AO AL Azienda Ospedaliera di ALESSANDRIA Santi Antonio e Biagio e Cesare Arrigo

Dipartimento Attività Integrate Ricerca e Innovazione AO AL - ASL AL

IL SEQUENZIAMENTO NGS NEL LABORATORIO DI MICROBIOLOGIA: VALIDAZIONE, IMPLEMENTAZIONE ED UTILITÀ CLINICA

11 NOVEMBRE *2023*

ore **9.00**

Salone di Rappresentanza
AON SS. Antonio e Biagio e Cesare Arrigo
Via Venezia 16, Alessandria

LA DIAGNOSTICA VIROLOGICA E L'APPLICAZIONE NGS
Lo stato dell'arte

Maria R. Capobianchi

UNICAMILLUS
.International Medical University in Rome

SACRO CUORE DON CALABRIA
Istituto Don Calabria
IRCCS Ospedale
Sacro Cuore Don Calabria
Presidio Ospedaliero Accreditato - Regione Veneto
I R C C S

Disclosures

Maria R. Capobianchi

Has received fees from:

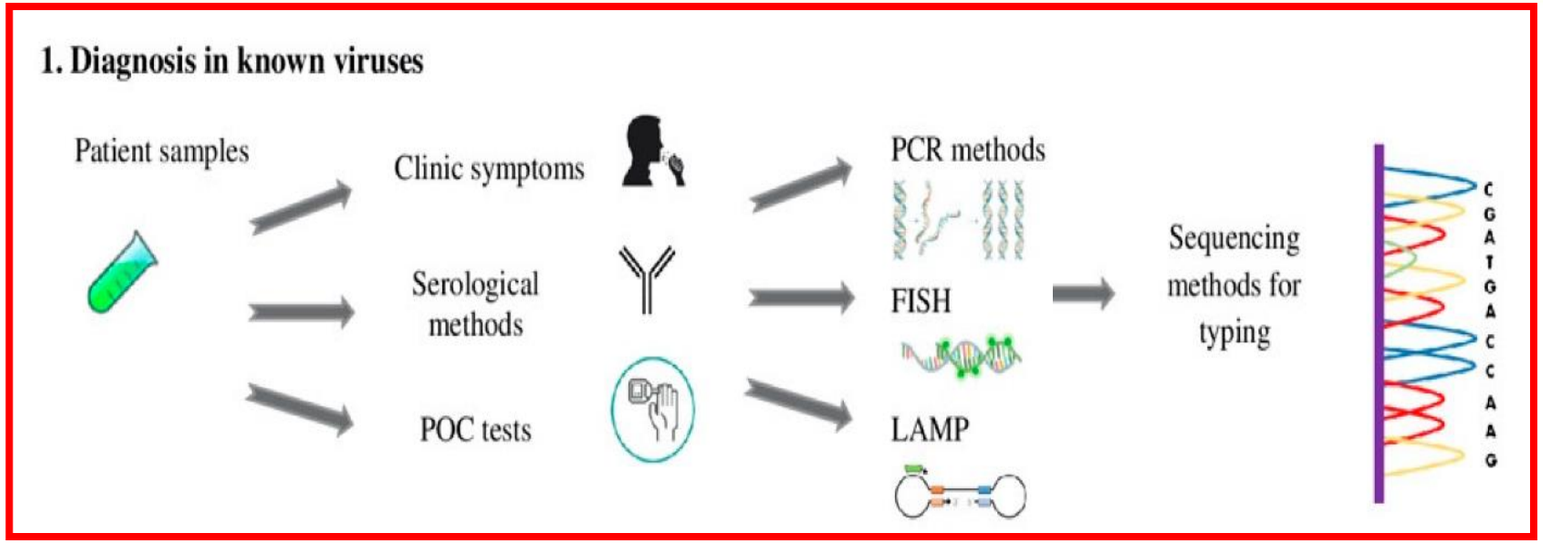
- Ma.CRO/Pfizer S.r.l. (educational activity)
- Proeventi S.r.l. /AB Analitica S.r.l. (educational activity)

Presentation outline

NGS applications to virology

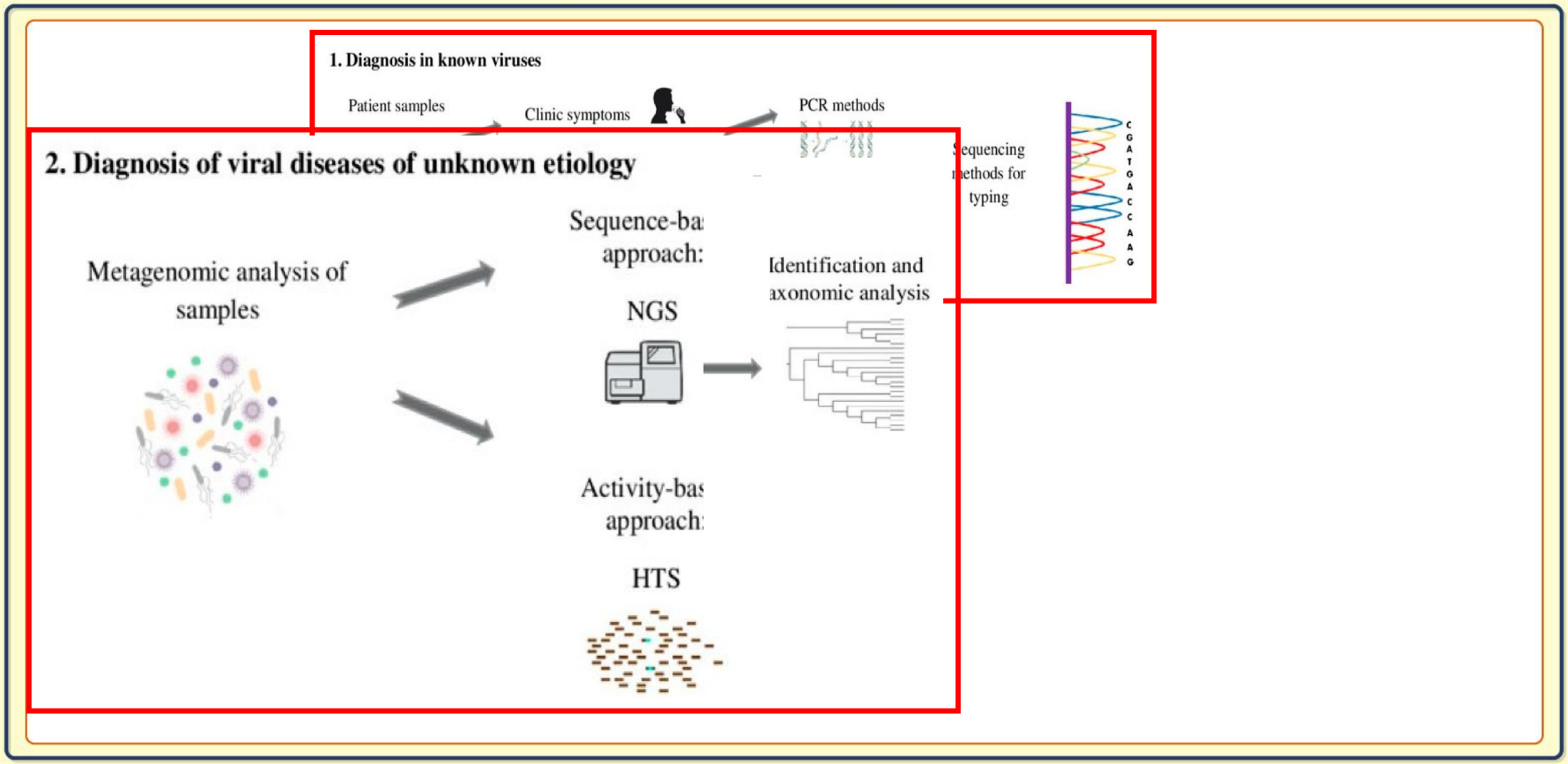
- Different NGS approaches address different issues
- Whole genome analysis
- Viral quasispecies and minority genomes
  - ✓Tropism and compartmentalization
  - ✓Quasispecies dynamics and implications for resistance
- Metagenomics
- NGS challenges; potential bias; standardization; ethical issues

Sequencing in the management of viral diseases



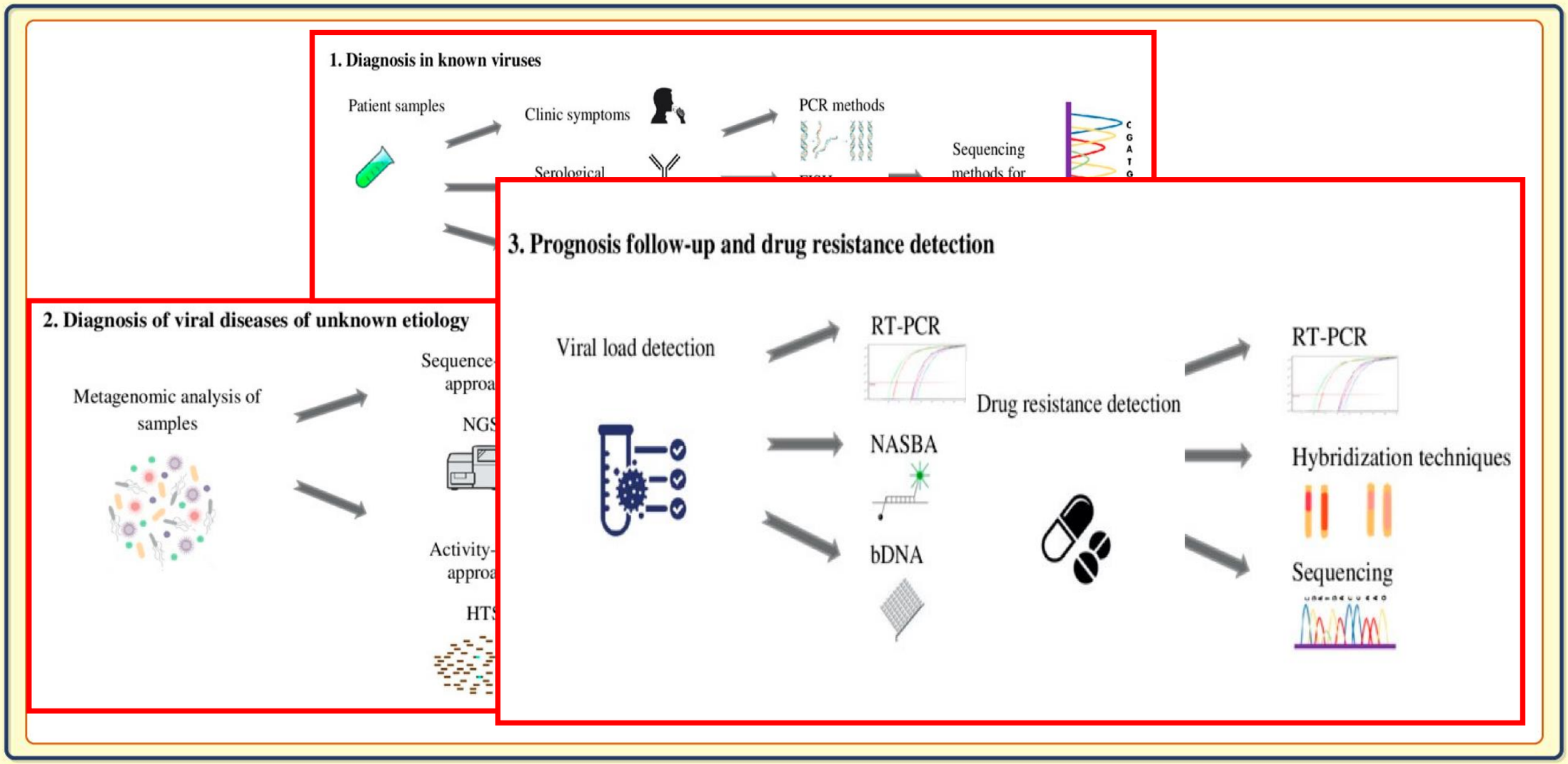
Ltindişet al. Diagnostics 2023 doi: 10.3390/diagnostics13081421

