P&S Mobile Genomics

Lecture 2:

Introduction to Sequencing

Dr. Mohammed Alser



ETH Zurich
Spring 2022
15 March 2022





Agenda for Today

- What is Genome Analysis?
- What is Intelligent Genome Analysis?
- How we Analyze Genome?

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What is Data Analysis?

"The purpose of COMPUTING is [to gain] insight, not numbers"

Richard Hamming

What is Genome Analysis?



What is Genome Analysis?



nature research

Search Q Login (S)

nature > subjects > genomic analysis

Genomic analysis





Genomic analysis is the identification, measurement or comparison of genomic features such as DNA sequence, structural variation, gene expression, or regulatory and functional element annotation at a genomic scale. Methods for genomic analysis typically require high-throughput sequencing or microarray hybridization and bioinformatics.

DNA Testing



Health + Ancestry
Service

\$199

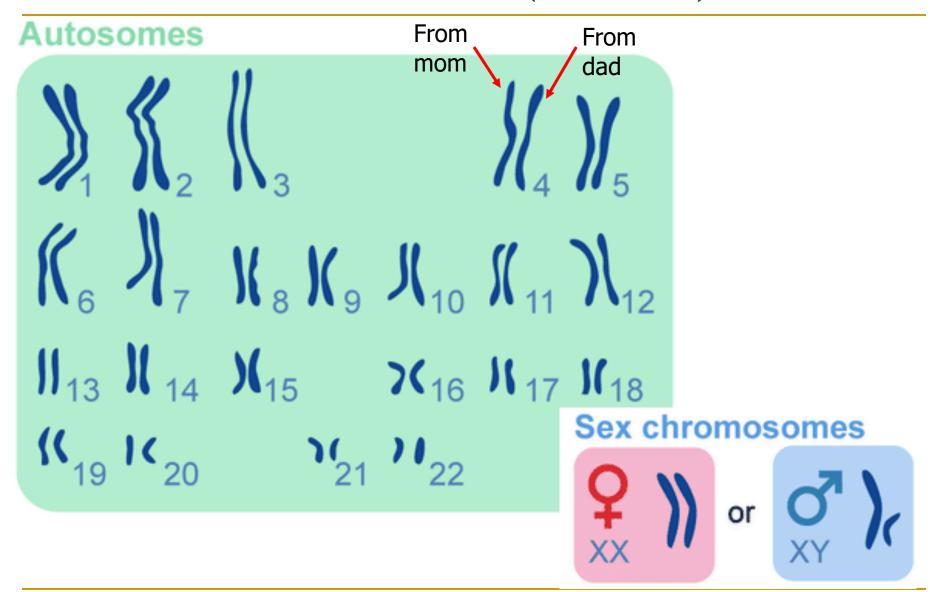
 Includes everything in Ancestry + Traits Service

PLUS

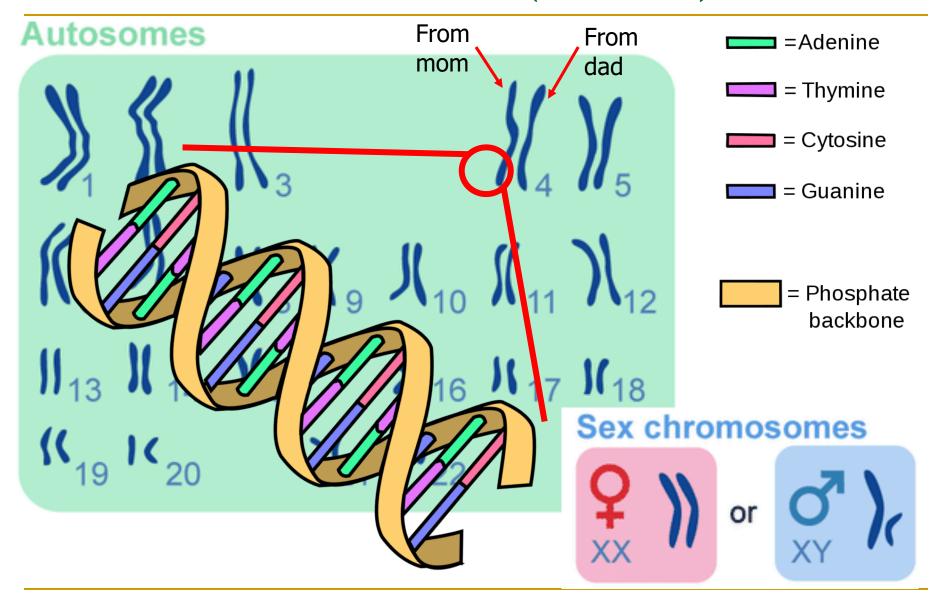
- 10+ Health Predisposition reports*
- 5+ Wellness reports
- 40+ Carrier Status reports*



Human Chromosomes (23 Pairs)



Human Chromosomes (23 Pairs)



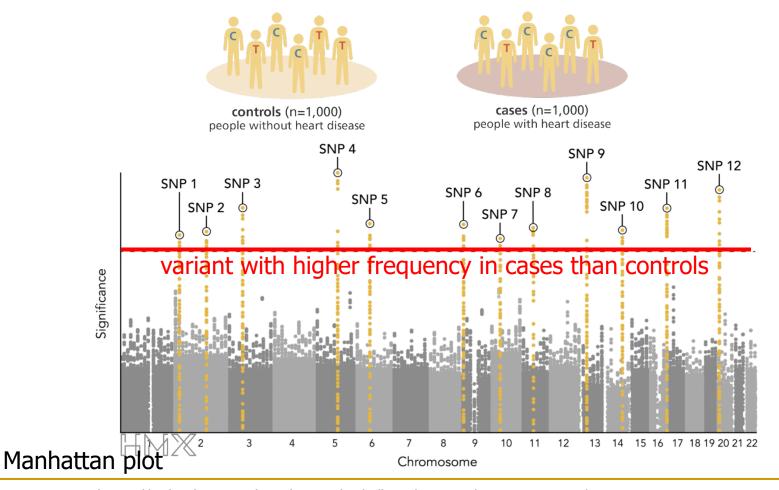
Finding SNPs Associated with Complex Trait

