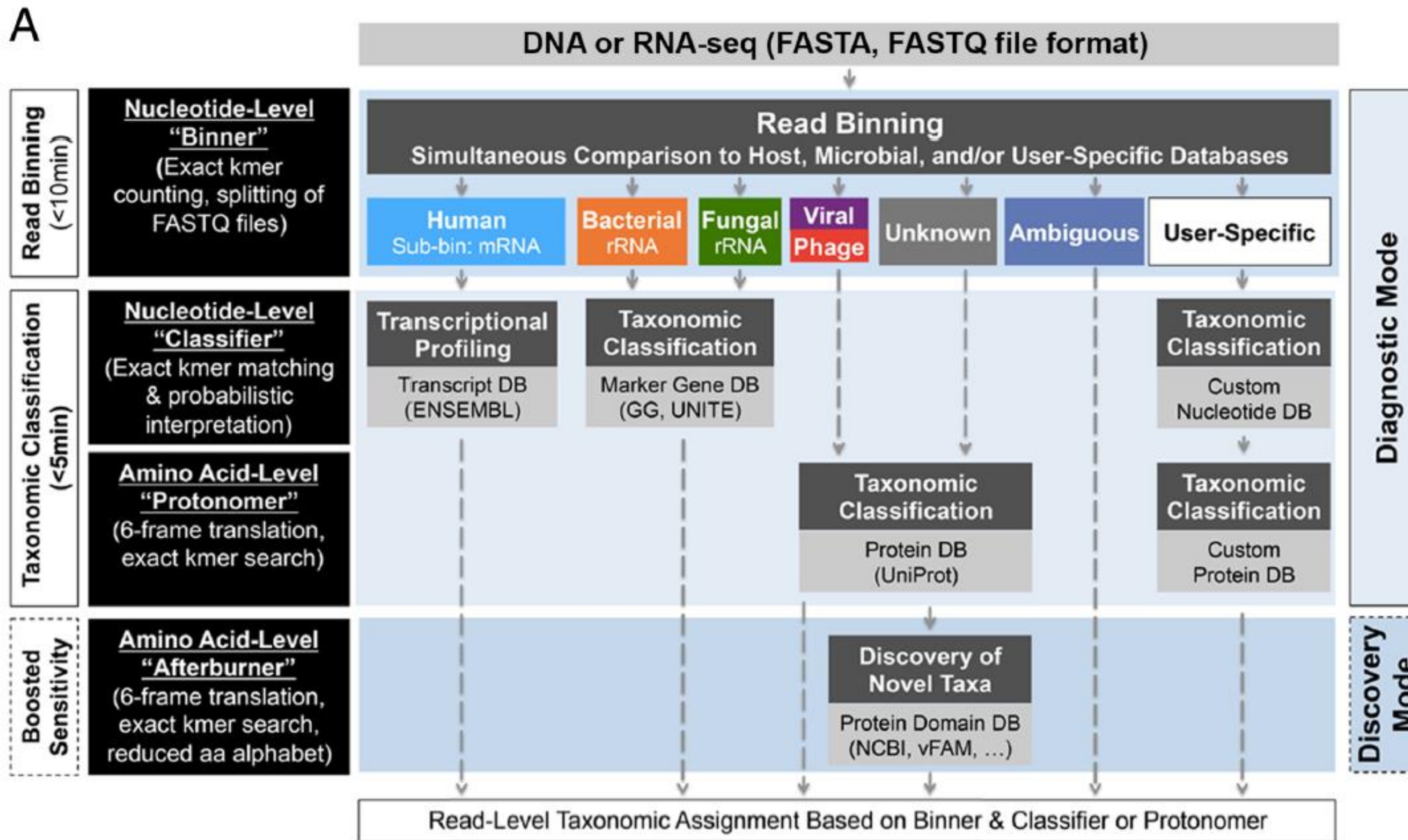
Results: We present Taxonomer, an ultrafast, web-tool for comprehensive metagenomics data analysis and interactive results visualization. Taxonomer is unique in providing integrated nucleotide and protein-based classification and simultaneous host messenger RNA (mRNA) transcript profiling. Using real-world case-studies, we show that Taxonomer detects previously unrecognized infections and reveals antiviral host mRNA expression profiles. To facilitate data-sharing across geographic distances in outbreak settings, Taxonomer is publicly available through a web-based user interface.