Sequencing in the management of viral diseases



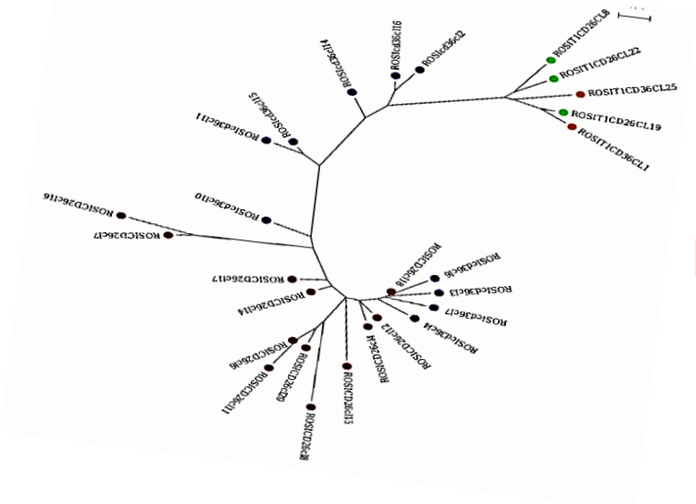
Ltindişet al. Diagnostics 2023 doi: 10.3390/diagnostics13081421

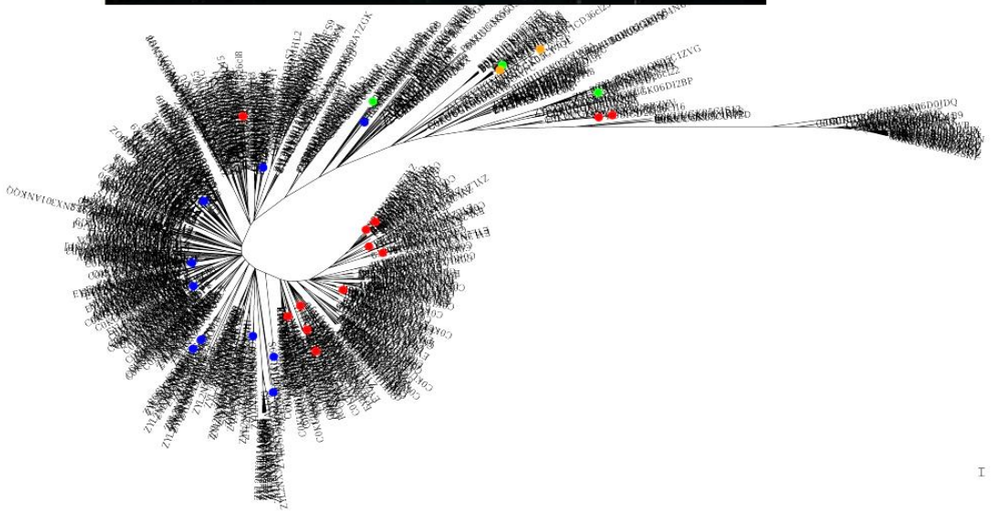
Sequencing in the management of viral diseases



Ltindişet al. Diagnostics 2023 doi: 10.3390/diagnostics13081421

NGS REVOLUTION





The NEW ENGLAND JOURNAL of MEDICINE

FOCUS ON RESEARCH

February 7, 2008 .

The New Age of Molecular Diagnostics for Microbial Agents

Richard Whitley, M.D.

Published in final edited form as:
J Clin Virol. 2014 September ; 61(1): 9–19. doi:10.1016/j.jcv.2014.06.013.

Deep Sequencing: Becoming a Critical Tool in Clinical Virology

Miguel E. QUIÑONES-MATEU[1,2,*], Santiago AVILA[3,4], Gustavo REYES-TERAN[3,4], and Miguel A. MARTINEZ[5]

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

OPINION
published: 0
doi: 10.3389/fcimb.2014.00025

The diagnosis of infectious diseases by whole genome next generation sequencing: a new era is opening

*Marc Lecuit[1,2,3,4] and Marc Eloit[5,6]**

Journal of Clinical Virology 58 (2013) 346–350

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Review

Next-generation sequencing technologies in diagnostic virology

Luisa Barzon *, Enrico Lavezzo, Giulia Costanzi, Elisa Franchin, Stefano Toppo, Giorgio Palù

VOLUME 7 | APRIL 2009 | 287

NATURE REVIEWS | MICROBIOLOGY

Application of 'next-generation' sequencing technologies to microbial genetics

Daniel MacLean, Jonathan D. G. Jones and David J. Studholme

OPEN ACCESS Freely available online

PLOS GENETICS

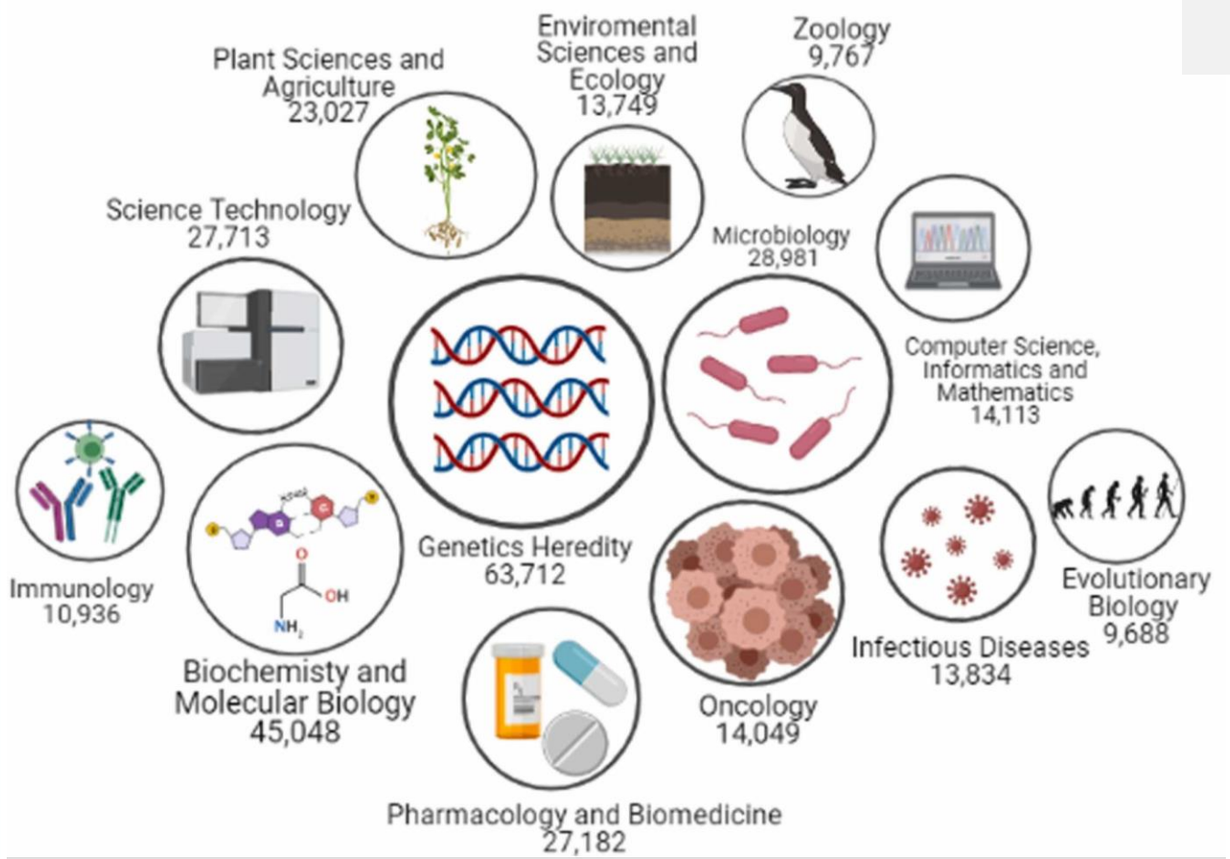
Perspective

The Next Generation Becomes the Now Generation

Diego A. Martinez*, Mary Anne Nelson

Department of Biology, University of New Mexico, Albuquerque, New Mexico, United States of America

