IL SEQUENZIAMENTO DI NUOVA GENERAZIONE NELLA DIAGNOSTICA DELLE PATOLOGIE GENETICHE ED INFETTIVE

Dott. Federico Anaclerio

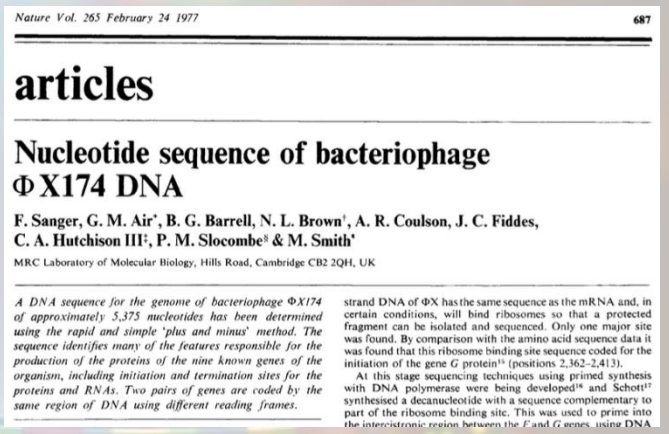
Center for Advanced Studies and Technology (CAST) - Laboratorio di Genetica Molecolare

Università G.D'Annunzio Chieti-Pescara

Via L.Polacchi 11, 66100, Chieti, Italy

EVOLUZIONE DEL SEQUENZIAMENTO

1977 – Il DNA è sequenziato per la prima volta da Frederick Sanger, Walter Gilbert e Allan Maxam in maniera indipendente. Viene ottenuta la sequenza completa dell'intero genoma del fago Φ-X174



Nature Vol. 265 February 24 1977 687

articles

Nucleotide sequence of bacteriophage ΦX174 DNA

F. Sanger, G. M. Air*, B. G. Barrell, N. L. Brown†, A. R. Coulson, J. C. Fiddes, C. A. Hutchison III‡, P. M. Slocombe§ & M. Smith*

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

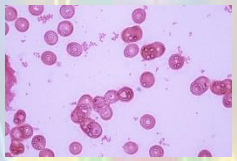
A DNA sequence for the genome of bacteriophage ΦX174 of approximately 5,375 nucleotides has been determined using the rapid and simple 'plus and minus' method. The sequence identifies many of the features responsible for the production of the proteins of the nine known genes of the organism, including 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.

strand DNA of ΦX has the same sequence as the mRNA and, in certain conditions, will bind ribosomes so that a protected fragment can be isolated and sequenced. Only one major site was found. By comparison with the amino acid sequence data it was found that this ribosome binding site sequence coded for the initiation of the gene *G* protein[15] (positions 2,362–2,413).

At this stage sequencing techniques using primed synthesis with DNA polymerase were being developed[16] and Schott[17] synthesised a decanucleotide with a sequence complementary to part of the ribosome binding site. This was used to prime into the intercistronic region between the *F* and *G* genes, using DNA

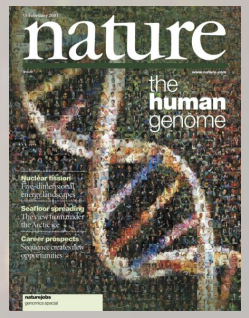
1977

1982: ***lambda virus***
DNA stretches up to 30-40Kbp
(Sanger et al.)

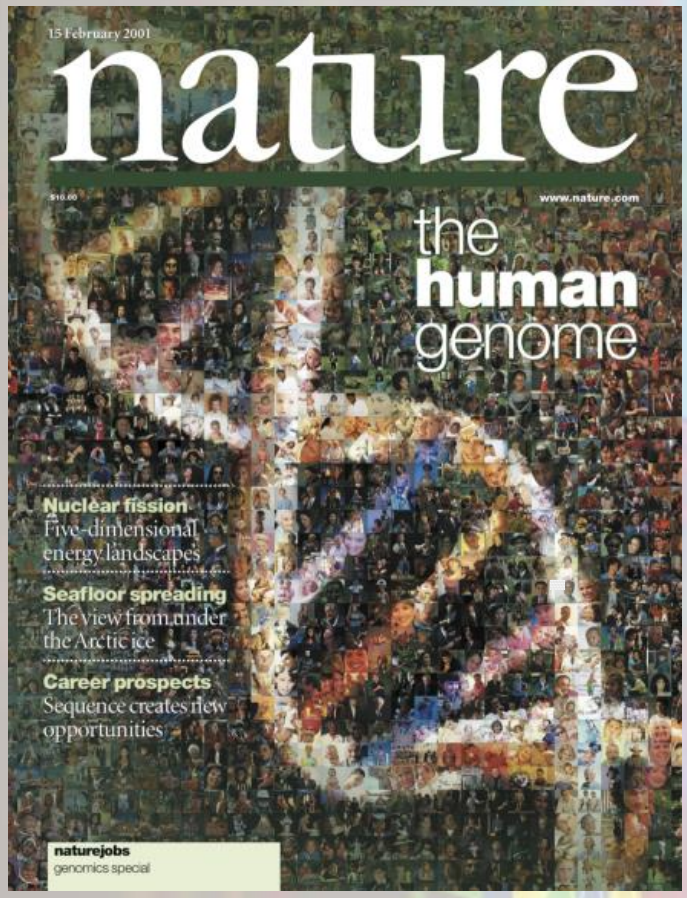


1994: ***H. Influenzae***
1.8 Mbp
(Fleischmann et al.)

2001: ***Homo Sapiens***



PROGETTO 1000 GENOMI

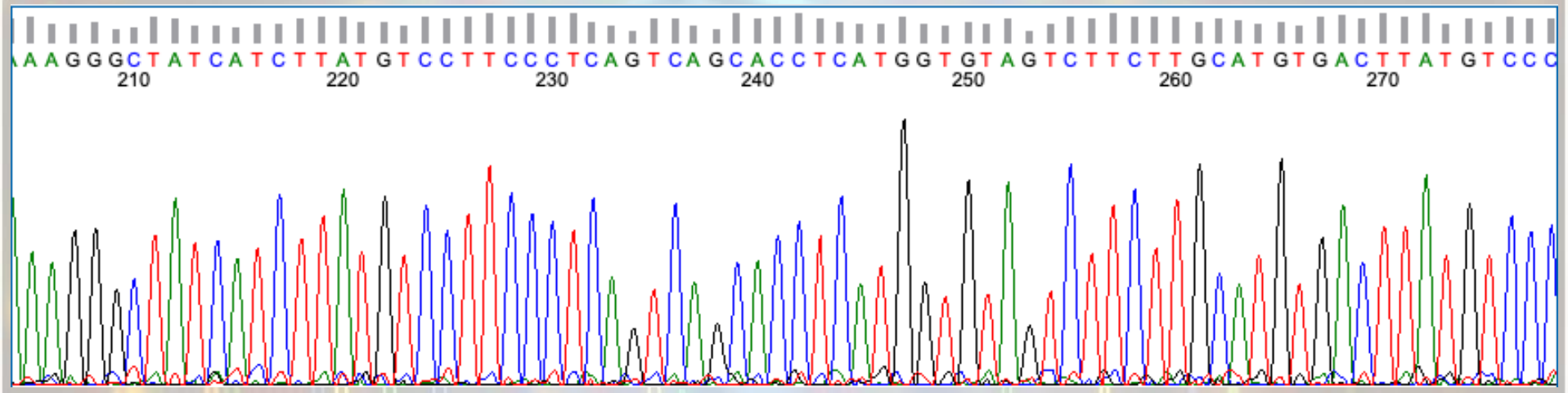


Non potremmo oggi parlare di **GENOMICA** e di **ANALISI DEI GENOMI** se non avessimo a disposizione le moderne tecnologie di **sequenziamento del DNA** con la possibilità di approfondite analisi correlate alla variabilità genomica o a specifiche patologie.

Negli ultimi anni lo sviluppo delle nanotecnologie in generale, e delle **piattaforme tecnologiche** per il **sequenziamento** degli acidi nucleici in particolare, hanno subito una straordinaria accelerazione che ha rivoluzionato gli orizzonti applicativi in moltissimi settori della ricerca pura e applicata, in primis la salute e la nutrizione umana.

20
40
60
80
AACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCAC
100
120
140
160
180
GCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTTTTGCAGCCGAT
200
220
240
260
280
CATCAGCACATCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAACACACGTCCAACTCAGTTTG
300
320
340
360
380
CCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGAGGTCTTATCAGAGGCACGTCAACATCTTAAAGATGGCACTTG
400
420
440
460
480
TGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAACTTGAACAGCCCTATGTGTTCATCAAACGTTCGGATGCTCGAACTGCACCTCATGGTCATG
500
520
540
560
580
TTATGGTTGAGCTGGTAGCAGAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTGTCCTTGTCCCTCATGTGGGCGAAATACCAGTG
600
620
640
660
680
GCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTGACTTAGGCGACGAGCT
700
720
740
760
780
TGGCACTGATCCTTATGAAGATTTTCAAGAAAACTGGAACACTAAACATAGCAGTGGTGTTACCCGTGAACTCATGCGTGAGCTTAACGGAGGGGCAT
800
820
840
860
880
ACACTCGCTATGTCGATAACAACTTCTGTGGCCCTGATGGCTACCCTCTTGAGTGCATTAAAGACCTTCTAGCACGTGCTGGTAAAGCTTCATGCACT
900
920
940
960
980
TTGTCCGAACAACTGGACTTTATTGACACTAAGAGGGGTGTATACTGCTGCCGTGAACATGAGCATGAAATTGCTTGGTACACGGAACGTTCTGAAAA
1.000
1.020
1.040
1.060
GAGCTATGAATTGCAGACACCTTTTGAAATTAAATTGGCAAAGAAATTTGACACCTTCAATGGGGAATGTCCAAATTTTGTATTTCCCTTAAATTCCA
1.080
1.100
1.120
1.140
1.160
TAATCAAGACTATTCAACCAAGGGTTGAAAAGAAAAAGCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTGCGTCACCAAATGAATGC
1.180
1.200
1.220
1.240
1.260
AACCAAATGTGCCTTTCAACTCTCATGAAGTGTGATCATTGTGGTGAAACTTCATGGCAGACGGGCGATTTTGTTAAAGCCACTTGCGAATTTTGTGG
1.280
1.300
1.320
1.340
1.360
CACTGAGAATTTGACTAAAGAAGGTGCCACTACTTGTGGTTACTTACCCCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTCACAATTCAGAAG
1.380
1.400
1.420
1.440
1.460
TAGGACCTGAGCATAGTCTTGCCGAATACCATAATGAATCTGGCTTGAAAACCATTCTTCGTAAGGGTGGTCGCACTATTGCCTTTGGAGGCTGTGTG
1.480
1.500
1.520
1.540
1.560
TTCTCTTATGTTGGTTGCCATAACAAGTGTGCCTATTGGGTTCCACGTGCTAGCGCTAACATAGGTTGTAACCATACAGGTGTTGTTGGAGAAGGTTC
1.580
1.600
1.620
1.640
1.660

SANGER SEQUENCING



SANGER SEQUENCING

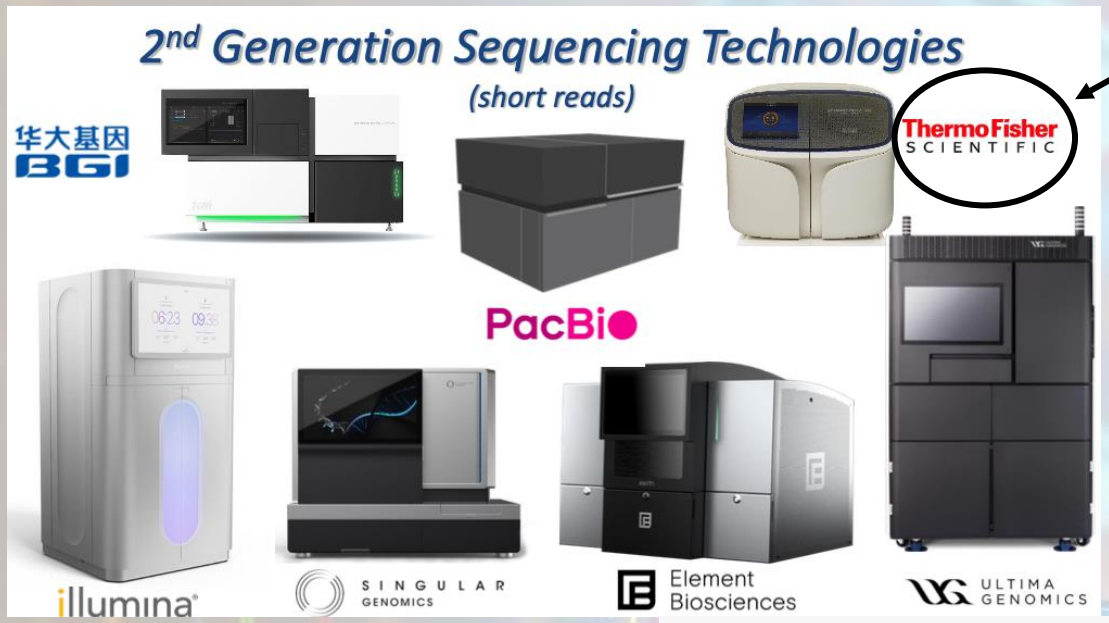
- Human genome: 3.000.000.000 bp
- Minimum coverage for an accurate analysis: 8X = 24.000.0000.000 nt

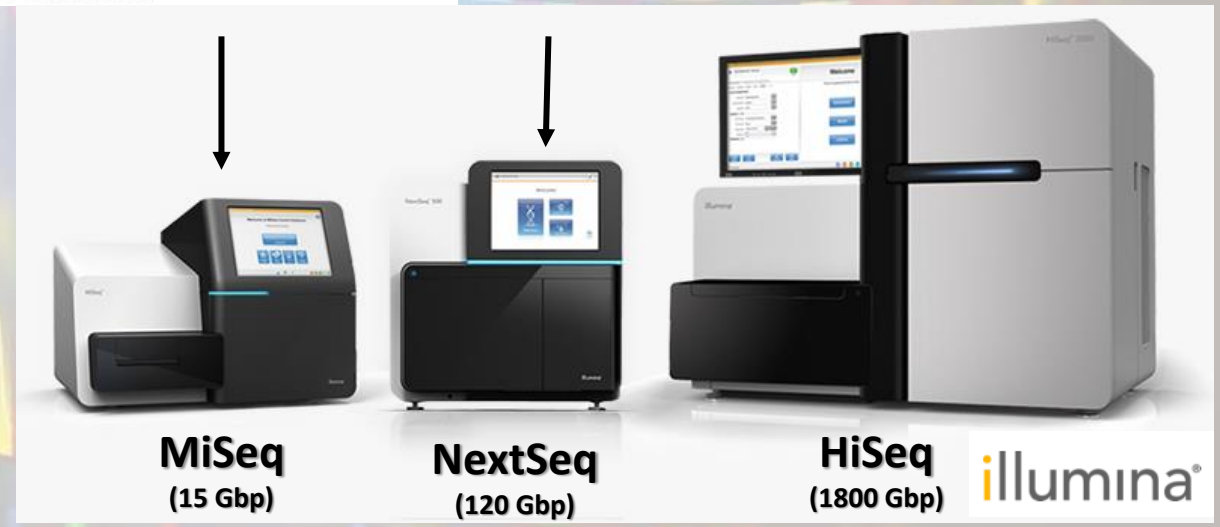
$$\frac{24.000.000.000}{1.600.000} = \textbf{15.000 days!}$$

