Tehnologiile genomice in cercetarea clinica neuropsihiatrica

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Sorina Mihaela Papuc

Planul prezentarii

- Genom uman - Genomica – elemente introductive
- Tehnici de investigatie genomica
- Cercetarea aplicativa in genetica neuropsihiatrica - exemple clinice -

Genom uman - Genomica – elemente introductive

SCIENCE

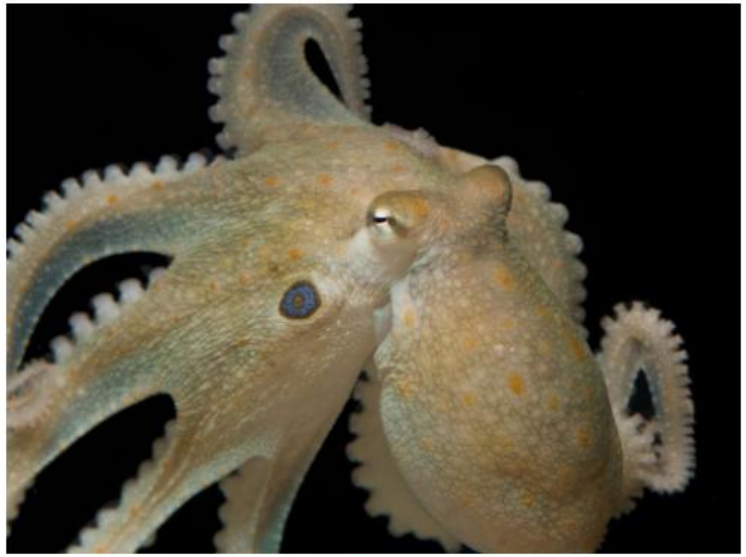
Ecstasy Turns Antisocial Octopuses Into Lovestruck Cuddle Buddies—Just Like Us

The genetic and neurological similarities between octopuses and humans shed light on how creatures became social beings

Rachael Lallensack

Former Assistant Editor, Science and Innovation

September 20, 2018



By studying the genome of a kind of octopus not known for its friendliness toward its peers, then testing its behavioral reaction to a popular mood-altering drug called MDMA or 'ecstasy,' scientists say they have found preliminary evidence of an evolutionary link between the social behaviors of the sea creature and humans, species separated b

https://www.smithsonianmag.com/science-nature/ecstasy-turns-antisocial-octopuses-lovestruck-cuddle-buddiesjust-us-180970363/

In deep water with Gül Dölen

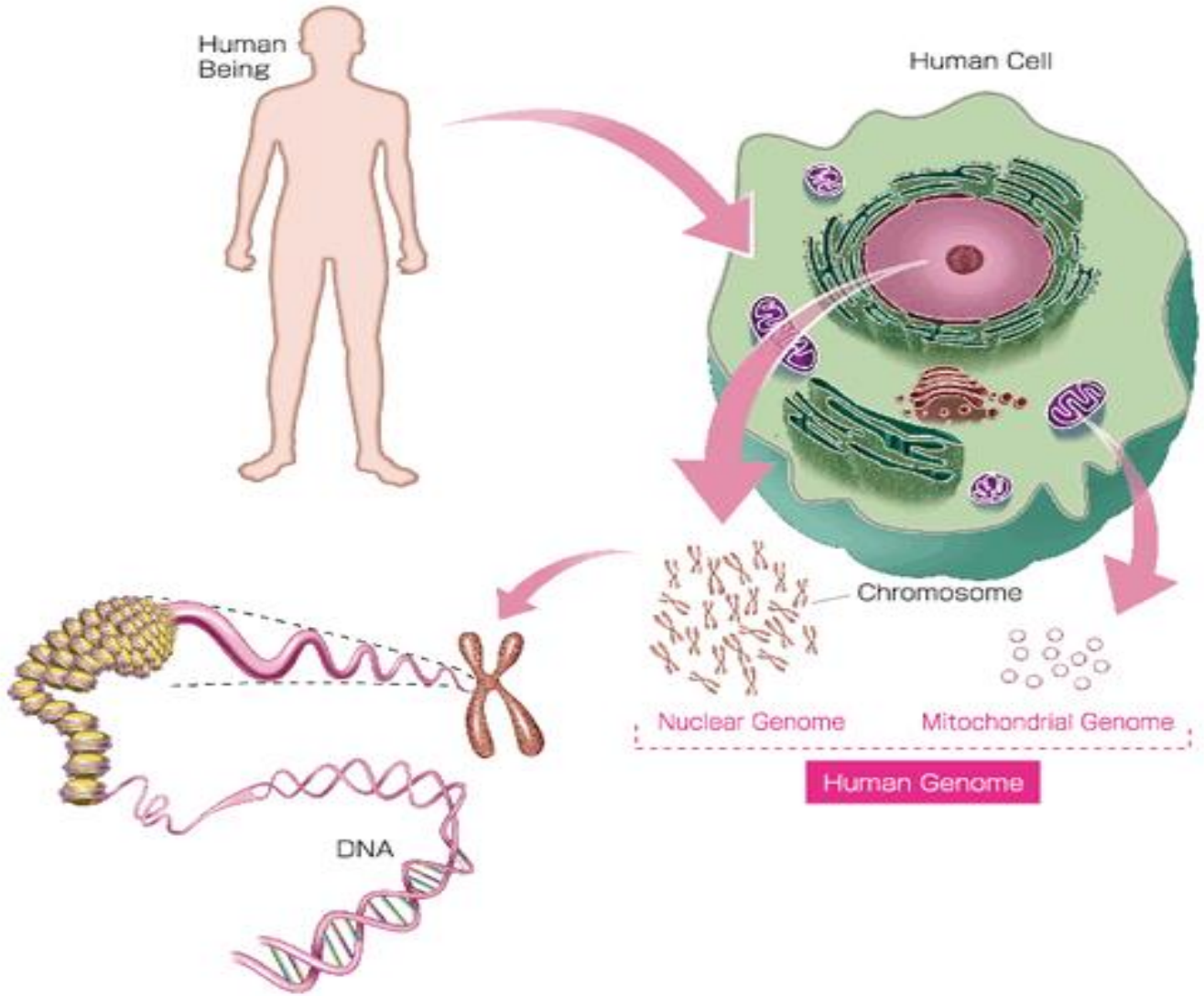
as both exhilarating and scary, and she quickly secured three private foundation grants, including a prestigious **Searle Scholarship**, to study autism via the brain circuitry of social reward — the positive feelings that motivate people and animals to be social. But the bigger money and recognition of National Institutes of Health (NIH) research grants proved more elusive. Her department and the university tweaked budgets to help keep her staff intact and the lab running, which felt "wonderful, on one hand," she says, but "on the other hand, it also felt like, God, I'm an immigrant — we don't rack up credit card debt." She wondered how she could really be perceived as a leader if she couldn't financially stabilize her team.

When Dölen reached her 10th NIH rejection, she began to fear she might not make it as a lab head, or even as a scientist. Her outside-the-box thinking had yielded impactful findings during her graduate and postdoc years, but her approach didn't seem to fly with the NIH, and she felt the mounting pressure to prove that she could run a world-class lab. "Even if the department isn't literally putting pressure on you to get it done or get out, it's implied," she says.

Grant rejections are part of doing science, and the need to chase money for researc

https://doi.org/10.53053/BKRC2077

GENOM – setul complet de ADN, incluzand totalitatea genelor, al unui organism

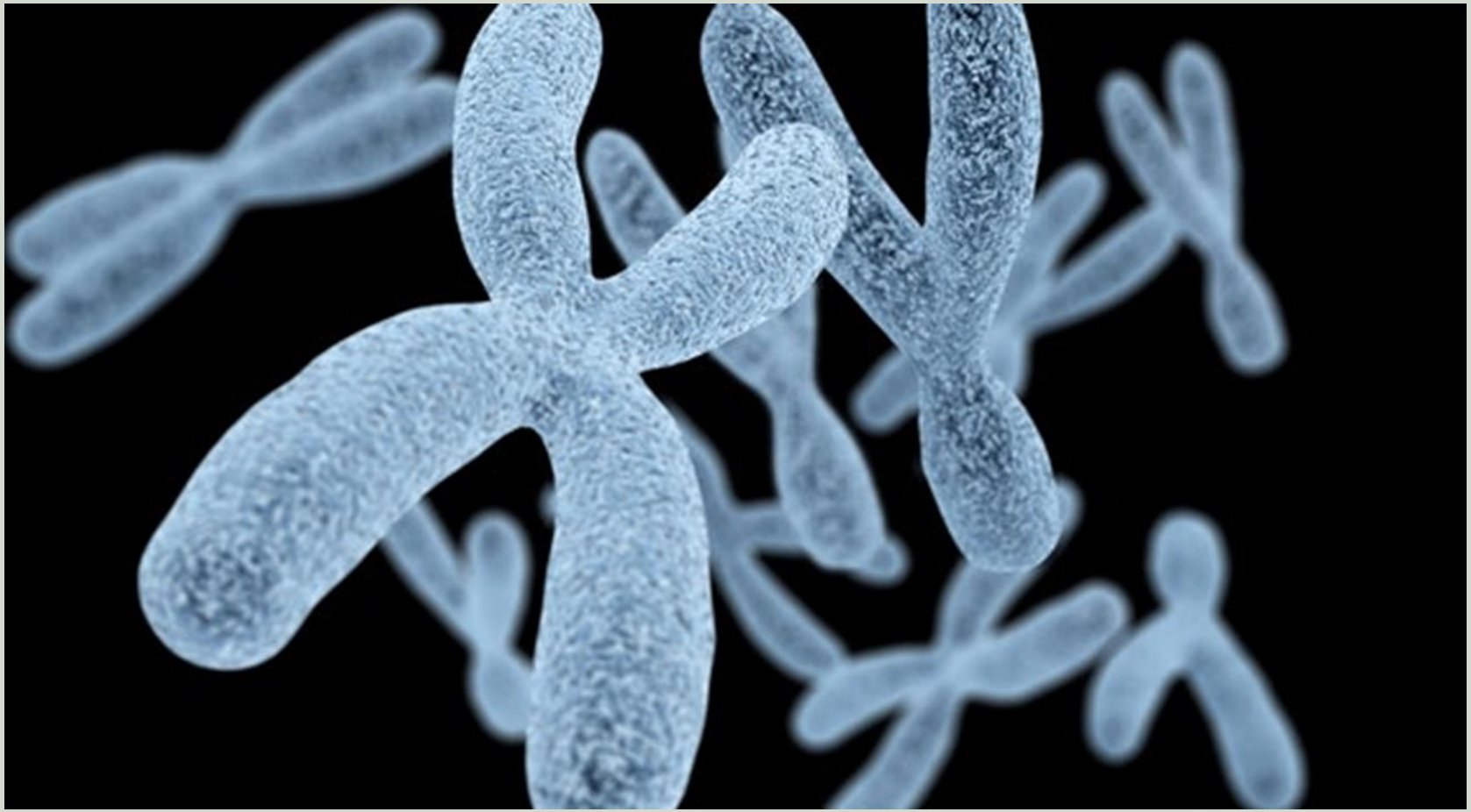


Genomul uman nuclear
– 24 molecule cromozomiale diferite;
> 3 miliarde de perechi de baze

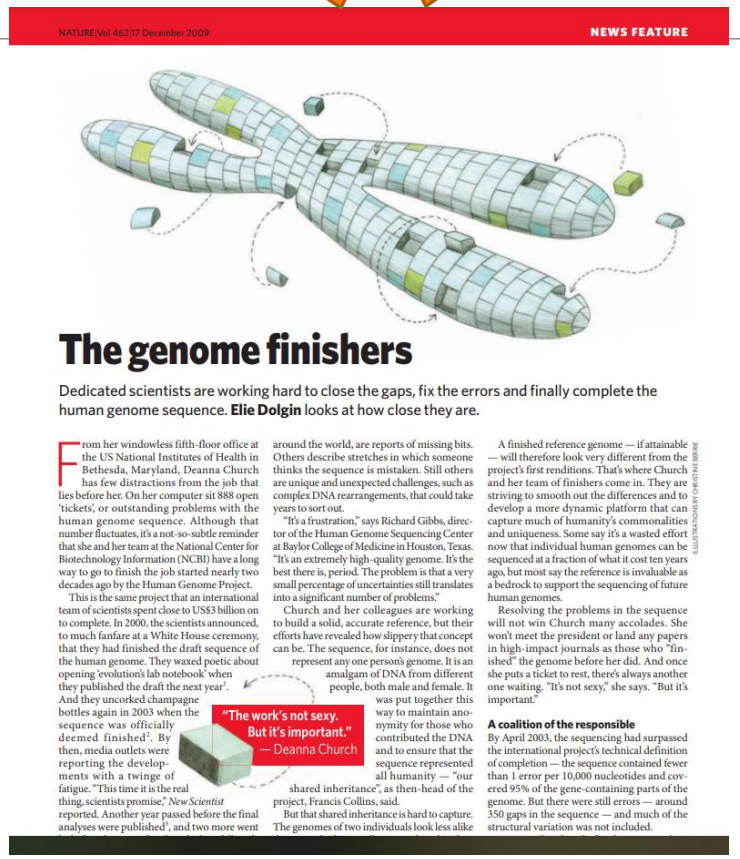
Genomul uman mitocondrial ~ 16.500 perechi de baze

https://mastergolflivestream.com

PROIECTE DEDICATE GENOMULUI UMAN



The Human Genome Project
1990-2003



NATURE|Vol 462|17 December 2009 NEWS FEATURE

The genome finishers

Dedicated scientists are working hard to close the gaps, fix the errors and finally complete the human genome sequence. **Elie Dolgin** looks at how close they are.

From her windowless fifth-floor office at the US National Institutes of Health in Bethesda, Maryland, Deanna Church has few distractions from the job that lies before her. On her computer sit 888 open 'tickets', or outstanding problems with the human genome sequence. Although that number fluctuates, it's a not-so-subtle reminder that she and her team at the National Center for Biotechnology Information (NCBI) have a long way to go to finish the job started nearly two decades ago by the Human Genome Project.

This is the same project that an international team of scientists spent close to US$3 billion on to complete. In 2000, the scientists announced, to much fanfare at a White House ceremony, that they had finished the draft sequence of the human genome. They waxed poetic about opening 'evolution's lab notebook' when they published the draft the next year[1]. And they uncorked champagne bottles again in 2003 when the sequence was officially deemed finished[2]. By then, media outlets were reporting the developments with a twinge of fatigue. "This time it is the real thing, scientists promise," *New Scientist* reported. Another year passed before the final analyses were published[3], and two more went around the world, are reports of missing bits. Others describe stretches in which someone thinks the sequence is mistaken. Still others are unique and unexpected challenges, such as complex DNA rearrangements, that could take years to sort out.

"It's a frustration," says Richard Gibbs, director of the Human Genome Sequencing Center at Baylor College of Medicine in Houston, Texas. "It's an extremely high-quality genome. It's the best there is, period. The problem is that a very small percentage of uncertainties still translates into a significant number of problems."

Church and her colleagues are working to build a solid, accurate reference, but their efforts have revealed how slippery that concept can be. The sequence, for instance, does not represent any one person's genome. It is an amalgam of DNA from different people, both male and female. It was put together this way to maintain anonymity for those who contributed the DNA and to ensure that the sequence represented all humanity — "our shared inheritance", as then-head of the project, Francis Collins, said.

"The work's not sexy. But it's important." — Deanna Church

But that shared inheritance is hard to capture. The genomes of two individuals look less alike

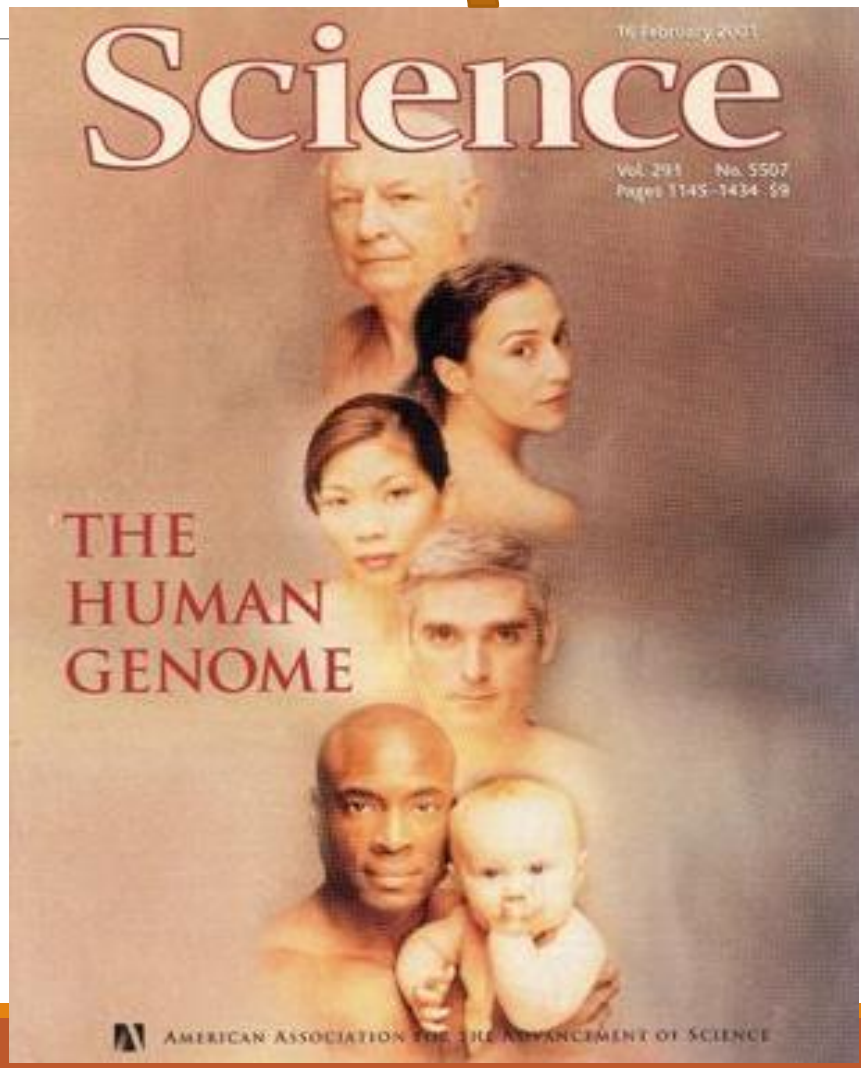
A finished reference genome — if attainable — will therefore look very different from the project's first renditions. That's where Church and her team of finishers come in. They are striving to smooth out the differences and to develop a more dynamic platform that can capture much of humanity's commonalities and uniqueness. Some say it's a wasted effort now that individual human genomes can be sequenced at a fraction of what it cost ten years ago, but most say the reference is invaluable as a bedrock to support the sequencing of future human genomes.