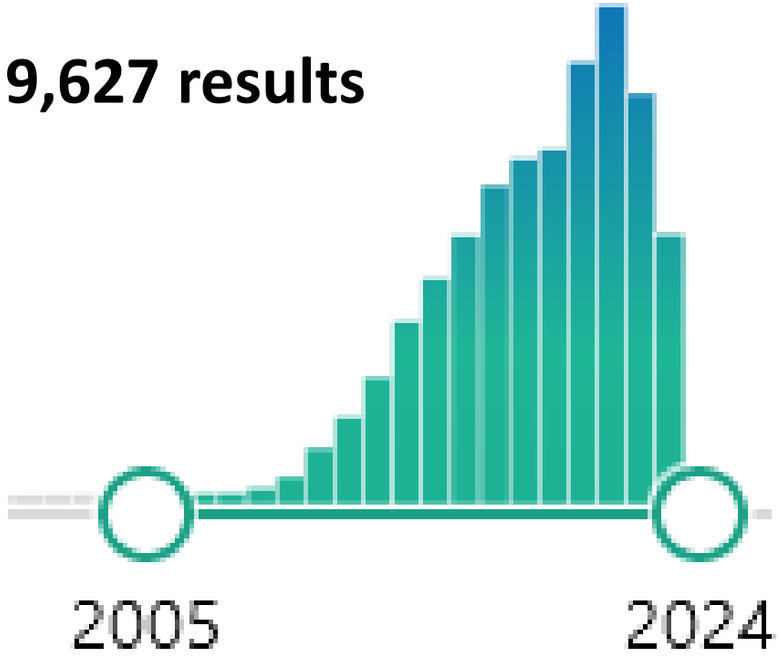
NGS applications in PubMed



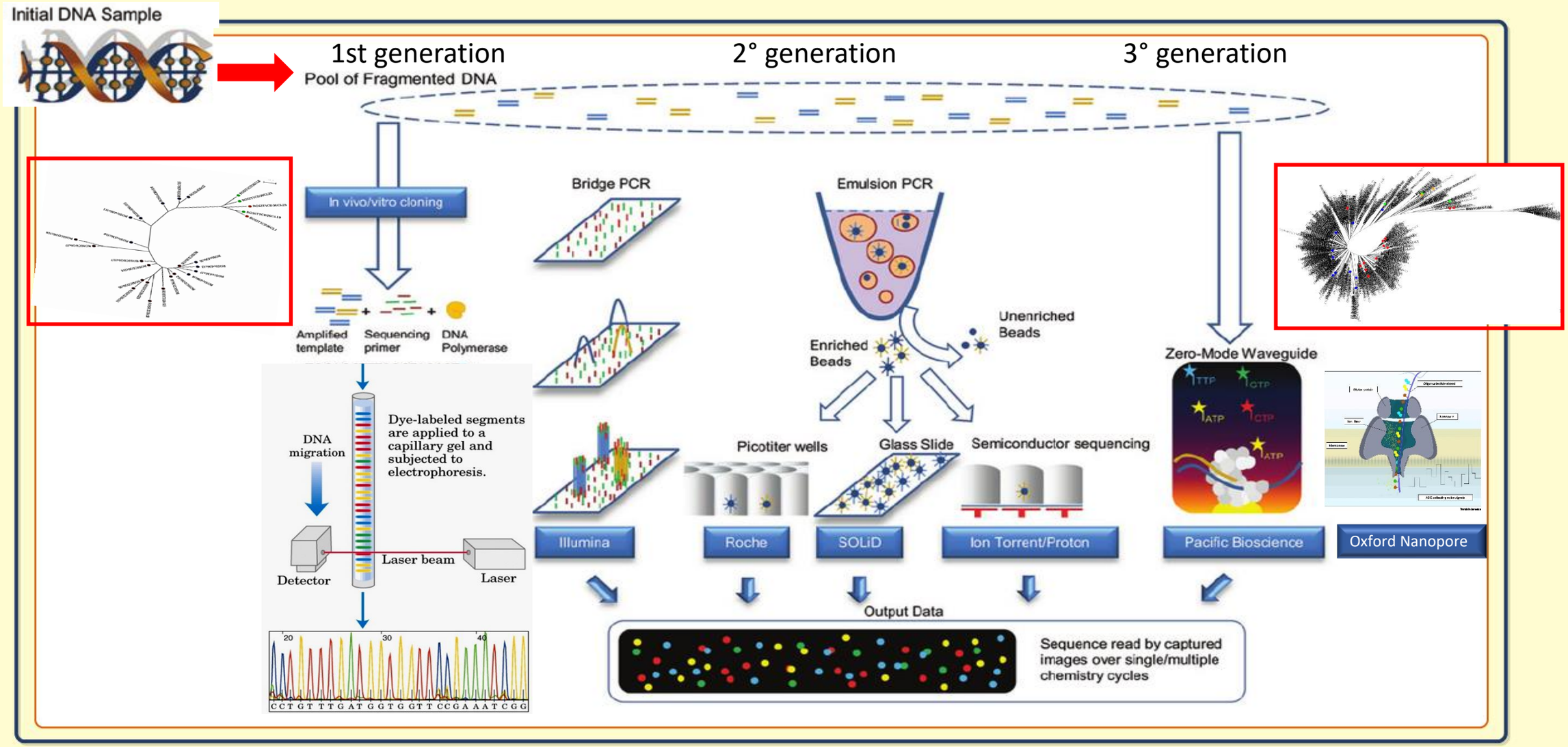
PubMed®

Search terms:
Next generation sequencing and virus

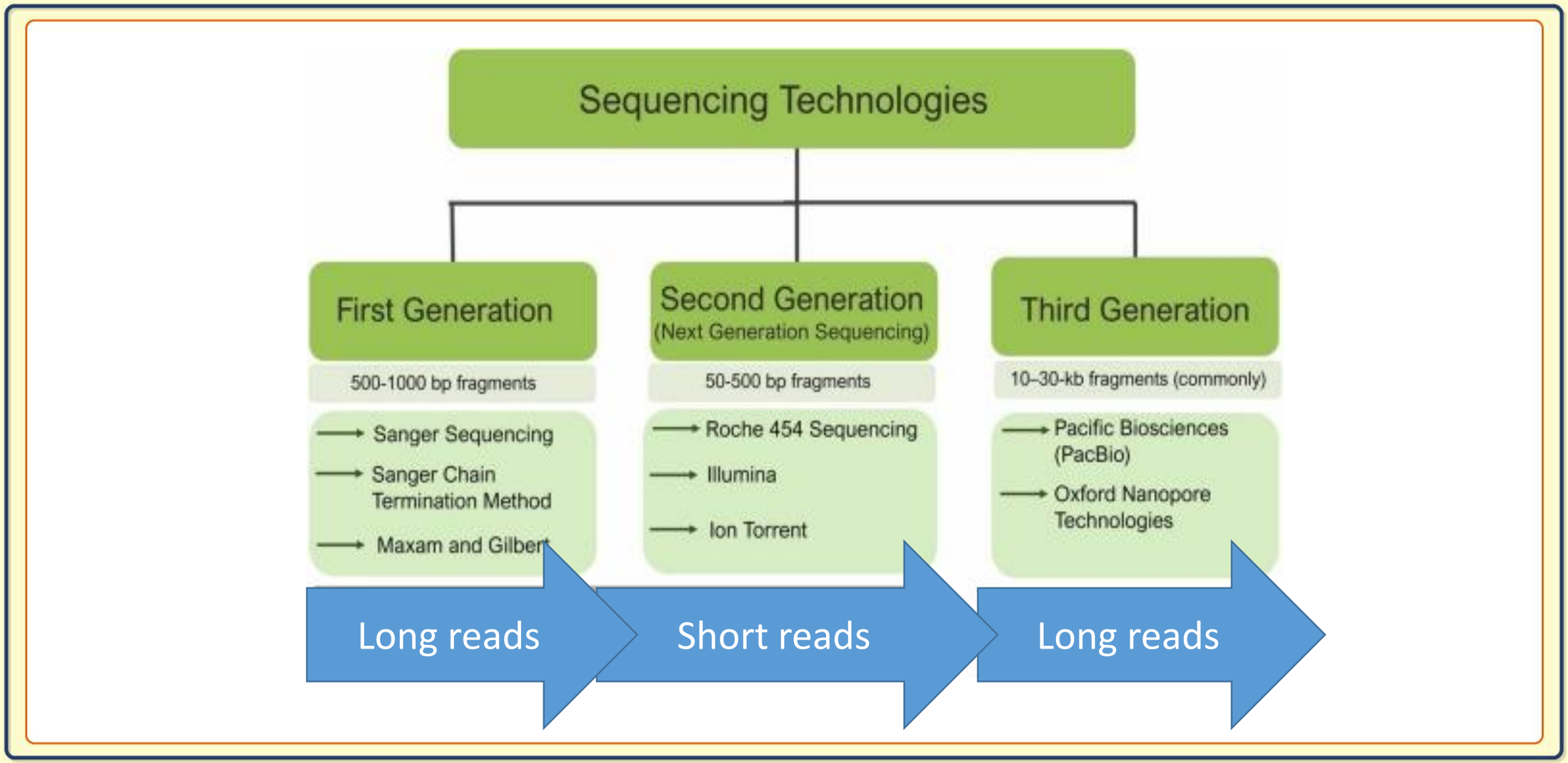
9,627 results



Akaçin İ et al. Microbiol Res 2022 doi: 10.1016/j.micres.2022.127154


Initial DNA Sample
1st generation
2° generation
3° generation
Pool of Fragmented DNA
In vivo/vitro cloning
Amplified template
Sequencing primer
DNA Polymerase
DNA migration
Dye-labeled segments are applied to a capillary gel and subjected to electrophoresis.
Laser beam
Detector
Laser
20
30
40
C C T G T T T G A T G G T G G T T C C G A A A T C G G
Bridge PCR
Emulsion PCR
Enriched Beads
Unenriched Beads
Picotiter wells
Glass Slide
Semiconductor sequencing
Zero-Mode Waveguide
Illumina
Roche
SOLiD
Ion Torrent/Proton
Pacific Bioscience
Oxford Nanopore
Output Data
Sequence read by captured images over single/multiple chemistry cycles

NGS platforms

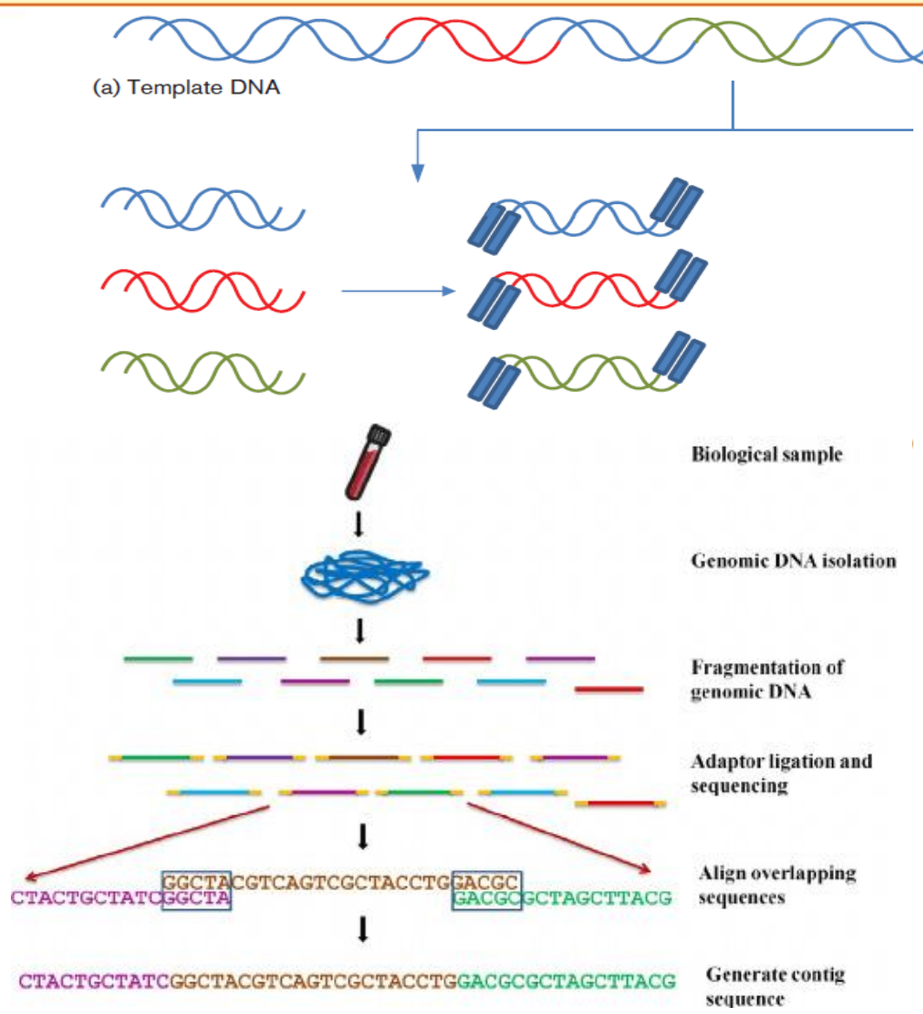


Mod. from Akaçin İ et al. Microbiol Res 2022 doi: 10.1016/j.micres.2022.127154

Different approaches in NGS:

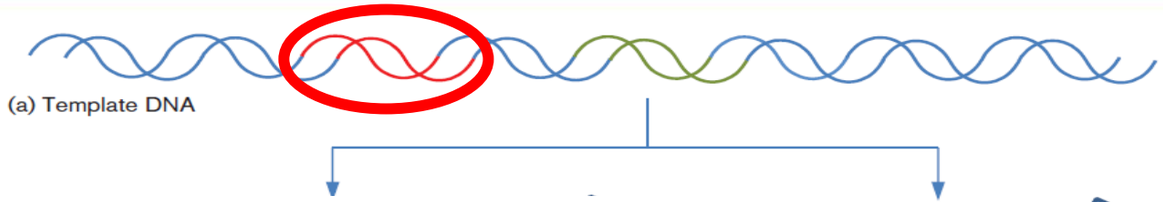
- Shotgun approach (unbiased)
- Amplicon approach
- Combined approach

Shotgun principle:

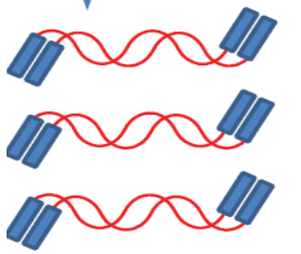


- Fragment DNA to obtain short random fragments
- Generate random library of amplified DNA fragments
- Sequence all the library fragments
- Bioinformatics elaboration:
  - ✓ Alignment; contig assembly
  - ✓ Blast against genomic databases
  - ✓ Classification of reads/contigs into known or new taxonomic entities