1st GENERATION SEQUENCING

- ***Buona qualità del dato sequenziato***

- ***Output di dati sufficiente per analisi bioinformatica***


2nd Generation Sequencing Technologies
(short reads)
华大基因
BGI
ThermoFisher
SCIENTIFIC
PacBio
illumina
SINGULAR
GENOMICS
Element
Biosciences
ULTIMA
GENOMICS


MiSeq
(15 Gbp)
NextSeq
(120 Gbp)
HiSeq
(1800 Gbp)
illumina

NEXT GENERATION SEQUENCING (NGS)

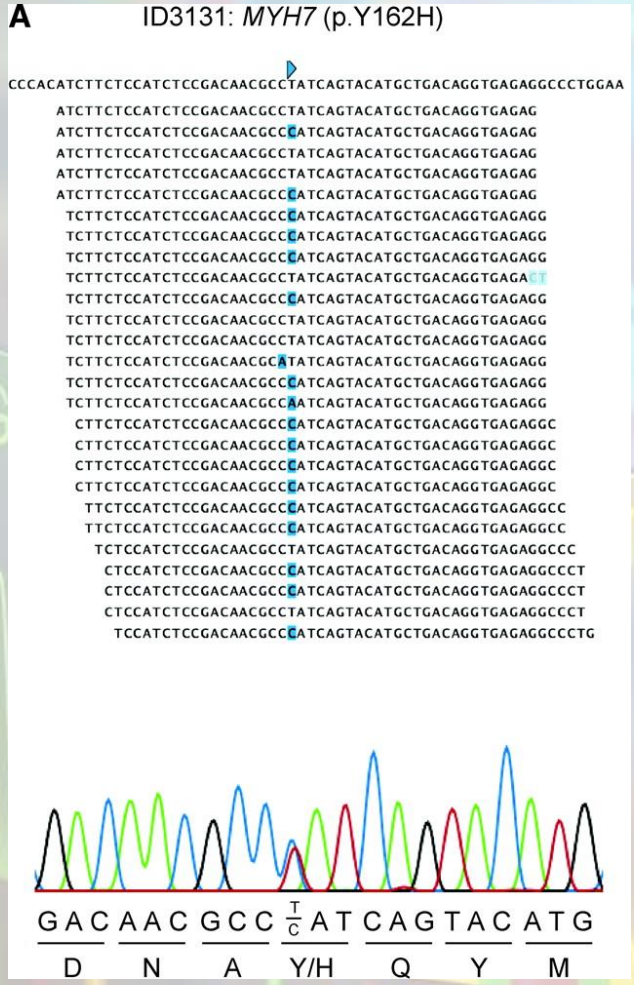
Il principale vantaggio è la possibilità tecnica di produrre un volume enorme di dati a **costi** più bassi ed in **tempi** estremamente più rapidi

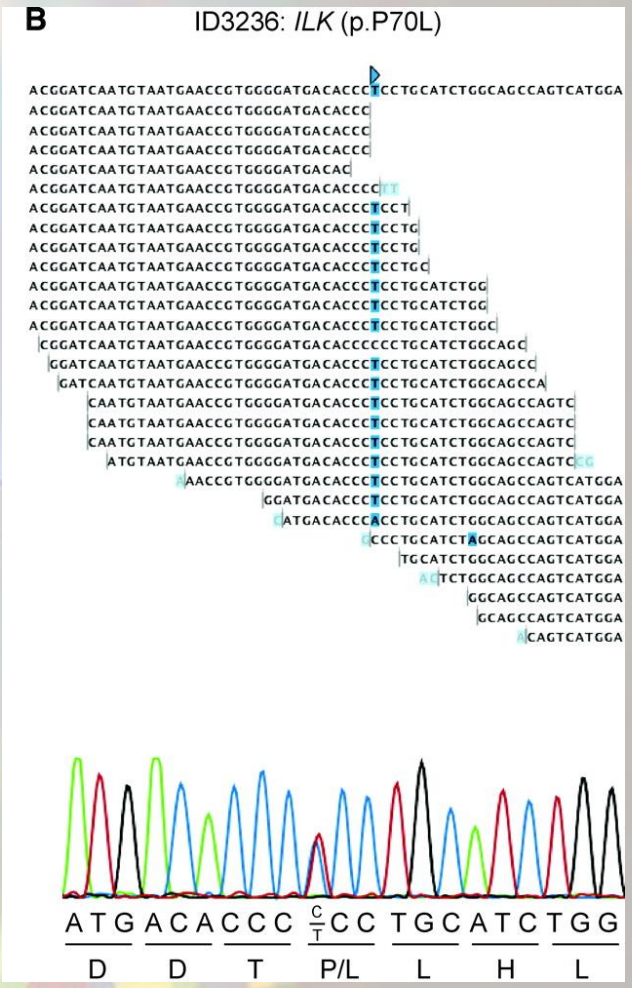
Con le attuali tecnologie è possibile produrre in **un solo giorno** lo stesso volume di dati prodotto complessivamente negli **ultimi 30 anni** attraverso il sequenziamento basato sul metodo di Sanger.

NGS vs SANGER Sequencing

NGS

Sanger



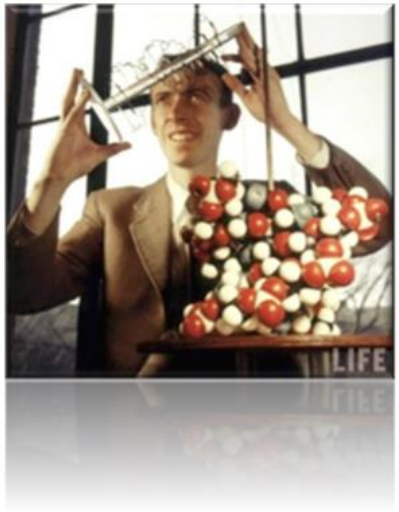


Sequencing the Human Genome

2001: Human Genome Project

13 years
$ 3 Billion

2007: James Watson

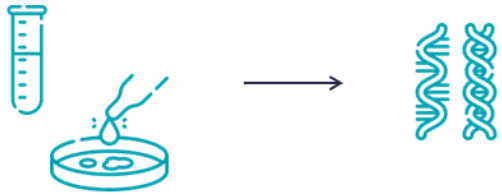


2 months
$ 2 Million

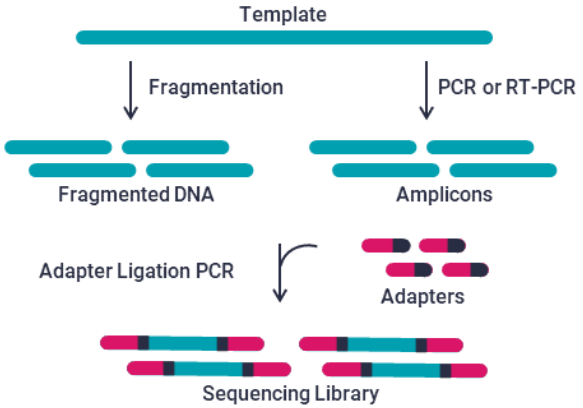
2023: Personal genome

1-2 days
$ 200-3,000

STEP 1:
Extraction



STEP 2:
Library Prep



STEP 3:
Sequencing

STEP 4:
Analysis

NGS – CAMPI DI APPLICAZIONI

I principali campi di applicazione delle piattaforme **NGS** sono:

- sequenziamento ***de novo,*** cioè sequenziamenti e ri-sequenziamenti **di genomi completi**;
- identificazione di siti **polimorfici e mutazionali;**
- analisi su larga scala del **trascrittoma (RNA-seq);**
- mappatura su scala genomica di **siti di interazione** tra DNA (RNA) e proteine e analisi epigenetiche dell'intero genoma;
- caratterizzazione dell'**esoma**, del **metiloma** e studio dell'**editing**;
- analisi **metagenomiche**.

TEST GENETICO

Analisi di specifici geni, del loro prodotto o della loro funzione, nonché ogni altro tipo di indagine del DNA o dei cromosomi, finalizzate ad individuare o ad escludere modificazioni del DNA verosimilmente associate a patologie genetiche, e comprendono:

test **DIAGNOSTICI** (conferma della clinica)

test **PRESINTOMATICI** (per malattie ad esordio tardivo)

test **IDENTIFICAZIONE DEI PORTATORI SANI**

test **PREDITTIVI** per suscettibilità ad una malattia (es. tumori familiari del colon o della mammella)

test **FARMACOGENETICI** (per la risposta individuale ai farmaci)

test **MEDICO-LEGALI** (es. accertamento esclusione paternità)

TEST GENETICO

✓ **CONSENSO INFORMATO** ALL'ESECUZIONE DEI TEST GENETICI

Secondo la legge italiana, richiedono esplicitamente il consenso informato i trattamenti terapeutici, chirurgici e diagnostici, compresi i test genetici, e le sperimentazioni cliniche (D.L. 24 giugno 2003, n. 211) e il paziente deve inoltre acconsentire al trattamento dei propri dati personali (D.L. 30 giugno 2003, n. 196). Come indicazione generale, un consenso scritto deve essere redatto in maniera chiara e comprensibile, deve contenere la descrizione dell'intervento medico, la spiegazione dei rischi prevedibili derivanti dall'esecuzione della prestazione prevista, illustrazioni delle tecniche e degli eventuali materiali da utilizzare, la descrizione dei benefici e dei rischi che possono derivare da eventuali e le potenziali complicazioni.

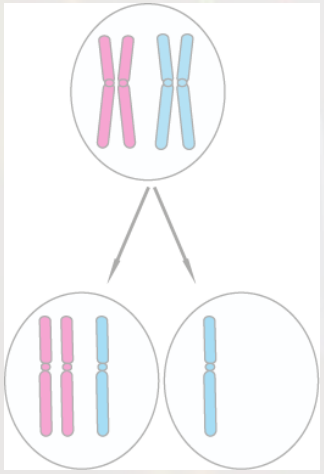
✓ **CONSULENZA GENETICA (PRE-TEST e POST-TEST):**

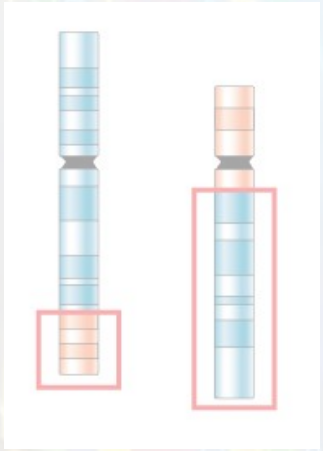
- comprendere le informazioni mediche, inclusa la diagnosi, la prognosi e le terapie disponibili
- rendersi conto del contributo ereditario alla malattia e del rischio di ricorrenza
- prendere le decisioni che sembrano appropriate in rapporto al rischio di ricorrenza, ai progetti familiari, agli standard etici e religiosi e ad agire in accordo con queste decisioni
- ottenere il miglior possibile adattamento alla malattia (in un soggetto affetto) o al rischio di ricorrenza

VARIANTI GENETICHE

Large scale

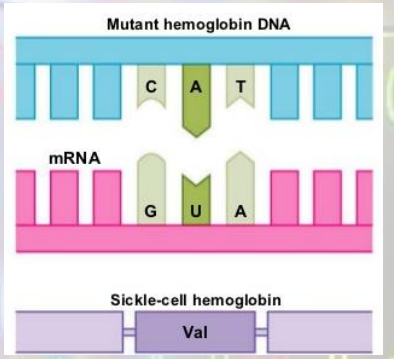
- Aneuploidy
- Structural rearrangements