	SNP1	SNP2	Blood Pressure
Individual #1	ACATGCCGACATT	TCATAGGCC	180
Individual #2	ACATGCCGACATT	TCATAAGCC	175
Individual #3	ACATGCCGACATT	TCATAGGCC	170
Individual #4	ACATGCCGACATT	TCATAAGCC	165
Individual #5	ACATGCCGACATT	TCATAGGCC	160
Individual #6	ACATGCCGACATT	TCATAGGCC	145
Individual #7	ACATGCCGACATT	TCATAAGCC	140
Individual #8	ACATGCCGACATT	TCATAAGCC	130
Individual #9	ACATGTCGACATT	TCATAGGCC	120
Individual #10	ACATGTCGACATT	TCATAAGCC	120
Individual #11	ACATGTCGACATT	TCATAGGCC	115
Individual #12	ACATGTCGACATT	TCATAAGCC	110
Individual #13	ACATGTCGACATT	TCATAGGCC	110
Individual #14	ACATGTCGACATT	TCATAAGCC	110
Individual #15	ACATGTCGACATT	TCATAGGCC	105
Individual #16	ACATGTCGACATT	TCATAAGCC	100

SNP: single nucleotide polymorphism

Genome-Wide Association Study (GWAS)

 Detecting genetic variants associated with phenotypes using two groups of people.



Similar Association Studies

PERSPECTIVE

https://doi.org/10.1038/s41588-019-0385-z



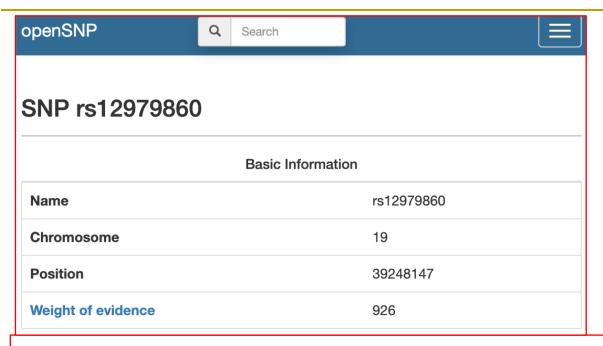
Opportunities and challenges for transcriptomewide association studies

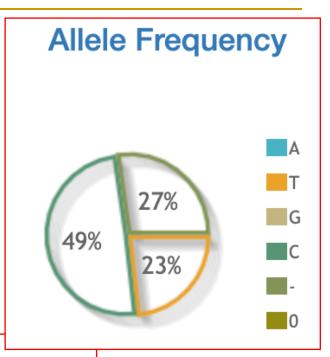
Michael Wainberg¹, Nasa Sinnott-Armstrong ¹, Nicholas Mancuso ¹, Alvaro N. Barbeira ¹, David A. Knowles ¹, David Golan², Raili Ermel⁷, Arno Ruusalepp^{7,8}, Thomas Quertermous ¹, Ke Hao ¹, Johan L. M. Björkegren ^{8,10,11,12*}, Hae Kyung Im ^{4*}, Bogdan Pasaniuc ^{3,13,14*}, Manuel A. Rivas ^{15*} and Anshul Kundaje ¹,2*

Transcriptome-wide association studies (TWAS) integrate genome-wide association studies (GWAS) and gene expression datasets to identify gene-trait associations. In this Perspective, we explore properties of TWAS as a potential approach to prioritize causal genes at GWAS loci, by using simulations and case studies of literature-curated candidate causal genes for schizophrenia, low-density-lipoprotein cholesterol and Crohn's disease. We explore risk loci where TWAS accurately prioritizes the likely causal gene as well as loci where TWAS prioritizes multiple genes, some likely to be non-causal, owing to sharing of expression quantitative trait loci (eQTL). TWAS is especially prone to spurious prioritization with expression data from non-trait-related tissues or cell types, owing to substantial cross-cell-type variation in expression levels and eQTL strengths. Nonetheless, TWAS prioritizes candidate causal genes more accurately than simple baselines. We suggest best practices for causal-gene prioritization with TWAS and discuss future opportunities for improvement. Our results showcase the strengths and limitations of using eQTL datasets to determine causal genes at GWAS loci.

Wainberg+, "Opportunities and challenges for transcriptome-wide

SNPs and Personalized Medicine





Links to SNPedia

Title	Summary
rs12979860 T/T	~20-25% of such hepatitis c patients respond to treatment
rs12979860 C/C	~80% of such hepatitis c patients respond to treatment
rs12979860 C/T	~20-40% of such hepatitis c patients respond to treatment

Personalized Medicine for Critically Ill Infants

- rWGS can be performed in 2-day (costly) or 5-day time to interpretation.
- Diagnostic rWGS for infants
 - Avoids morbidity
 - Reduces hospital stay length by 6%-69%
 - Reduces inpatient cost by \$800,000-\$2,000,000.

Article | Open Access | Published: 04 April 2018

Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization

Lauge Farnaes, Amber Hildreth, Nathaly M. Sweeney, Michelle M. Clark, S Chowdhury, Shareef Nahas, Julie A. Cakici, Wendy Benson, Robert H. Kal Richard Kronick, Matthew N. Bainbridge, Jennifer Friedman, Jeffrey J. Go Ding, Narayanan Veeraraghavan, David Dimmock & Stephen F. Kingsmore

npj Genomic Medicine 3, Article number: 10 (2018) | Cite this article

Article | Open Access | Published: 05 May 2020

Clinical utility of 24-h rapid trio-exome sequencing for critically ill infants

Huijun Wang, Yanyan Qian, Yulan Lu, Qian Qin, Guoping Lu, Guoqiang Cheng, Ping Zhang, Lin Yang, Bingbing Wu \boxtimes & Wenhao Zhou \boxtimes

npj Genomic Medicine 5, Article number: 20 (2020) | Cite this article

Personalized Medicine in UK

"From 2019, all seriously ill children in UK will be offered whole genome sequencing as part of their care"



Much Larger Structural Variations!