Resolving the problems in the sequence will not win Church many accolades. She won't meet the president or land any papers in high-impact journals as those who "finished" the genome before her did. And once she puts a ticket to rest, there's always another one waiting. "It's not sexy," she says. "But it's important."

A coalition of the responsible

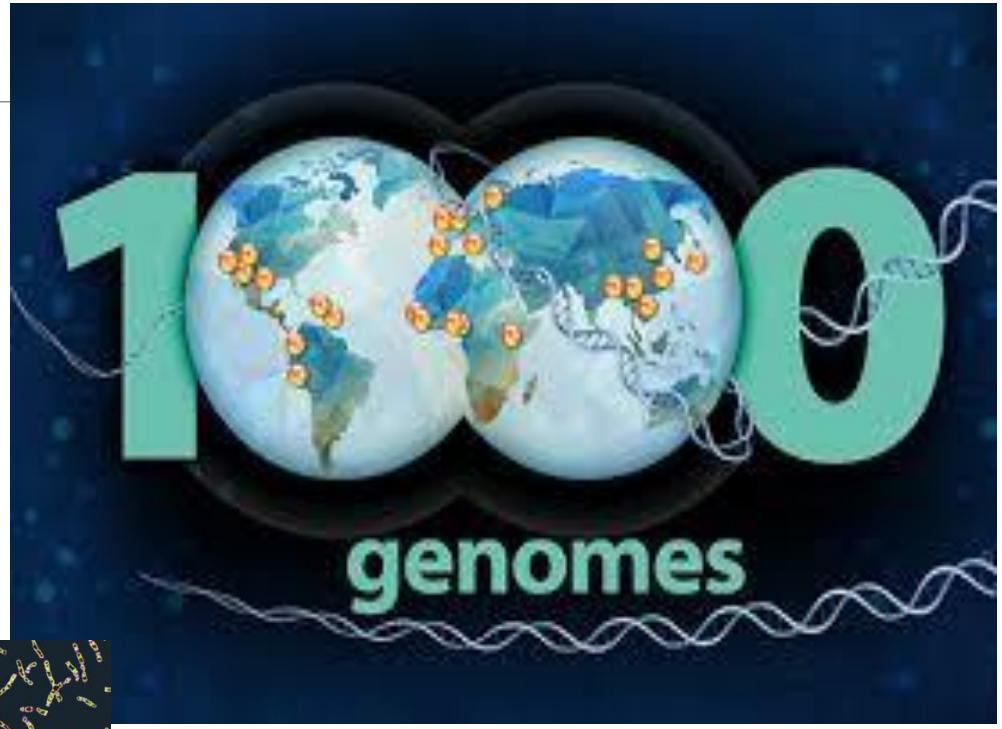
By April 2003, the sequencing had surpassed the international project's technical definition of completion — the sequence contained fewer than 1 error per 10,000 nucleotides and covered 95% of the gene-containing parts of the genome. But there were still errors — around 350 gaps in the sequence — and much of the structural variation was not included.

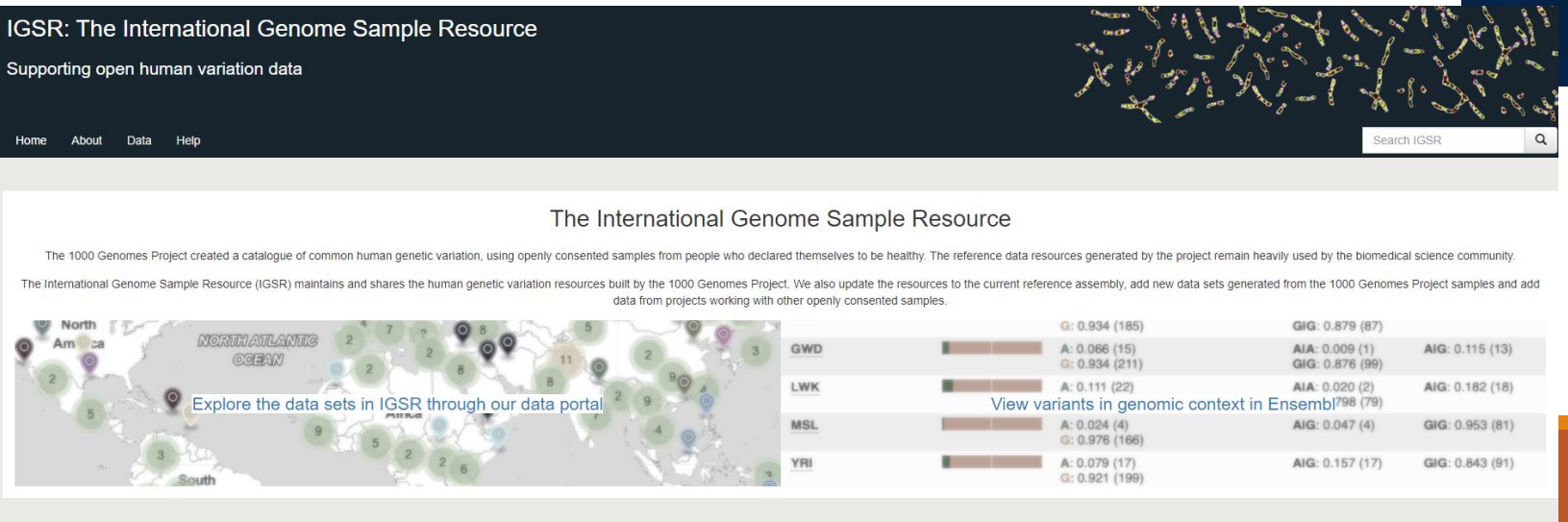
ILLUSTRATION BY CHRISTIAN BOHR



”The supersonic age of genomics”

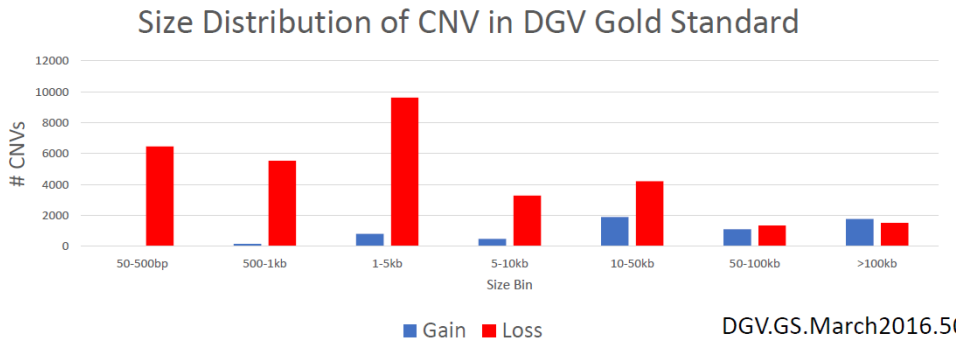
- Identificarea variatiilor de secventa cu frecventa de minim 1% in populatia studiata.
- Primul proiect care si-a propus secventierea genomului unui numar mare de indivizi cu scopul de a studia variabilitatea genetica umana



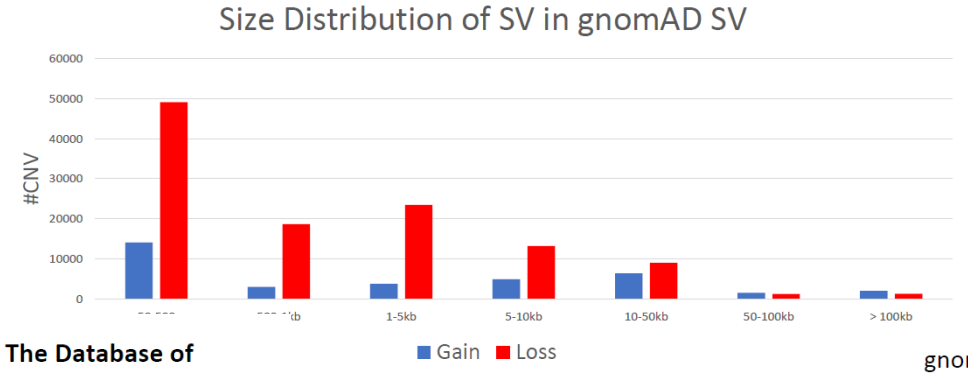


http://www.internationalgenome.org/about /

Genomul uman prezinta o variabilitate inter-individuala importanta



DGV.GS.March2016.50percent.GainLossSep.Final.hg19.gff3



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https://gnomad.broadinstitute.org/

http://dgv.tcag.ca/dgv/app/home

Nucleic Acids Research Advance Access published October 29, 2013

Nucleic Acids Research, 2013, 1–7
doi:10.1093/nar/gkt958

The database of genomic variants: a curated collection of structural variation in the human genome

Jeffrey R. MacDonald[1], Robert Ziman[1], Ryan K. C. Yuen[1], Lars Feuk[2,*] and Stephen W. Scherer[1,3,*]

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Received August 16, 2013; Revised September 25, 2013; Accepted September 27, 2013

Nucleic Acids Res. 2014 Jan;42. **The Database of Genomic Variants: a curated collection of structural variation in the human genome.**
MacDonald JR[1], Ziman R, Yuen RK, Feuk L, Scherer SW.

AOP, published online 3 February 2015; doi:10.1038/nrg3871

ANALYSIS

A copy number variation map of the human genome

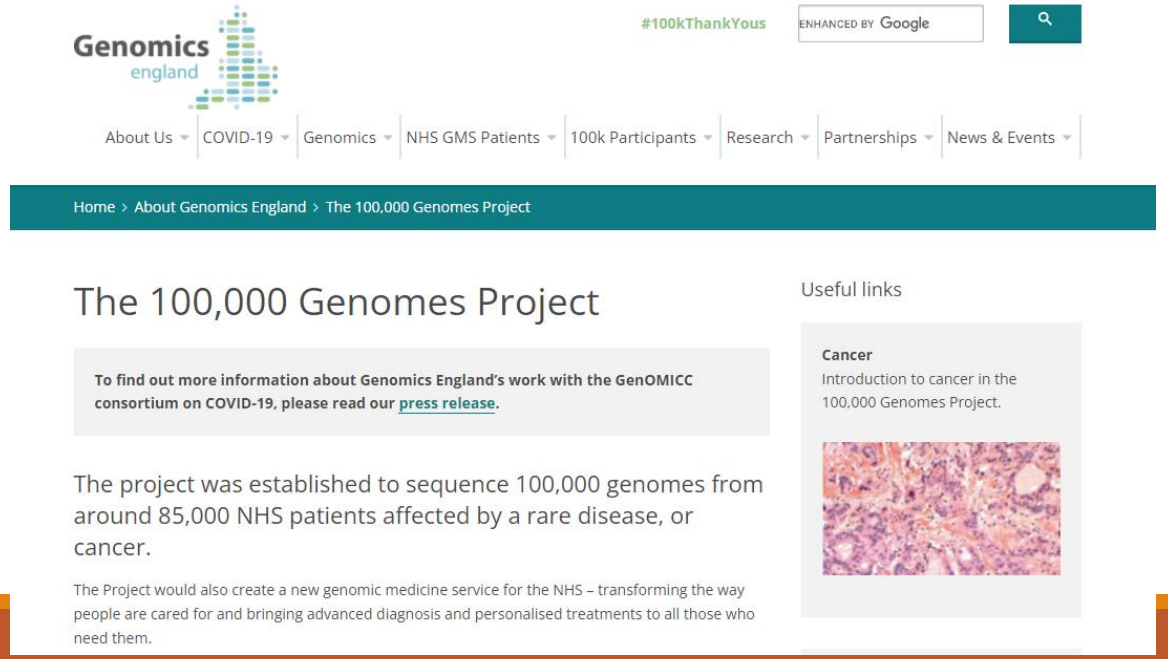
Mehdi Zarrei[1], Jeffrey R. MacDonald[1], Daniele Merico[1] and Stephen W. Scherer[1,2]

Abstract | A major contribution to the genome variability among individuals comes from deletions and duplications — collectively termed copy number variations (CNVs) — which alter the diploid status of DNA. These alterations may have no phenotypic effect, account for adaptive traits or can underlie disease. We have compiled published high-quality data on healthy individuals of various ethnicities to construct an updated CNV map of the human genome. Depending on the level of stringency of the map, we estimated that 4.8–9.5% of the genome contributes to CNV and found approximately 100 genes that can be completely deleted without producing apparent phenotypic consequences. This map will aid the interpretation of new CNV findings for both clinical and research applications.

Nat Rev Genet. 2015 Mar;16(3):172-83. doi: 10.1038/nrg3871.
A copy number variation map of the human genome.
Zarrei M[1], MacDonald JR[1], Merico D[1], Scherer SW[2].

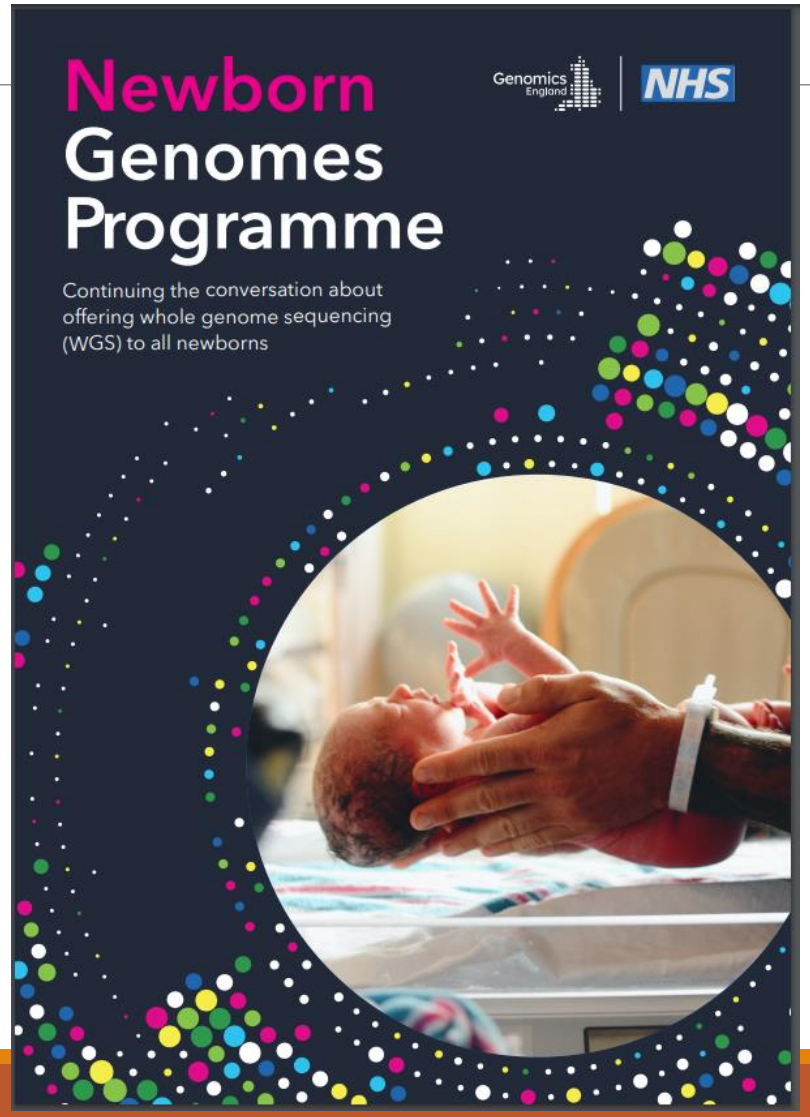
“The supersonic age of genomics”

85.000 de pacienti cu boli rare si cancere si membri ai familiilor acestora, totalizand 100.000 probe cu intregul genom secventiat.
Lansat in 2012 - finalizat in 2018



https://www.genomicsengland.co.uk/about-genomics-england/the-100000-genomes-project/

“The supersonic age of genomics”



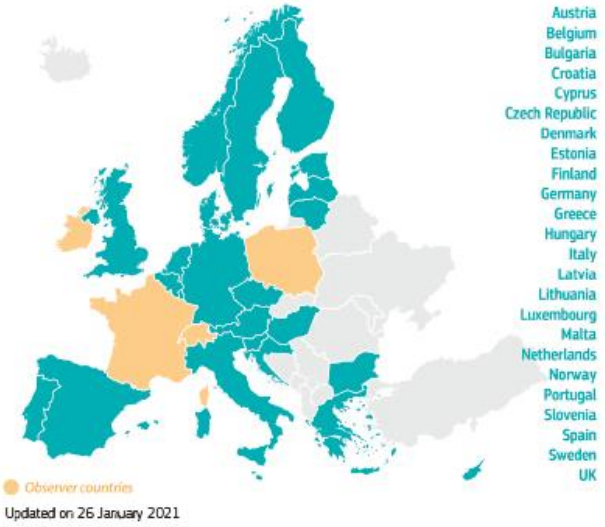
Anual, aproximativ 3000 de nou nascuti pot beneficia de interventii
medicale majore datorita investigatiei prin secventierea intregului genom (WGS)

1+MG and beyond

Initiativa *One million genomes* - 24 tari din spatiul european. Scop: cumularea datelor de secventiere de la 1 million de indivizi pana 2022 si acces comun.