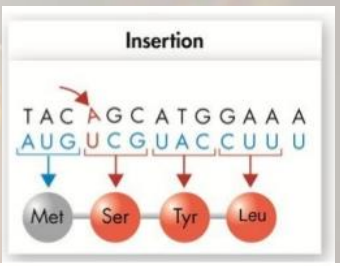


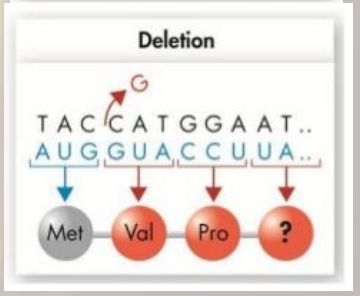


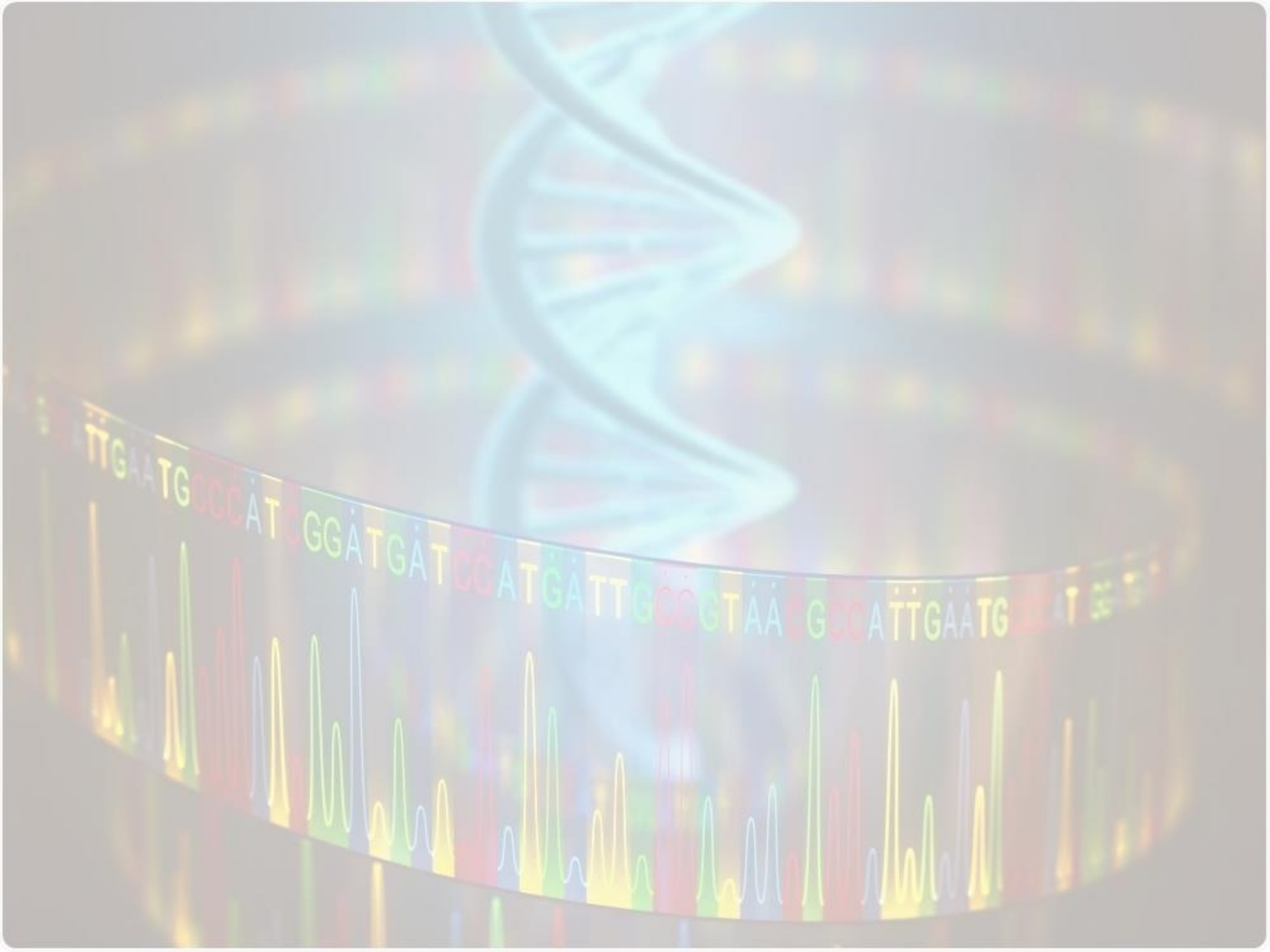
Smaller scale

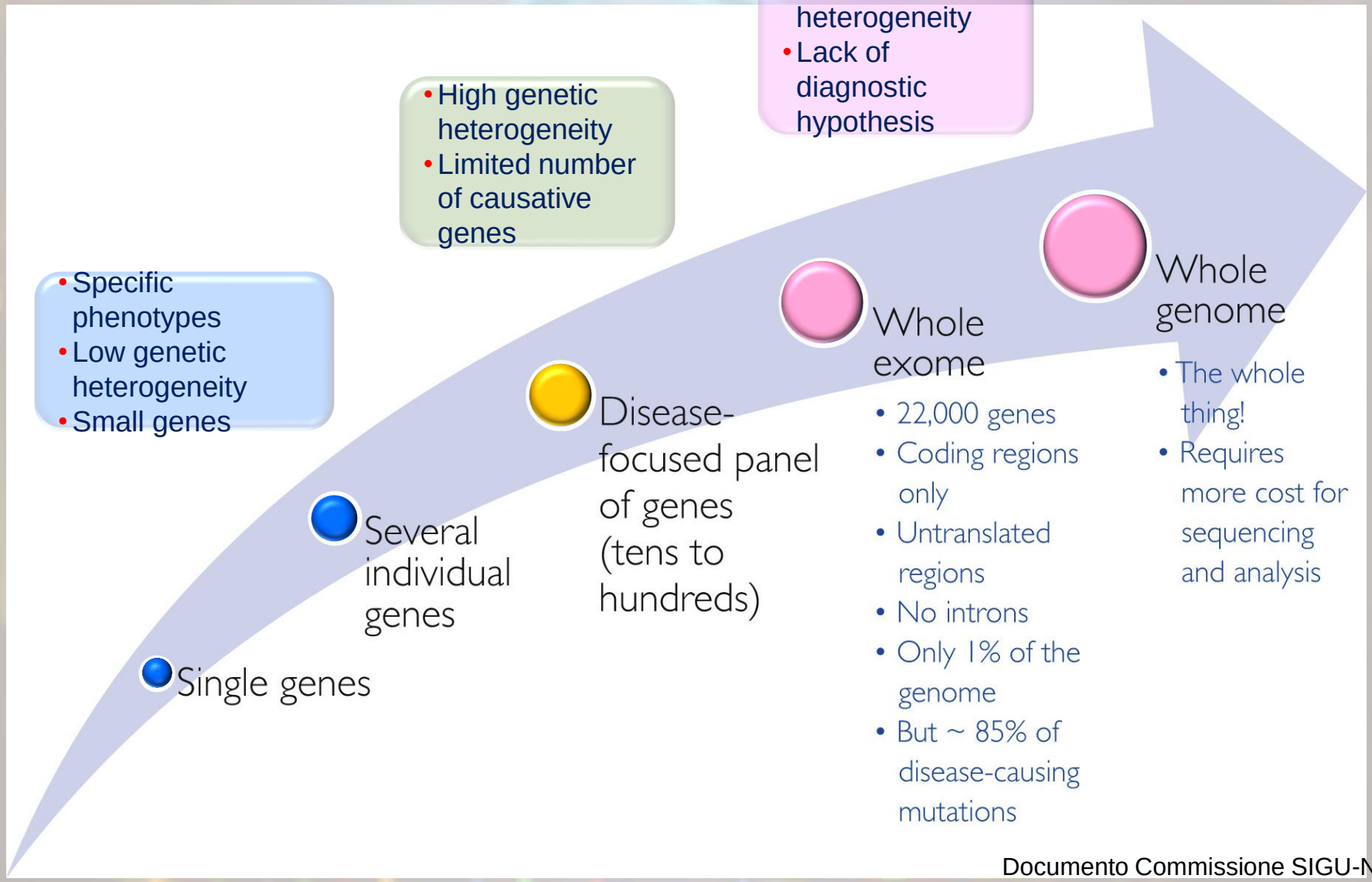
- Base substitutions
- Small insertions and deletions











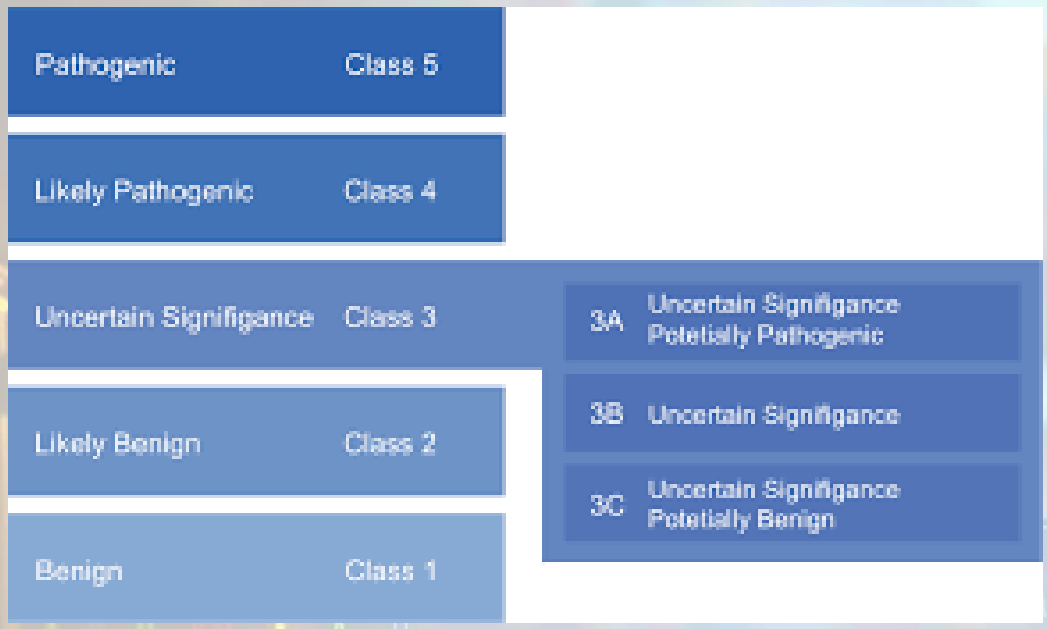
Documento Commissione SIGU-NGS

INTERPRETAZIONE DEI DATI

- La fase limite dell'analisi del DNA con approcci NGS è rappresentata dall'analisi bioinformatica dei dati ottenuti.

- L'uso di grandi pannelli genici e, ancor più, il sequenziamento dell'intero esoma o dell'intero genoma solleva problemi specifici per l'interpretazione della patogenicità delle varianti identificate e, quindi, per la valutazione del rischio.

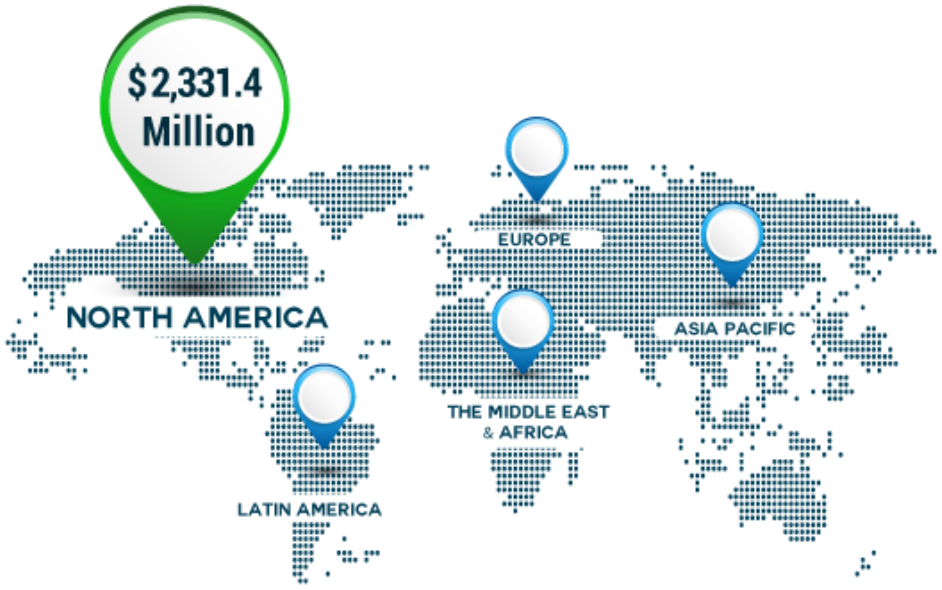
Come vengono utilizzati gli SNPs in ambito medico?



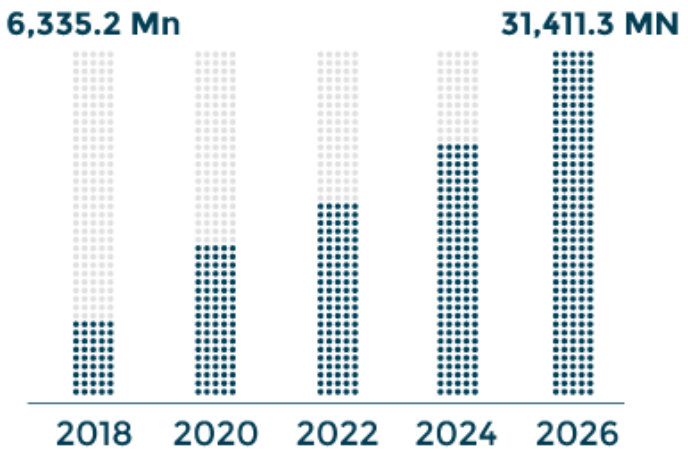
Le varianti a significato incerto (**VUS**) sono un problema in rapida crescita nei test genetici, con un tasso di nuove interpretazioni di VUS che supera di gran lunga quello delle interpretazioni patogeniche e benigne.