Amplicon principle: single amplicon



- Generate sequence-targeted amplicons (lenght depending on system)
- Sequence all the amplicons



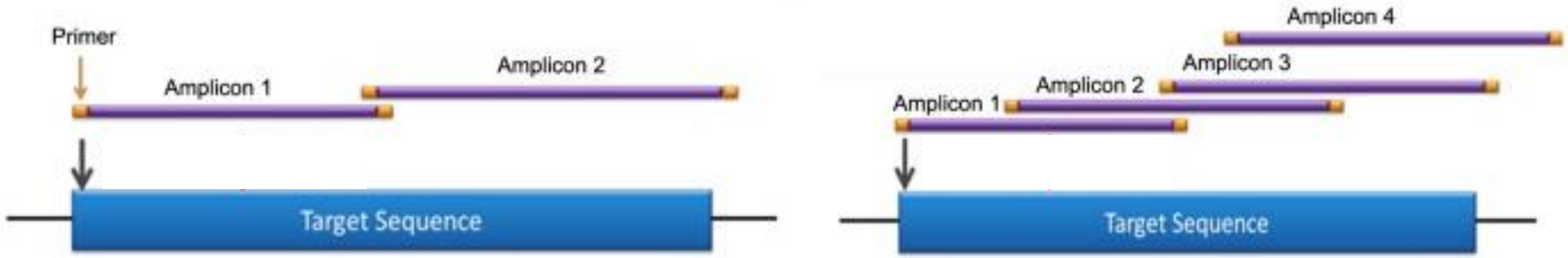
Analysis of variability in the target region

```
read 1          GCTAGCAATCA          O = 10
read 2            AGCAATCACCG        O = 7
read 3              CAATCACCGTT      O = 5
read 4               AATCACCGTTC     O = 4
read 5               AATGCAGCCAT     O = 3
read 6               AATGCAGCCAT     O = 3

contig . . .GTCGCTAGCAATC
```

Amplicon principle: multiplex overlapping amplicons

- Use multiplex PCR to generate overlapping amplicons spanning a long region, eventually the whole genome



- Use bioinformatics pipelines to combine the overlapping amplicons and build the long sequence, up to the whole genome

Different approaches in NGS:

- Shotgun approach (unbiased) :
  - Fragment DNA to obtain short random fragments
  - Generate random library of amplified DNA fragments
  - Sequence all the library fragments
    - Blast reads against known sequence database to recognize known agents
    - Build contigs to obtain full length genomes
- Amplicon approach
  - Generate sequence-targeted amplicons (lenght depending on system snd scope)
  - Sequence all the amplicons
    - Align overlapping amplicons to reconstruct whole genome
    - Analyze intra-amplicon variability
- Combined approach:
  - Generate a long sequence-targeted amplicon
  - Fragment the long amplicon
  - Continue with the shotgun approach
  - Bionformatics to build contigs and assembly of full length genome

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  - ✓Quasispecies dynamics and implications for resistance
- Metagenomics
- NGS pitfalls; potential bias; standardization; ethical issues

Example of full genome sequencing with combined amplicon-shotgun approach

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 25, Number 9, 2009

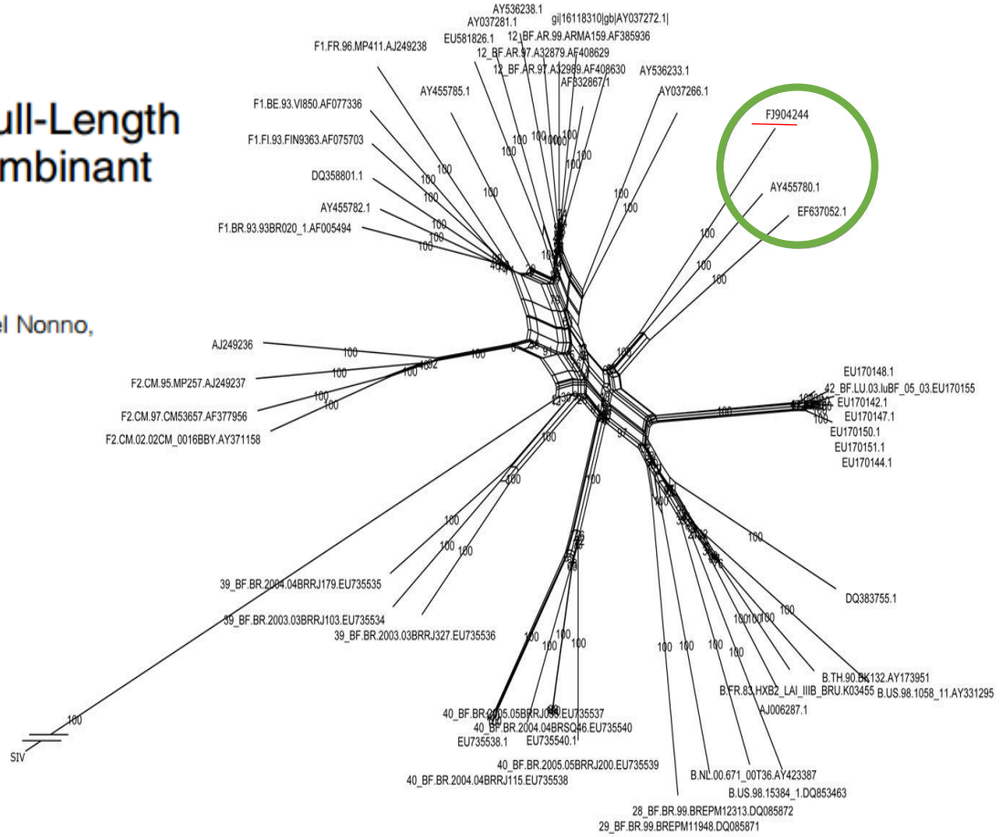
DOI: 10.1089/aid.2009.0083

SEQUENCE NOTE

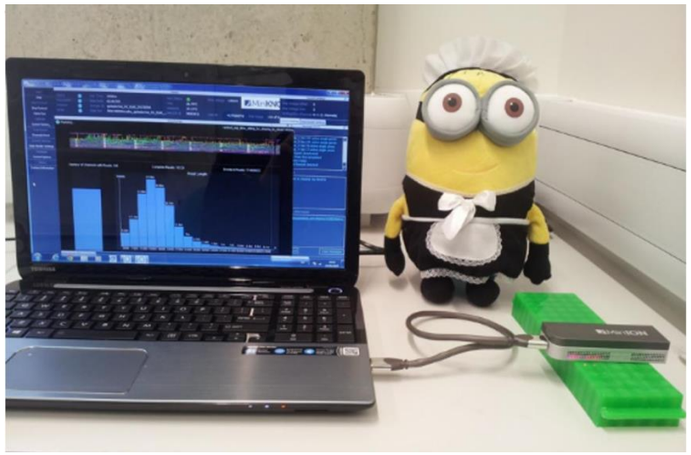
Use of Massive Parallel Pyrosequencing for Near Full-Length Characterization of a Unique HIV Type 1 BF Recombinant Associated with a Fatal Primary Infection

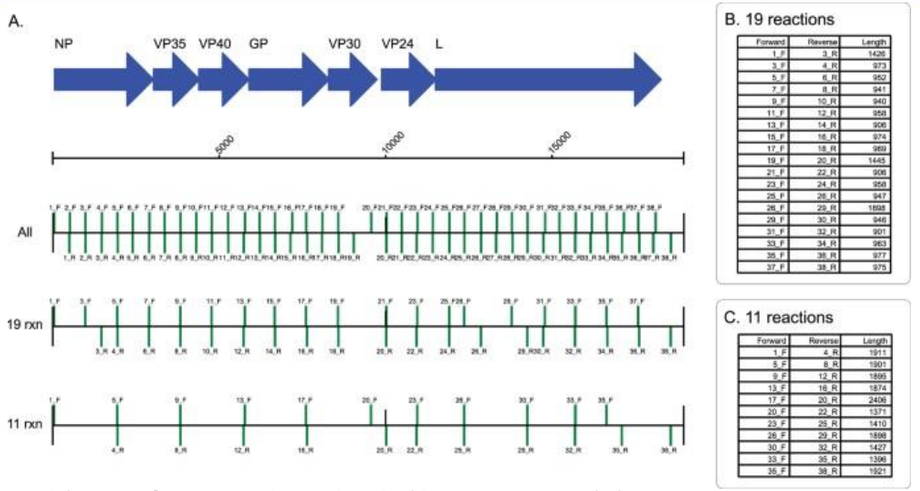
Alessandro Bruselles, Gabriella Rozera, Barbara Bartolini, Mattia Prosperi, Franca Del Nonno, Pasquale Narciso, Maria R. Capobianchi, and Isabella Abbate

- Two long overlapping amplicons encompassing the whole HIV genome (total lenght: about 10kb)
- Fragment and sequenc with whotgun appriach
- Bioinformatic elaboration to obtain whole HIV genome

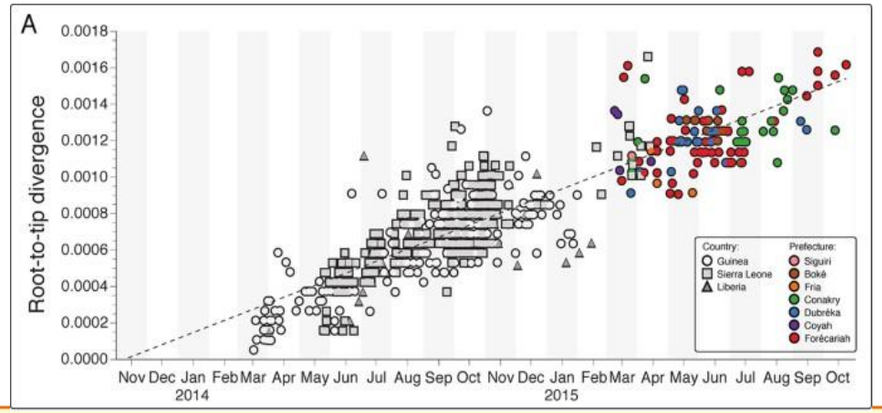


Deployment of the portable genome surveillance system in Guinea for Ebola surveillance





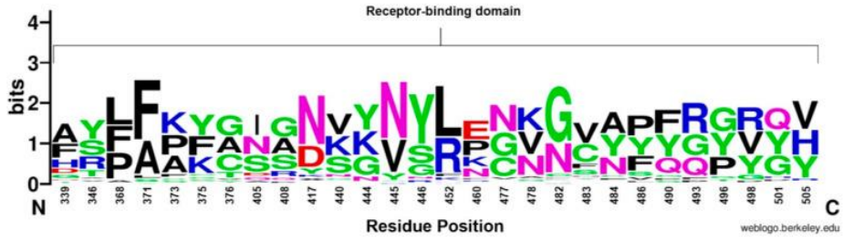
- Minion Oxford Nanopore: all equipment fits in <50kg of standard airline travel luggage
- Adding a genome sequencing capability to an EVD diagnostic laboratory
- Results in <2 days from sampling
- Remote bioinformatics analysis
- Combining genome sequences with epidemiological investigations helps confirm or confute transmission chains and inform outbreak control efforts
- Data were made available to virology community in real-time through Internet



Quick J, et al. Nature 2016 doi: 10.1038/nature16996

NGS applications to virology: whole genome analysis

- A previously unknown human coronavirus (hCoV-19) was first detected in late 2019 in patients in Wuhan.
- On 10. January 2020, the first virus genomes and associated data were publicly shared via GISAID.
- As the pandemic progresses, scientists from around the globe are tracking the virus and its genome sequences to ensure optimal virus diagnostic tests, to track and trace the ongoing outbreak and to identify potential intervention options.