AUTISM

Weiss, *N Eng J Med* 2008 Deletion of 593 kb



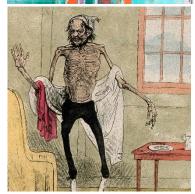
SCHIZOPHRENIA

McCarthy, *Nat Genet* 2009 Duplication of 593 kb



OBESITY

Walters, *Nature* 2010 Deletion of 593 kb



UNDERWEIGHT

Jacquemont, *Nature* 2011 Duplication of 593 kb



Deletion in the short arm of chromosome 16 (16p11.2)



Duplication in the short arm of chromosome 16 (16p11.2)

Recommended Reading

nature reviews genetics

Explore our content > Journal information >

nature > nature reviews genetics > review articles > article

Review Article | Published: 15 November 2019

Structural variation in the sequencing era

Steve S. Ho, Alexander E. Urban & Ryan E. Mills ⊠

Nature Reviews Genetics 21, 171–189(2020) | Cite this article

15k Accesses | 16 Citations | 309 Altmetric | Metrics

Ho+, "Structural variation in the sequencing era", Nature Reviews Genetics, 2020

Agenda for Today

What is Genome Analysis?

- What is Intelligent Genome Analysis?
- How we Analyze Genome?

What is Intelligent Genome Analysis?

Fast genome analysis

Bandwidth

□ Real-time analysis

Using intelligent architectures

□ Specialized HW with less data movement

Energy-efficiency & Latency

DNA is a valuable asset

□ Controlled-access analysis

Privacy

Population-scale genome analysis

Sequence anywhere at large scale!

Scalability

Avoiding erroneous analysis

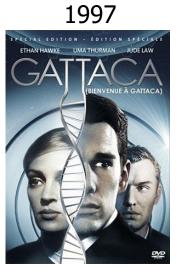
□ *E.g., your father is not your father*

Accuracy

Does intelligent genome analysis really matter?

Fast Genome Analysis?

 Fast genome analysis in mere seconds using limited computational resources (i.e., personal computer or small hardware).

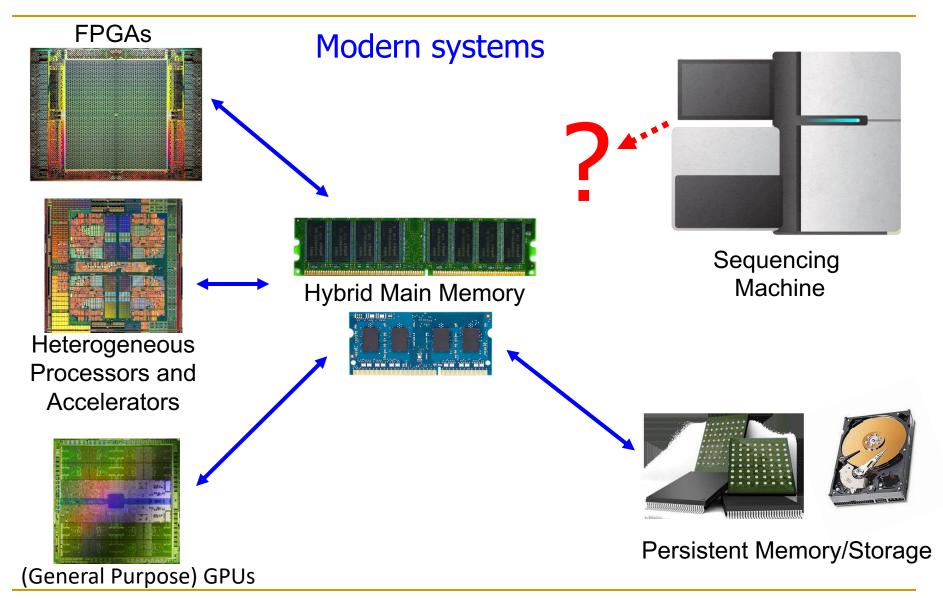




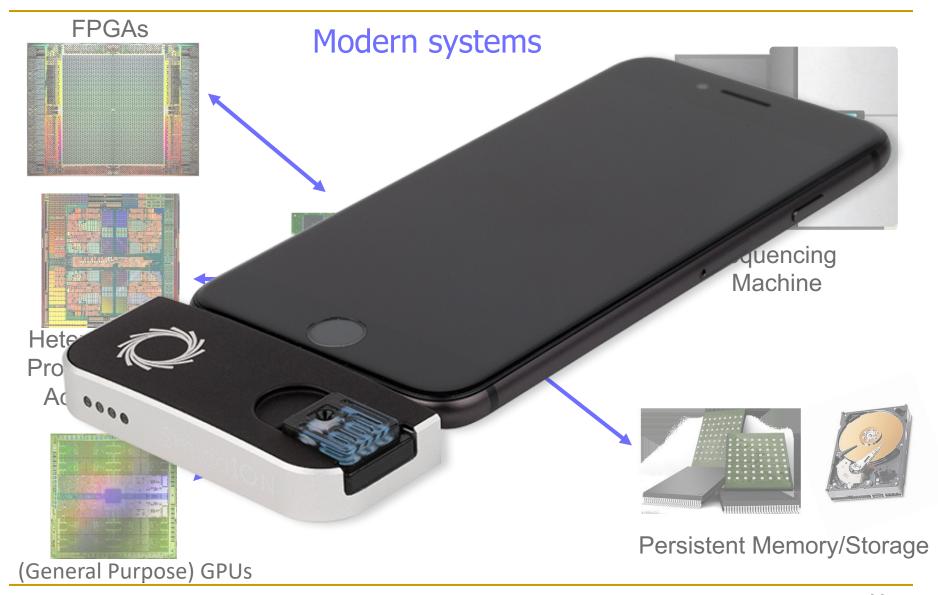




Intelligent Architecture?