Conclusions: Taxonomer enables rapid, accurate, and interactive analyses of metagenomics data on personal computers and mobile devices.

Keywords: Metagenomics, Microbiome, Pathogen detection, Infectious disease diagnostics

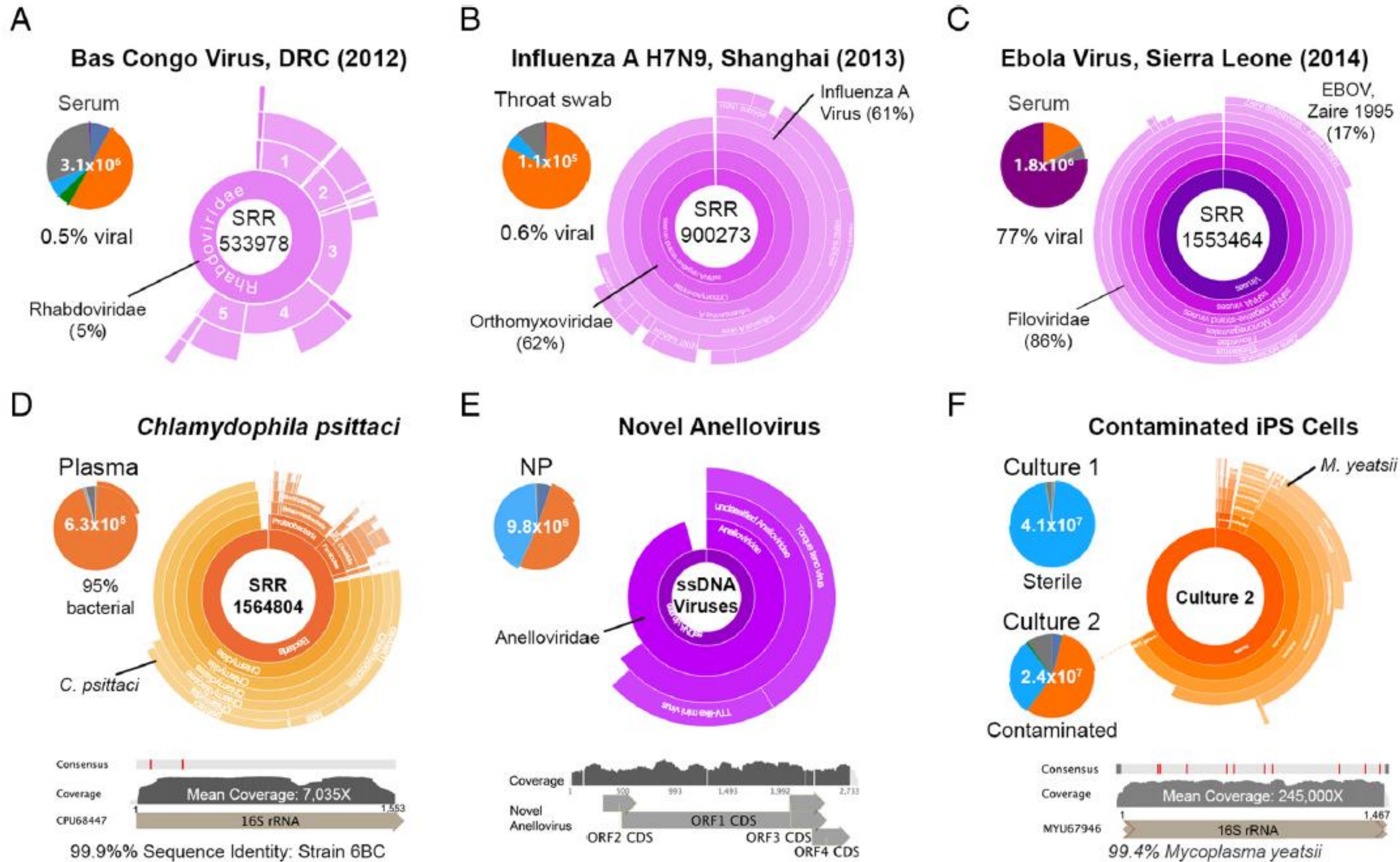
Dizi analizi temelli yöntemler

- Enfeksiyon hastalıklarının sistem biyolojisi çözümü için iş akış modülleri



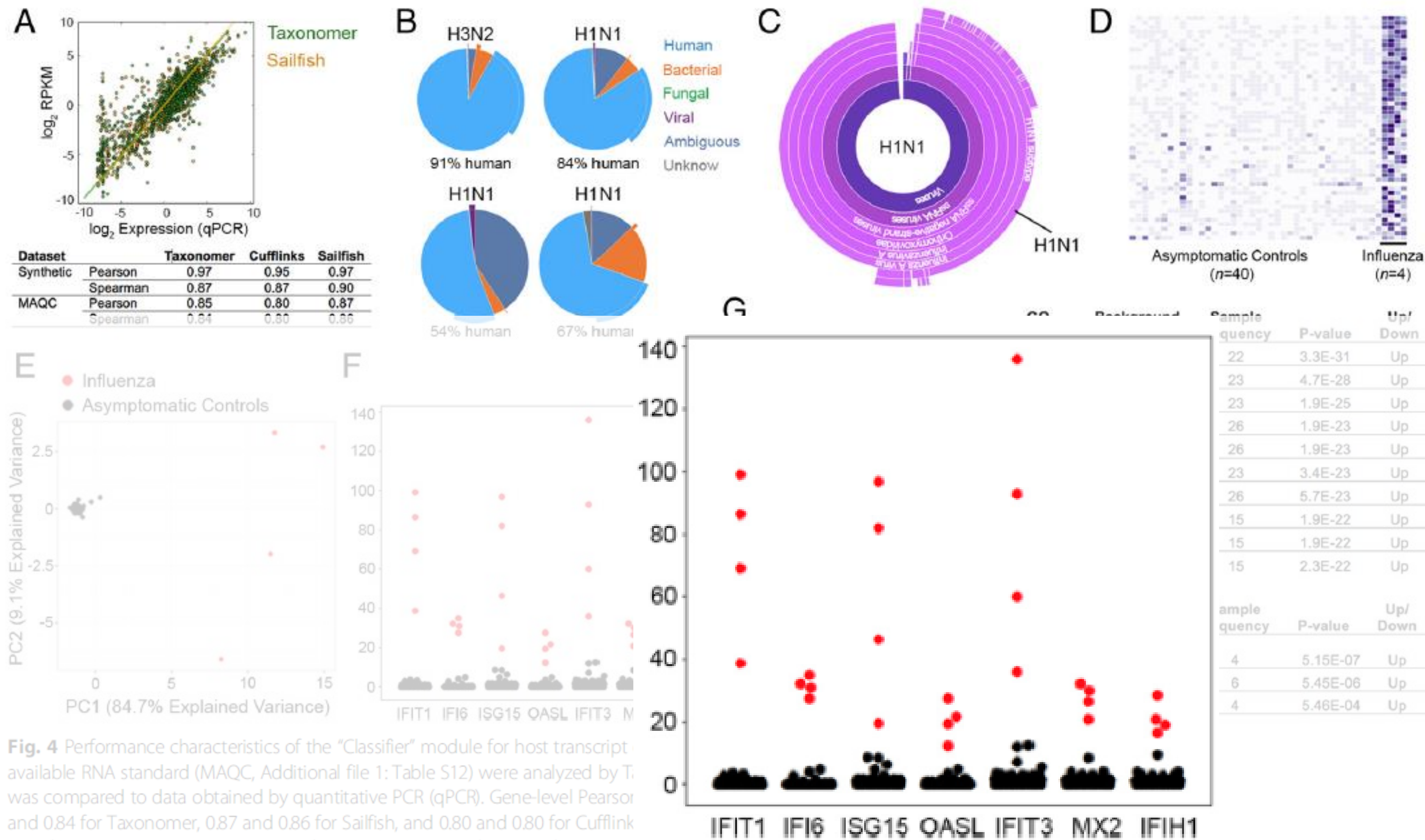
Dizi analizi temelli yöntemler

- Hiyerarşik, taksonomik sınıflama ile ayrıştırılan dizilerin bir araya getirilmesi



Dizi analizi temelli yöntemler

- Taksonomik sınıflandırma ile birlikte gen ekspresyon paternlerinin belirlenmesi



Dataset		Taxonomer	Cufflinks	Sailfish
Synthetic	Pearson	0.97	0.95	0.97
	Spearman	0.87	0.87	0.90