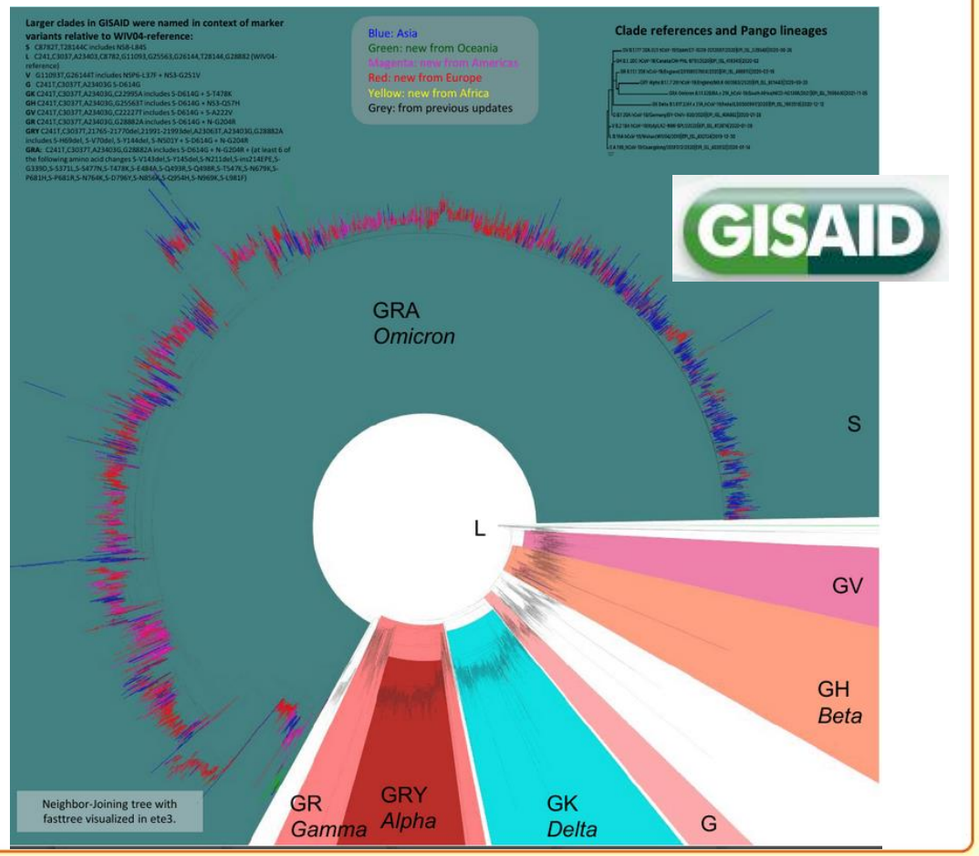


WebLogo representation of amino acid substitutions within the RBD of SARS-CoV-2

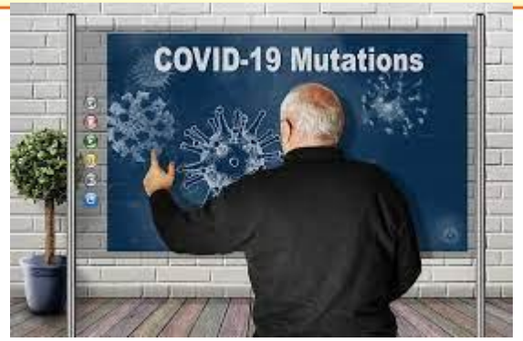
Gerardi V, et al. Vaccines 2023 doi: 10.3390/vaccines11030668

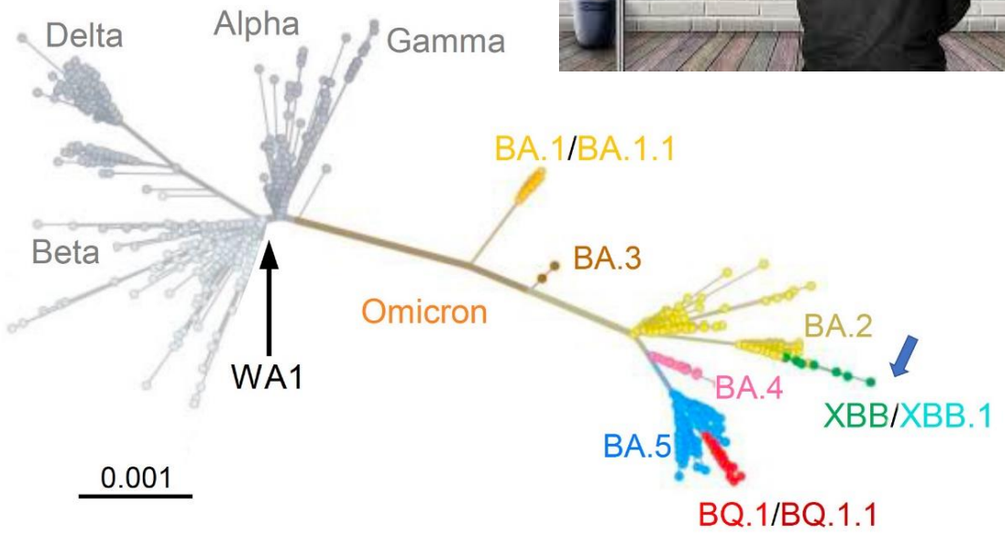
Sequence data shared via GISAID by November 7, 2023

- **16,176,162** genome sequence submissions
- **14,391,034 full genomes**

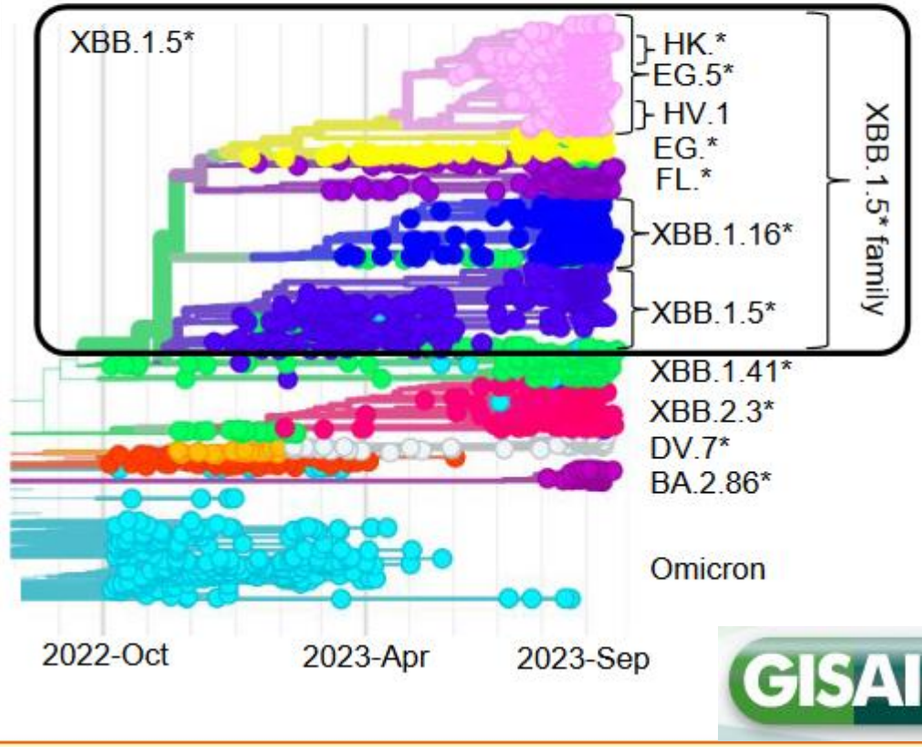


https://www.epicov.org/epi3/frontend#lightbox-125956593





Recently approved updated vaccines use XBB.1.5 strains as basis. The majority of current strains in circulation globally continue to belong to the broader XBB.1.5* family.



GISAID

https://erictopol.substack.com/ 23 dic 2022

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July 2010 | Volume 6 | Issue 7 | e1001005

OPEN ACCESS Freely available online

PLoS PATHOGENS

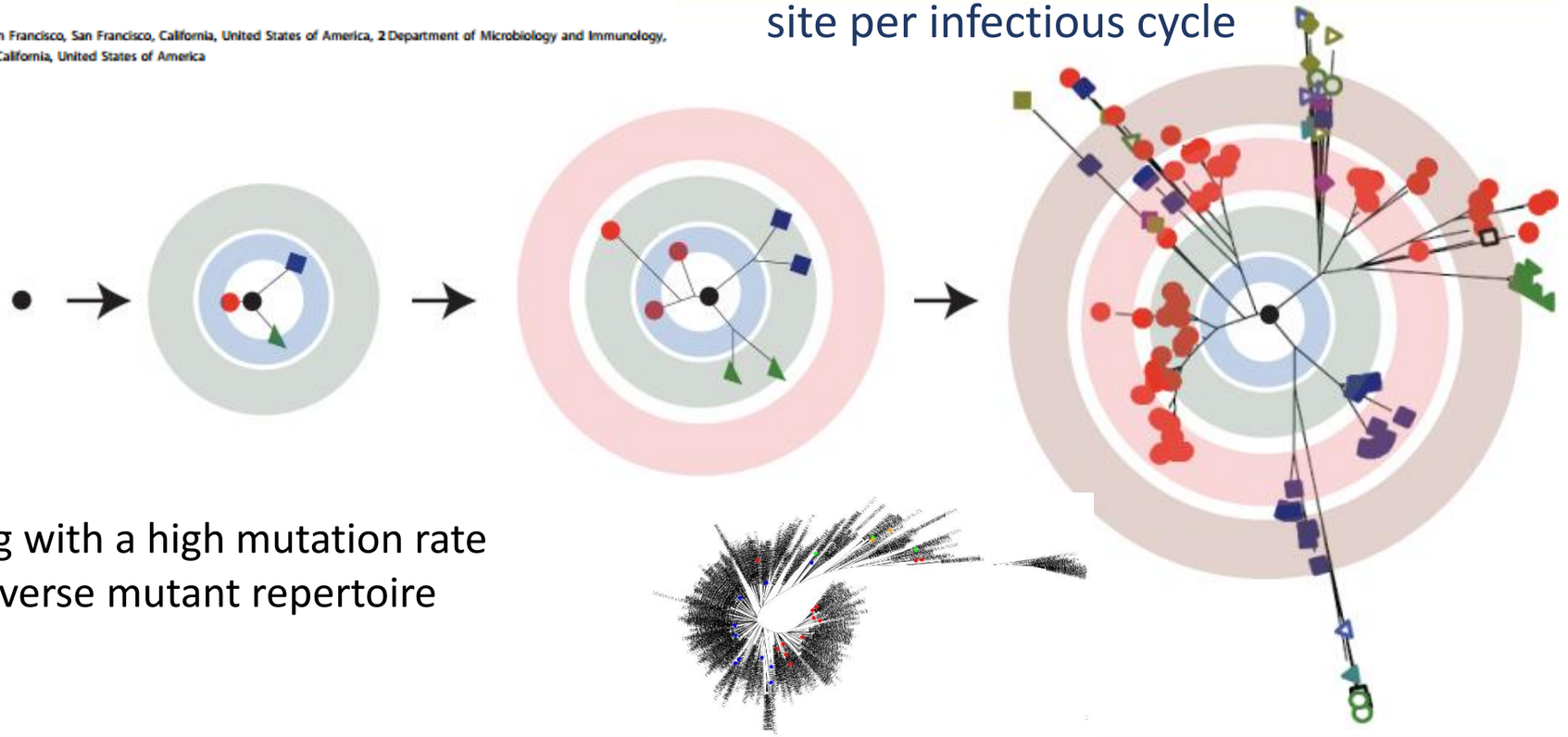
Review

Quasispecies Theory and the Behavior of RNA Viruses

Adam S. Lauring[1], Raul Andino[2]*

1 Department of Medicine, University of California, San Francisco, San Francisco, California, United States of America, 2 Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, California, United States of America