Intelligent Architecture?



Privacy-Preserving Genome Analysis?

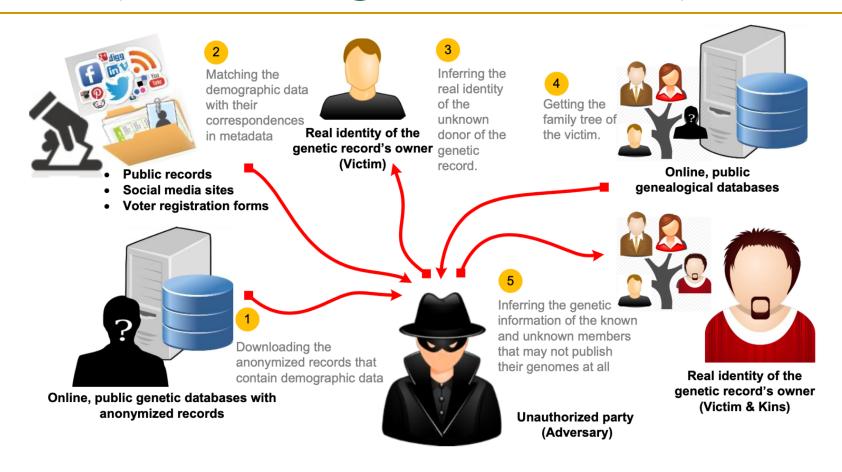


Fig. 5. A completion attack.

Alser+, "Can you really anonymize the donors of genomic data in today's digital world?" 10th International Workshop on Data Privacy Management (DPM), 2015.

Can you Really Anonymize the Donors?

(Position Paper) Can You Really Anonymize the Donors of Genomic Data in Today's Digital World?

Mohammed Alser, Nour Almadhoun, Azita Nouri, Can Alkan, and Erman Ayday

Computer Engineering Department, Bilkent University, 06800 Bilkent, Ankara, Turkey

Abstract. The rapid progress in genome sequencing technologies leads to availability of high amounts of genomic data. Accelerating the pace of biomedical breakthroughs and discoveries necessitates not only collecting millions of genetic samples but also granting open access to genetic databases. However, one growing concern is the ability to protect the privacy of sensitive information and its owner. In this work, we survey a wide spectrum of cross-layer privacy breaching strategies to human genomic data (using both public genomic databases and other public non-genomic data). We outline the principles and outcomes of each technique, and assess its technological complexity and maturation. We then review potential privacy-preserving countermeasure mechanisms for each threat.

Keywords: Genomics, Privacy, Bioinformatics



Alser+, "Can you really anonymize the donors of genomic data in today's digital world?" 10th International Workshop on Data Privacy Management (DPM), 2015.

Privacy-Preserving DNA Test

Our DNA Test, Reports, and Technology

- Whole Genome Sequencing. Decode 100% of your DNA with Whole Genome Sequencing and fully unlock your genetic blueprints.
- Privacy First DNA Testing. Begin your journey of discovery without risking the privacy of your most personal information.
- Nebula Research Library. Receive new reports every week that are based on the latest scientific discoveries.
- Genome Exploration Tools. Use powerful, browser-based genome exploration tools to answer any questions about your DNA.
- Deep Genetic Ancestry. Discover more about your ancestry with full Y chromosome and mitochondrial DNA sequencing and analysis.
- Genomic Big Data Access. Download your FASTQ, BAM, and VCF files and dive deeper into your Whole Genome Sequencing data.
- Ready for Diagnostics. Our Whole Genome Sequencing data is of the highest quality and can be used by physicians and genetic counselors.



30x Whole Genome Sequencing DNA Test

\$299Normally \$1000
Save 70%!

A genetic test that decodes 100% of your DNA with very high accuracy. 30x Whole Genome Sequencing offers the best value for money and is the best choice for most people.

100x Whole Genome Sequencing DNA Test

\$999Normally \$3500
Save 70%!

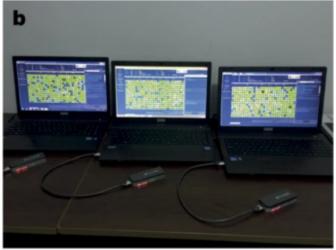
A genetic test that decodes 100% of your DNA with extremely high accuracy. 100x Whole Genome Sequencing is recommended for the discovery of rare genetic mutations.

Get Sequenced

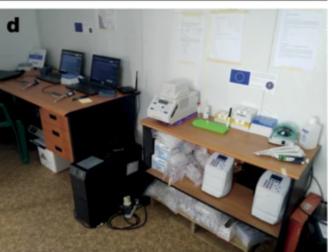
Rapid Surveillance of Disease Outbreaks?

Figure 1: Deployment of the portable genome surveillance system in Guinea.