MAQC	Pearson	0.85	0.80	0.87
	Spearman	0.84	0.80	0.86

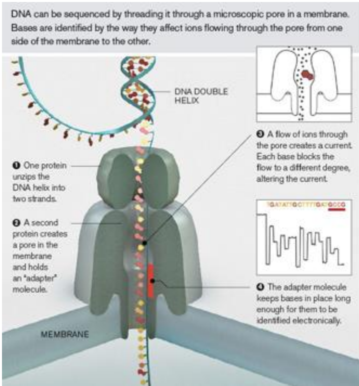
Fig. 4 Performance characteristics of the "Classifier" module for host transcript ... available RNA standard (MAQC, Additional file 1: Table S12) were analyzed by T... was compared to data obtained by quantitative PCR (qPCR). Gene-level Pearso... and 0.84 for Taxonomer, 0.87 and 0.86 for Sailfish, and 0.80 and 0.80 for Cufflink... data from routine respiratory samples from patients with influenza infection (n ... of this strain as influenza A(H1N1)pdm09 (*top right sample* from **a**). **d** Differential gene-level mRNA expression profiles from four patients with influenza A virus compared to asymptomatic controls (n = 40; top 50 differentially expressed genes are shown). Expression profiles for 17 genes were significantly higher in influenza-positive patients (Additional file 1: Table S5). **e** Expression profiles for the 17 most differentially expressed genes differentiate cases from controls (principal component analysis, PC1 and PC2 explaining 93.8 % of the total variance). **f** Normalized expression levels for individual patients of seven of the top 17 genes. Gene ontology assignments for enrichment of biological processes (**g**) and molecular functions (**h**) are shown

Dizi analizi temelli yöntemler

Tek Molekül Dizileme Sistemleri

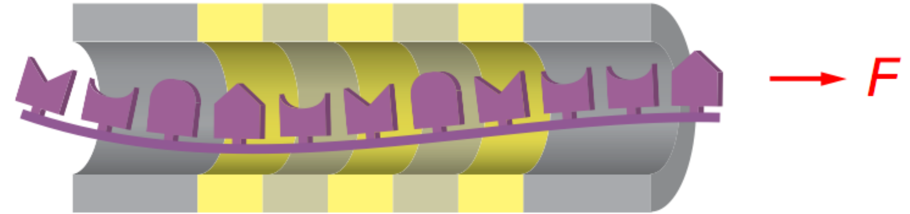
- “İkinci yeni nesil” ya da “üçüncü nesil” zenginleştirme aşamalarına gerek kalmadan tek DNA molekülü ile dizi analizi yapılabilmektedir.