RNA viruses: error prone nature of the RNA-dependent RNA polymerase 7×10^{-4} to 5.4×10^{-3} mutations per site per infectious cycle

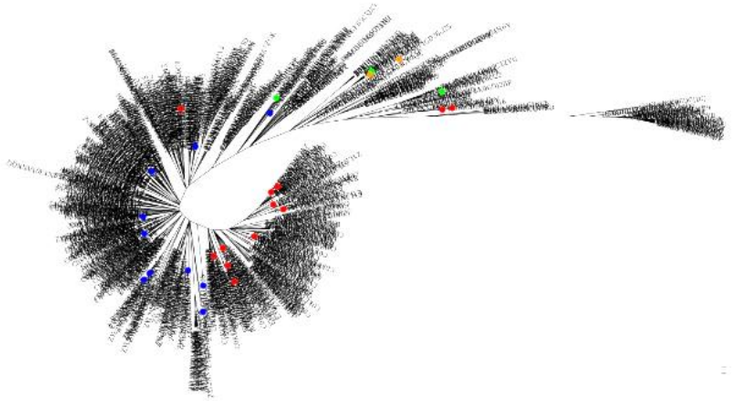


A virus replicating with a high mutation rate will generate a diverse mutant repertoire

NGS applications to virology

Powerful tool to describe intra-host variability (HCV, HIV, HBV)

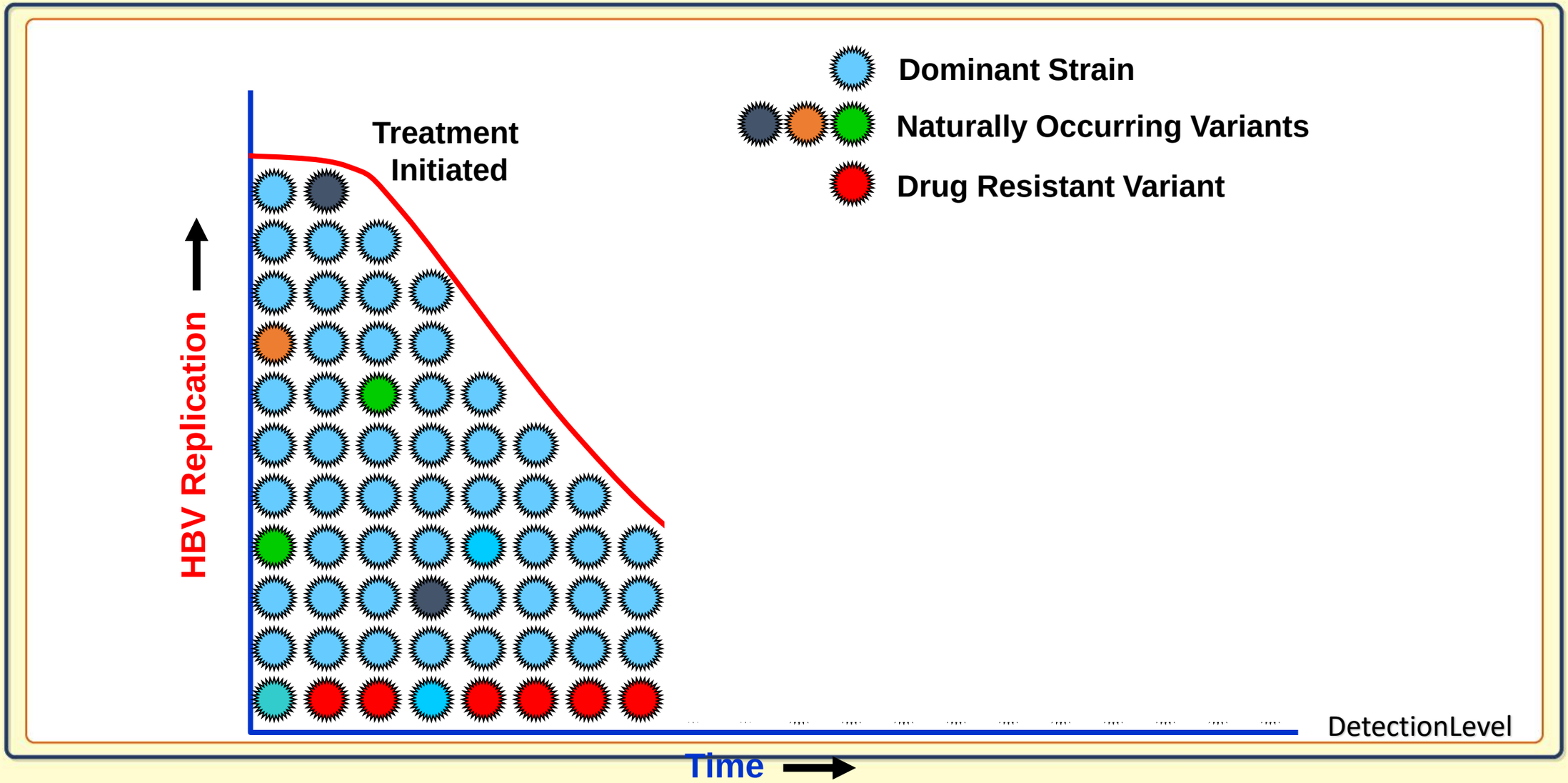
- High resolution power for minority variants
  - compartmentalization of viral quasispecies
  - tropism analysis (HIV)
  - resistance-associated mutations



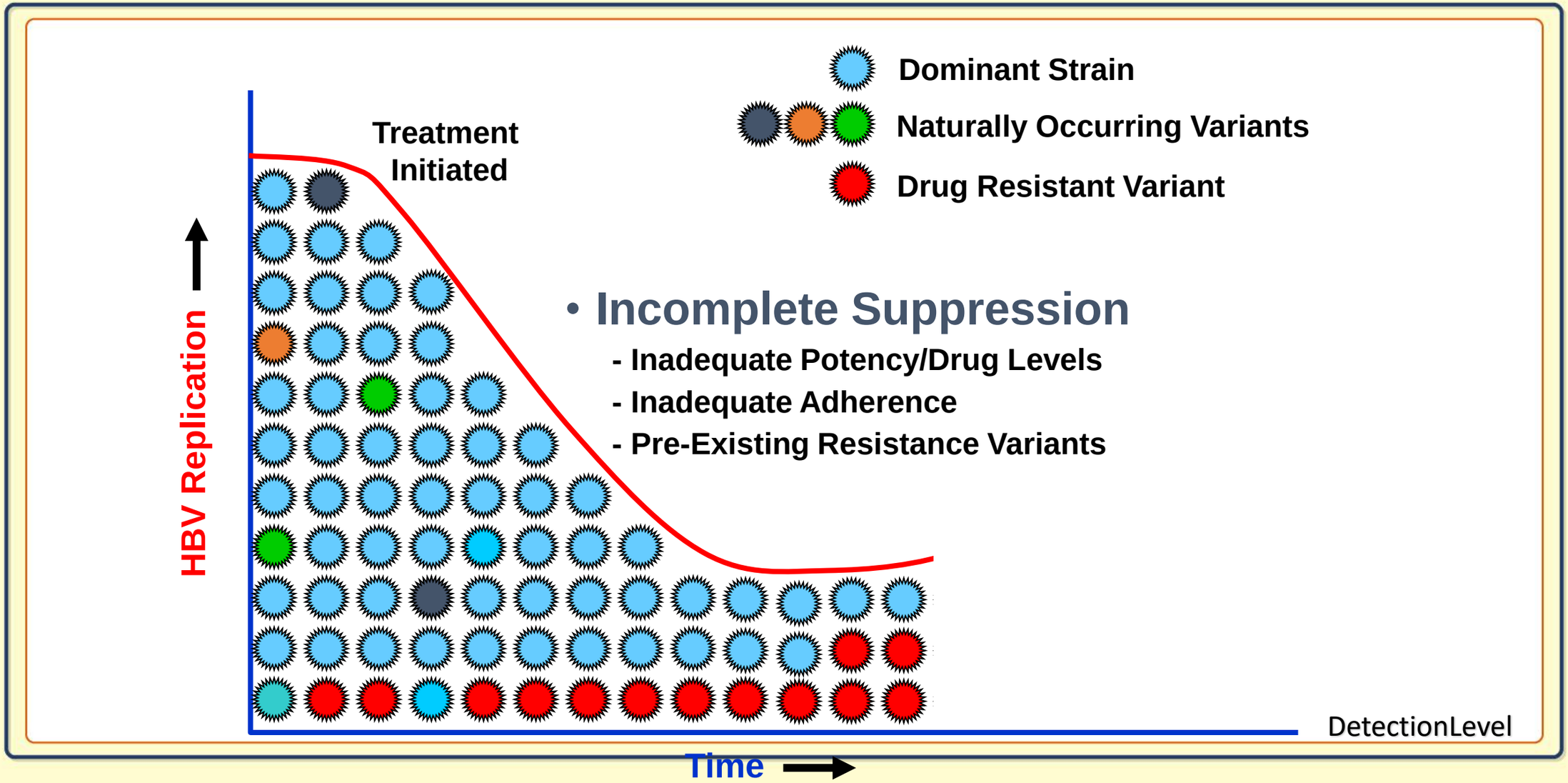
- Viral dynamics
  - during natural history
  - after therapeutic intervention

- Trace the evolution of viral properties (i.e. HIV tropism)
- Determine if amino acid substitutions can be correlated with treatment failure (emergence of resistance)
- Determine if any baseline polymorphisms lead to reduced therapy efficacy
- Determine if early detection of resistance-associated substitutions may anticipate treatment failure