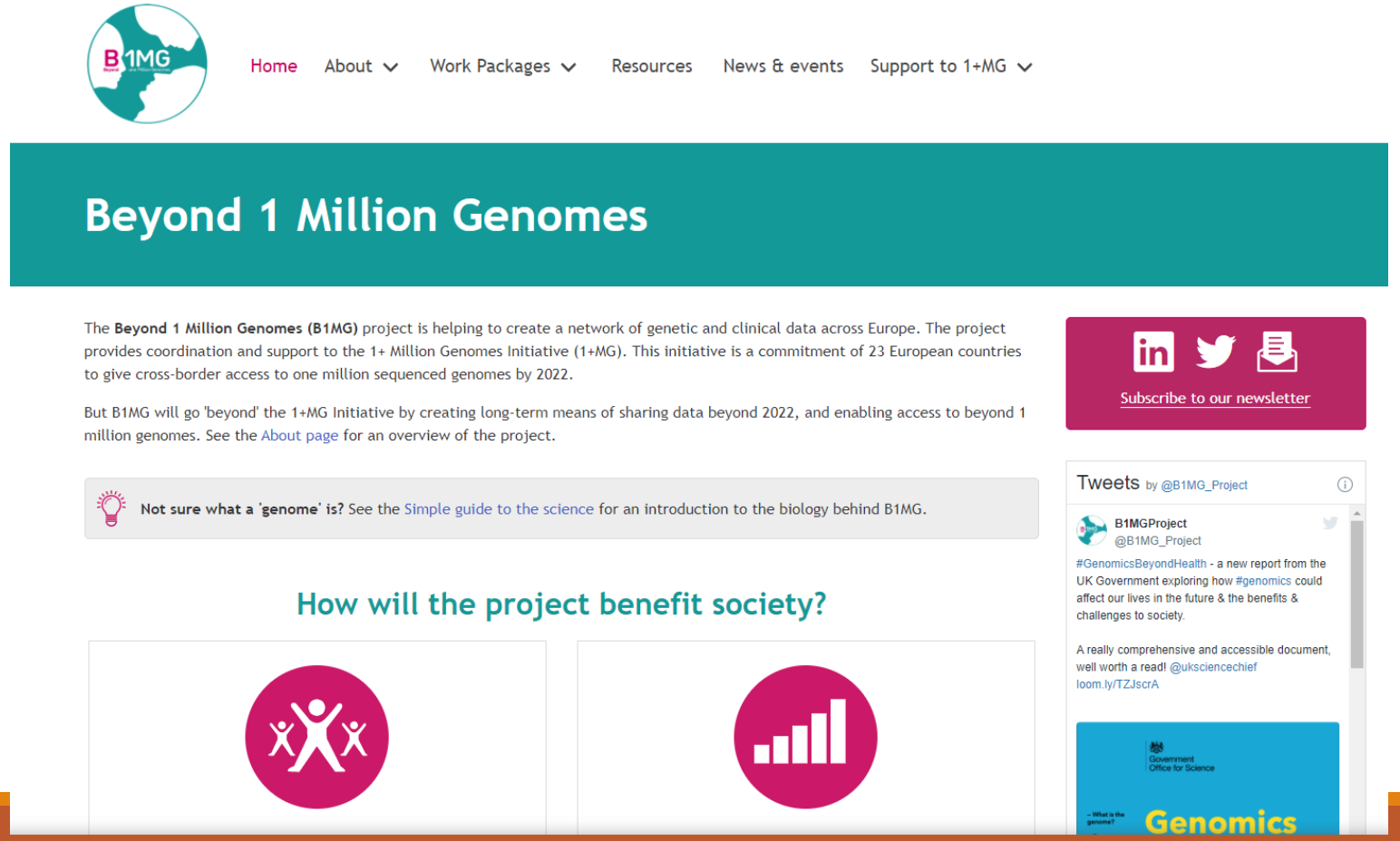
https://digital-strategy.ec.europa.eu/en/policies/1-million-genomes

24 countries have
signed the 1+MG Declaration
since 2018



“1+MG and beyond”

B1MG va continua intiativa 1+MG prin crearea de facilitate de partajare / accesare a datelor care sa fie functionale pe termen lung



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Genome assembly T2T-CHM13v2.0

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Reference sequence	RefSeq GCF_009914755.1
Submitted sequence	GenBank GCA_009914755.4
Taxon	*Homo sapiens* (human)
Synonym	hs1
Assembly type	haploid
Submitter	T2T Consortium
Date	Jan 24, 2022

SPECIAL SECTION COMPLETING THE HUMAN GENOME

RESEARCH ARTICLE

2022

HUMAN GENOMICS

The complete sequence of a human genome

Sergey Nurk[1†], Sergey Koren[1†], Arang Rhie[1†], Mikko Rautiainen[1†], Andrey V. Bzikadze[2], Alla Mikheenko[3], Mitchell R. Vollger[4], Nicolas Altemose[5], Lev Uralsky[6,7], Ariel Gershman[8], Sergey Aganezov[9‡], Savannah J. Hoyt[10], Mark Diekhans[11], Glennis A. Logsdon[4], Michael Alonge[9], Stylianos E. Antonarakis[12], Matthew Borchers[13], Gerard G. Bouffard[14], Shelise Y. Brooks[14], Gina V. Caldas[15], Nae-Chyun Chen[9], Haoyu Cheng[16,17], Chen-Shan Chin[18], William Chow[19], Leonardo G. de Lima[13], Philip C. Dishuck[4], Richard Durbin[19,20], Tatiana Dvorkina[3], Ian T. Fiddes[21], Giulio Formenti[22,23], Robert S. Fulton[24], Arkarachai Fungtammasan[18], Erik Garrison[11,25], Patrick G. S. Grady[10], Tina A. Graves-Lindsay[26], Ira M. Hall[27], Nancy F. Hansen[28], Gabrielle A. Hartley[10], Marina Haukness[11], Kerstin Howe[19], Michael W. Hunkapiller[29], Chirag Jain[1,30], Miten Jain[11], Erich D. Jarvis[22,23], Peter Kerpedjiev[31], Melanie Kirsche[9], Mikhail Kolmogorov[32], Jonas Korlach[29], Milinn Kremitzki[26], Heng Li[16,17], Valerie V. Maduro[33], Tobias Marschall[34], Ann M. McCartney[1], Jennifer McDaniel[35], Danny E. Miller[4,36], James C. Mullikin[14,28], Eugene W. Myers[37], Nathan D. Olson[35], Benedict Paten[11], Paul Peluso[29], Pavel A. Pevzner[32], David Porubsky[4], Tamara Potapova[13], Evgeny I. Rogaev[6,7,38,39], Jeffrey A. Rosenfeld[40], Steven L. Salzberg[9,41], Valerie A. Schneider[42], Fritz J. Sedlazeck[43], Kishwar Shafin[11], Colin J. Shew[44], Alaina Shumate[45], Ying Sims[19], Arian F. A. Smit[46], Daniela C. Soto[44], Ivan Sovic[29,46], Jessica M. Storer[45], Aaron Streets[5,47], Beth A. Sullivan[48], Françoise Thibaud-Nissen[42], James Torrance[19], Justin Wagner[35], Brian P. Walenz[1], Aaron Wenger[29], Jonathan M. D. Wood[19], Chunlin Xiao[42], Stephanie M. Yan[49], Alice C. Young[14], Samantha Zarate[9], Urvashi Surti[50], Rajiv C. McCoy[49], Megan Y. Dennis[44], Ivan A. Alexandrov[1,7,51], Jennifer L. Gerton[13,52], Rachel J. O'Neill[10], Winston Timp[8,41], Justin M. Zook[35], Michael C. Schatz[9,49], Evan E. Eichler[4,53✉], Karen H. Miga[11,54✉], Adam M. Phillippy[1✉]

Since its initial release in 2000, the human reference genome has covered only the euchromatic fraction of the genome, leaving important heterochromatic regions unfinished. Addressing the remaining 8% of the genome, the Telomere-to-Telomere (T2T) Consortium presents a complete 3.055 billion–base pair sequence of a human genome, T2T-CHM13, that includes gapless assemblies for all chromosomes except Y, corrects errors in the prior references, and introduces nearly 200 million base pairs of sequence containing 1956 gene predictions, 99 of which are predicted to be protein coding. The completed regions include all centromeric satellite arrays, recent segmental duplications, and the short arms of all five acrocentric chromosomes, unlocking these complex regions of the genome to variational and functional studies.

The current human reference genome was released by the Genome Reference Consortium (GRC) in 2013 and most recently patched in 2019 (GRCh38.p13) (*1*). This reference traces its origin to the publicly funded Human Genome Project (*2*) and has been continually improved over the past two decades. Unlike the competing Celera effort (*3*) and most modern sequencing projects based on "shotgun" sequence assembly (*4*), the GRC assembly was constructed from sequenced bacterial artificial chromosomes (BACs) that were ordered and oriented along the human genome by means of radiation hybrid, genetic linkage, and fingerprint maps. However, limitations of BAC cloning led to an underrepresentation of repetitive sequences, and the opportunistic assembly of BACs derived from multiple individuals resulted in a mosaic of haplotypes. As a result, several GRC assembly gaps are unsolvable because of incompatible structural polymorphisms on their flanks, and many other repetitive and polymorphic regions were left unfinished or incorrectly assembled (*5*).

The GRCh38 reference assembly contains 151 mega–base pairs (Mbp) of unknown sequence distributed throughout the genome, including pericentromeric and subtelomeric regions, recent segmental duplications, ampliconic gene arrays, and ribosomal DNA (rDNA) arrays, all of which are necessary for fundamental cellular processes (Fig. 1A). Some of the largest reference gaps include human satellite (HSat) repeat arrays and the short arms of all five acrocentric chromosomes, which are represented in GRCh38 as multimegabase stretches of unknown bases (Fig. 1, B and C). In addition to these apparent gaps, other regions of GRCh38 are artificial or are otherwise incorrect. For example, the centromeric alpha satellite arrays are represented as computationally generated models of alpha satellite monomers to serve as decoys for resequencing analyses (*6*), and sequence assigned to the short arm of chromosome 21 appears falsely duplicated and poorly assembled (*7*). When compared with other human genomes, GRCh38 also shows a genome-wide deletion bias that is indicative of incomplete assembly (*8*). Despite finishing efforts from both the Human Genome Project (*9*) and GRC (*1*) that improved the quality of the reference, there was limited

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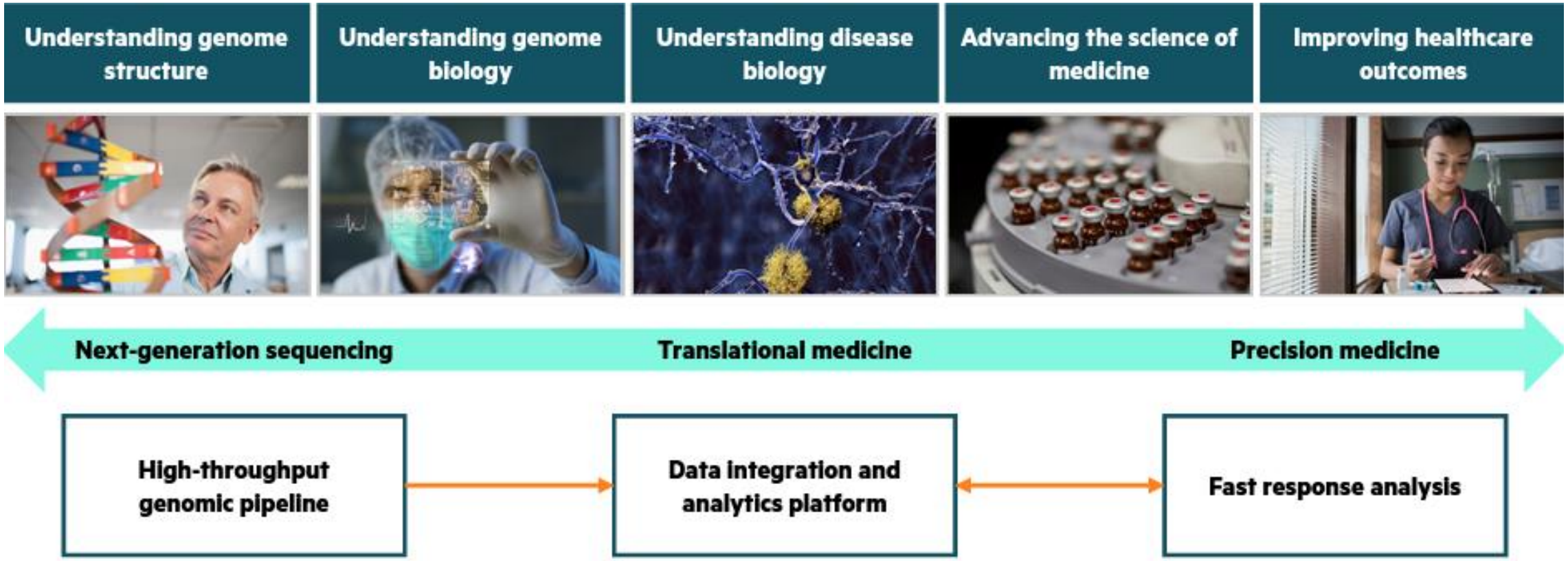
†These authors contributed equally to this work. ‡Present address: Oxford Nanopore Technologies Inc., Lexington, MA, USA.

Genomica


Understanding genome structure
Understanding genome biology
Understanding disease biology
Advancing the science of medicine
Improving healthcare outcomes
Next-generation sequencing
Translational medicine
Precision medicine
High-throughput genomic pipeline
Data integration and analytics platform
Fast response analysis

Variantele genetice/ genomice

Variantele genetice in bolile de neurodezvoltare (BND)

BND au o componenta genetica importanta

Arhitectura genetica este frecvent complexa si heterogena (exemple bolile din spectrul autist – TSA sau dizabilitatea intelectuala -DI)

In BND a fost descrisa o gama larga de anomalii genetice, de la variatii de secventa de mici dimensiuni pana la modificari cromozomiale importante, cu consecinte functionale extrem de diverse.

Efect fenotipic individual major - defecte genetice cu rol important etiopatogenetic

Efect fenotipic individual minor - defecte genetice cu rol de factori de risc (corelat cu prezenta altor factori de risc)

Variantele genetice in bolile de neurodezvoltare (BND)

Variantele genetice cu efect fenotipic individual major - sunt modificari in general *de novo*, cu penetranta inalta si consecinte fenotipice detrimentale

Fiecare dintre aceste anomalii este insa rara, fiind detectata la un procent restrans de pacienti (in special in TSA)

Variantele genetice in bolile de neurodezvoltare (BND)

Variantele comune au un efect fenotipic individual minor, actionand insa prin cumularea efectului cu cel al altor variante comune.

Sunt variante frecvente in populatie

Bolile neuropsihiatrice pot avea la baza o interactiune complexa intre variante rare si comune.

Box 5:

Bold Predictions for Human Genomics by 2030

Some of the most impressive genomics achievements, when viewed in retrospect, could hardly have been imagined ten years earlier. Here are ten bold predictions for human genomics that might come true by 2030. While most are unlikely to be fully attained, achieving one or more of these would require individuals to strive for something that currently seems out of reach. These predictions were crafted to be both inspirational and aspirational in nature, provoking discussions about what might be possible at The Forefront of Genomics in the coming decade.

1. Generating and analyzing a complete human genome sequence will be routine for any research laboratory, becoming as straightforward as carrying out a DNA purification.
2. The biological function(s) of every human gene will be known; for non-coding elements in the human genome, such knowledge will be the rule rather than the exception.
3. The general features of the epigenetic landscape and transcriptional output will be routinely incorporated into predictive models of the impact of genotype on phenotype.
4. Research in human genomics will have moved beyond population descriptors based on historic social constructs such as race.
5. Studies involving analyses of genome sequences and associated phenotypic information for millions of human participants will be regularly featured at school science fairs.
6. The regular use of genomic information will have transitioned from boutique to mainstream in all clinical settings, making genomic testing as routine as complete blood counts (CBCs).
7. The clinical relevance of all encountered genomic variants will be readily predictable, rendering the diagnostic designation "variant of uncertain significance (VUS)" obsolete.
8. A person's complete genome sequence along with informative annotations can be securely and readily accessible on their smartphone.
9. Individuals from ancestrally diverse backgrounds will benefit equitably from advances in human genomics.
10. Genomic discoveries will lead to curative therapies involving genomic modifications for dozens of genetic diseases.

Nature. 2020 October ;
586(7831): 683–692.
doi:10.1038/s41586-020-2817-4.