NEXT-GENERATION SEQUENCING (NGS) MARKET

North America Next-Generation Sequencing Market Size (US$ Mn), 2018



Global Next-Generation Sequencing (NGS) Market Size (US$ Mn), 2018 to 2026



Global Next-Generation Sequencing (NGS) Market Share, By Type, 2018

Copyrights © Fortune Business Insights | www.fortunebusinessinsights.com

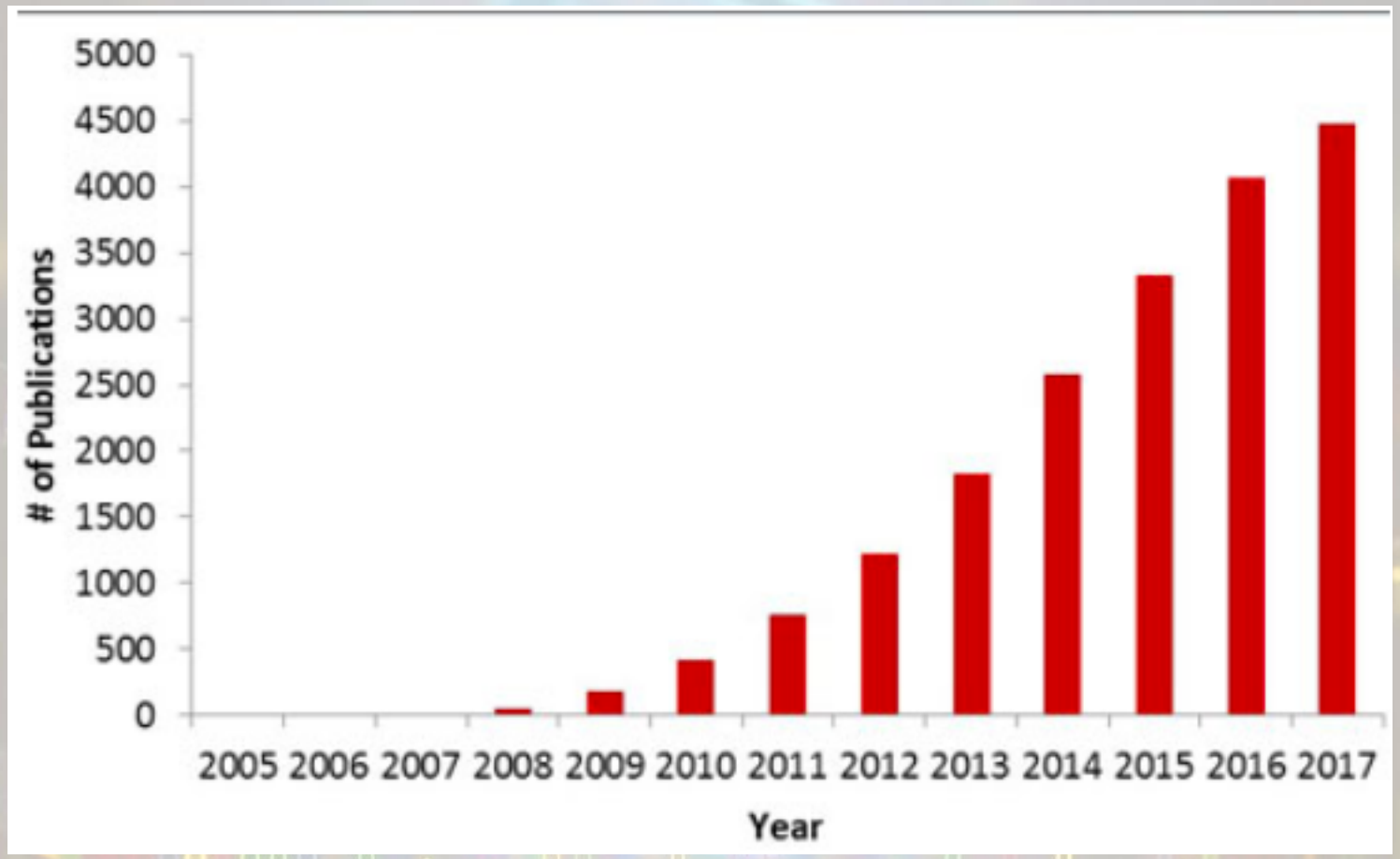
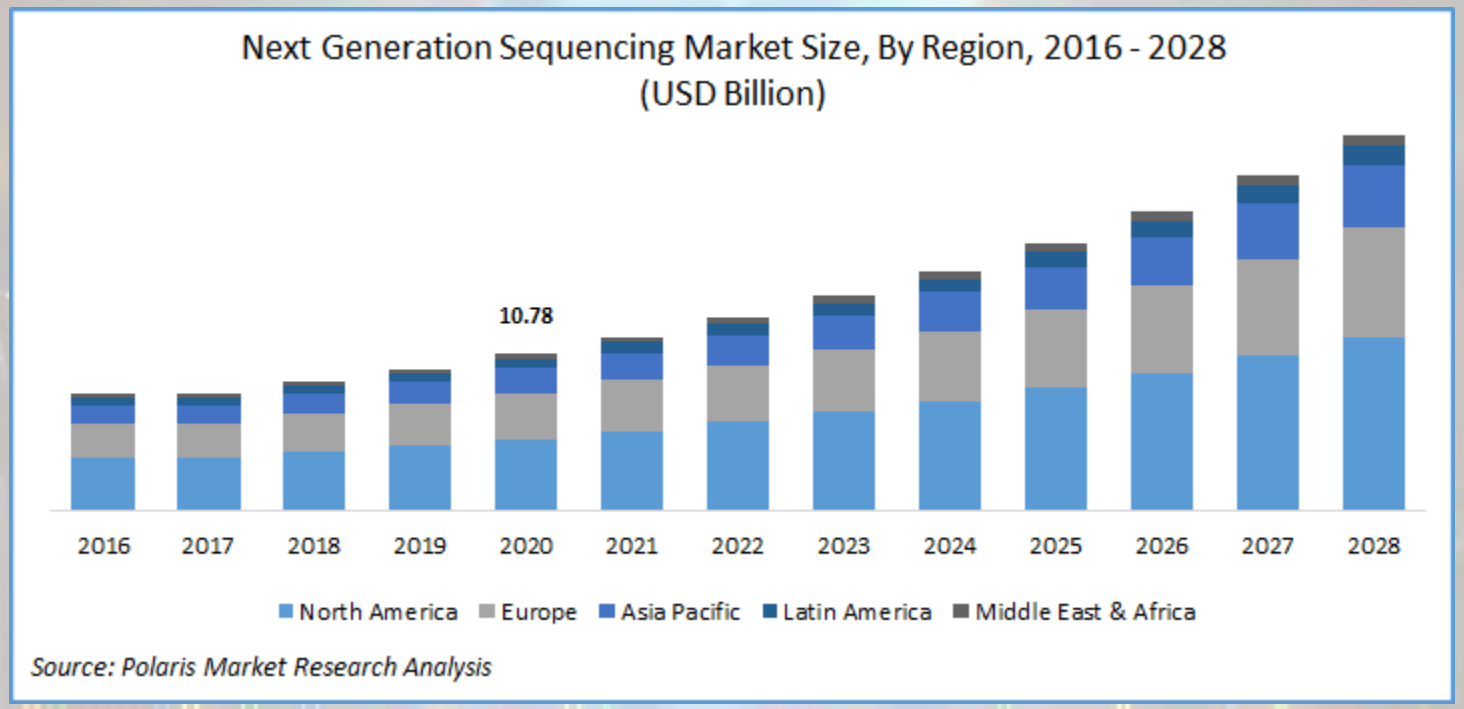
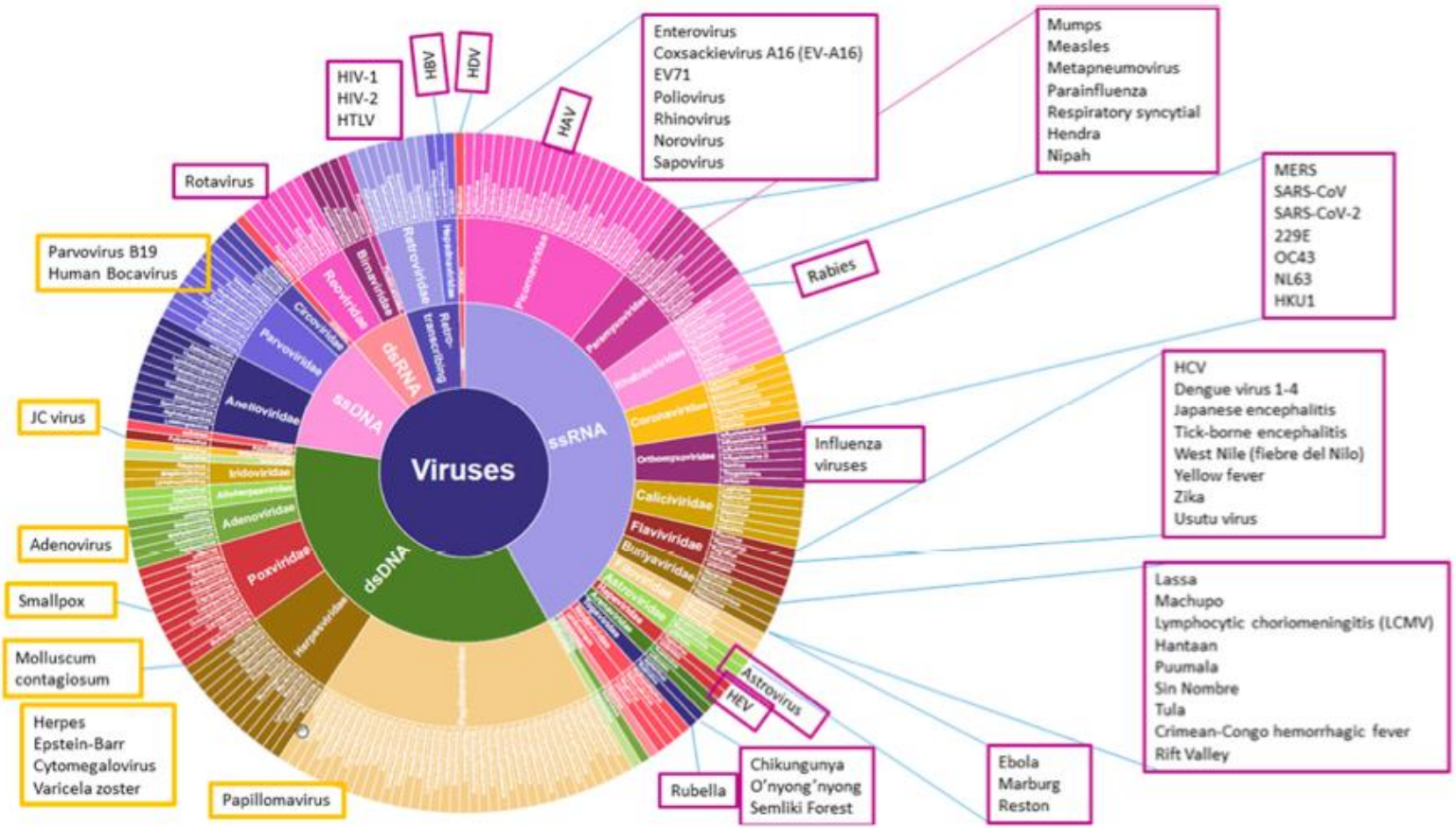
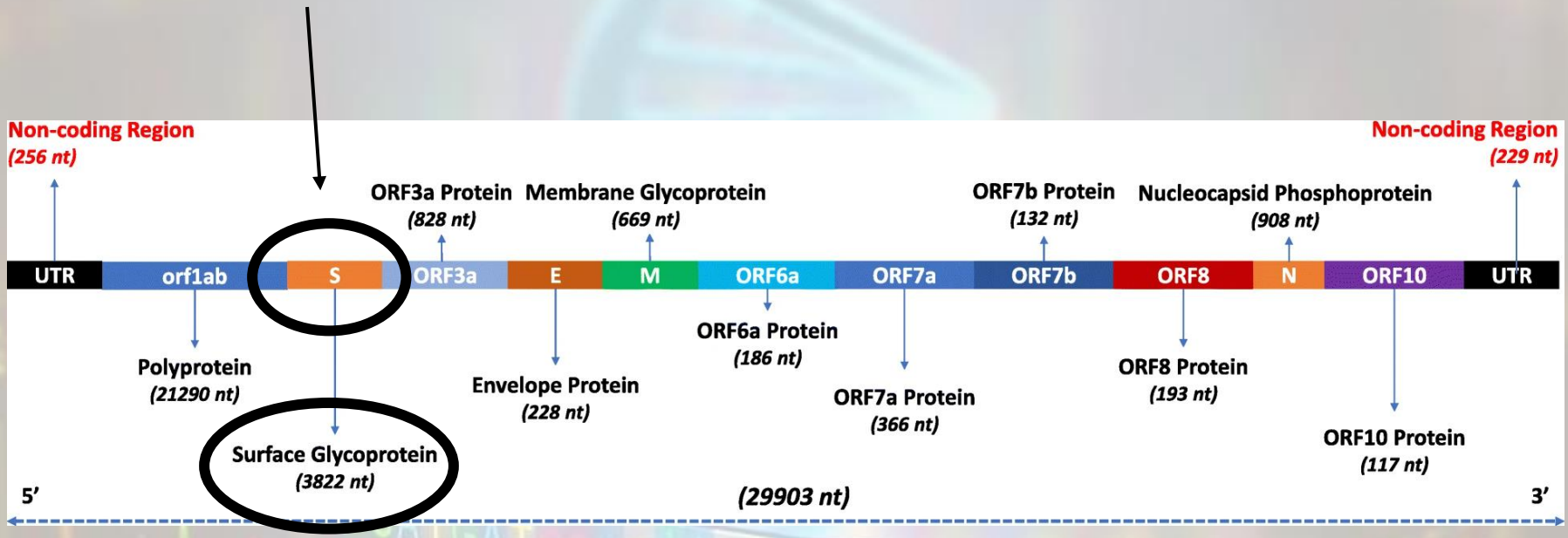


Image Courtesy of: https://www.maxpixel.net/Dna-Network-Research-Chemistry-Medical-Biology-3539309(link is external).

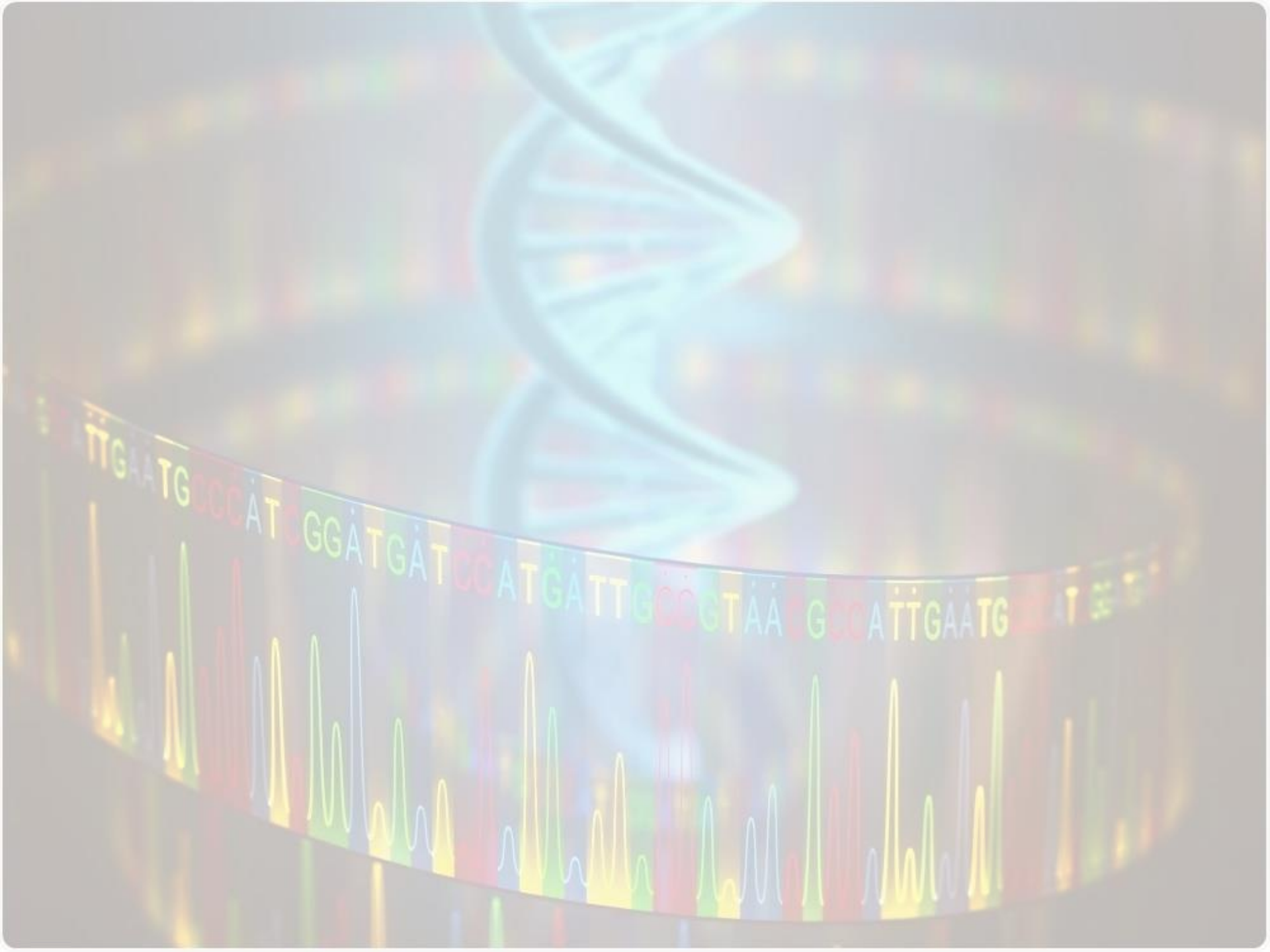

Next Generation Sequencing Market Size, By Region, 2016 - 2028
(USD Billion)
10.78
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
North America
Europe
Asia Pacific
Latin America
Middle East & Africa
Source: Polaris Market Research Analysis