Oxford Nanopore Technology



My Twitter feed just exploded. Oxford Nanopore, long the sleeper project to watch in the field of mapping DNA, just announced two products that could dramatically change the field of DNA sequencing: a new DNA sequencer that may be able to handle a human genome in 15 minutes, and a USB thumb drive DNA sequencer that can read DNA directly from blood with no prep work.

IBM DNA Transistor Technology



Dizi analizi temelli yöntemler

Tek Molekül Dizileme Sistemleri

- Oxford Nanopore tarafından geliştirilen MinION nanapore dizi analizi cihazıyla, direkt olarak örnekten chikungunya, Ebola virus ve HCV altı saatten kısa bir sürede tespit edebilmişlerdir.

Greninger *et al. Genome Medicine* (2015) 7:99
DOI 10.1186/s13073-015-0220-9

METHOD Open Access

Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis

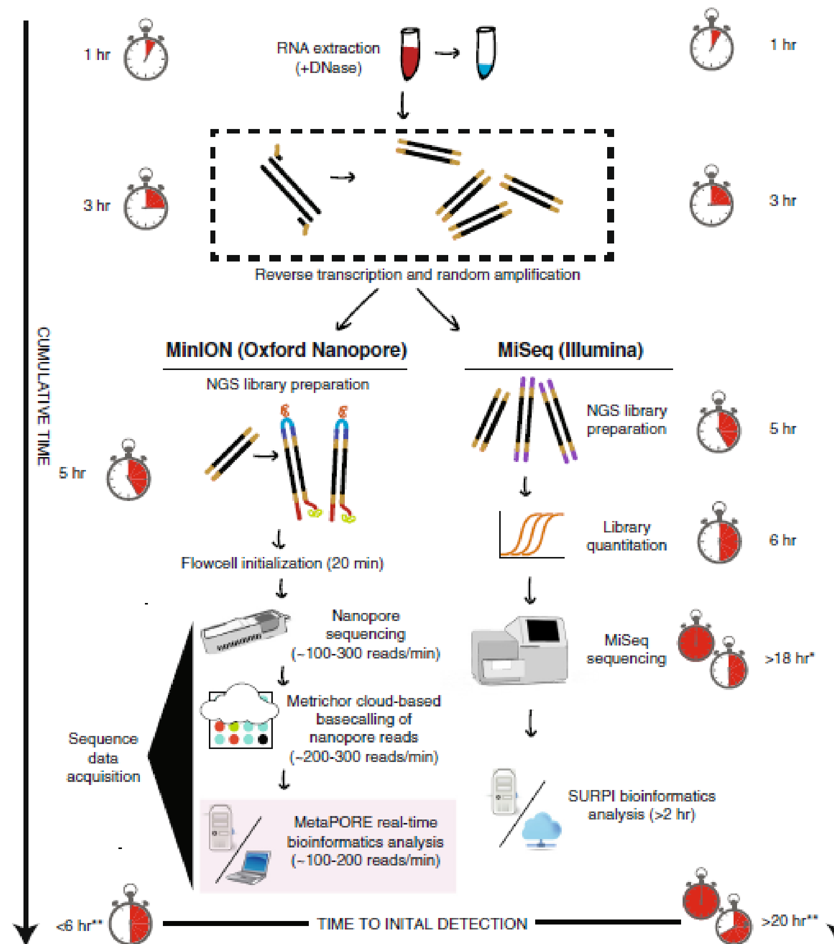
Alexander L. Greninger[1,2], Samia N. Naccache[1,2†], Scot Federman[1,2†], Guixia Yu[1,2], Placide Mbala[3,6], Vanessa Bres[4], Doug Stryke[1,2], Jerome Bouquet[1,2], Sneha Somasekar[1,2], Jeffrey M. Linnen[4], Roger Dodd[5], Prime Mulembakani[6], Bradley S. Schneider[6], Jean-Jacques Muyembe-Tamfum[3], Susan L. Stramer[5] and Charles Y. Chiu[1,2,7*]