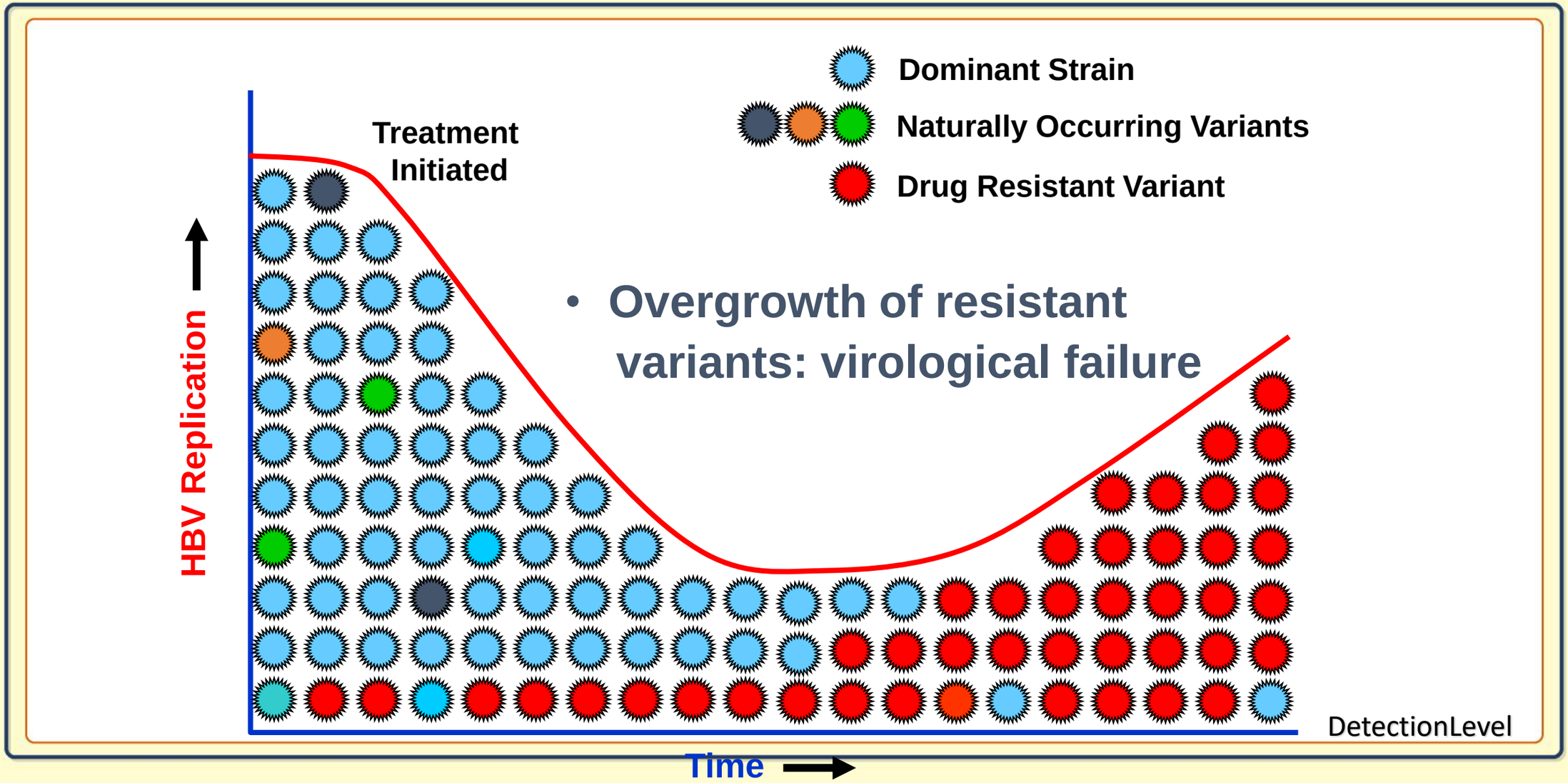
Incomplete suppression of virus replication leads to selection of mutants



Incomplete suppression of virus replication leads to selection of mutants



Incomplete suppression of virus replication leads to selection of mutants



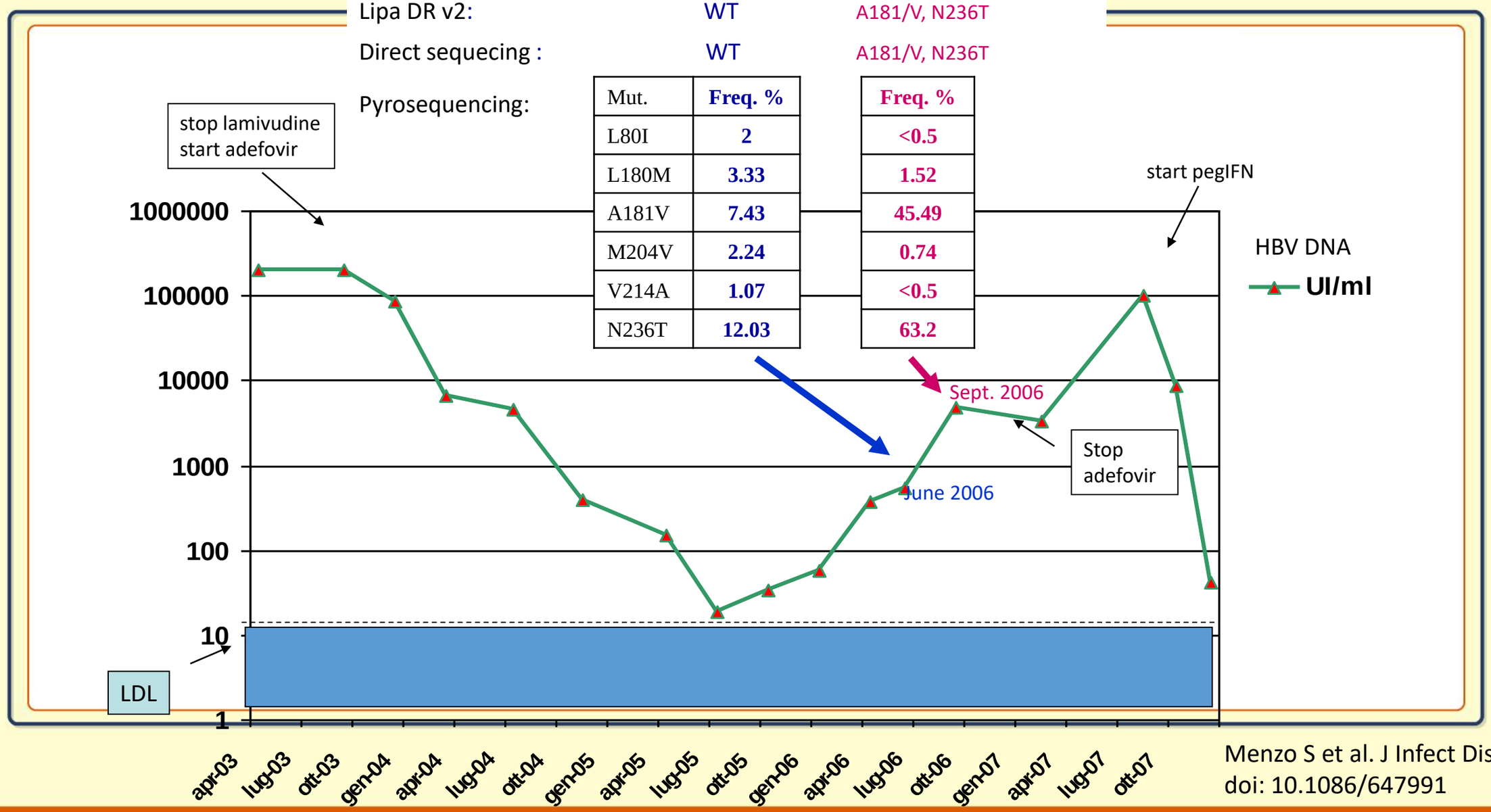
Dynamics of HBV resistance mutations in a patient failing treatment after switch lamivudine to adefovir

Lipa DR v2: WT A181/V, N236T

Direct sequecing : WT A181/V, N236T

Pyrosequencing:

Mut.	Freq. %	Freq. %
L80I	2	<0.5
L180M	3.33	1.52
A181V	7.43	45.49
M204V	2.24	0.74
V214A	1.07	<0.5
N236T	12.03	63.2



Menzo S et al. J Infect Dis. 2009
doi: 10.1086/647991

Contents lists available at ScienceDirect

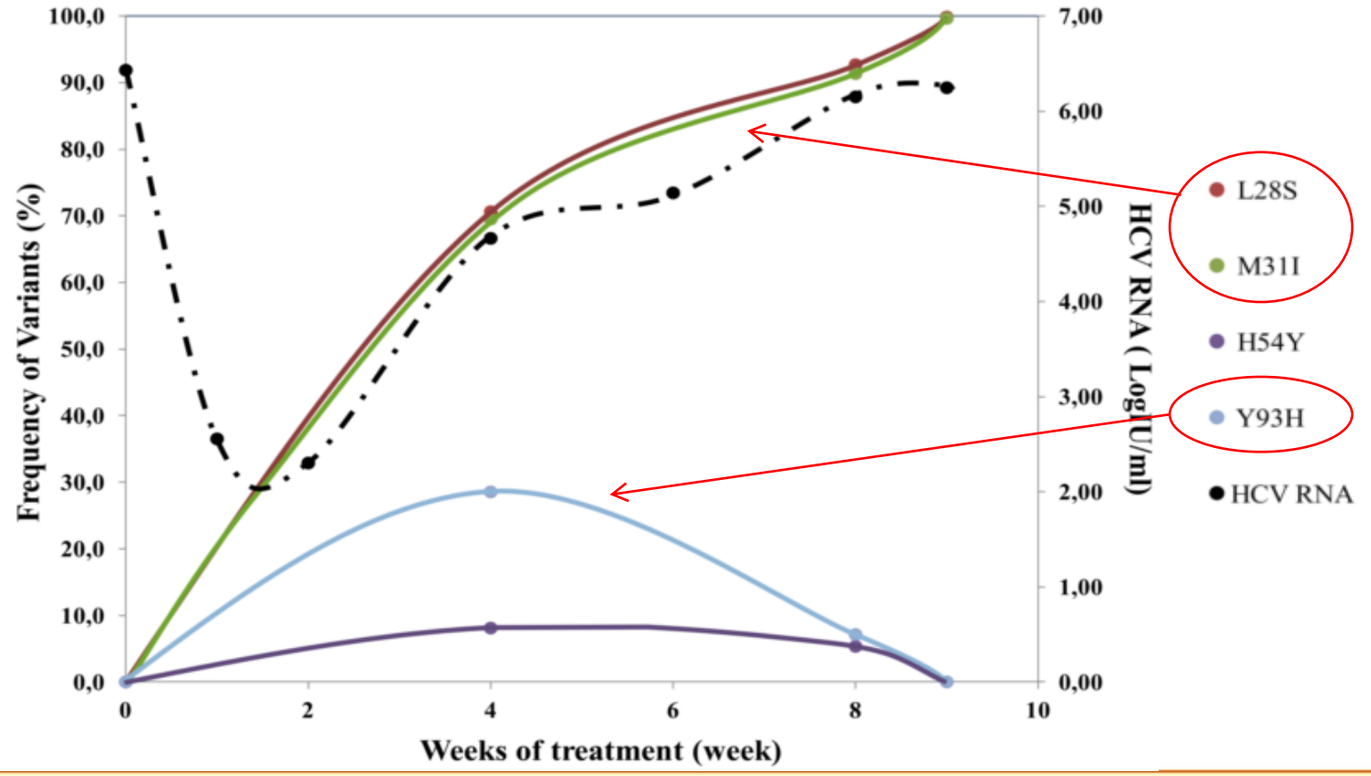
Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