Take-home message

- Genomul uman este o structura dinamica cu o arhitectura complexa

- Datele existente despre genomul uman au creat resurse extrem de valoroase atat pentru cercetare cat si pentru medicina clinica

Tehnici de investigatie genomica

Tehnici de investigatie genomica

- **Tehnologii microarray**

  Microarray cromozomial - detecteaza CNV

  - Hibridizare comparative genomica bazata pe microarray – array-CGH
  - SNP array

  Microarray de genotipare - detecteaza SNP

  - SNP array

- **Secventiere de generatie urmatoare -** NGS (Next-generation sequencing)

Tehnologiile microarray

Microarray cromozomial

- Hibridizare comparativa genomica bazata pe microarray – array-CGH- detecteaza CNV-uri
- SNP array - detecteaza CNV-uri si regiuni de homozigotie cu numar normal de copii ADN
- Platforme hibride CGH+SNP - detecteaza CNV-uri si regiuni de homozigotie cu numar normal de copii ADN

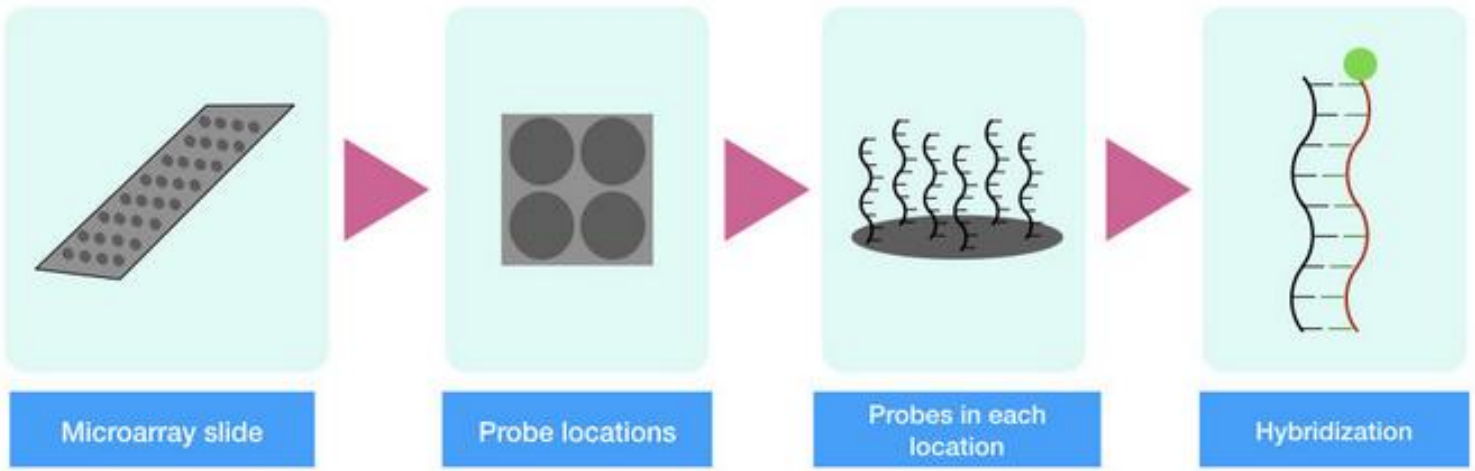
Microarray de genotipare - detecteaza SNP

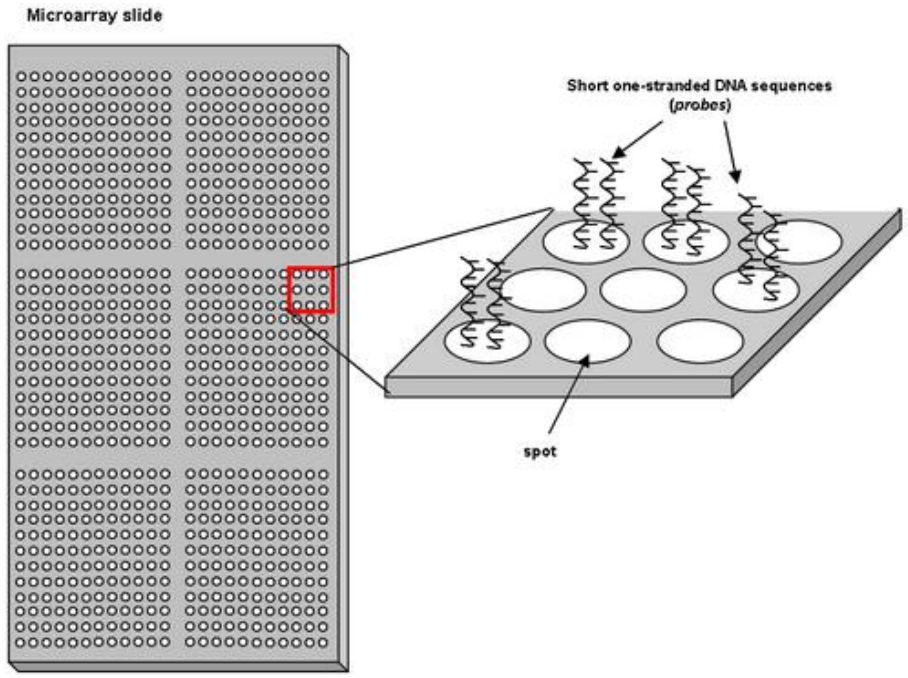
- SNP array

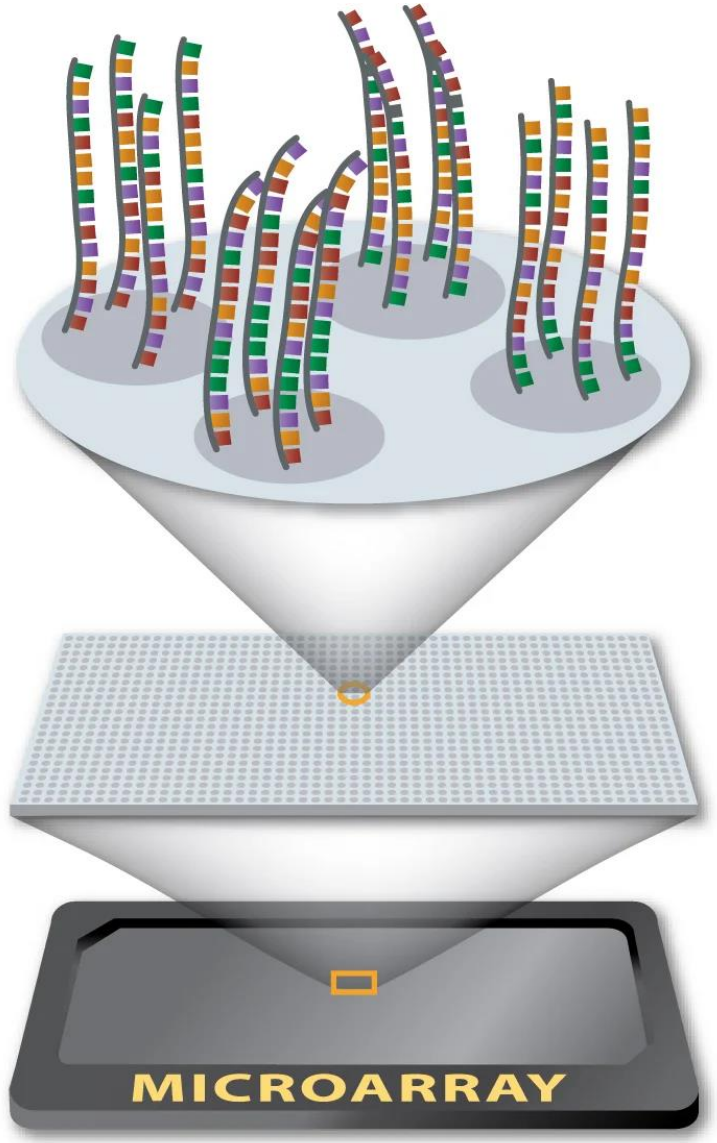
Array CGH – Platforma Agilent Technologies

Scanner microarray Agilent SureScan, Agilent Technologies

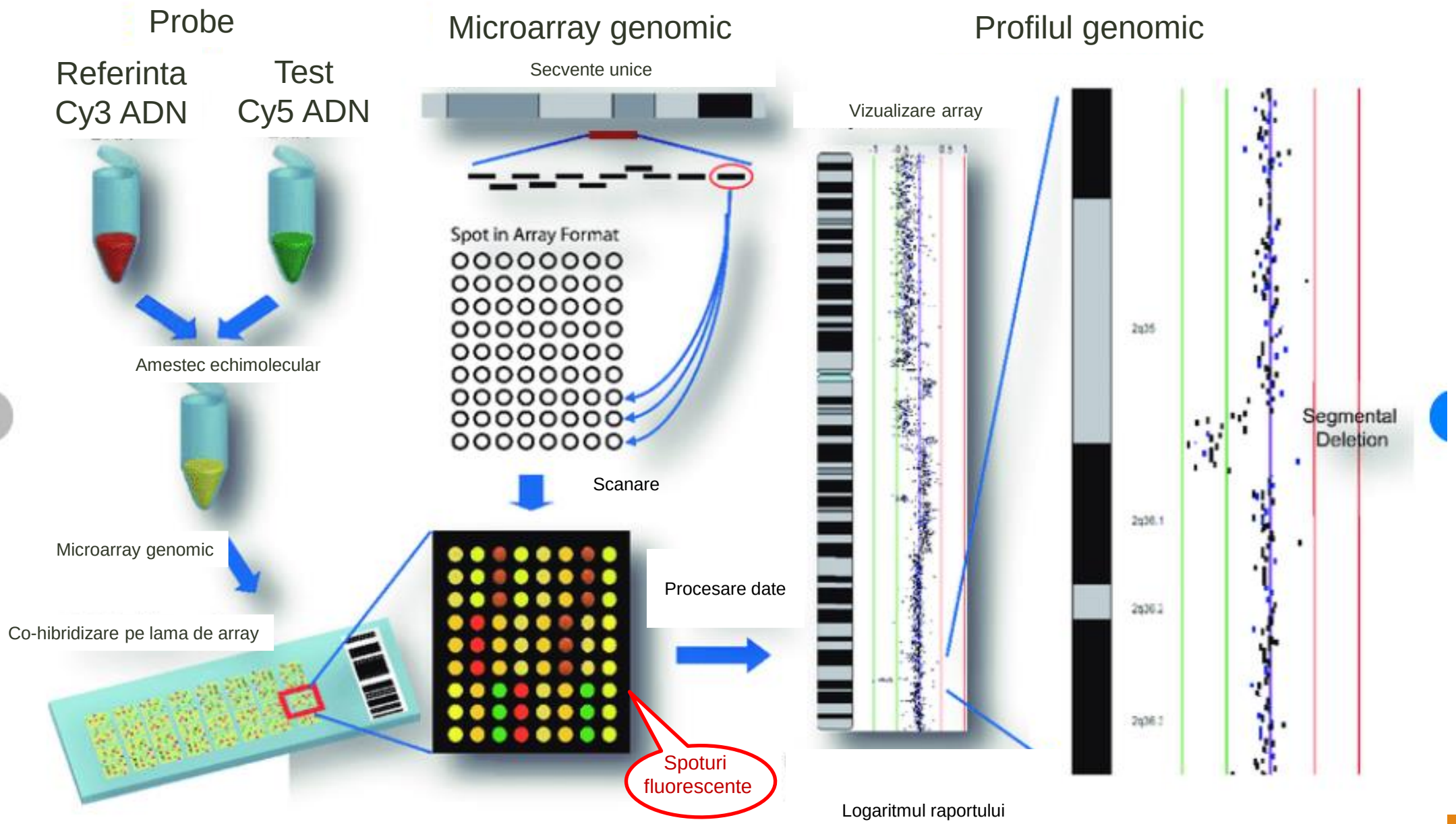
Array CGH – Platforma Agilent Technologies



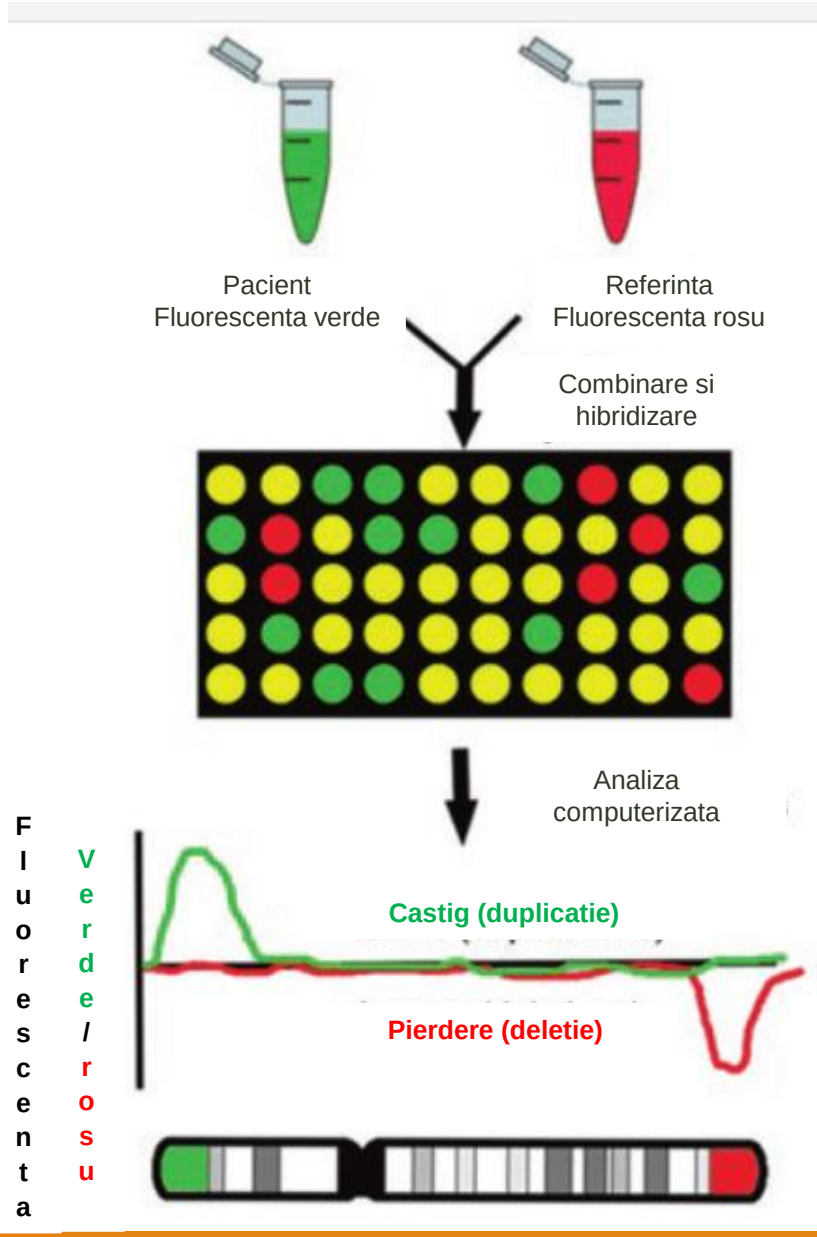




Array CGH – Platforma Agilent Technologies



Raj Chari et al., 2006



$\log_2$=Ri/Gi

0

0,583

-1

Karampetsou et al., 2014

Analiza datelor si clasificarea CNV-urilor

Clasificarea contributiei CNV-urilor la etiopatogeneza diferitelor afectiuni umane si interpretarea semnificatiei clinice pentru fenotipul patologic al pacientului investigat

50 YEARS OMIM
Human Genetics Knowledge for the World

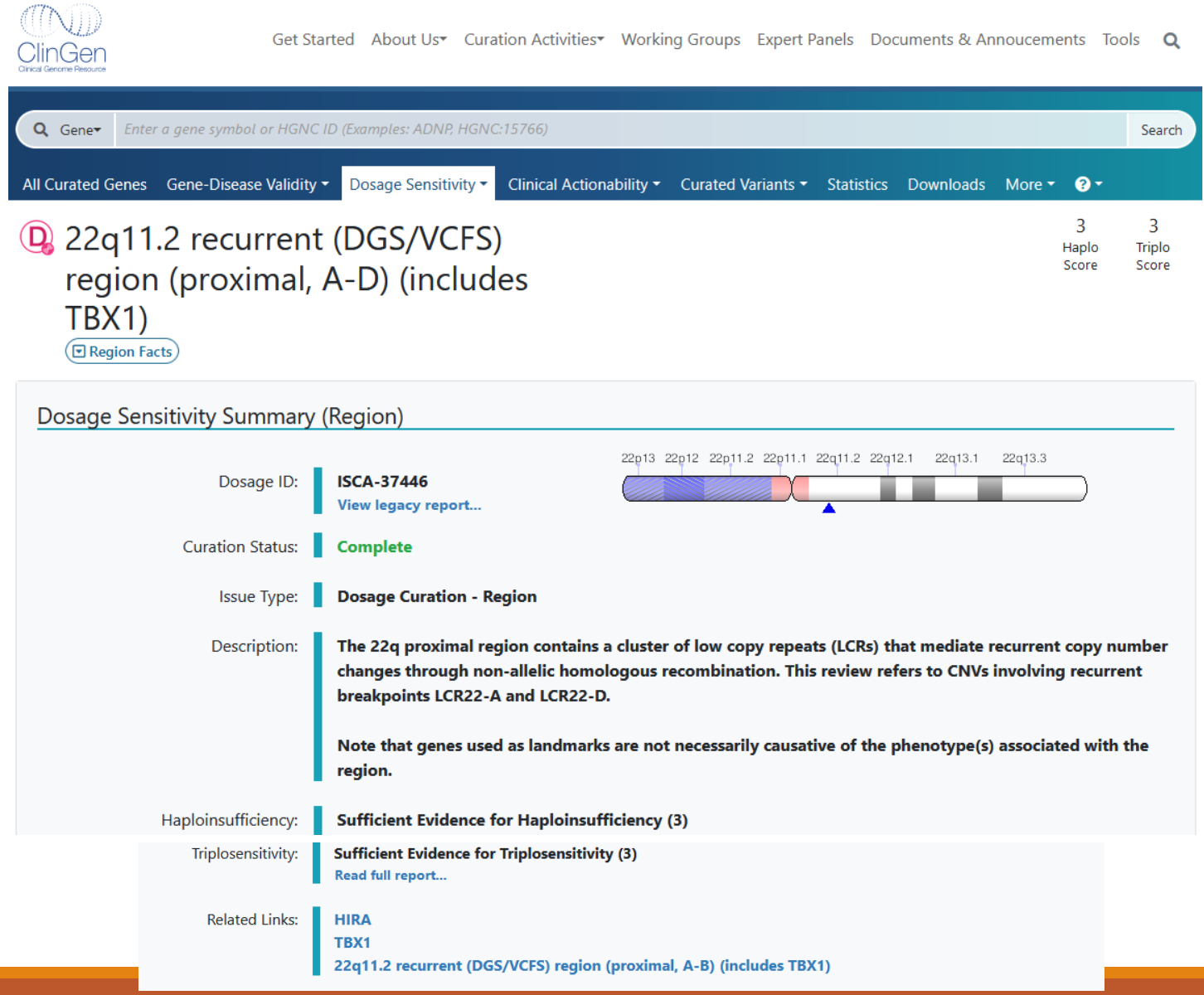
OMIM®

Online Mendelian Inheritance in Man®

An Online Catalog of Human Genes and Genetic Disorders

Updated February 16, 2022

Search OMIM for clinical features, phenotypes, genes, and more...