Quick+, "Real-time, portable genome sequencing for Ebola surveillance", Nature, 2016

Scalable SARS-CoV-2 Testing







HOME | ABOU

Search

Comments (I)

Swab-Seq: A high-throughput platform for massively scaled up SARS-CoV-2 testing

Doshua S. Bloom, Eric M. Jones, De Molly Gasperini, De Nathan B. Lubock, Laila Sathe, Chetan Munugala, De A. Sina Booeshaghi, De Oliver F. Brandenberg, De Longhua Guo, De James Boocock, De Scott W. Simpkins, Isabella Lin, Nathan LaPierre, Duke Hong, Yi Zhang, Gabriel Oland, Bianca Judy Choe, Sukantha Chandrasekaran, Evann E. Hilt, De Manish J. Butte, De Robert Damoiseaux, De Aaron R. Cooper, De Yi Yin, De Lior Pachter, De Omai B. Garner, De Jonathan Flint, De Eleazar Eskin, De Chongyuan Luo, De Sriram Kosuri, De Leonid Kruglyak, De Valerie A. Arboleda

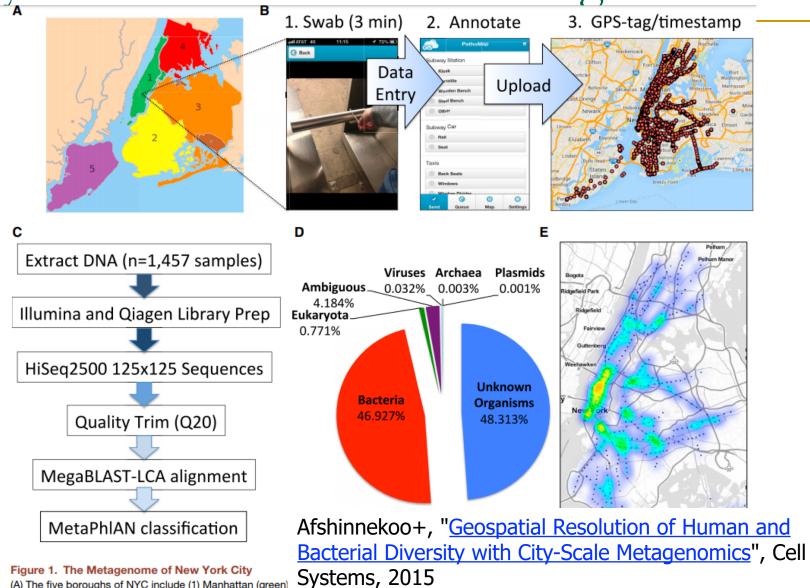
doi: https://doi.org/10.1101/2020.08.04.20167874

Bloom+, "Swab-Seq: A high-throughput platform for massively scaled up SARS-CoV-2 testing", medRxiv, 2020

Population-Scale Microbiome Profiling



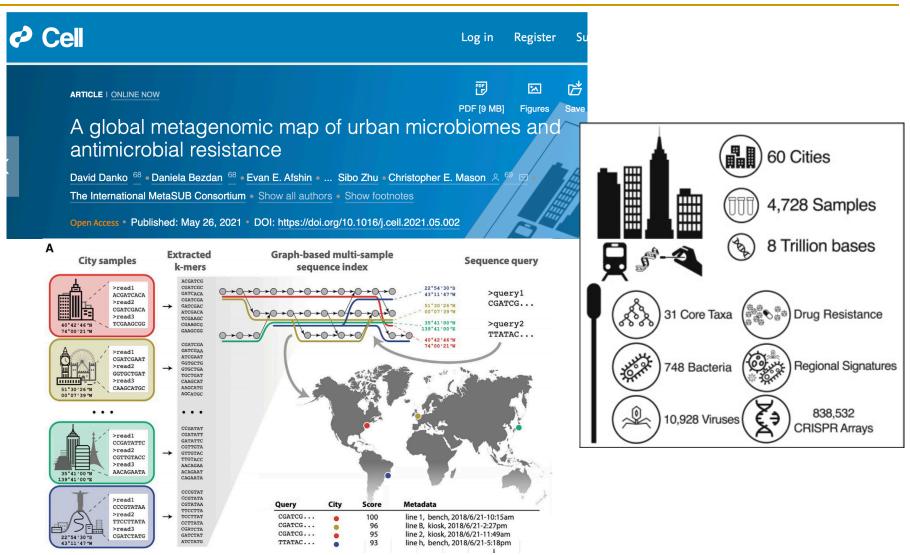
City-Scale Microbiome Profiling



(A) The five boroughs of NYC include (1) Manhattan (green)

(B) The collection from the 466 subway stations of NYC across the 24 subway lines involved three main steps: (1) collection with Copan Elution swabs, (2) data entry into the database, and (3) uploading of the data. An image is shown of the current collection database, taken from http://pathomap.giscloud.com. (C) Workflow for sample DNA extraction, library preparation, sequencing, quality trimming of the FASTQ files, and alignment with MegaBLAST and MetaPhIAn to discern taxa present

Population-Scale Microbiome Profiling



Danko+, "A global metagenomic map of urban microbiomes and antimicrobial resistance", Cell, 2021



Plague in New York Subway System?

Plague (Yersinia Pestis)



What Is It?

Published: December, 2018

Plague is caused by Yersinia pestis bacteria. It can be a life-threatening infection if not treated promptly. Plague has caused several major epidemics in Europe and Asia over the last 2,000 years. Plague has most famously been called "the Black Death" because it can cause skin sores that form black scabs. A plague epidemic in the 14th century killed more than one-third of the population of Europe within a few years. In some cities, up to 75% of the population died within days, with fever and swollen skin sores.

Plague in New York Subway System?

Plague (Yersi₁[®]

What Is It?

Published: December, 2018

Plague is caused by Yersinia treated promptly. Plague has last 2,000 years. Plague has cause skin sores that form b than one-third of the popul the population died within

The New Hork Times Bubonic Plague in the Subway System? Don't Worry About It



In October, riders were not deterred after reports that an Ebola-infected man had ridden the subway just before he fell ill. Robert Stolarik for The New York Times

https://www.nytimes.com/2015/02/07/nyregion/bubonic-plague-in-the-subway-system-dont-worry-about-it.html

The findings of Yersinia Pestis in the subway received wide coverage in the lay press, causing some alarm among New York residents

Failure of Bioinformatics



data. Rob Knight, a professor in the department of pediatrics at the University of California, San Diego, calls this type of error "a failure of bioinformatics," in that Mason had assumed the gene fragments were unique to the pathogens, when in fact they can also be detected in other

Living in a microbial world

Charles Schmidt

Nature Biotechnology, volume 35, pages401–403 (2017)

https://www.nature.com/articles/nbt.3868

There is a critical need for **fast** and **accurate** genome analysis.