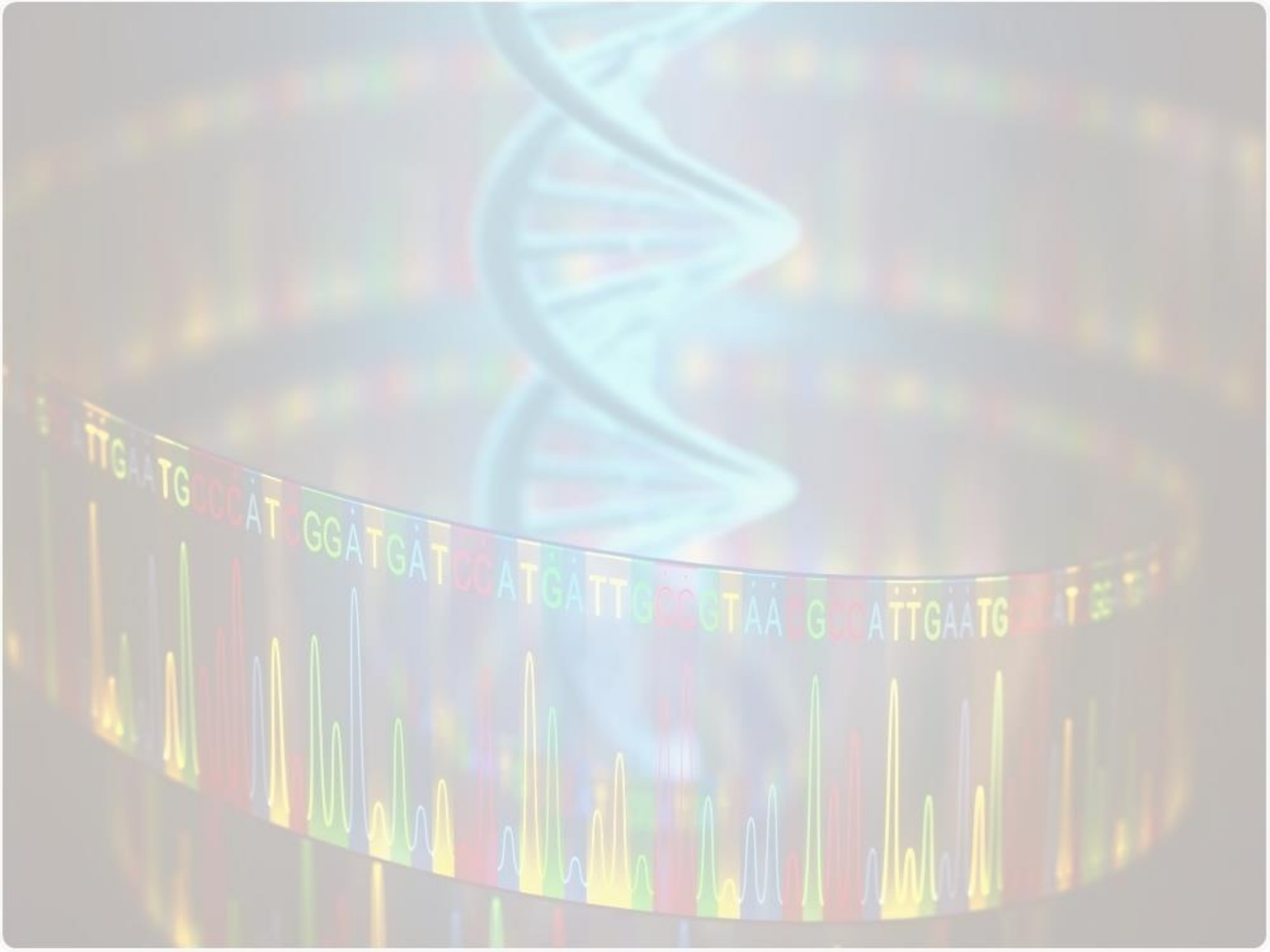


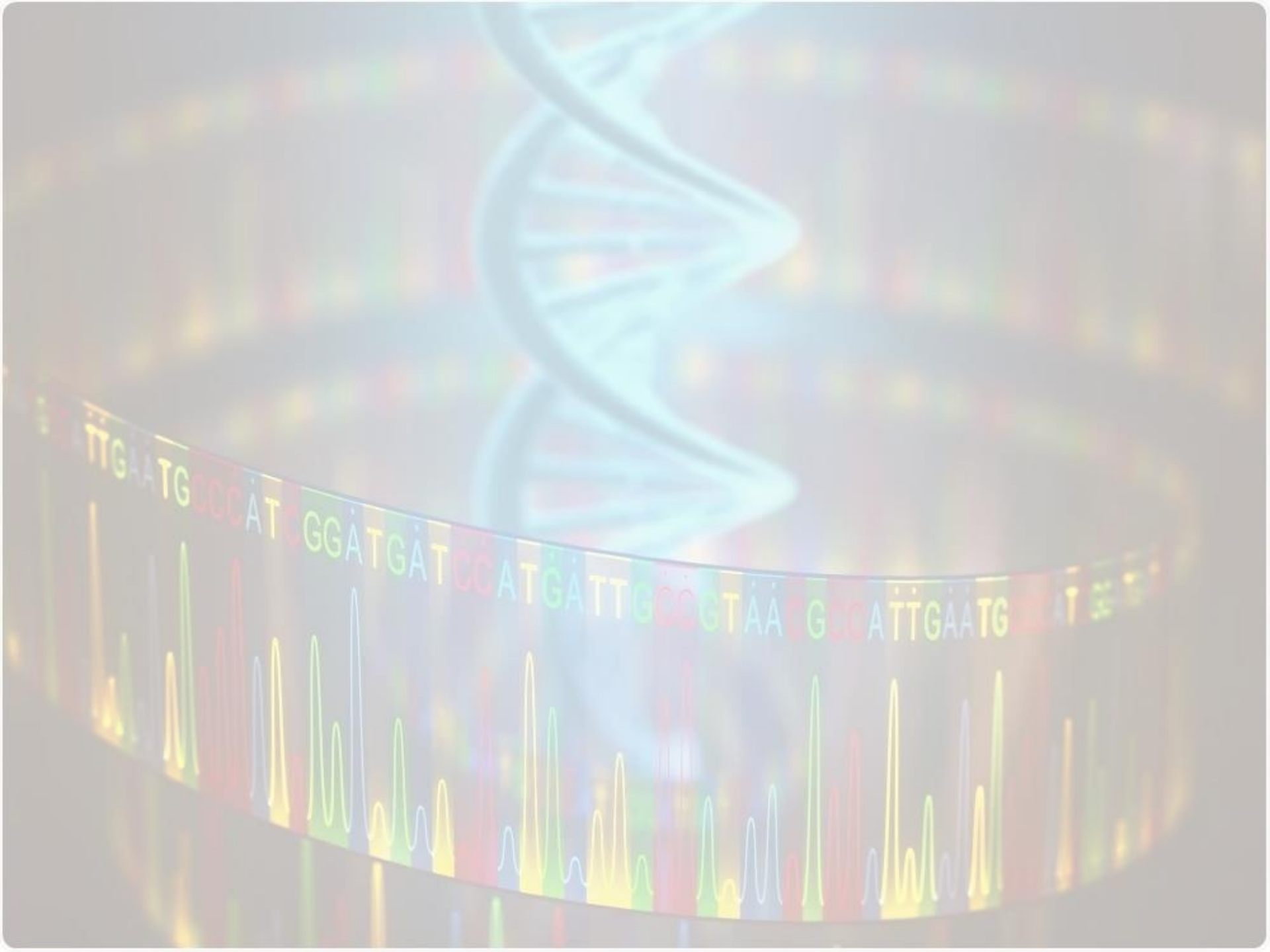
Quer J, Colomer-Castell S, Campos C, et al. Next-Generation Sequencing for Confronting Virus Pandemics. *Viruses*. 2022;14(3):600. Published 2022 Mar 14. doi:10.3390/v14030600

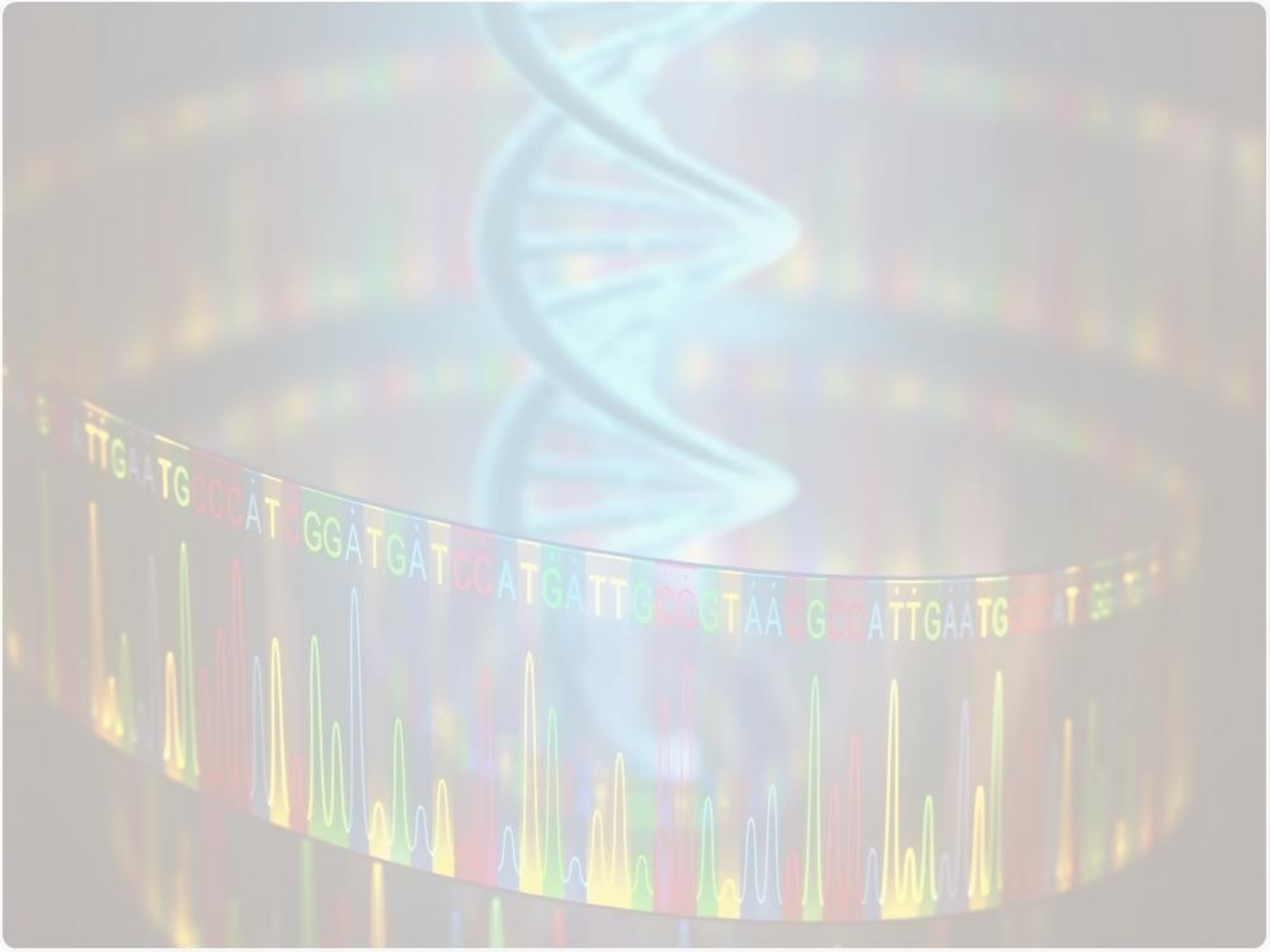


SARS-CoV-2 GENOME

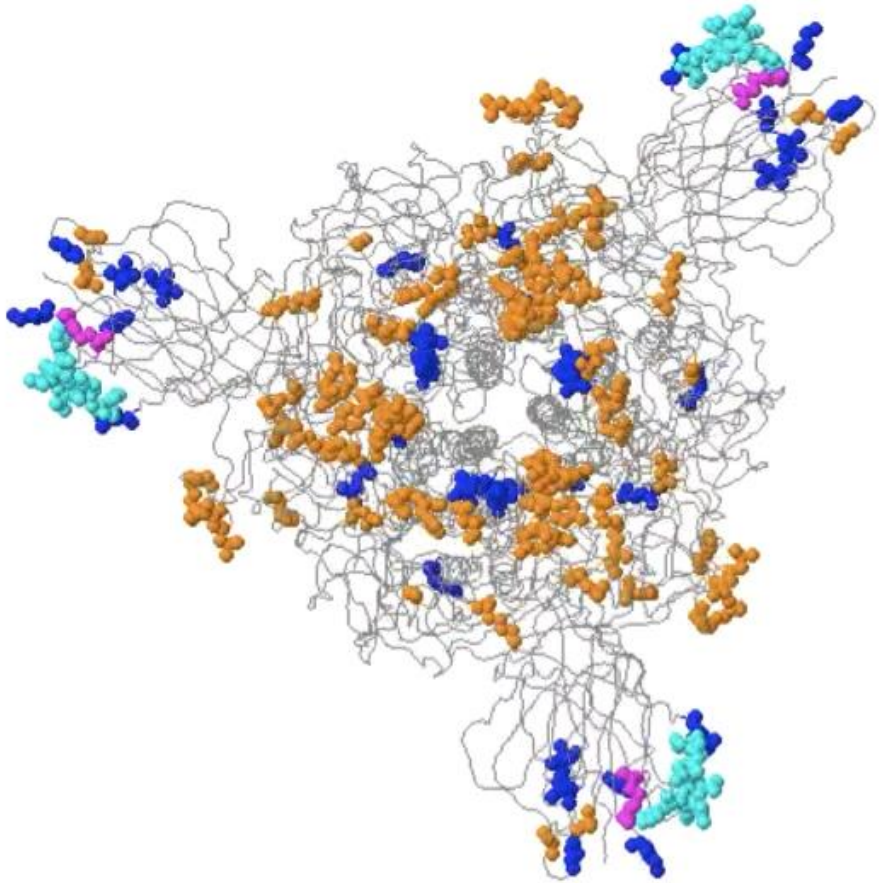


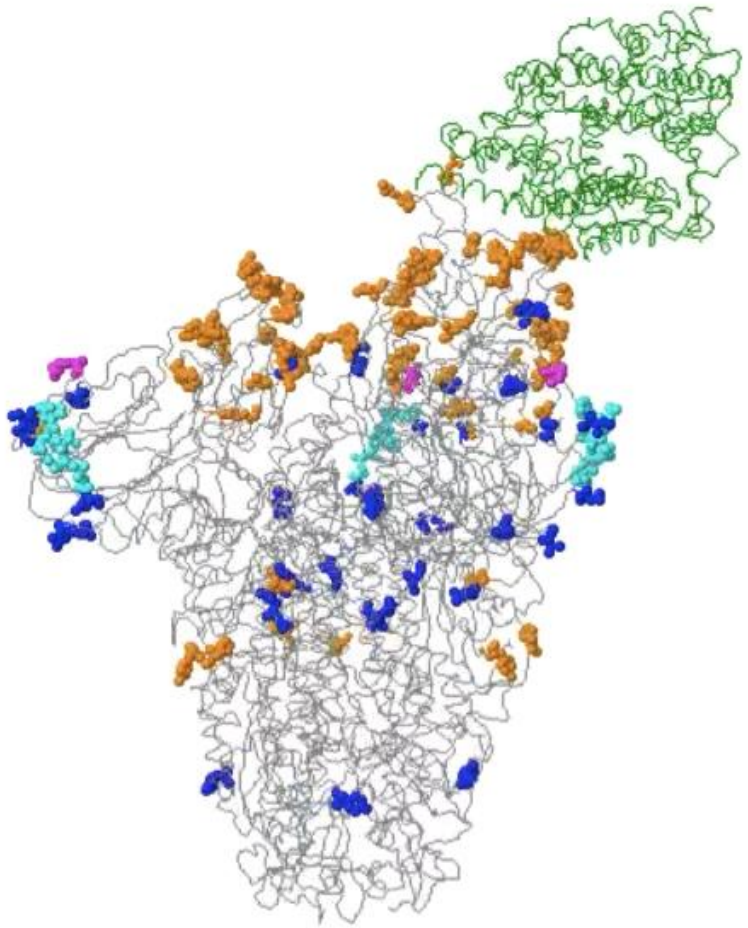






CH.1.1 (ORTHRUS)