Analiza datelor si clasificarea CNV-urilor

Clasificare:

- Benign / Probabil benign
- VOUS
- Patologic / Probabil patologic

Guideline > Genet Med. 2011 Jul;13(7):680-5. doi: 10.1097/GIM.0b013e3182217a3a.

American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants

Hutton M Kearney [1], Erik C Thorland, Kerry K Brown, Fabiola Quintero-Rivera, Sarah T South, Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee

Affiliations + expand

PMID: 21681106 DOI: 10.1097/GIM.0b013e3182217a3a

ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013

Sarah T. South PhD, Charles Lee PhD, Allen N. Lamb PhD, Anne W. Higgins PhD & Hutton M. Kearney PhD on behalf of ; for the Working Group for the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee

Genetics in Medicine **15**, 901–909 (2013) | Cite this article

> Genet Med. 2020 Feb;22(2):245-257. doi: 10.1038/s41436-019-0686-8. Epub 2019 Nov 6.

Technical standards for the interpretation and reporting of constitutional copy–number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen)

Erin Rooney Riggs [1], Erica F Andersen [2 3], Athena M Cherry [4], Sibel Kantarci [5], Hutton Kearney [6], Ankita Patel [7], Gordana Raca [8], Deborah I Ritter [9], Sarah T South [10], Erik C Thorland [6], Daniel Pineda-Alvarez [11], Swaroop Aradhya [4 11], Christa Lese Martin [12]

Analiza datelor si clasificarea CNV-urilor

CONSENSUS STATEMENT

ACMG PRACTICE GUIDELINES | Genetics inMedicine

Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions

G. Bradley Schaefer, MD[1] and Nancy J. Mendelsohn, MD[2]; for the Professional Practice and Guidelines Committee

OPEN

Check for updates

International consensus recommendations on the diagnostic work-up for malformations of cortical development

Renske Oegema[1]✉, Tahsin Stefan Barakat[2], Martina Wilke[2], Katrien Stouffs[3], Dina Amrom[4,5], Eleonora Aronica[6,7], Nadia Bahi-Buisson[8], Valerio Conti[9], Andrew E. Fry[10,11], Tobias Geis[12], David Gomez Andres[13], Elena Parrini[9], Ivana Pogledic[14], Edith Said[14,15], Doriette Soler[16,17], Luis M. Valor[18], Maha S. Zaki[19], Ghayda Mirzaa[20,21], William B. Dobyns[20,21], Orly Reiner[21], Renzo Guerrini[9], Daniela T. Pilz[22], Ute Hehr[23], Richard J. Leventer[24], Anna C. Jansen[25], Grazia M. S. Mancini[2,26] and Nataliya Di Donato[27]✉

ARTICLE

Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies

David T. Miller,[1,*] Margaret P. Adam,[2,3] Swaroop Aradhya,[4] Leslie G. Biesecker,[5] Arthur R. Brothman,[6] Nigel P. Carter,[7] Deanna M. Church,[8] John A. Crolla,[9] Evan E. Eichler,[10] Charles J. Epstein,[11] W. Andrew Faucett,[2] Lars Feuk,[12] Jan M. Friedman,[13] Ada Hamosh,[14] Laird Jackson,[15] Erin B. Kaminsky,[2] Klaas Kok,[16] Ian D. Krantz,[17] Robert M. Kuhn,[18] Charles Lee,[19] James M. Ostell,[8] Carla Rosenberg,[20] Stephen W. Scherer,[21] Nancy B. Spinner,[17] Dimitri J. Stavropoulos,[22] James H. Tepperberg,[23] Erik C. Thorland,[24] Joris R. Vermeesch,[25] Darrel J. Waggoner,[26] Michael S. Watson,[27] Christa Lese Martin,[2] and David H. Ledbetter[2,*]

The American Journal of Human Genetics *86*, 749–764, May 14, 2010 749

SYSTEMATIC REVIEW | Genetics inMedicine

Open

Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders

Siddharth Srivastava, MD[1], Jamie A. Love-Nichols, MS, MPH[1], Kira A. Dies, ScM[1], David H. Ledbetter, PhD[2], Christa L. Martin, PhD[2], Wendy K. Chung, MD, PhD[3,4], Helen V. Firth, DM, FRCP[5,6], Thomas Frazier, PhD[7], Robin L. Hansen, MD[8], Lisa Prock, MD, MPH[1,9], Han Brunner, MD[10,11,12], Ny Hoang, MS[13,14,15], Stephen W. Scherer, PhD[14,15,16,17], Mustafa Sahin, MD PhD[1], David T. Miller, MD PhD[18] and the NDD Exome Scoping Review Work Group

GENETICS in MEDICINE | Volume 21 | Number 11 | November 2019

Secventiere de generatie urmatoare (generatia 2, 3, 4…)

First Generation

Sanger Sequencing
Maxam and Gilbert
Sanger Chain-termination

- Infer nucleotide identity using dNTPs then visualize with electrophoresis
- 500-1000 bp fragments

Second Generation
(Next Generation Sequencing)

454, Solexa,
Ion Torrent
Illumina

- High throughput from the parallelization of sequencing reactions
- ~50-500 bp fragments

Short-read sequencing

Third Generation

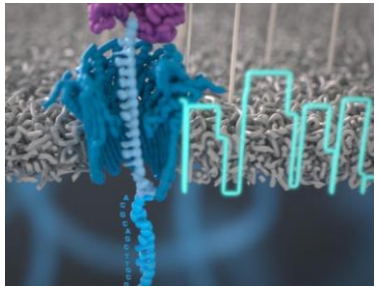
PacBio
Oxford Nanopore

- Sequence native DNA in real time with single-molecule resolution
- Tens of kb fragments, on average

Fourth Generation

MinION

GridION$_{X5}$



NANOPORE

Sequence native DNA in real time with single-molecule resolution
Up to 4 Mb fragments

Long-read sequencing

https://www.pacb.com/blog/the-evolution-of-dna-sequencing-tools/

Oxford Nanopore Technologies

BGI provides a comprehensive array of genomic sequencing solutions to meet your diverse research needs.

Offering the widest range of sequencing services available today, with applications in human, plant and animal, and microbial research, we can provide innovative, affordable, and reliable solutions for virtually any genomic challenge our customers and collaborators may face. Our facilities feature the industry's latest next-generation sequencing technologies and quality management systems.

Whole Human Genome Sequencing

Whole Exome Sequencing

Target Region Sequencing

Plant/Animal/Microbial Whole Genome Resequencing

Secventierea de generatie urmatoare

- **NGS denumita si Massively Parallel Sequencing** investigheaza, intr-un singur experiment si intr-o perioada scurta de timp, cu un pret scazut / nucleotide comparativ cu metodele clasice, numeroase tinte.
- **Tinte genomice**:
  - intreg genomul (Whole Genome sequencing)
  - intreg exomul (Whole Exome Sequencing)
  - paneluri de gene (Genes Panels Seqencing)
- **Analiza datelor furnizeaza informatii despre mutatii punctiforme,** indels, CNV si alte modificari structurale genomice

Secventierea de generatia a 2-a

illumina®


Ion PGM System

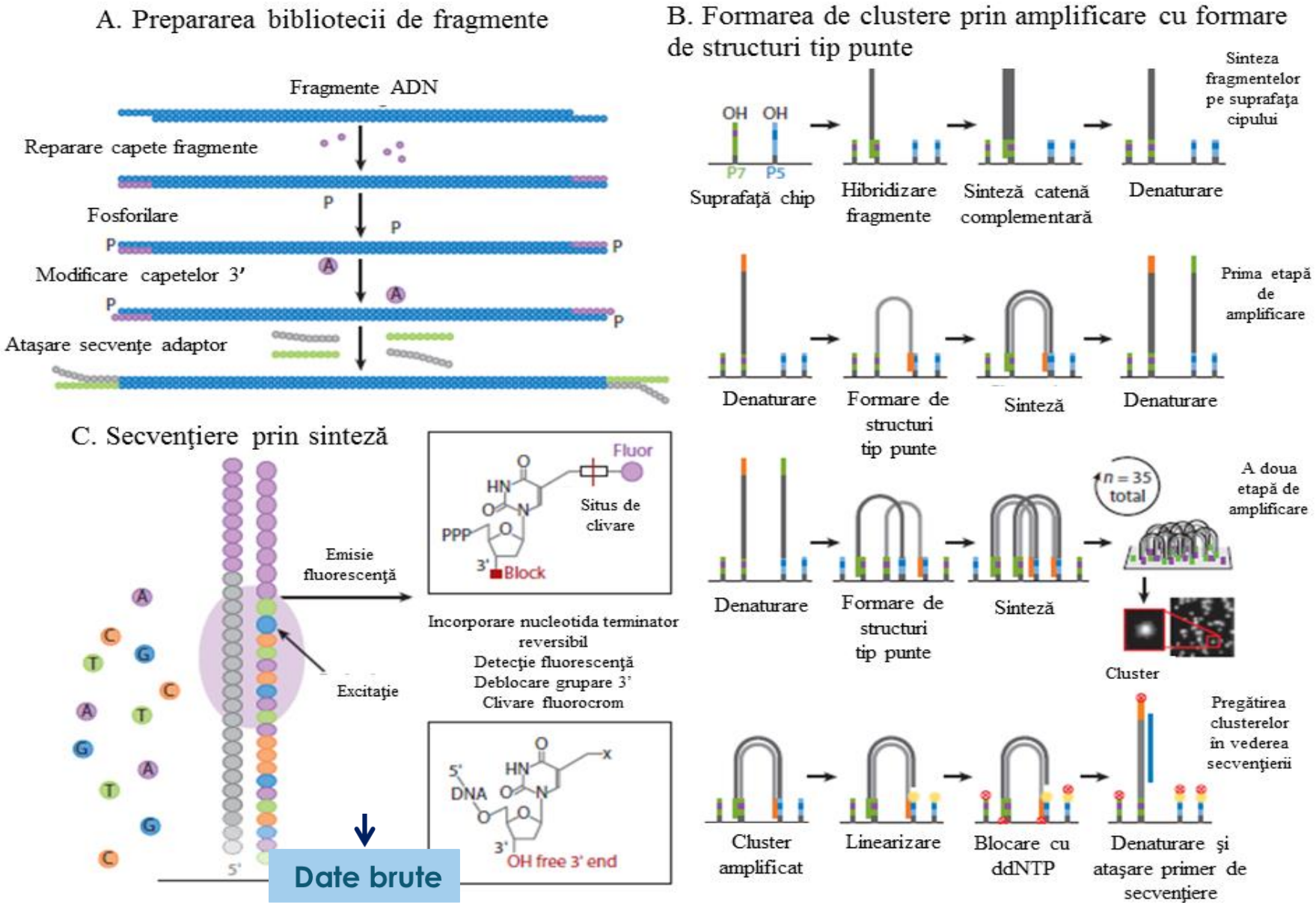

Ion GeneStudio S5 System


Ion GeneStudio S5 Plus System


Ion GeneStudio S5 Prime System

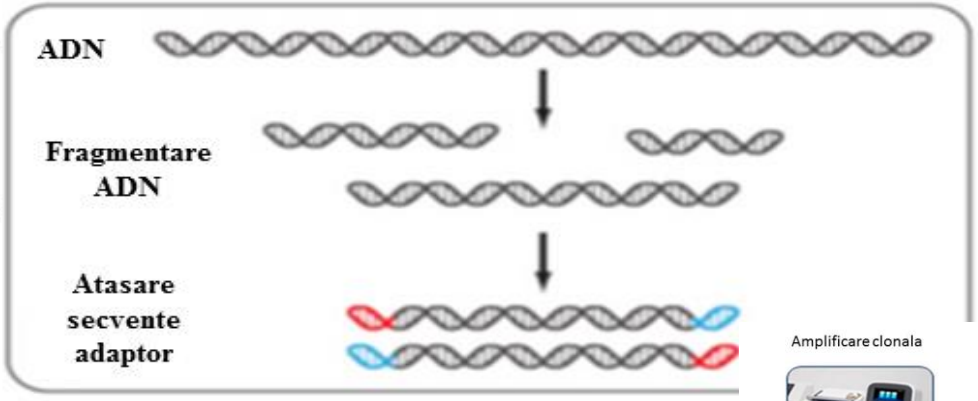
ThermoFisher SCIENTIFIC

illumina®

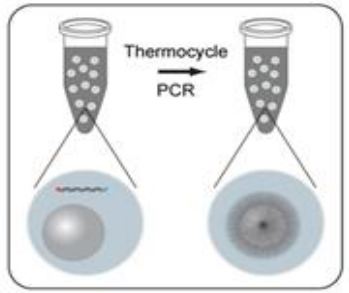


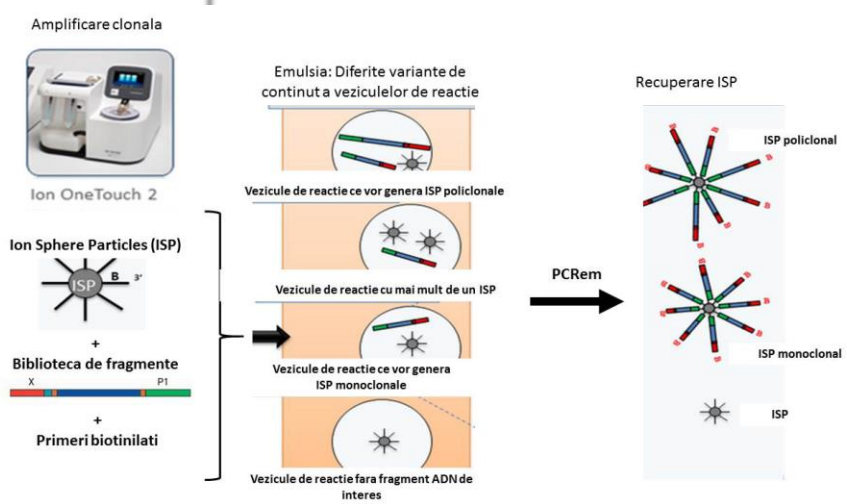
Prezentarea schematizata a principului secventierii de noua generatie - tehnologia Illumina (adaptare dupa Mardis, 2013)

A. Prepararea bibliotrcii de fragmente

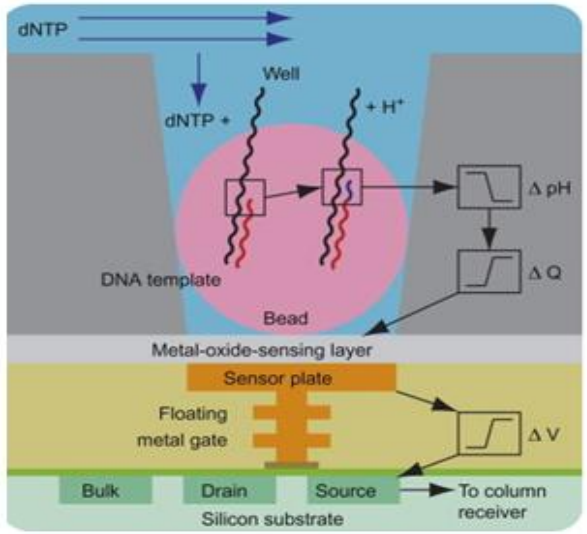


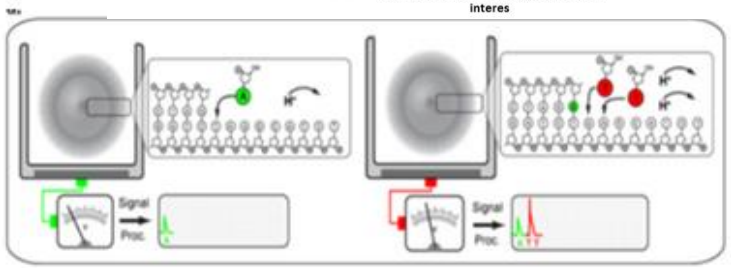
B. Amplificare clonala a fragmentelor prin PCR de emulsie