Achieving Intelligent Genome Analysis?

How and where to enable

fast, accurate, cheap,

privacy-preserving, and exabyte scale analysis of genomic data?

Agenda for Today

- What is Genome Analysis?
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Genome Analysis



No machine can read the *entire* content of a genome

TAATGTAGCTATACTGAACGTTATCTAGGGGAAAGATTGAAGGGGAGCTCTAAGGTCAACACCACCACCACTTCCCAGAAAGCTTCTTCA......



Genome Analysis



no machine can read the *entire* content of a genome



>CCT GACC CATGT GAAG ACTA AAGTA

Why?!

SAFARI

CAAG

TCTT

CATTG

AAAA

ATTT

AAAA

ATGG GAAA

Suggested Readings

nature methods

Explore content > About the journal > Publish with us >

Published: November 2009

Next-generation sequencing library preparation: simultaneous fragmentation and tagging using in vitro transposition

Fraz Syed ✓, Haiying Grunenwald & Nicholas Caruccio

Nature Methods 6, i–ii (2009) | Cite this article

16k Accesses | 4 Citations | 5 Altmetric | Metrics

https://www.nature.com/articles/nmeth.f.272

Suggested Readings

nature biotechnology

Explore content > About the journal > Publish with us >

nature > nature biotechnology > review articles > article

Published: 09 October 2008

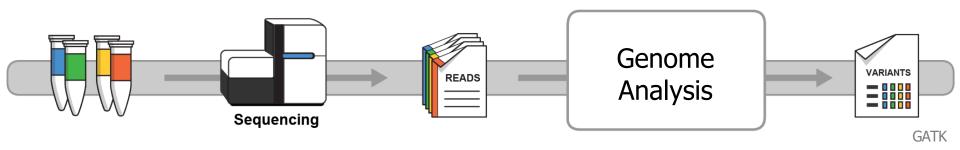
Next-generation DNA sequencing

Nature Biotechnology 26, 1135–1145 (2008) Cite this article

149k Accesses | 2645 Citations | 79 Altmetric | Metrics

https://www.nature.com/articles/nbt1486

Genome Sequencer is a Chopper



CCCCCTATATATACGTACTAGTACGT

ACGACTTTAGTACGTACGT TATATACGTACTAGTACGT

ACGTACG CCCCTACGTA
TATATATACGTACTAGTACGT

ACGACTTTAGTACGTACGT TATATATACGTACTAAAGTACGT TATATATACGTACTAGTACGT

ACG TTTTTAAAACGTA
TATATATACGTACTACGT

ACGAC GGGGAGTACGT



1x10¹² bases*



44 hours*



<1000 \$

* NovaSeq 6000

High-Throughput Sequencers



Illumina MiSeq



Illumina NovaSeq 6000



Pacific Biosciences Sequel II



Pacific Biosciences RS II





Oxford Nanopore MinION



... and more! All produce data with different properties.

Oxford Nanopore Sequencers NANOPORE











MinION Mk1B

MinION Mk1C

GridION Mk1

PromethION 24/48

	MinION Mk1B	MinION Mk1C	GridION Mk1	PromethION 24	PromethION 48
Read length	> 2Mb	> 2Mb	> 2Mb	> 2Mb	> 2Mb
Yield per flow cell	r flow cell 50 Gb		50 Gb 50 Gb		220 Gb
Number of flow cells per device			5	24	48
Yield per device	<50 Gb	<50 Gb	<250 Gb	<5.2 Tb	<10.5 Tb
Starting price	\$1,000	\$4,990	\$49,995	\$195,455	\$327,455