Classificazione varianti

Al 4 aprile 2023 le classi delle varianti sono state cosi aggiornate:

VBM: Variant Being Monitored

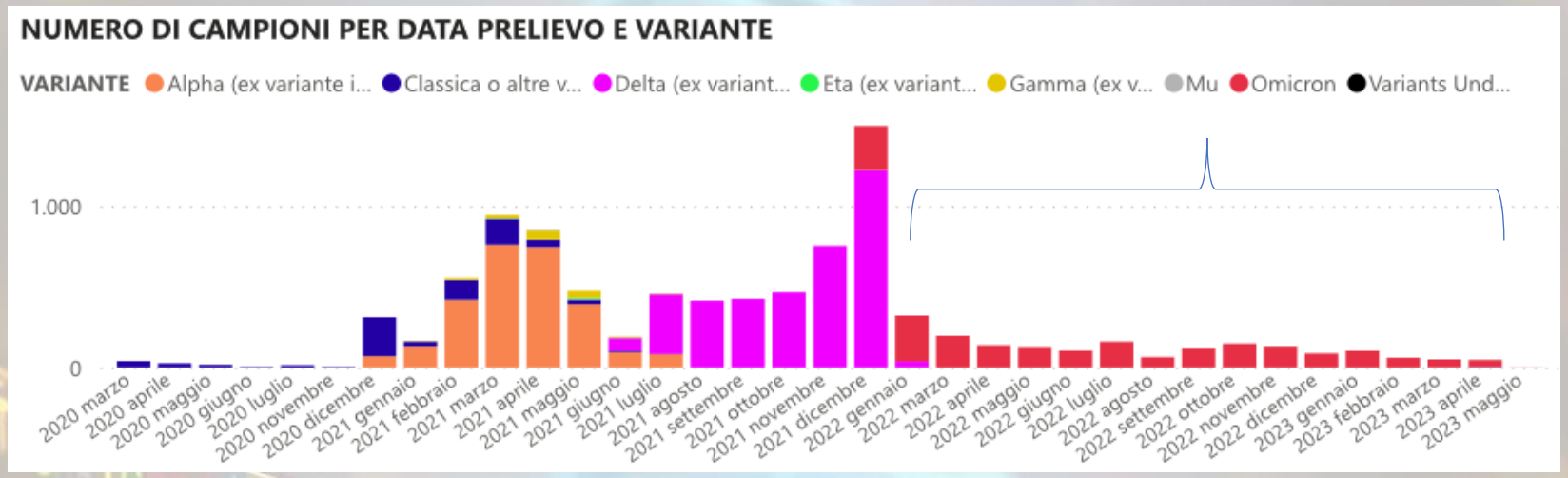
- Omicron BA.2.3.20
- Omicron BF.7
- Omicron XBC
- Omicron BN.1
- Omicron CH.1.1

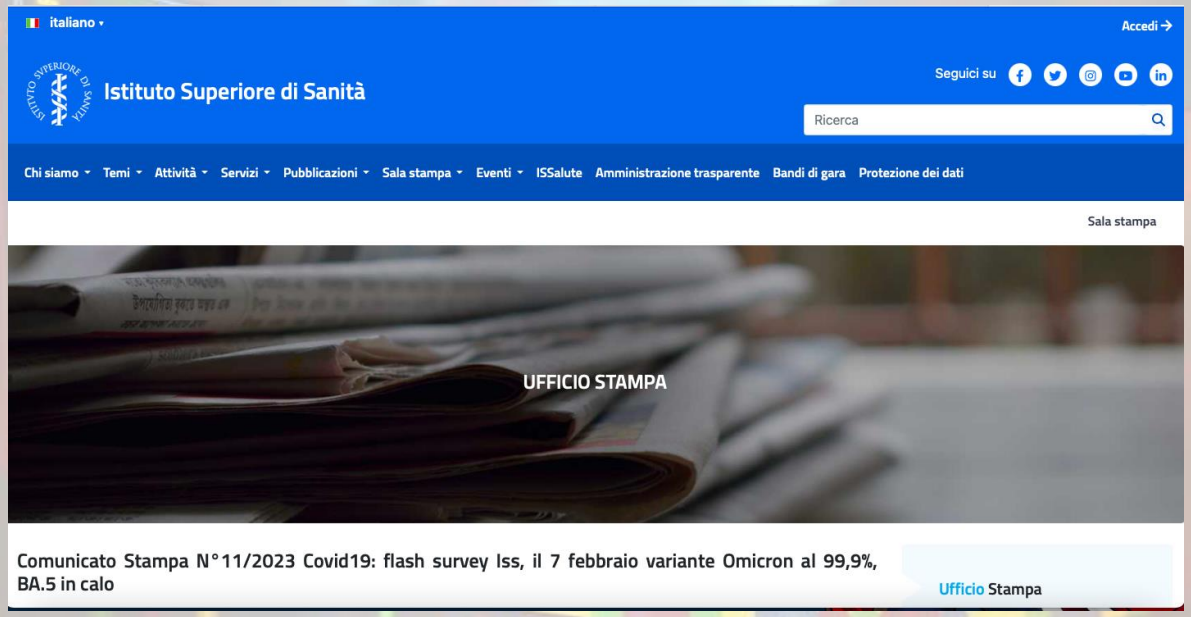
VOI: Variant Of Interest

- Omicron BA.2.75
- Omicron BQ.1
- Omicron XBB
- Omicron XBB.1.5

VOC: Variant Of Concern

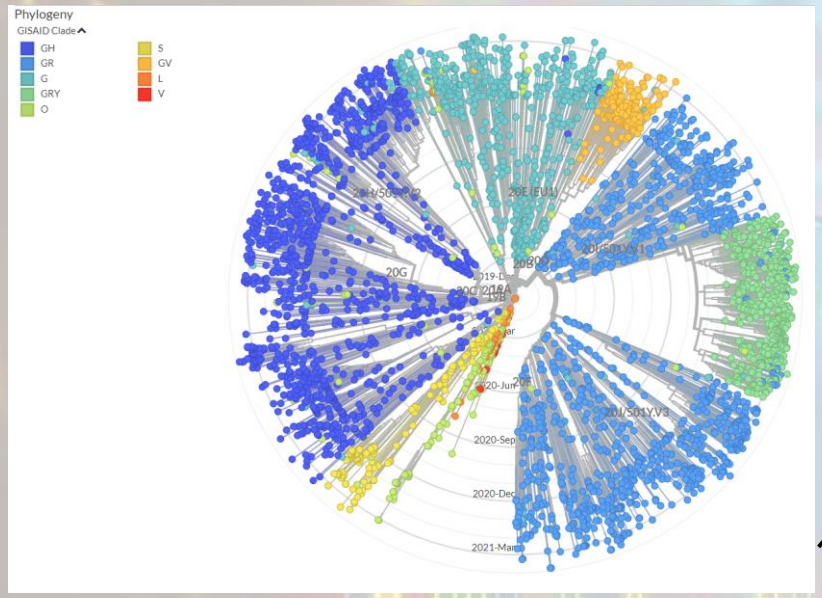
- Omicron BA.2
- Omicron BA.4
- Omicron BA.5

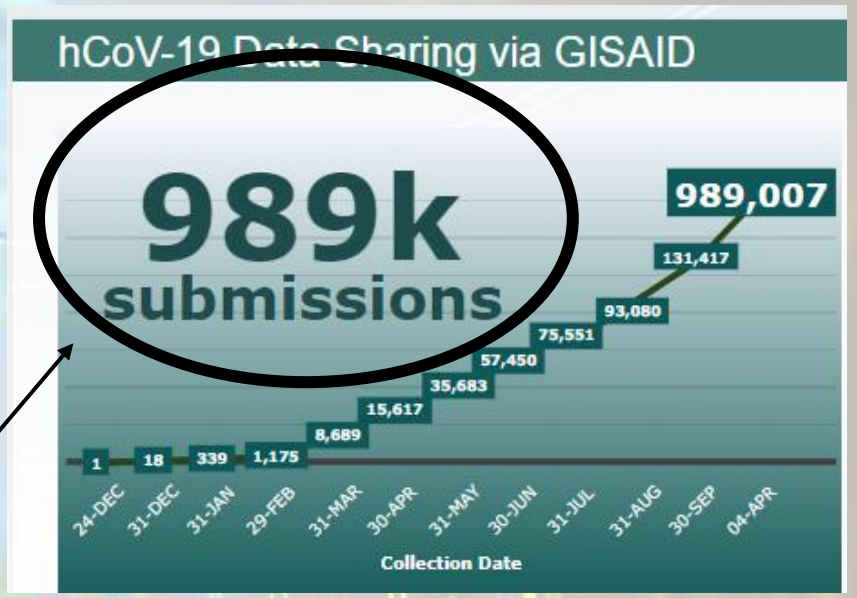

NUMERO DI CAMPIONI PER DATA PRELIEVO E VARIANTE
VARIANTE
Alpha (ex variante i...
Classica o altre v...
Delta (ex variant...
Eta (ex variant...
Gamma (ex v...
Mu
Omicron
Variants Und...
1.000
0
2020 marzo
2020 aprile
2020 maggio
2020 giugno
2020 luglio
2020 novembre
2020 dicembre
2021 gennaio
2021 febbraio
2021 marzo
2021 aprile
2021 maggio
2021 giugno
2021 luglio
2021 agosto
2021 settembre
2021 ottobre
2021 novembre
2021 dicembre
2022 gennaio
2022 marzo
2022 aprile
2022 maggio
2022 giugno
2022 luglio
2022 agosto
2022 settembre
2022 ottobre
2022 novembre
2022 dicembre
2023 gennaio
2023 febbraio
2023 marzo
2023 aprile
2023 maggio


italiano
Accedi
Istituto Superiore di Sanità
Seguici su
Ricerca
Chi siamo
Temi
Attività
Servizi
Pubblicazioni
Sala stampa
Eventi
ISSalute
Amministrazione trasparente
Bandi di gara
Protezione dei dati
Sala stampa
UFFICIO STAMPA
Comunicato Stampa N°11/2023 Covid19: flash survey Iss, il 7 febbraio variante Omicron al 99,9%, BA.5 in calo
Ufficio Stampa

Approccio di sequenziamento

- GISAID-NextStrain, sviluppato precedentemente per monitorare l'evoluzione di altri virus (influenza, Zika, West Nile, Ebola, ecc.)
- https://www.gisaid.org
- Portale pubblicamante accessibile per depositare, accedere ed esplorare i dati di sequenziamento di SARS-CoV-2
- Quasi 1 milione di genomi depositati ad aprile 2021





GISAID

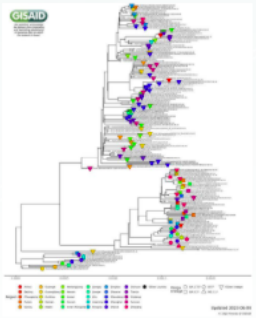
About us | Database Features | Events | Collaborations | Resources | Help | Login

In Focus

Since lifting the zero-COVID policy, data from China continues to resemble globally seen patterns

The genomic surveillance of data from China with preliminary phylogenetic analyses over recent months demonstrated a pattern of variant introduction and emergence risk which is substantially similar to that observed globally. Therefore, the daily updates will no longer be shown in the In Focus section. Future GISAID analyses for China will be viewable on the dedicated page accessible here.

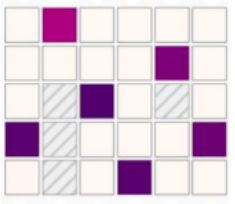
Global public health relies on timely genomic surveillance efforts in all countries and regions of the world to detect new evolutionary trends early on.



Representative genome sequences from China in the global context

中国新冠疫情 ▸

Lineage comparison ▸



hCoV-19 data sharing via GISAID

15,650,257

genome sequence submissions

METAGENOMICA

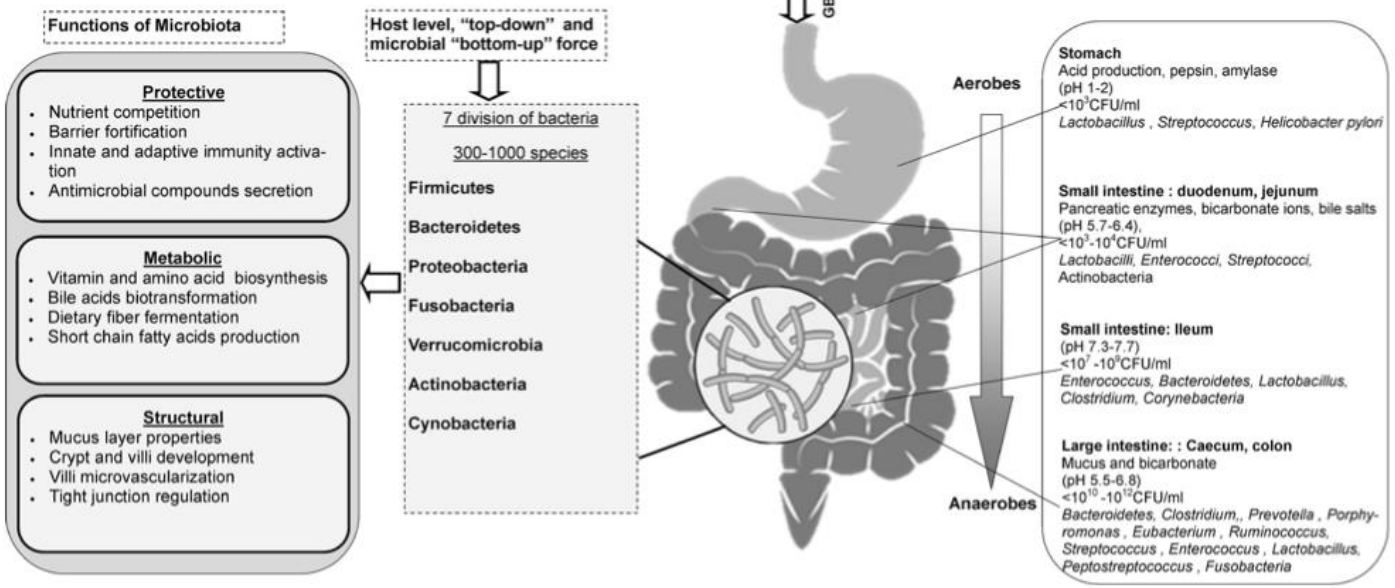
La metagenomica è uno dei campi applicativi più interessanti e promettenti delle tecnologie NGS, consiste nel **sequenziare contemporaneamente** in un piccolo campione biologico tutti gli organismi presenti nel campione, ottenendo un quadro dettagliato della diversità microbica.