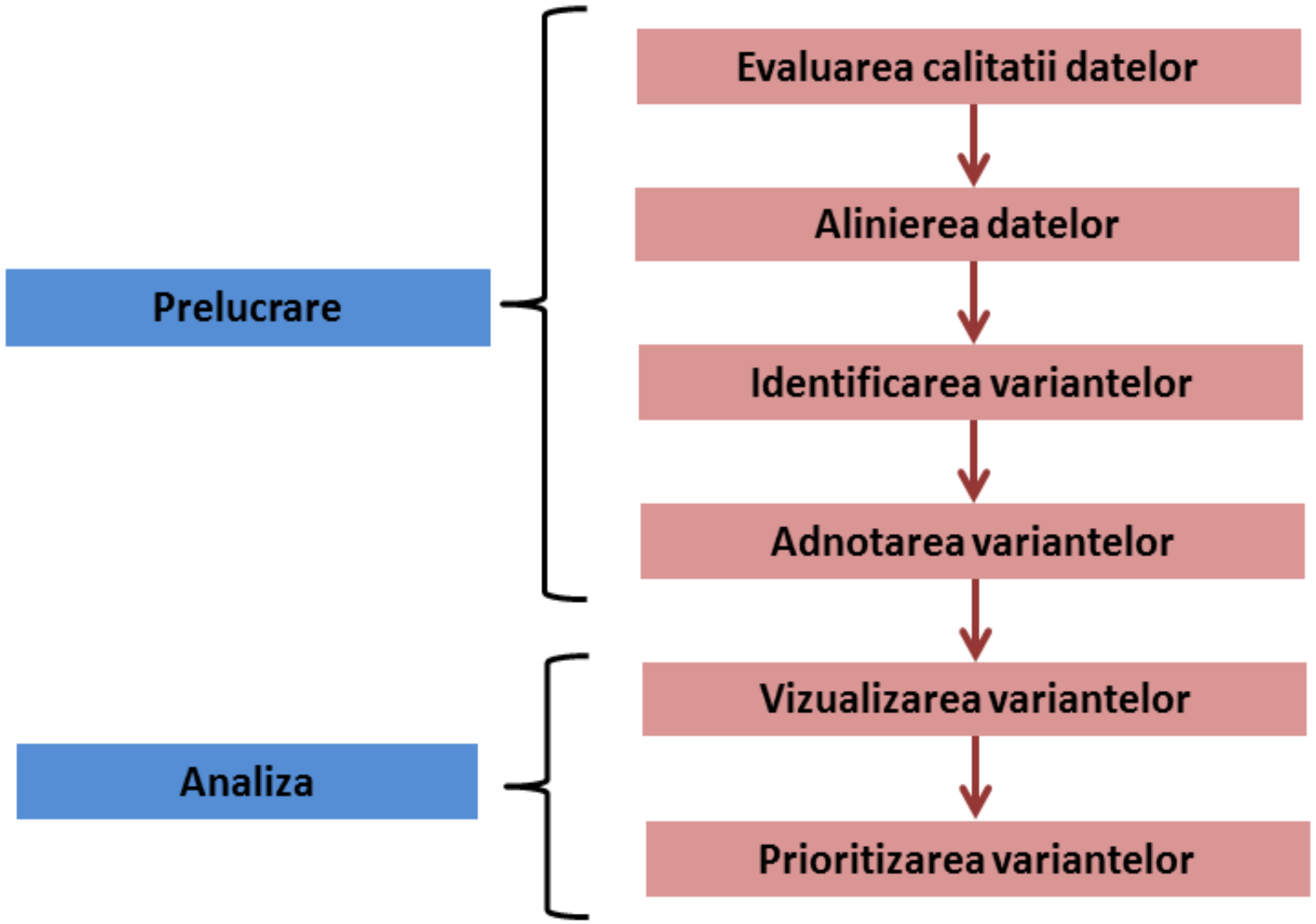
C. Secventiere bazata pe semiconductori





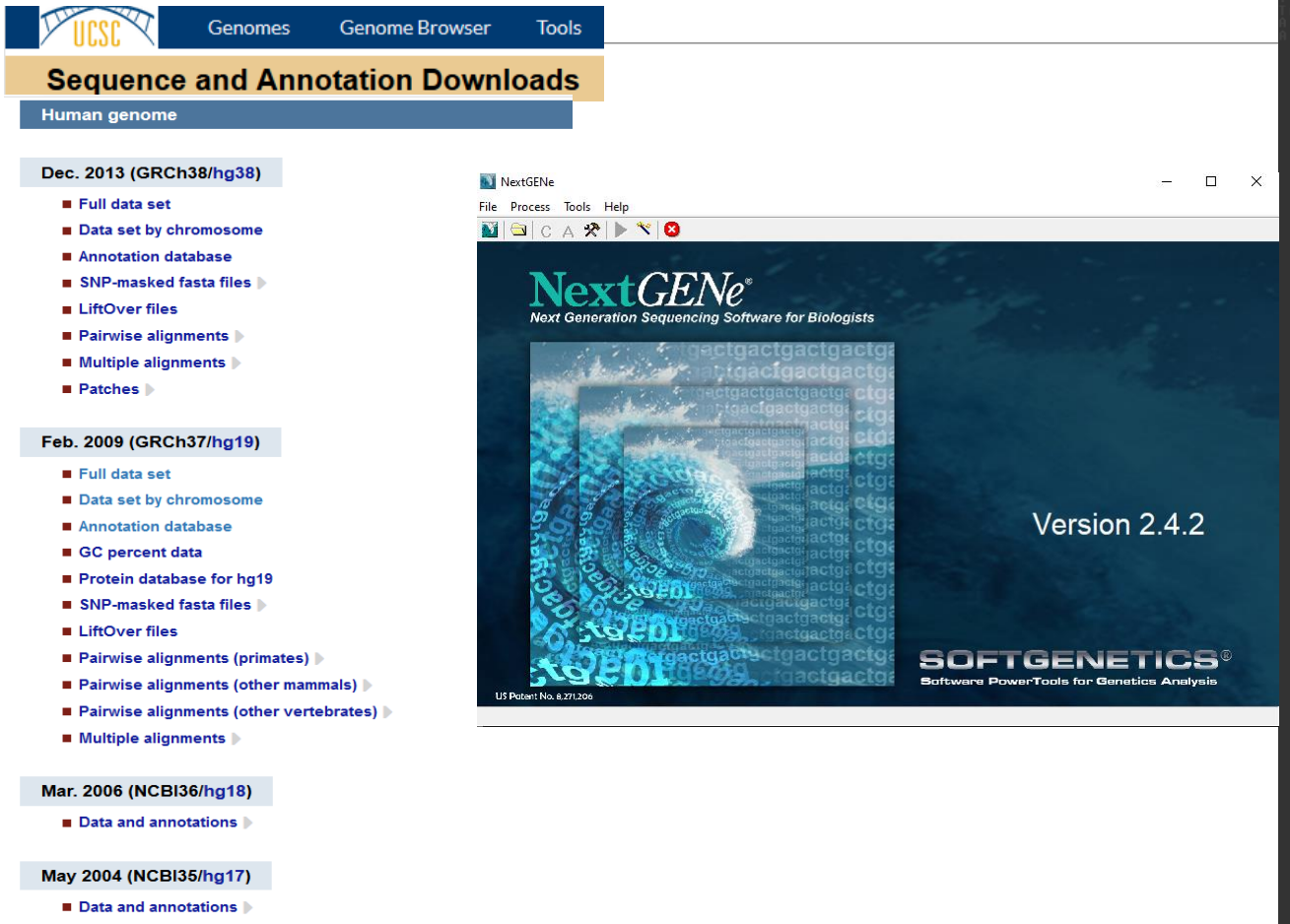
Prezentarea schematizata a principului secventierii de noua generatie - tehnologia Thermo Fischer Scientific (adaptare dupa http://www.biorigami.com/tag=principe-ion-torrent-pgm)

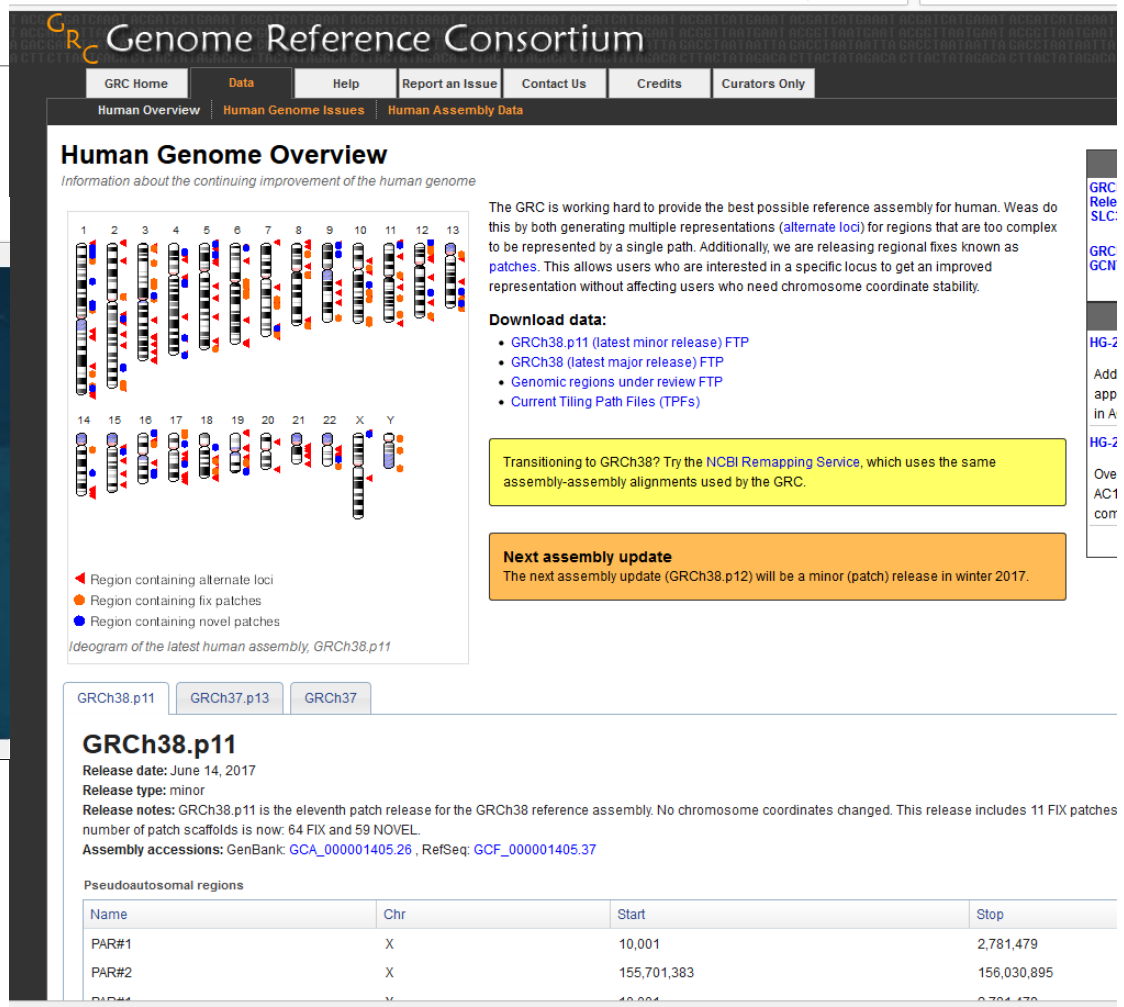
ANALIZA DATELOR NGS



ALINIEREA DATELOR NGS

Surse referinte: UCSC (University of Santa Cruz) – hg si
GRC (Genome Reference Consortium) - GRCh



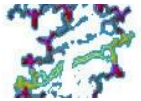


Reference alignment algoritms: Bowtie/Bowtie2, BWA, mrFAST, SOAP....

ADNOTAREA VARIANTELOR

Resurse pentru adnotarea variantelor populationale:

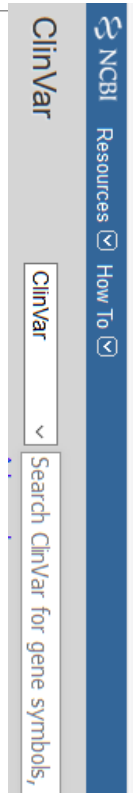
NCBI dbSNP Short Genetic Variations



ExAC Browser (Exome Aggregation Consortium)

gnomAD Genome Aggregation Database

NHLBI Exome Sequencing Project (ESP) Exome Variant Server



Resurse pentru adnotarea variantelor cu semnificatie clinica

COSMIC Catalogue Of Somatic Mutations In Cancer

HGMD® The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff

MY CANCER GENOME Genetically Informed Cancer Medicine

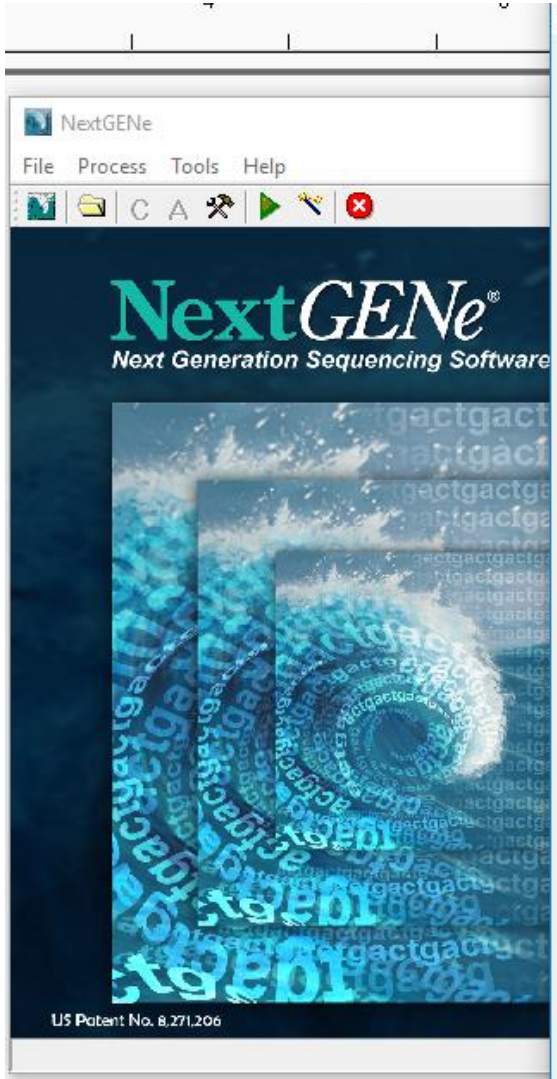
Aplicatii pentru evaluarea impactului functional:

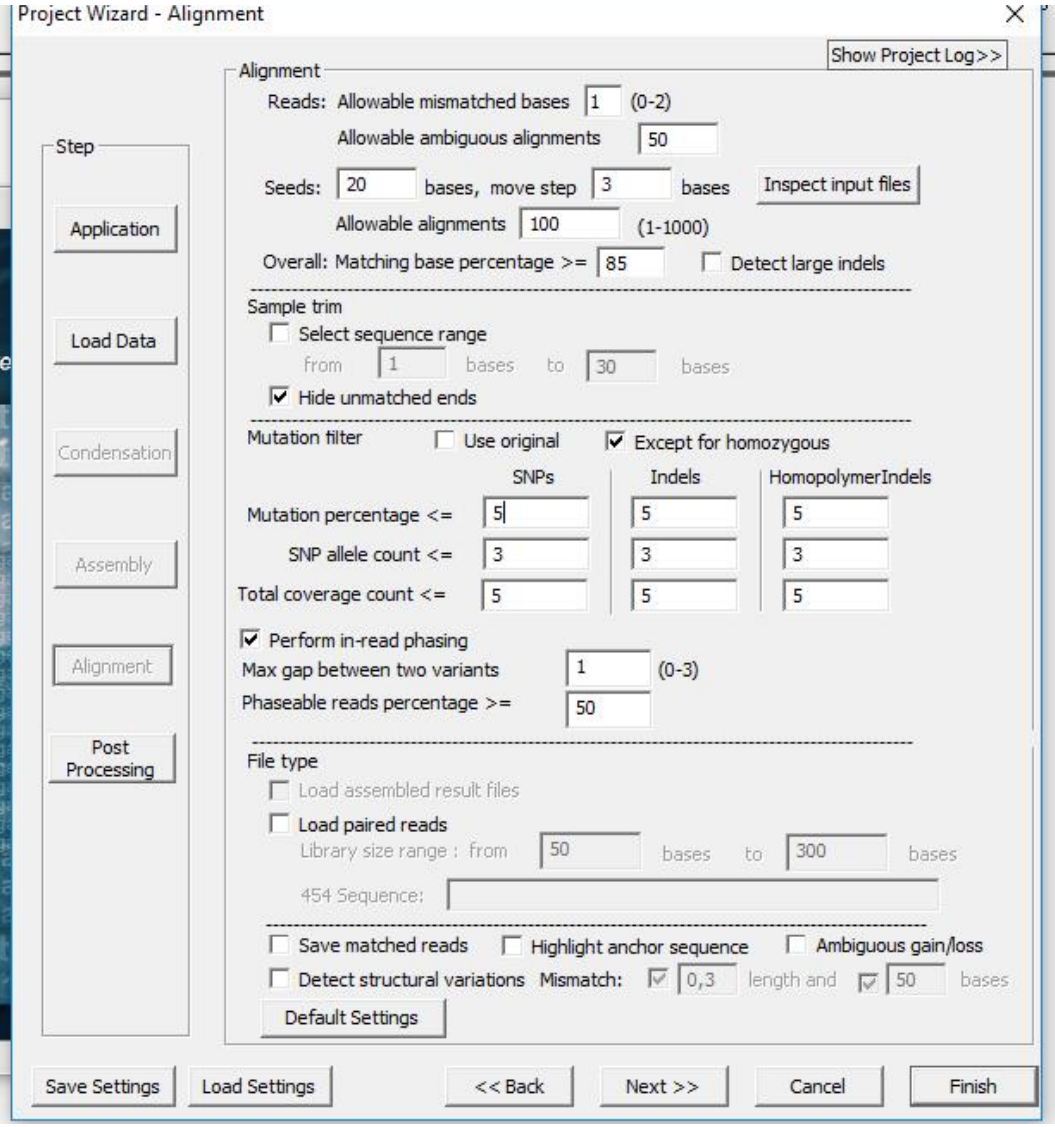
dbNSFP database for nonsynonymous SNPs' functional predictions