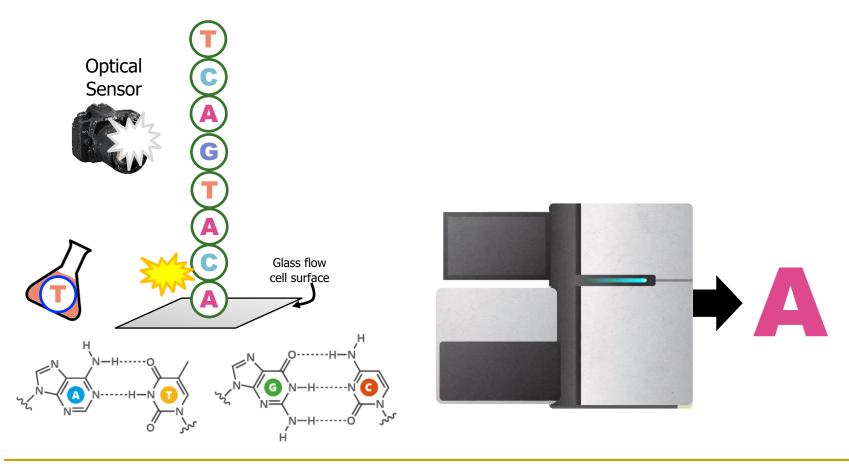
Illumina Sequencers



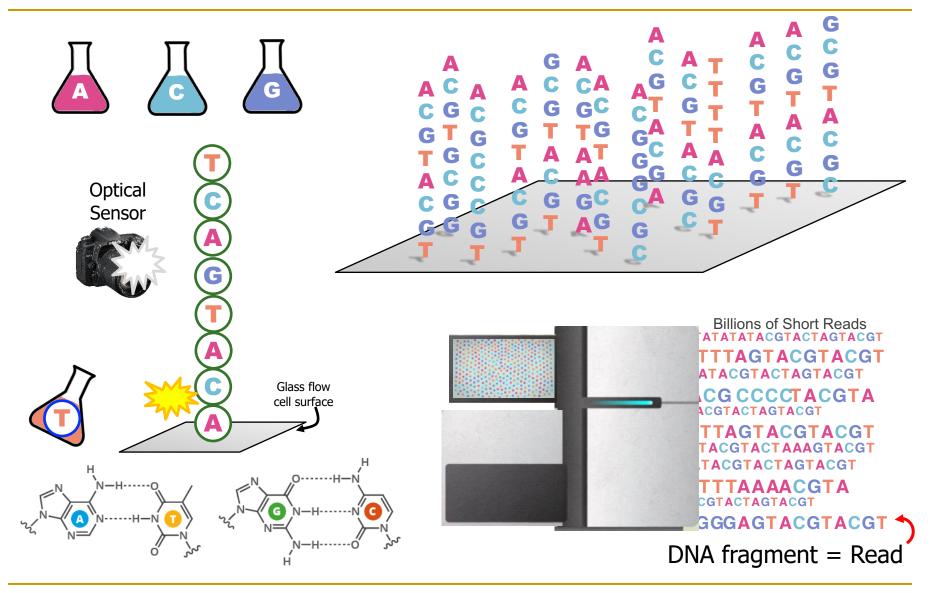


Run time	9.5–19 hrs	4–24 hrs	4–55 hrs	12–30 hrs	24-48 hrs	13-44 hrs
Max. reads per run	4 million	25 million	25 million	400 million	1 billion	20 billion
Max. read length	2 × 150 bp	2 × 150 bp	2 × 300 bp	2 × 150 bp	2 × 150 bp	2 x 250
Max. output	1.2 Gb	7.5 Gb	15 Gb	120 Gb	300 Gb	6000 Gb
Estimated price	\$19,900	\$49,500	\$128,000	\$275,000	\$335,000	\$985,000

How Does Illumina Machine Work?



How Does Illumina Machine Work?



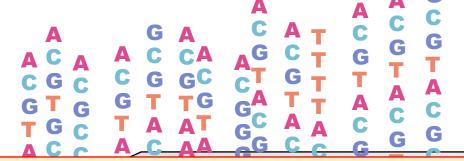
How Does Illumina Machine Work?





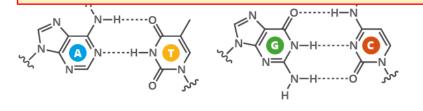






Check Illumina virtual tour:

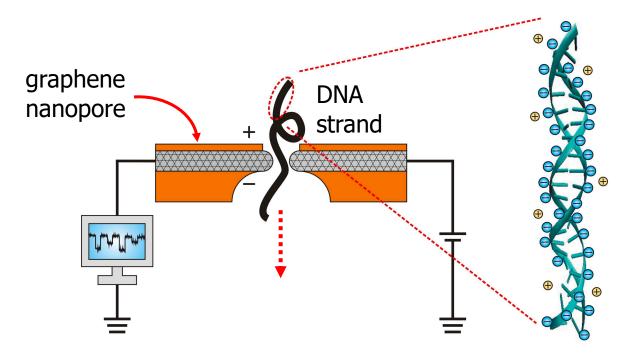
https://emea.illumina.com/systems/sequencing-platforms/iseq/tour.html



TTTAAAACGTA
CGTACTAGTACGT
GGGAGTACGTACGT

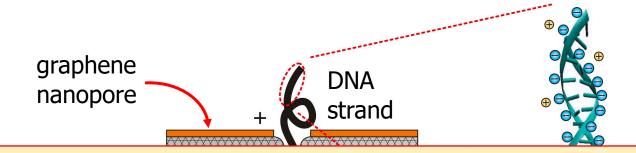
DNA fragment = Read

How Does Nanopore Machine Work?



- Nanopore is a nano-scale hole (<20nm).</p>
- In nanopore sequencers, an ionic current passes through the nanopores
- When the DNA strand passes through the nanopore, the sequencer measures the the change in current
- This change is used to identify the bases in the strand with the help of different electrochemical structures of the different bases

How Does Nanopore Machine Work?



Check Nanopore virtual tour:

https://nanoporetech.com/resource-centre/minion-video

measures the the change in current

 This change is used to identify the bases in the strand with the help of different electrochemical structures of the different bases

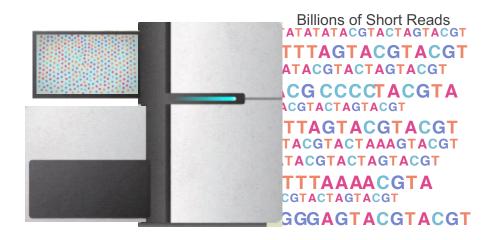


Common Disadvantages!

Regardless the sequencing machine,

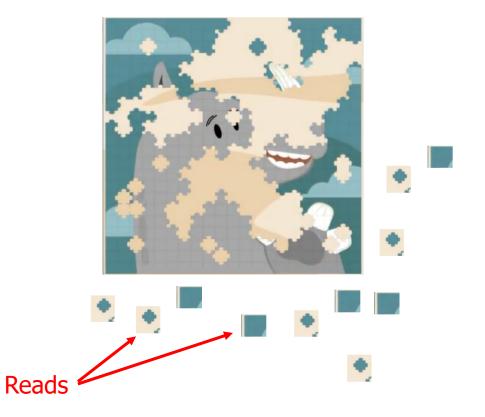
reads still lack information about their order and location

(which part of genome they are originated from)



Solving the Puzzle





https://www.pacb.com/smrt-science/smrt-sequencing/hifi-reads-for-highly-accurate-long-read-sequencing/

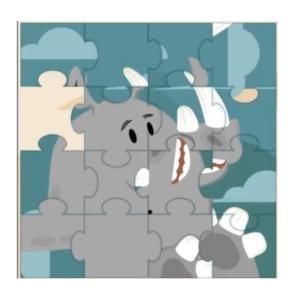


HTS Sequencing Output

Small pieces of a puzzle short reads (Illumina)



Large pieces of a puzzle long reads (ONT & PacBio)



Which sequencing technology is the best?