Abstract

We report unbiased metagenomic detection of chikungunya virus (CHIKV), Ebola virus (EBOV), and hepatitis C virus (HCV) from four human blood samples by MinION nanopore sequencing coupled to a newly developed, web-based pipeline for real-time bioinformatics analysis on a computational server or laptop (MetaPORE). At titers ranging from 10^7–10^8 copies per milliliter, reads to EBOV from two patients with acute hemorrhagic fever and CHIKV from an asymptomatic blood donor were detected within 4 to 10 min of data acquisition, while lower titer HCV virus (1×10^5 copies per milliliter) was detected within 40 min. Analysis of mapped nanopore reads alone, despite an average individual error rate of 24 % (range 8–49 %), permitted identification of the correct viral strain in all four isolates, and 90 % of the genome of CHIKV was recovered with 97–99 % accuracy. Using nanopore sequencing, metagenomic detection of viral pathogens directly from clinical samples was performed within an unprecedented <6 hr sample-to-answer turnaround time, and in a timeframe amenable to actionable clinical and public health diagnostics.

Dizi analizi temelli yöntemler

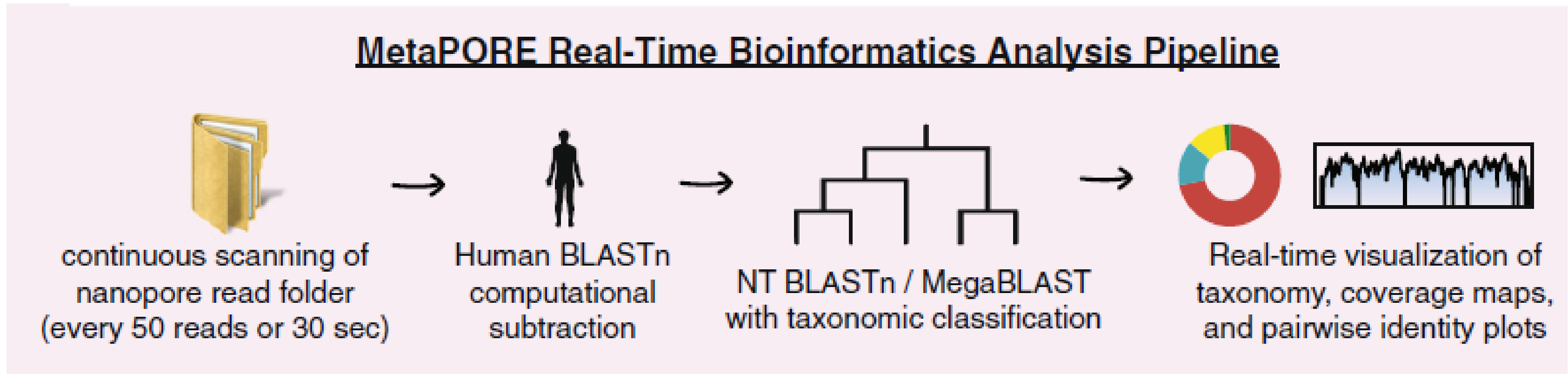
Tek Molekül Dizileme Sistemleri



Sistem Biyolojisi

Tek Molekül Dizileme Sistemleri

- MetaPORE bioinformatics pipeline



Dizi analizi temelli yöntemler

- Dual RNA-seq; çeşitli farklılıktan yararlanılarak konak ve patojen transkriptleri aynı anda ancak ayrı ayrı incelenebilir.