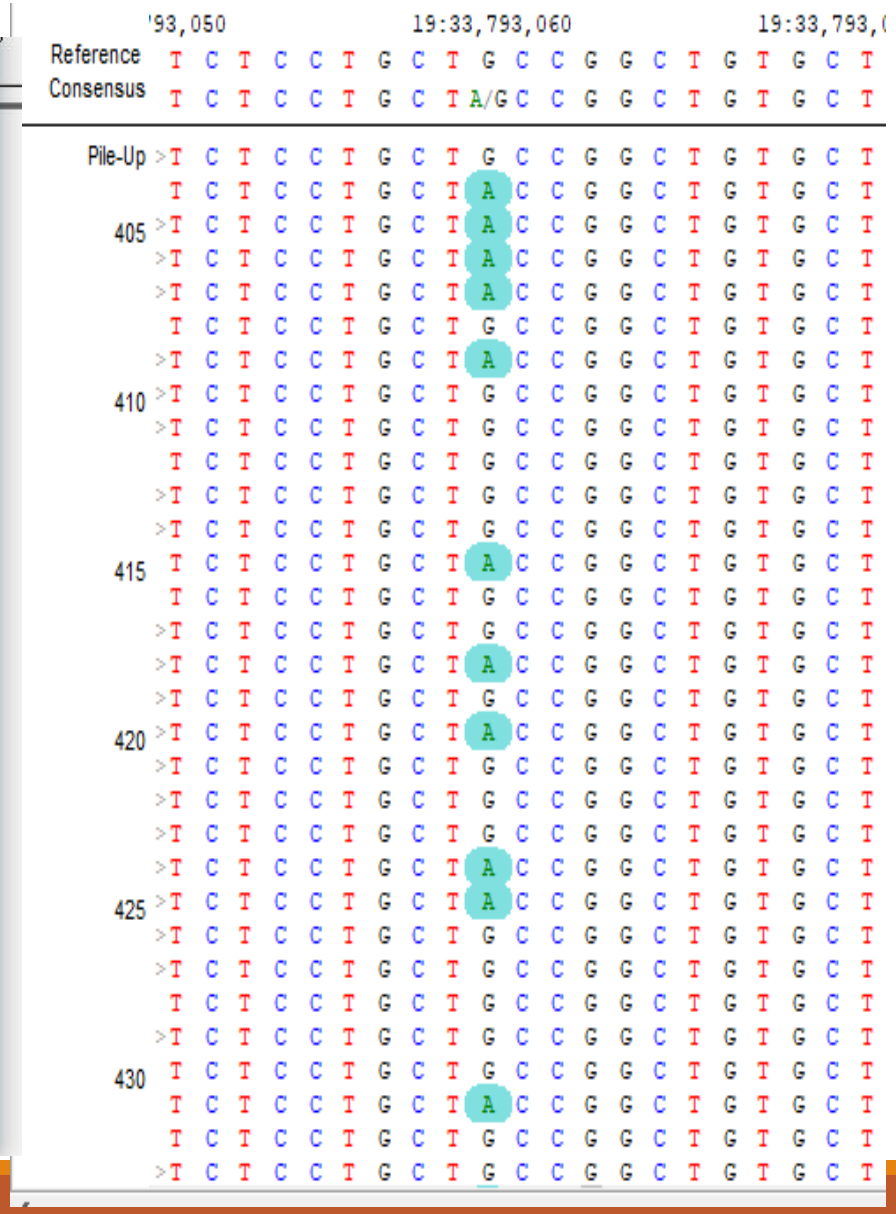
CADD (Combined Annotation Dependent Depletion)

Ensembl Variant Effect Predictor

Detectarea variantelor





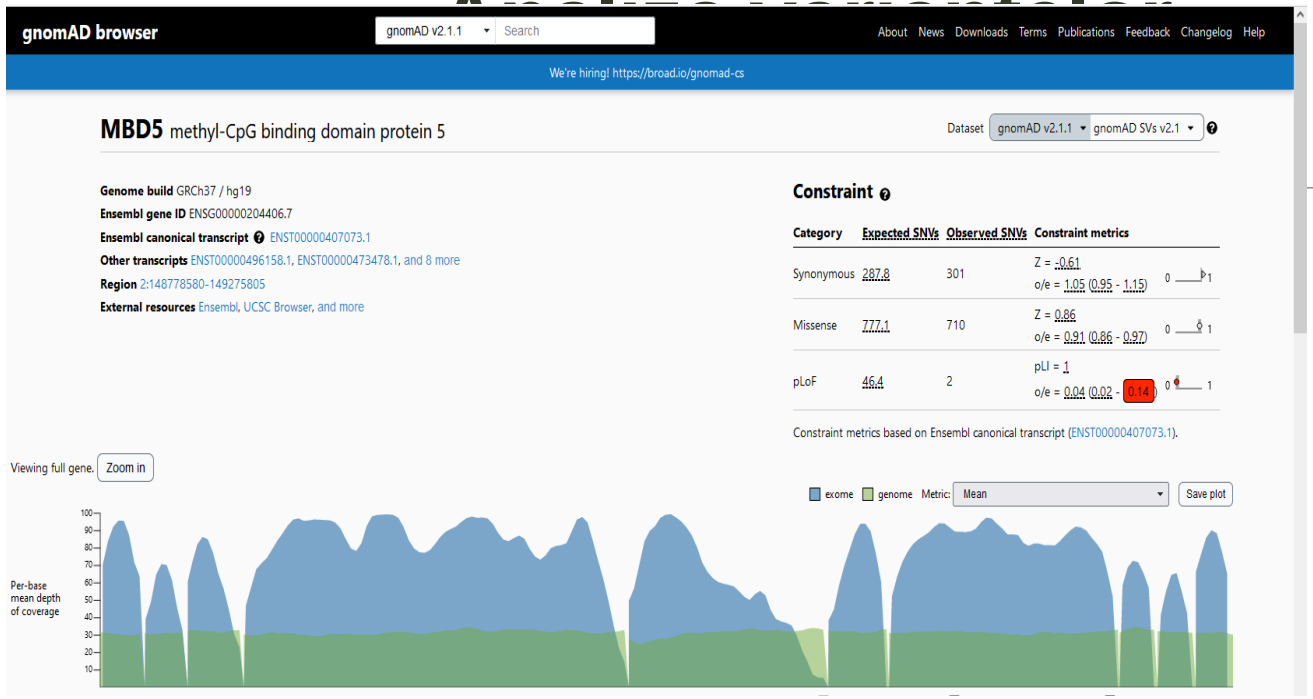


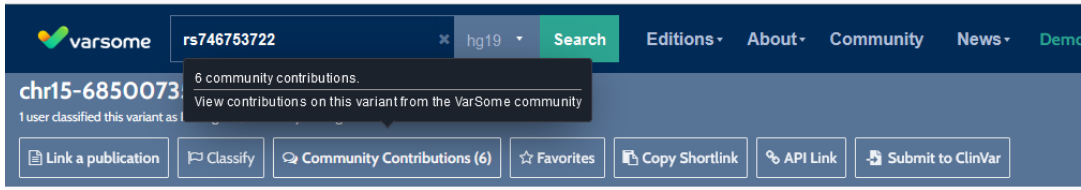
Analiza variantelor

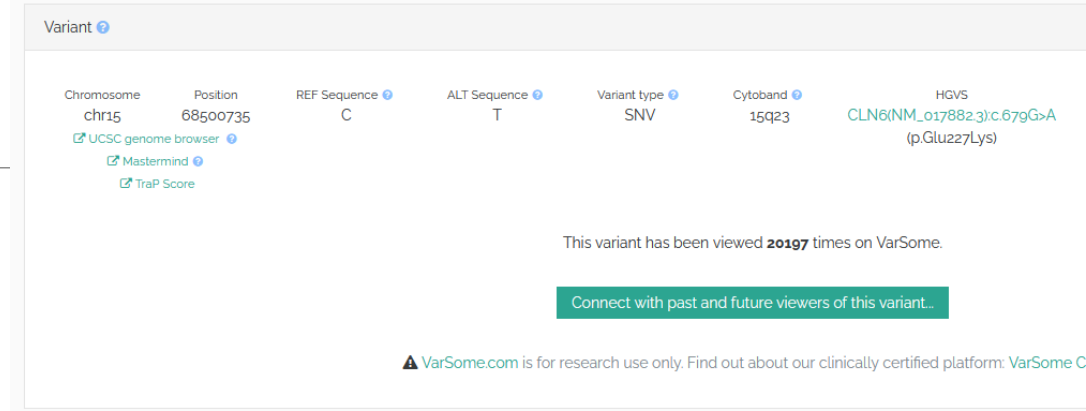
Prioritizarea variantelor:

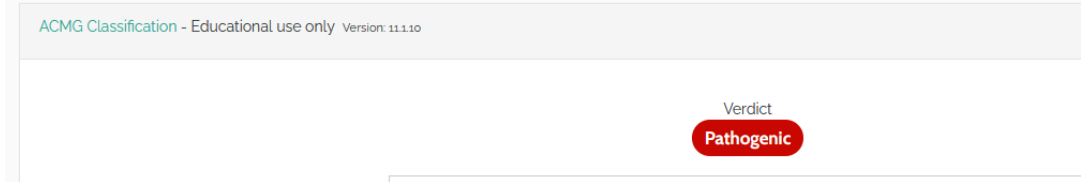
- - pe baza scorului MAF,
- - pe baza localizarii (intronice/exonice) sau tipului (silentioase/modificare aa)
- - pe baza informatiilor din diverse baze de date (ClinVar, HGMD) si a datelor din literature.

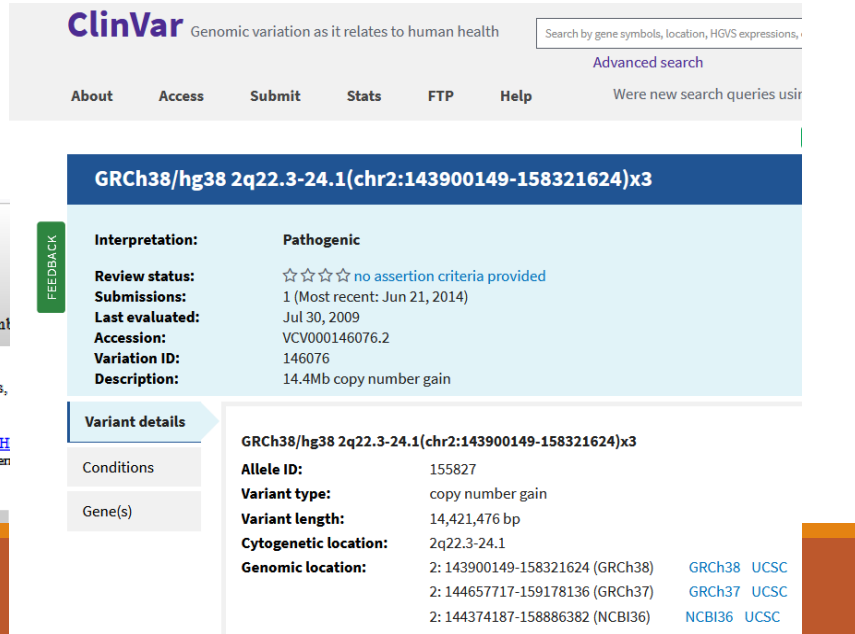
Analiza variantelor

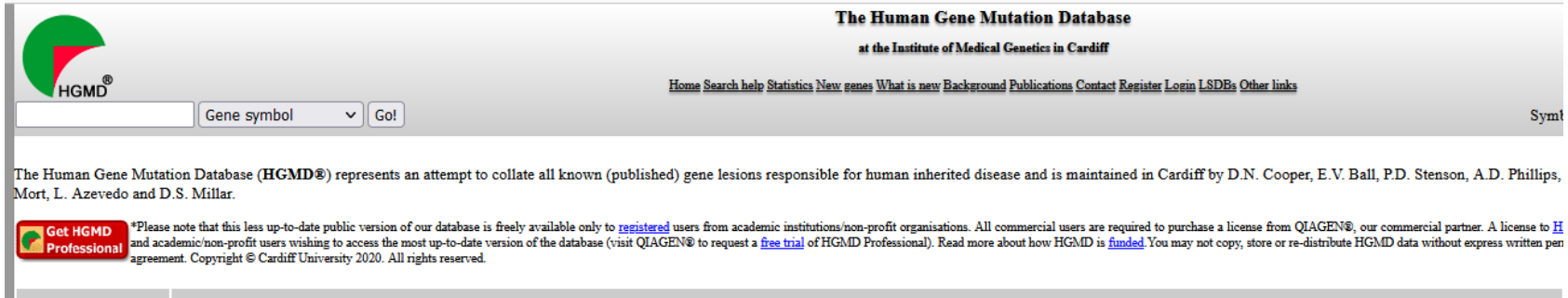












Interpretarea semnificației clinice a variantelor

Clasificare:

- Benign / Probabil benign
- VUS
- Patologic / Probabil patologic

Genet Med. Author manuscript; available in PMC 2015 Nov 1.
Published in final edited form as:
Genet Med. 2015 May; 17(5): 405–424.
Published online 2015 Mar 5. doi: 10.1038/gim.2015.30
PMCID: PMC4544753
NIHMSID: NIHMS697486
PMID: 25741868

Author Manuscript

Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, Chair, ACMG, Nazneen Aziz, CAP, Julie Gastier-Foster, AMP, Wayne W. Grody, ACMG, Karl Voelkerding, CAP, and Heidi L. Rehm

ACGS
Association for Clinical Genomic Science

ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020

Sian Ellard[1,2], Emma L Baple[2,3,4], Alison Callaway[5], Ian Berry[6], Natalie Forrester[7], Clare Turnbull[4], Martina Owens[1], Diana M Eccles[8], Stephen Abbs[9], Richard Scott[4,10], Zandra C Deans[11], Tracy Lester[12], Jo Campbell[13], William G Newman[14,15], Simon Ramsden[14] and Dominic J McMullan[16]

ACMG PRACTICE GUIDELINES | Genetics inMedicine

Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions

G. Bradley Schaefer, MD[1] and Nancy J. Mendelsohn, MD[2]; for the Professional Practice and Guidelines Committee

J Mol Diagn. 2017 Jan; 19(1): 4–23.
doi: 10.1016/j.jmoldx.2016.10.002
PMCID: PMC5707196
PMID: 27993330

J Mol Diagn

Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists

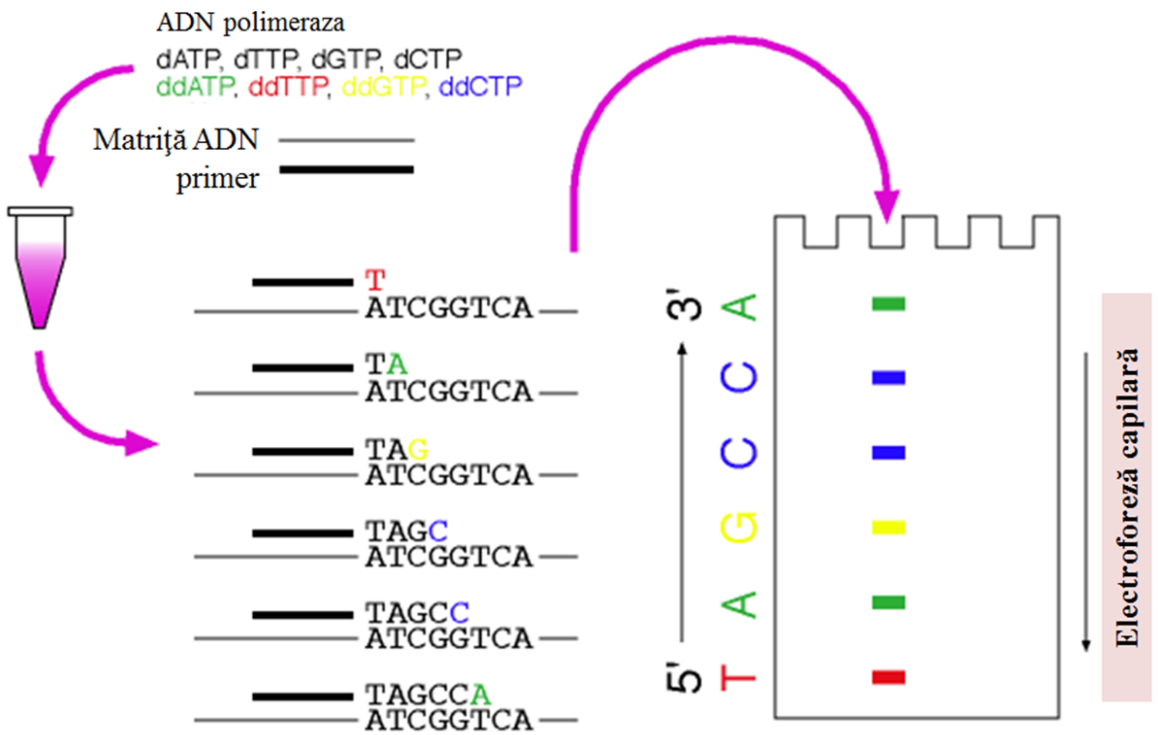
Marilyn M. Li,*†* Michael Datto,*‡ Eric J. Duncavage,*§ Shashikant Kulkarni,*¶ Neal I. Lindeman,*‖ Somak Roy,*** Apostolia M. Tsimberidou,*†† Cindy L. Vnencak-Jones,*‡‡ Daynna J. Wolff,*§§ Anas Younes,*¶¶ and Marina N. Nikiforova***