Se si considera che la gran parte dei microorganismi presenti in natura è tuttora sconosciuta e **non coltivabile in laboratorio** si comprendono le enormi possibilità di questo approccio.

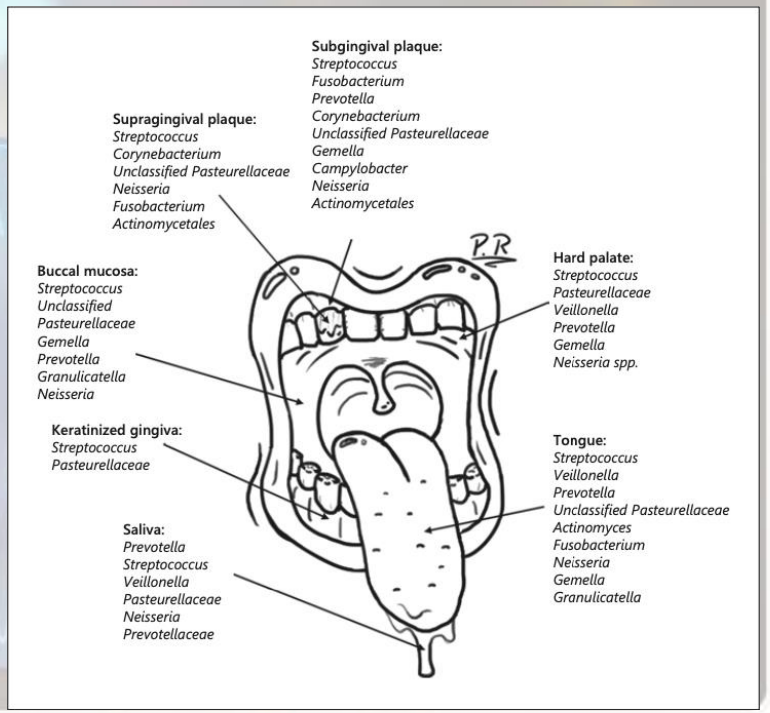
La caratterizzazione del **microbioma umano** può far luce sulle interazioni tra l'uomo e la sua flora microbica e le sue implicazioni nell'origine delle patologie.

Il nostro corpo è costituito da circa 10^{13} cellule, ma contiene un numero dieci volte maggiore (circa 10^{14}) di cellule batteriche.

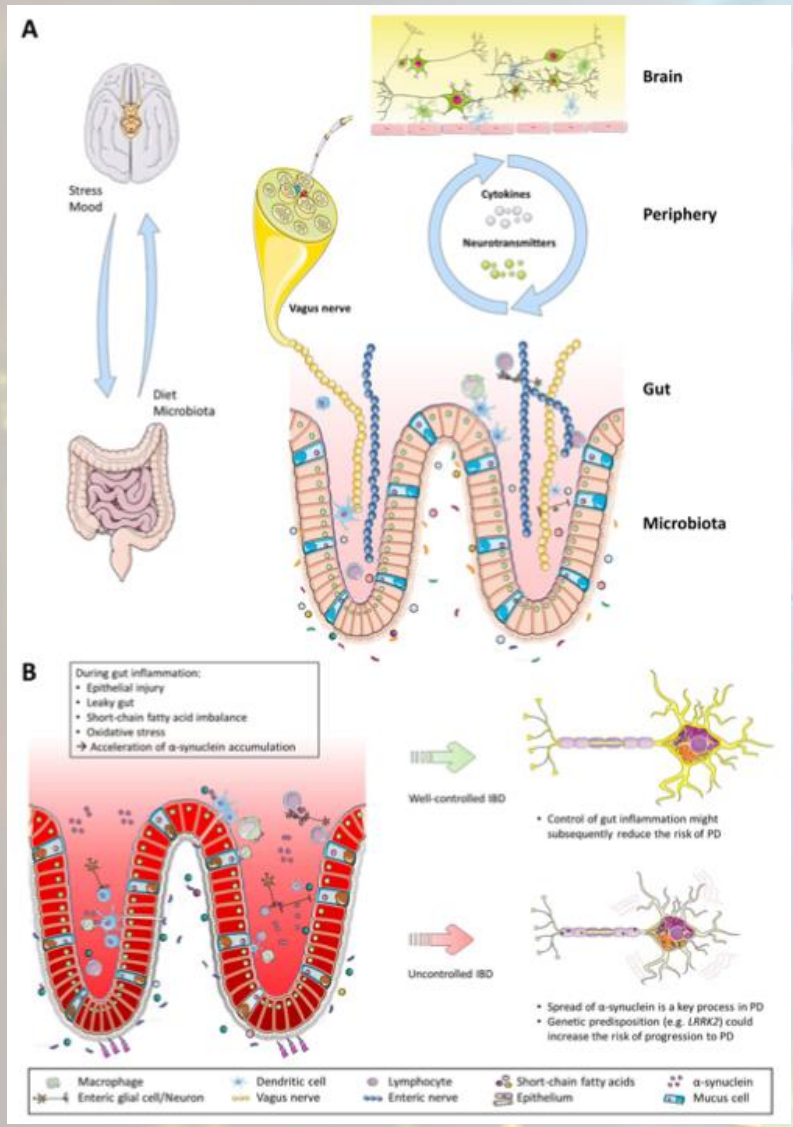
GUT MICROBIOTA



ORAL MICROBIOTA



FIRST PROJECT



RESEARCH ARTICLE Open Access

Comparison study of differential abundance testing methods using two large Parkinson disease gut microbiome datasets derived from 16S amplicon sequencing

Zachary D. Wallen*

AMERICAN SOCIETY FOR MICROBIOLOGY | mSystems®
RESEARCH ARTICLE
Host-Microbe Biology

Gut Microbiota and Metabolome Alterations Associated with Parkinson's Disease

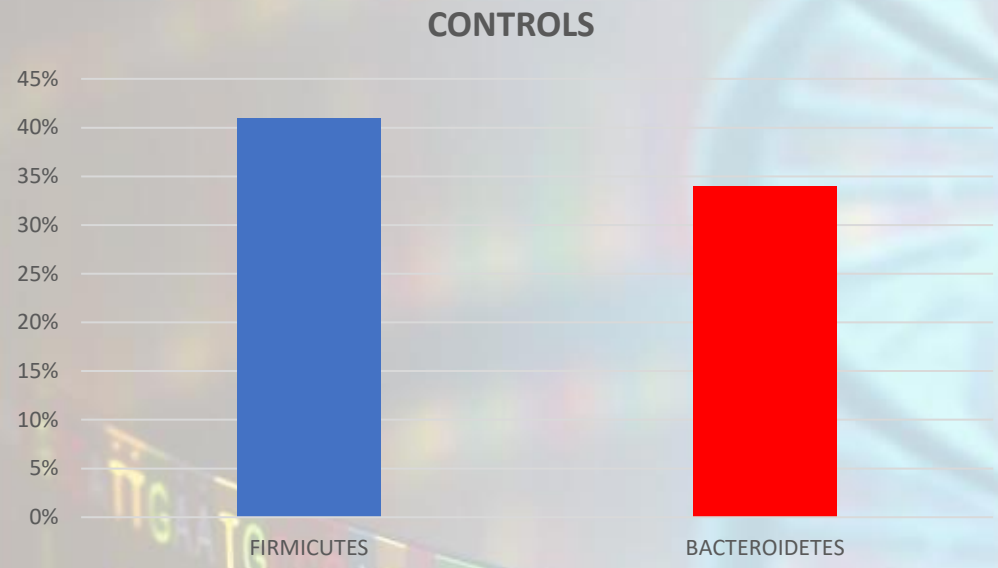
Sarah Vascellari,[a] Vanessa Palmas,[a] Marta Melis,[b] Silvia Pisanu,[a] Roberto Cusano,[c] Paolo Uva,[c] Daniela Perra,[a] Veronica Madau,[a] Marianna Sarchioto,[b,d*] Valentina Oppo,[b] Nicola Simola,[e] Micaela Morelli,[e] Maria Laura Santoru,[f] Luigi Atzori,[f] Maurizio Melis,[b] Giovanni Cossu,[b] Aldo Manzin[a]

Lin et al. Journal of Neuroinflammation (2019) 16:129
https://doi.org/10.1186/s12974-019-1528-y
Journal of Neuroinflammation
RESEARCH Open Access

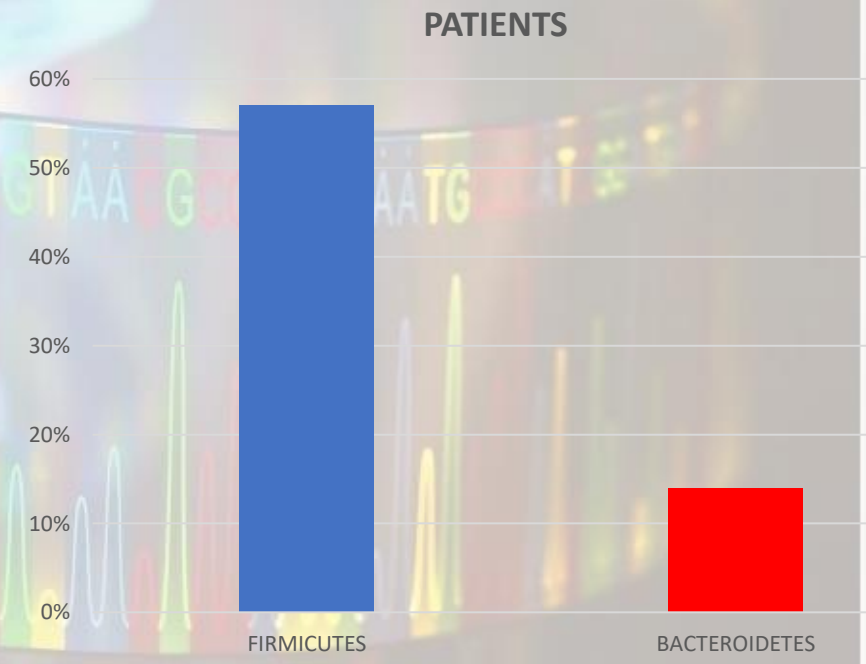
Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease

Chin-Hsien Lin[1*†], Chieh-Chang Chen[2,3†], Han-Lin Chiang[4], Jyh-Ming Liou[2], Chih-Min Chang[5], Tzu-Pin Lu[6], Eric Y. Chuang[7,8], Yi-Cheng Tai[9], Chieh Cheng[1], Han-Yi Lin[1] and Ming-Shiang Wu[2]

RESULTS OF THE FIRST PROJECT



Rapporto PHYLUM
Firmicutes / Bacteroidetes



Under physiological conditions of well-being, there is eubiosis, understood as a balance between symbiotic microorganisms, commensals, pathobionts, and the immune system. An imbalance between the two main phyla of the microbiota results in dysbiosis, which is a composition of the microbiota in which the weight of pathogenic species with proinflammatory properties is increased.

SCIENTIFIC REPORTS
nature research

OPEN

Increased abundance of proteobacteria in aggressive Crohn's disease seven years after diagnosis

Received: 13 March 2019
Accepted: 23 August 2019
Published online: 17 September 2019

M. K. Vester-Andersen[1,2], H. C. Mirsepasi-Lauridsen[3], M. V. Prosberg[1], C. O. Mortensen[5], C. Träger[5], K. Skovsen[5], T. Thorkilgaard[5], C. Nøjgaard[1], I. Vind[1], K. A. Krogfelt[3,4], N. Sørensen[6], F. Bendtsen[1] & A. M. Petersen[1,7]

SECOND PROJECT

Cell Host & Microbe
Article

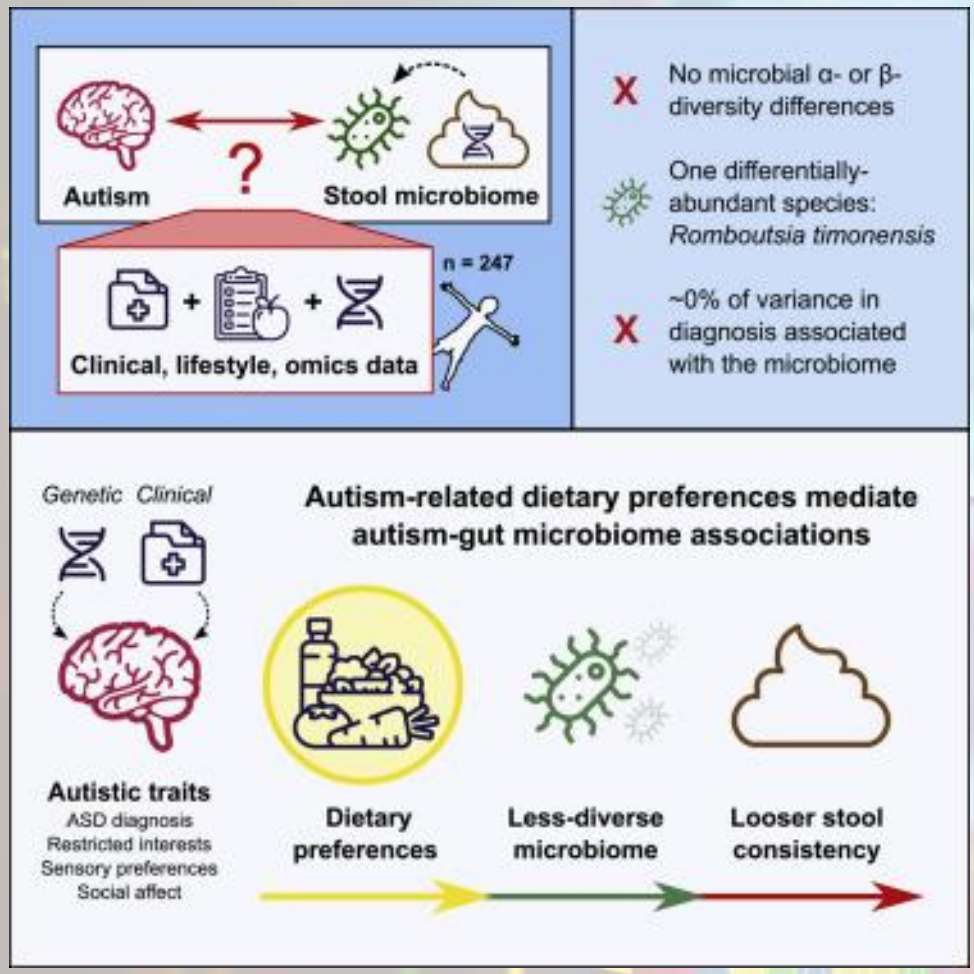
Cell PRESS

Transient Inability to Manage Proteobacteria Promotes Chronic Gut Inflammation in TLR5-Deficient Mice