REVIEWS

Dual RNA-seq of pathogen and host

Alexander J. Westermann, Stanislaw A. Gorski and Jörg Vogel

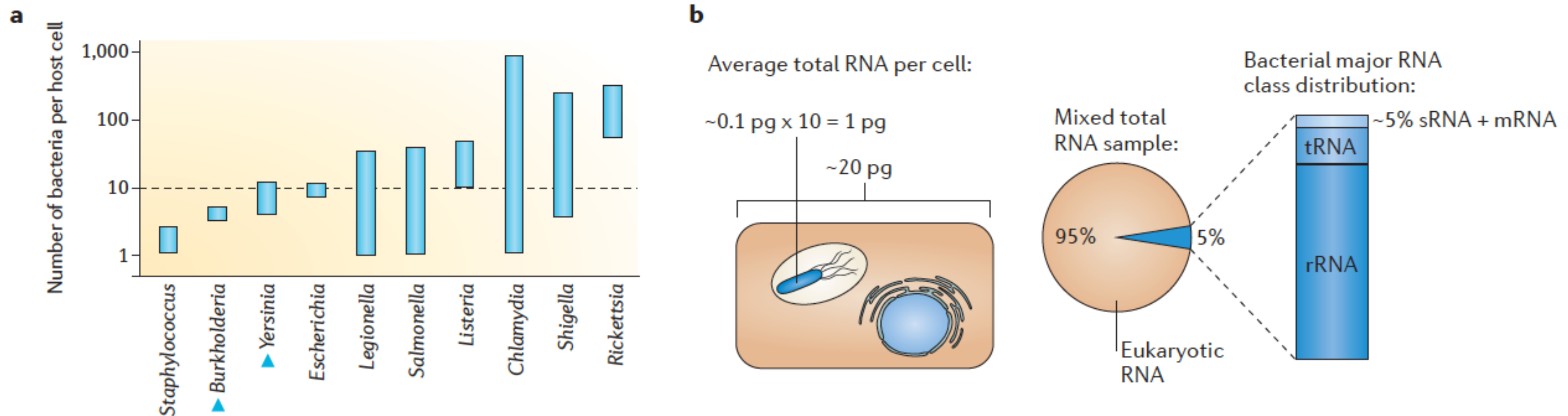
Abstract | A comprehensive understanding of host–pathogen interactions requires a knowledge of the associated gene expression changes in both the pathogen and the host. Traditional, probe-dependent approaches using microarrays or reverse transcription PCR typically require the pathogen and host cells to be physically separated before gene expression analysis. However, the development of the probe-independent RNA sequencing (RNA-seq) approach has begun to revolutionize transcriptomics. Here, we assess the feasibility of taking transcriptomics one step further by performing 'dual RNA-seq', in which gene expression changes in both the pathogen and the host are analysed simultaneously.

Pathogen-associated molecular patterns (PAMPs). General small molecular motifs that are present on microorganisms and engage host innate immune receptors, in particular Toll-like receptors. Examples

Eukaryotic host cells are subject to infection by agents of varying complexity, from viruses to bacteria to eukaryotic parasites such as fungi and protozoa. Infection initiates a dynamic cascade of events that culminates in altered gene expression patterns in both interacting organisms. These changes lead to the adaptation and persistence of the pathogen or to its clearance from the parallel. The major benefit of such an approach is the potential to monitor gene expression in two organisms to a high level of accuracy and depth. Given the sensitivity of this approach, it could potentially be used to sequence the transcriptomes of a small number of cells at the initial site of infection, a feat which is yet to be achieved in infection biology. Most importantly, a dual approach

Dizi analizi temelli yöntemler

- Dual RNA-seq; çeşitli farklılıktan yararlanılarak konak ve patojen transkriptleri aynı anda ancak ayrı ayrı incelenebilir.



c

Separate RNA-seq

Organism	Total RNA	Enriched RNA
Pathogen	4–10 million reads	2–5 million reads
Host	>2,000 million reads	≥100 million reads

Dual RNA-seq

Organism	Total RNA	Enriched RNA
Pathogen	≥ 400 million reads	≥200 million reads
Host	>2,000 million reads	≥100 million reads

Sistem Biyolojisi

- Bu yüzyılda mikroorganizmalarla mücadelede;

  nihayi bir çözümün bulunabilmesi için yeni antimikrobiyol arayışlarının yerine

  sistem biyolojisi yaklaşımı ile konak, patojen ve çevre etkileşimleri ortaya konacaktır.