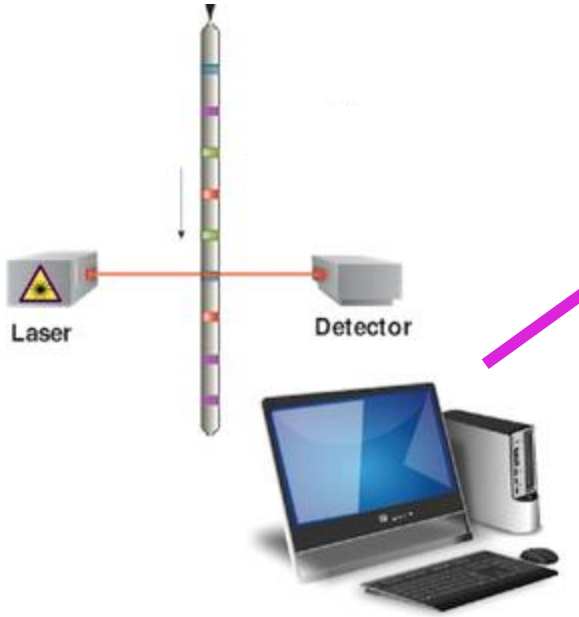
METODE DE CONFIRMARE SAU COMPLEMENTARE TEHNOLOGIILOR GENOMICE

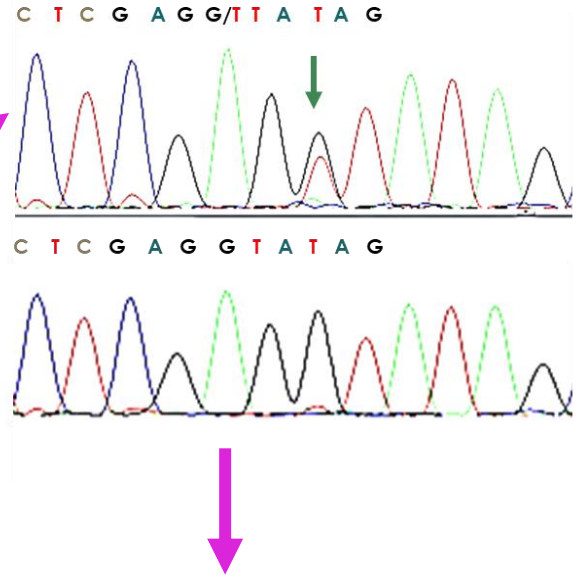
Applied Biosystems 3500 Genetic Analyzer

Microscop motorizat Axio Imager.Z1 Zeiss

Secventierea clasica Sanger- analiza de secventa a unor fragmente de maxim 1000 perechi de baze. Interogarea tintita a unei regiuni de interes si identificarea de variante patologice







Reprezentare schematizata a principiului secvențierii Sanger
(adaptat după http://oregonstate.edu/instruct/bb331/lecture05/FigG4.html)

CTCGAGg/tTATAG

Analiza cromozomilor umani
Microscopie optica in lumina directa

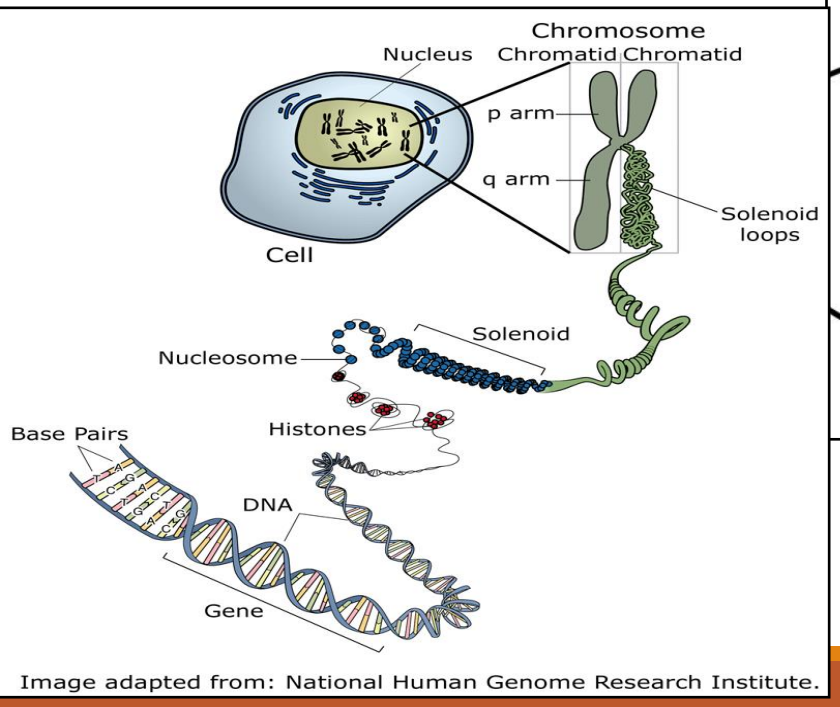
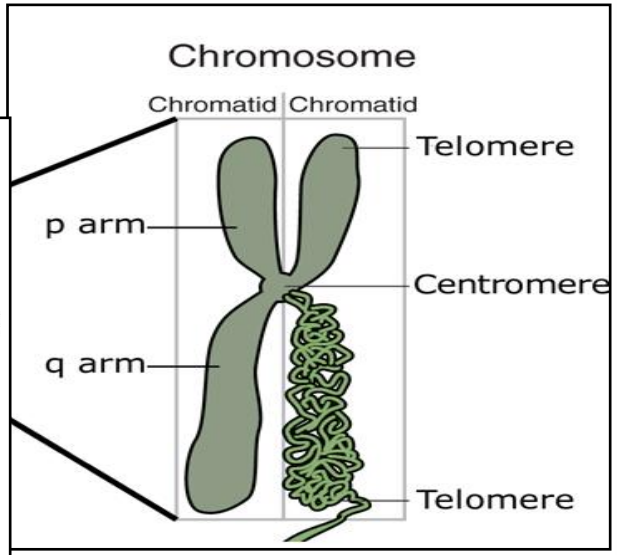
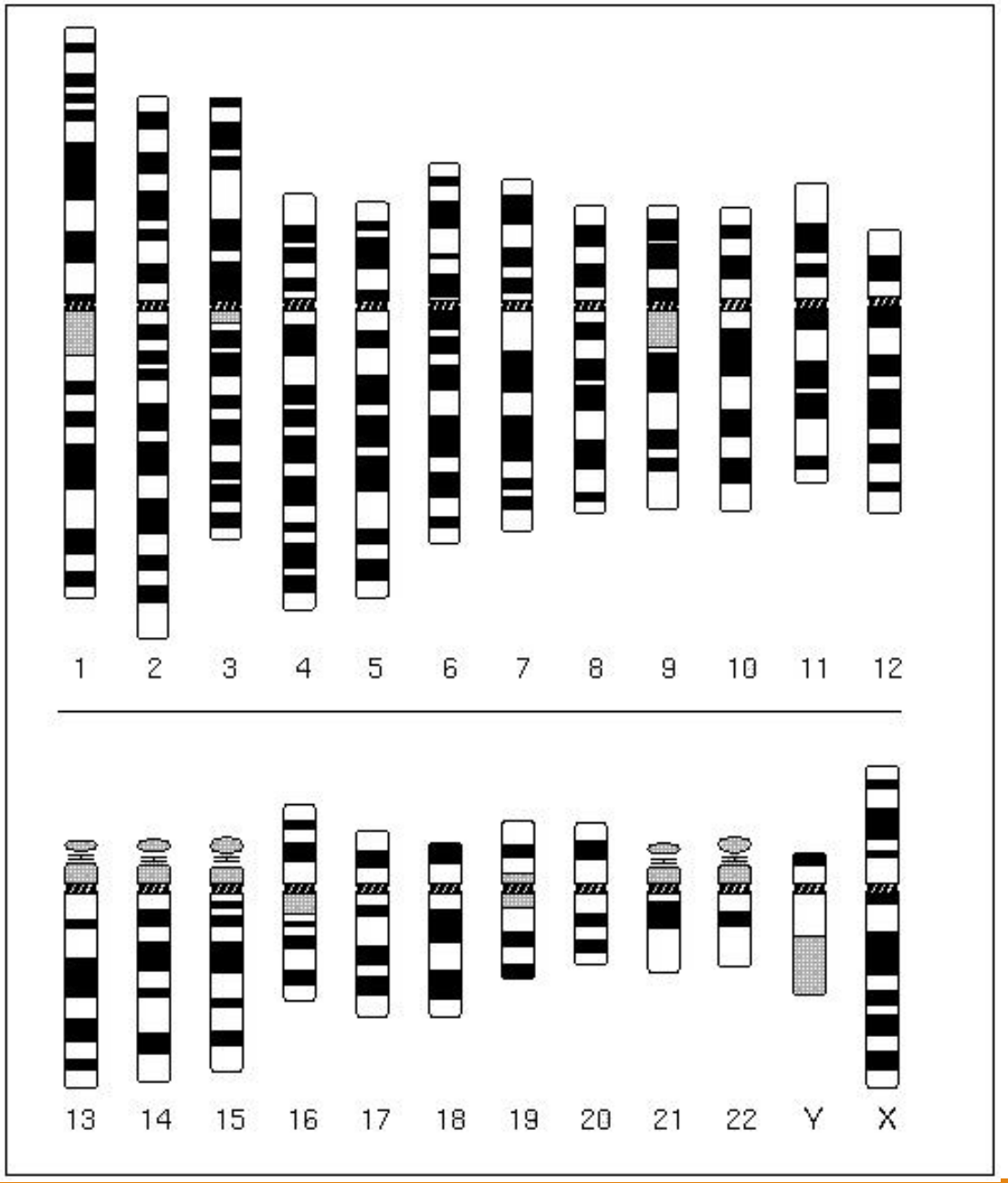
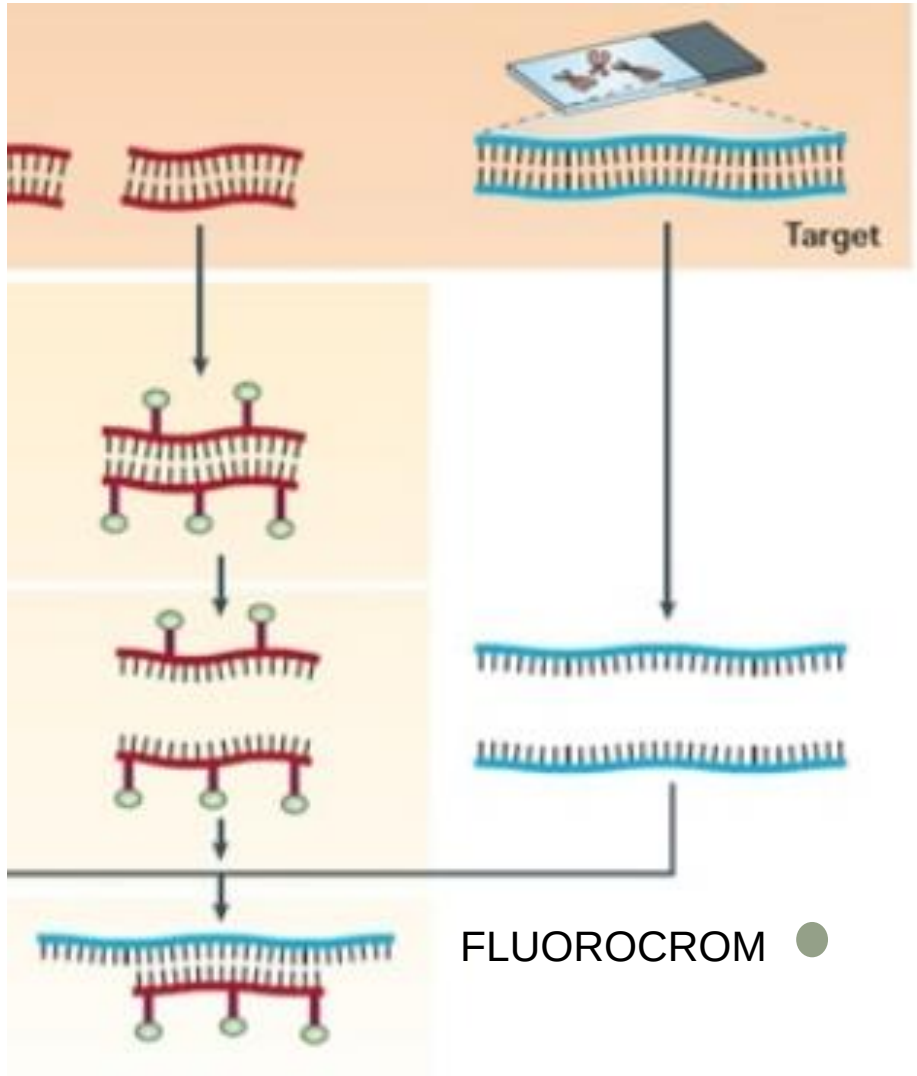


Image adapted from: National Human Genome Research Institute.





Analiza cromozomilor umani
Hibridizare fluorescenta *in situ*



O'Connor, C. (2008) Fluorescence in situ hybridization (FISH). *Nature Education* 1(1):171

Take-home message

Tehnologiile genomice:

au revolutionat cercetarea si practica medicala prin schimbarea modului de abordare de la “single gene” la “genome-wide”;

au permis investigarea genetica pe grupuri mari de indivizi intr-un timp relativ scurt si cu preturi in continua scadere;

au permis generarea unor volume impresionante de date care au dus la acumularea rapida de informatii atat cu privire la variatiile prezente in populatia generala cat si la variatii rare asociate cu diverse patologii umane.

Cercetarea aplicativa in genetica neuropsihiatrica - exemple clinice -

Aplicatii array-CGH
Exemple clinice

Proiect EEA “Improving quality of life for Autism Spectrum Disorders patients by promoting strategies for early diagnosis and preventive measures”

Abordarea multidisciplinara a unui grup de pacienti cu tulburari de spectru autist neinvestigati anterior si cautarea variantelor genetice / genomice relevante clinic

Parteneri:

Spitalul Clinic de Psihiatrie Prof Dr Alex Obregia: evaluare clinica si studii imagistice cerebrale

Institutul National Victor Babes: tehnologii genetice si genomice (screening bazat pe PCR si MLPA pentru detectia defectelor responsabile pentru sindromul X fragil, Hibridizare comparativa genomica bazata pe microarray – array-CGH - pentru detectia dezechilibrelor genomice)

Universitatea Oslo: tehnologii genomice (NGS si microarray de genotipare)

Spectrum Disorders patients by promoting strategies for early diagnosis and preventive measures”

Analiza comuna a datelor – date clinice, rezultate MRI cerebral, profile genomice obtinute prin array-CGH, rezultatele testelor privind statusul genei FMR1, date NGS si de genotipare.

Identificarea variantelor genetice si genomice; evaluarea contributiei variantelor noi sau rar raportate la etiopatogeneza TSA.

Corelatii genotip-fenotip, evaluare clinico-evolutiva.

Trei exemple clinice de dezechilibre genomice detectate prin array-CGH

Aplicatii NGS
Exemple clinice

Proiect ERA NET “Multi-OMICS interrogation of cerebral cortical malformations”

Proiectul HETEROMICS:

Caracterizarea genetica comprehensiva a unei noi cohorte de pacienti cu heterotopii si corelarea cu datele clinice si imagistice

Parteneri:
Consortiu European format din: Inserm UMR-S 839, Sorbonne University Coordonator proiect , Max Planck Institute of Psychiatry, Geneva Institute, Inserm U1249, Koc University, INCD Victor Babes si Spitalul Clinic de Psihiatrie Prof Dr Alexandru Obregia.

Doua exemple clinice cu variatii de secventa detectate prin NGS

Take-home message

Tehnologiile genomice se dovedesc extrem de utile in investigarea pacientilor cu afectiuni de neurodezvoltare in special in contextul unor fenotipuri complexe;

Identificarea etiologiei genetice in bolile de neurodezvoltare este importanta atat in cercetare – intelegerea mecanismelor de patogeneza - cat si in clinica prin imbunatatirea ingrijirii si calitatii vietii pacientului.

MULTUMIM!

Acknowledgment:
The research leading to these results has received partial funding from the EEA Grant 2014-2021, under the project contract No 6/2019;
This work was supported by a grant of the Romanian National Authority for Scientific Research Innovation, CCCDI-UEFISCDI, project number COFUND-ERANET E-RARE 3-HETER-OMICS-2, within PNCDI III
WES was performed with access for WES infrastructure and technical support from Medical Genetics Center team, CRH Craiova