Frederic A. Carvalho,[1,2,3] Omry Koren,[4] Julia K. Goodrich,[4] Malin E.V. Johansson,[5] Ilke Nalbantoglu,[6] Jesse D. Aitken,[1] Yueju Su,[1] Benoit Chassaing,[1] William A. Walters,[7] Antonio González,[8] Jose C. Clemente,[9] Tyler C. Cullender,[4] Nicolas Barnich,[11] Arlette Darfeuille-Michaud,[11] Matam Vijay-Kumar,[1] Rob Knight,[9,10] Ruth E. Ley,[4] and Andrew T. Gewirtz[1,*]

[1]Center for Inflammation, Immunity, and Infection and Department of Biology, Georgia State University, Atlanta, GA 30303, USA

THIRD PROJECT



- Study of the oral and intestinal microbiome in Autism patients

Trends in Cognitive Sciences

CellPress
REVIEWS

Feature Review

The Microbiome in Psychology and Cognitive Neuroscience

Amar Sarkar,[1,2,3,*] Siobhán Harty,[1,4] Soili M. Lehto,[5,6,7] Andrew H. Moeller,[8] Timothy G. Dinan,[9,10] Robin I.M. Dunbar,[1] John F. Cryan,[10,11] and Philip W.J. Burnet[12]

International Journal of
Molecular Sciences

MDPI

Review

The Possible Role of the Microbiota-Gut-Brain-Axis in Autism Spectrum Disorder

Piranavie Srikantha and M. Hasan Mohajeri *

Department of medicine, University of Zurich, Winterthurerstrasse 190, 8057 Zürich, Switzerland; piranavie.srikantha@uzh.ch
* Correspondence: mhasan.mohajeri@uzh.ch; Tel.: +41-79-938-1203

Received: 27 March 2019; Accepted: 28 April 2019; Published: 29 April 2019

check for updates

Neuroscience 324 (2016) 131–139

NEUROSCIENCE

IBRO INTERNATIONAL BRAIN RESEARCH ORGANIZATION

REVIEW

C. Doenyas / Neuroscience 374 (2018) 271–286

Gut Microbiota, Inflammation, and Probiotics on Neural Development in Autism Spectrum Disorder

Ceymi Doenyas
Koc University, Istanbul, Turkey

REVIEW

THE MICROBIOTA–GUT–BRAIN AXIS AND ITS POTENTIAL THERAPEUTIC ROLE IN AUTISM SPECTRUM DISORDER

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v22.i1.361

World J Gastroenterol 2016 January 7; 22(1): 361-368
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2016 Baishideng Publishing Group Inc. All rights reserved.

REVIEW

Gut microbiota in autism and mood disorders

Francesca Mangiola, Gianluca Ianiro, Francesco Franceschi, Stefano Fagiuoli, Giovanni Gasbarrini, Antonio Gasbarrini

SCIENTIFIC REPORTS

OPEN

Analysis of gut microbiota profiles and microbe-disease associations in children with autism spectrum disorders in China

Received: 14 March 2018
Accepted: 24 August 2018
Published online: 18 September 2018

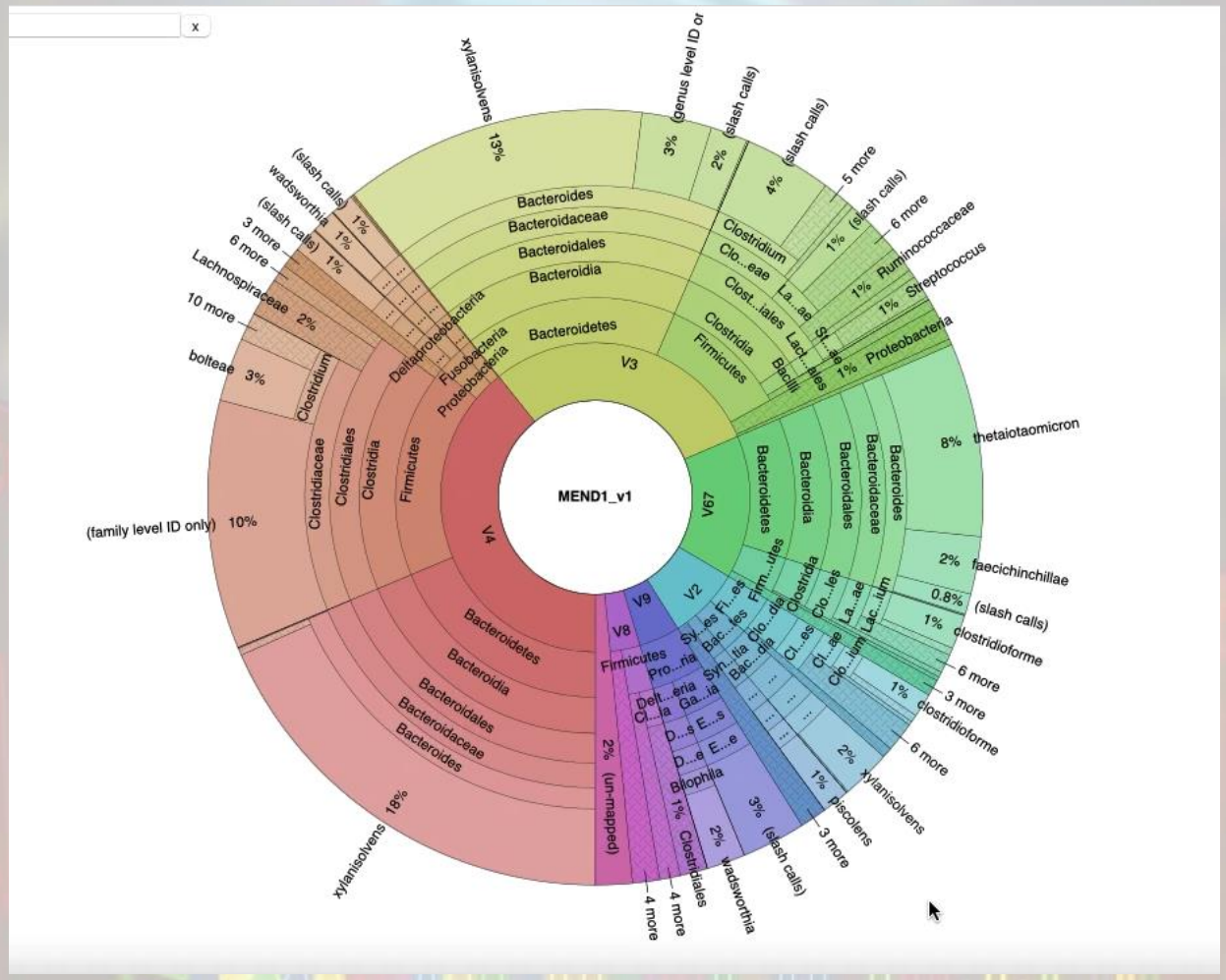
Mengxiang Zhang[1,2], Wei Ma[3], Juan Zhang[4], Yi He[1,2] & Juan Wang[1,2]

Review

Biological Psychiatry

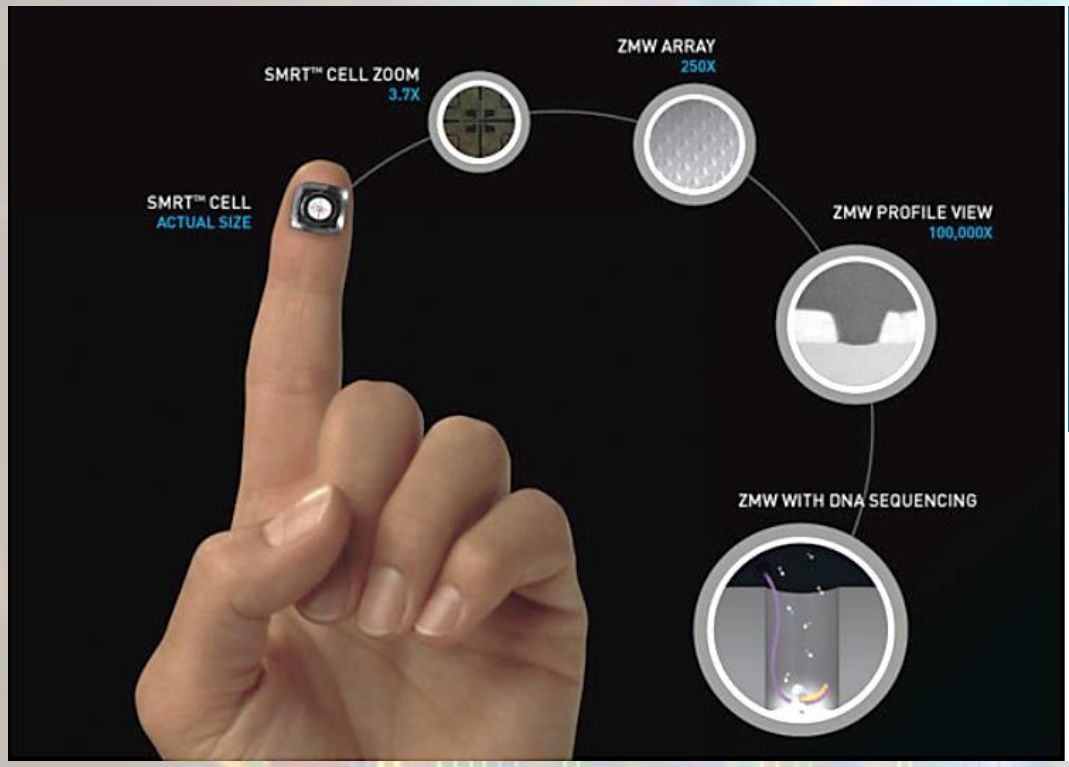
Emerging Roles for the Gut Microbiome in Autism Spectrum Disorder

Helen E. Vuong and Elaine Y. Hsiao

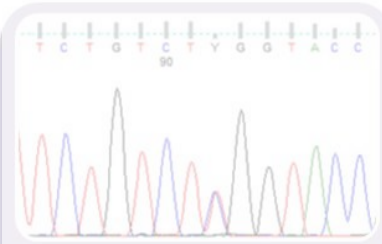

x
MEND1_v1
V3
V67
V2
V9
V8
V4
xylanisolvens 13%
3% (genus level ID or
2% (slash calls)
4% (slash calls)
5 more
1% (slash calls)
6 more
1% Ruminococcaceae
1% Streptococcus
1% Proteobacteria
Bacteroides
Bacteroidaceae
Bacteroidales
Bacteroidia
Bacteroidetes
Clostridium
Clo...eae
Clost...iales
Clostridia
Firmicutes
La...ae
Lact...ales
Bacilli
8% thetaiotaomicron
2% faecichinchillae
0.8% (slash calls)
1% clostridioforme
6 more
3 more clostridioforme
6 more
2% xylanisolvens
1% piscolens
3 more
3% (slash calls)
2% wadsworthia
Clostridiales
1% Blautia
4 more
2% (un-mapped)
4 more
xylanisolvens 18%
Bacteroides
Bacteroidaceae
Bacteroidales
Bacteroidia
Bacteroidetes
Clostridia
Clostridiales
Clostridiaceae
(family level ID only) 10%
bolteae 3%
Clostridium
10 more
Lachnospiraceae 2%
6 more
3 more
(slash calls) 1%
wadsworthia 1%
(slash calls) 1%
Deltaproteobacteria
Fusobacteria
Proteobacteria

What's next?

3rd Generation Sequencing Technologies - NGS



What's next?

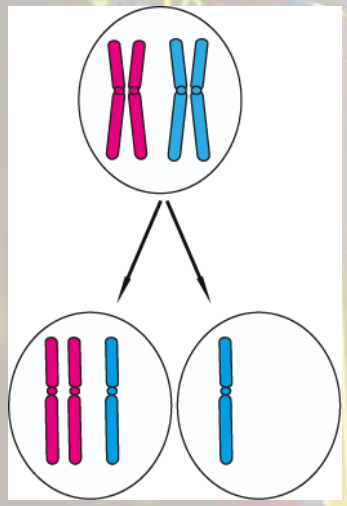


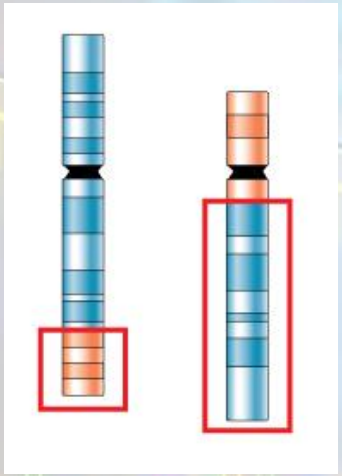
	Past	Current	Future
Analysis method	Sanger or direct sequencing	Targeted-NGS WES	WGS Long-read sequencing
Assessed features	SNVs Small del/ins/indels	SNVs Small del/ins/indels CNVs	SNVs Small/large del/ins/indels CNVs Trinucleotide repeat expansion
Fragment lengths *Technical accuracy*	500 - 1000 bp *Very high*	50 – 150 bp *High*	50-150 bp; > 500 bp *Low*
Functional interpretation	Literature	Computational Pathogenicity prediction	Computational Pathogenicity prediction Multi-omics tools

Russo R, Marra R, Rosato BE, Iolascon A, Andolfo I. Front. Physiol. 2020

Large scale

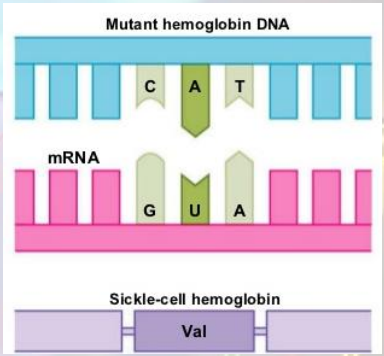
- Aneuploidy
- Structural rearrangements

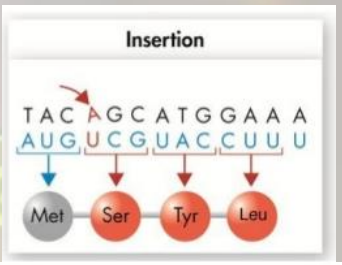


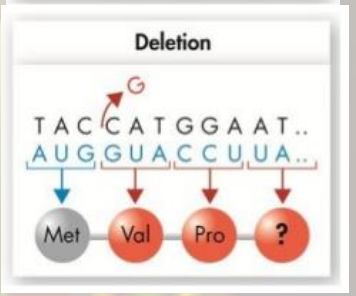


Smaller scale

- Base substitutions
- Small insertions and deletions







Russo R, Marra R, Rosato BE, Iolascon A, Andolfo I. Front. Physiol. 2020

Laboratorio di Genetica Molecolare

Center for Advanced Studies and Technology (CAST)

Università G.D'Annunzio Chieti-Pescara

Via L.Polacchi 11, 66100, Chieti, Italy

Grazie per l'attenzione