□ 100-300 bp

□ 500-2M bp

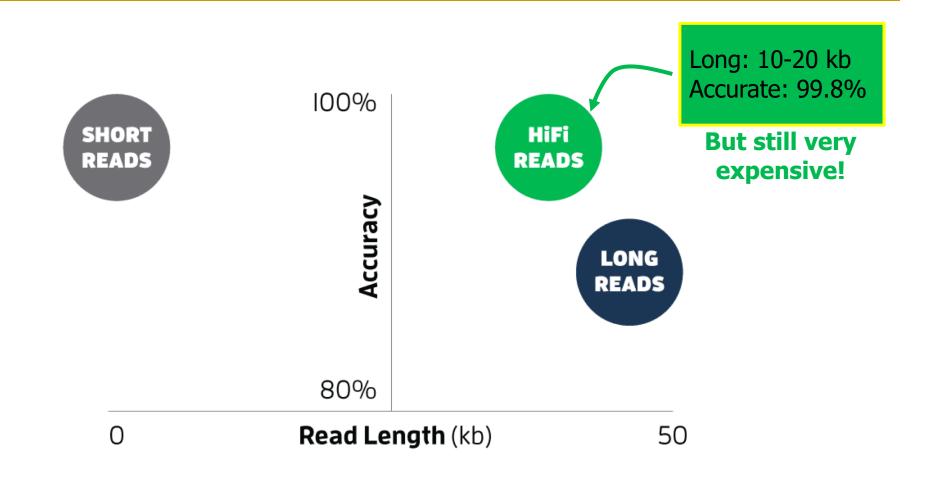
 \square low error rate (~0.1%)

☐ high error rate (~15%)

https://www.pacb.com/smrt-science/smrt-sequencing/hifi-reads-for-highly-accurate-long-read-sequencing/



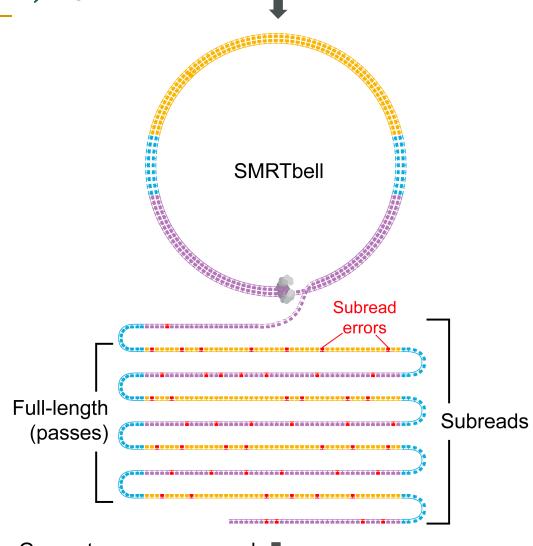
HiFi Reads (PacBio)



Wenger+, "<u>Accurate circular consensus long-read sequencing improves variant</u> detection and assembly of a human genome", *Nature Biotechnology*, 2019



HiFi Reads (PacBio)



Generate consensus read



Changes in sequencing technologies can render some read mapping algorithms irrelevant

Read Mapping in 111 pages!

In-depth analysis of 107 read mappers (1988-2020)

Mohammed Alser, Jeremy Rotman, Dhrithi Deshpande, Kodi Taraszka, Huwenbo Shi, Pelin Icer Baykal, Harry Taegyun Yang, Victor Xue, Sergey Knyazev, Benjamin D. Singer, Brunilda Balliu, David Koslicki, Pavel Skums, Alex Zelikovsky, Can Alkan, Onur Mutlu, Serghei Mangul

"<u>Technology dictates algorithms: Recent developments in read alignment</u>" Genome Biology, 2021

Source code

Alser et al. Genome Biology (2021) 22:249 https://doi.org/10.1186/s13059-021-02443-7

Genome Biology

REVIEW Open Access

Technology dictates algorithms: recent developments in read alignment



Mohammed Alser^{1,2,3†}, Jeremy Rotman^{4†}, Dhrithi Deshpande⁵, Kodi Taraszka⁴, Huwenbo Shi^{6,7}, Pelin Icer Baykal⁸, Harry Taegyun Yang^{4,9}, Victor Xue⁴, Sergey Knyazev⁸, Benjamin D. Singer^{10,11,12}, Brunilda Balliu¹³, David Koslicki^{14,15,16}, Pavel Skums⁸, Alex Zelikovsky^{8,17}, Can Alkan^{2,18}, Onur Mutlu^{1,2,3†} and Serghei Mangul^{5*†}

Feedback From Our Community!



James Ferguson

@Psy Fer

This is awesome! I've got my evening reading sorted.



Stéphane Le Crom @slecrom

Very complete article on the evolution of read alignment algorithms. #NGS #genomics



Svetlana Gorokhova
@SGorokhova

An impressive overview of read alignment methods over the last three decades



BContrerasMoreira @BrunoContrerasM · Sep 10

Replying to @mealser @GenomeBiology and 3 others

Buen hilo de repaso sobre la evolución de los algoritmos de alineamiento de secuencias a medida que ha mejorado la tecnología de secuenciación

Looking forward, Will we be able to read the entire genome sequence?

P&S Mobile Genomics

Lecture 2:

Introduction to Sequencing

Dr. Mohammed Alser



ETH Zurich
Spring 2022
15 March 2022