2015

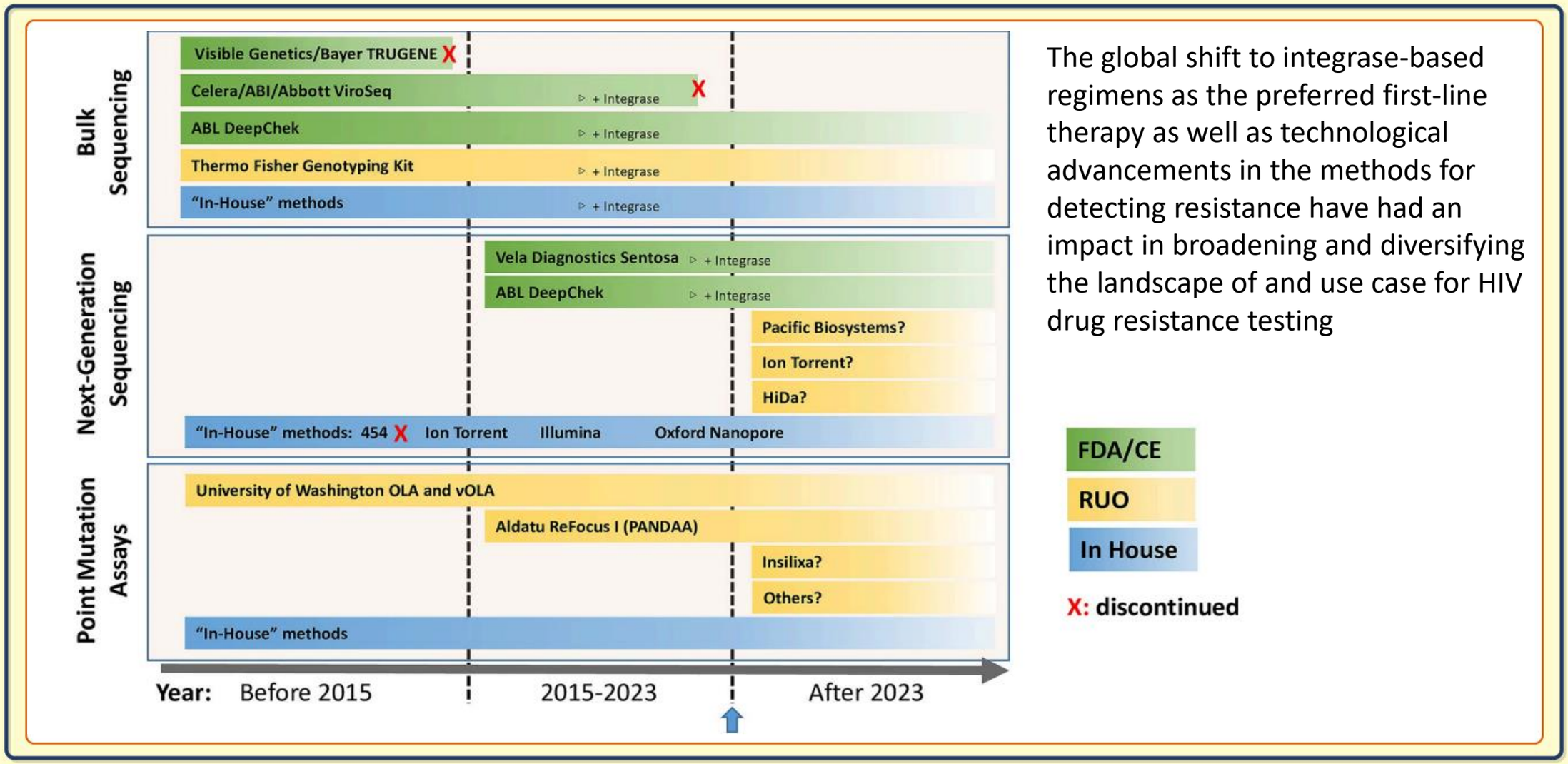
Dynamics of HCV genotype 4 resistance-associated variants during virologic escape with pIFN/RBV + daclatasvir: A case study using ultra deep pyrosequencing

Barbara Bartolini[a], Raffaella Lionetti[a], Emanuela Giombini[a], Catia Sias[a], Chiara Taibi[a], Marzia Montalbano[a], Gianpiero D'Offizi[a], Fiona McPhee[b], Eric A. Hughes[c], Nannan Zhou[b], Giuseppe Ippolito[a], Anna Rosa Garbuglia[a], Maria R. Capobianchi[a,*]



L28S (novel RAV) and M31I progressively accumulate and become fixed in the rebounding virus; Y93H and H54Y are transient

HIV drug resistance technology landscape



The global shift to integrase-based regimens as the preferred first-line therapy as well as technological advancements in the methods for detecting resistance have had an impact in broadening and diversifying the landscape of and use case for HIV drug resistance testing

FDA/CE

RUO

In House

X: discontinued

Parkin N, et al. PLOS Glob Public Health 2023 doi: 10.1371/journal. pgph.0001948

- NGS will become the new standard for genotypic HIV-1 drug resistance testing.
- Lower per test cost due to multiplexing
- High sensitivity for low abundancy variants, allows to antepone the discovery of genotyic resistance
- **Caution in overvaluing the low-frequency variant**
- NGS platforms have the ability to generate almost limitless numbers of sequence reads starting with a PCR product
- This gives the illusion that it is possible to analyze minor variants in a viral population
- However, including a PCR step obscures the sampling depth of the viral population, the key parameter needed to understand the utility of the data set for finding minor variants.
- Identification and quantification of low-frequency mutations remain challenging despite improvements in sequencing and in the baseline error rate of next-generation sequencing technologies
- Standardization and external quality assessment strategies/programs are urgently needed for the implementation of NGS-based genotypic HIV-1 drug resistance testing.

Metzner KJ. Curr Opin HIV AIDS. 2022 doi: 10.1097/COH.0000000000000737

Dalmat R et al. bioRxiV 2018 doi.org/10.1101/414995

Zhou S. Viruses 2020 doi: 10.3390/v12080850

Manyana S et al. Viruses 2021 doi: 10.3390/v13061125

Perspective

Fact and Fiction about 1%: Next Generation Sequencing and the Detection of Minor Drug Resistant Variants in HIV-1 Populations with and without Unique Molecular Identifiers

Shuntai Zhou [1,*] and Ronald Swanstrom [1,2]

How to Fool Yourself into Thinking Your NGS Protocol Gives You 1% Sensitivity

- *"I climbed Mt. Everest, it's that hill over there."*
- *"Really? It doesn't look very high."*
- *"Yes, but there is a sign on top that says 'Mt. Everest'."*

Zhou S. Viruses 2020 doi: 10.3390/v12080850

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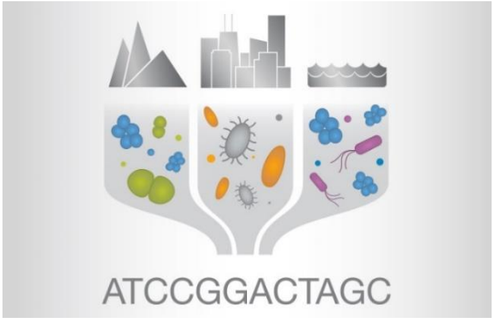
- Whole genome analysis
- Viral quasispecies and minority genomes
  - ✓Tropism and compartmentalization
  - ✓Quasispecies dynamics and implications for resistance
- Metagenomics
- NGS challenges; potential bias; standardization; ethical issues

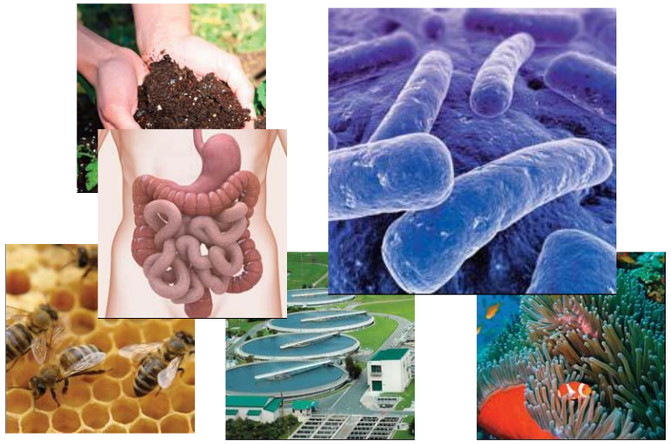
Metagenomics (mNGS)

Contemporary analysis of all genomes present in a sample

Applications

- Pathogen discovery
- Description of microbial communities (virome, microbiome)
- Analysis of microbial variability
- Diagnostics → the future lab





Metagenomic next-generation sequencing (mNGS) is a **game-changing technology** for infectious disease diagnosis as nearly all pathogens – viruses, bacteria, fungi, and parasites – can be detected in a single assay

NGS allows to interrogate clinical samples for the presence of infectious agent(s) in a completely unbiased manner

However, significant challenges confront clinical microbiology laboratories attempting to implement metagenomics using traditional clinical work flows

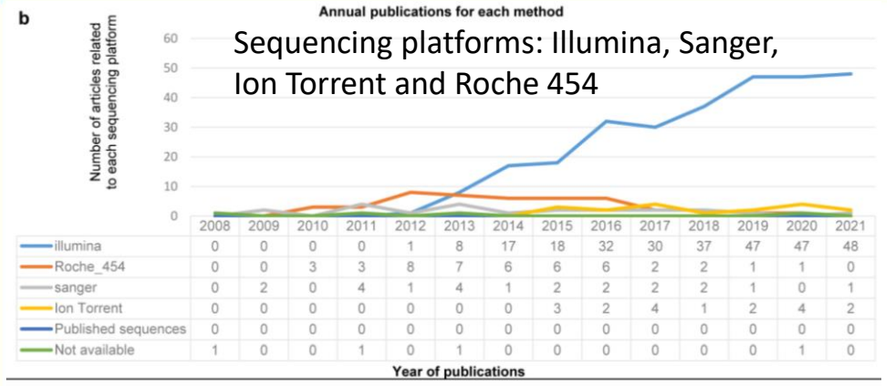
RESEARCH ARTICLE | VIROME

Cryptic and abundant marine viruses at the evolutionary origins of Earth's RNA virome

AHMED A. ZAYED, JAMES M. WAINAINA, GUILLERMO DOMINGUEZ-HUERTA, ERIC PELLETIER, JIARONG GUO, MOHAMED MOHSSEN, FUNING TIAN, AKBAR ADJIE PRATAMA, BENJAMIN BOLDUC, [...] MATTHEW B. SULLIVAN +23 authors Authors Info & Affiliations

SCIENCE • 7 Apr 2022 • Vol 376, Issue 6589 • pp. 156-162 • DOI: 10.1126/science.abm5847

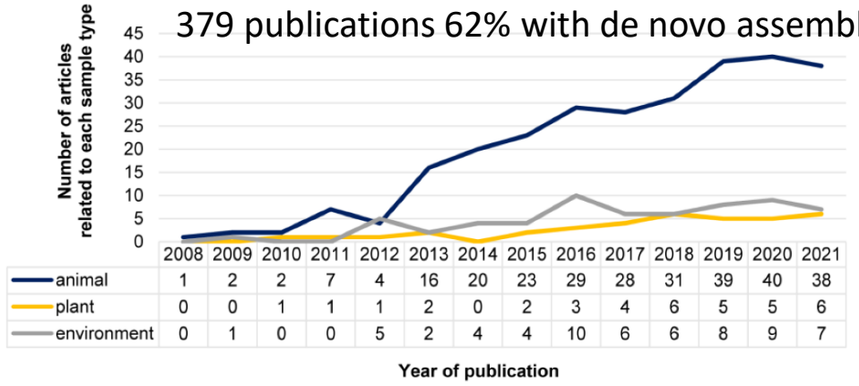
Earth virome (animal, plant, environment) and novel virus discovery: a review, 2022



Sequencing platforms: Illumina, Sanger, Ion Torrent and Roche 454

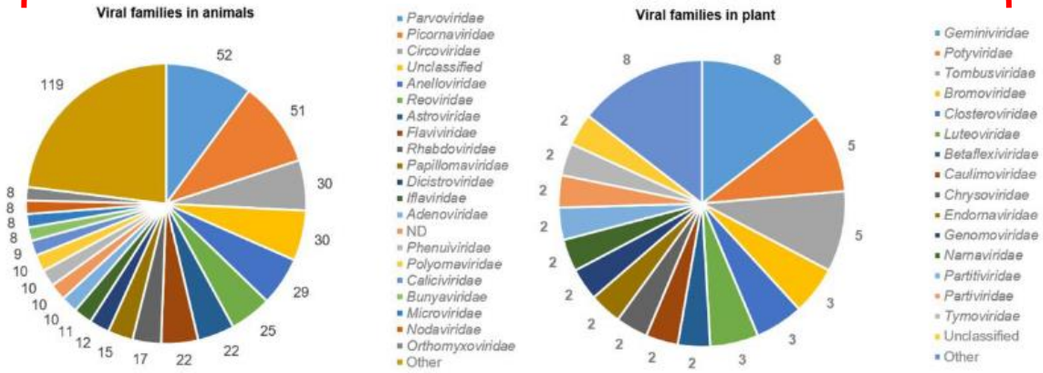
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
illumina	0	0	0	0	1	8	17	18	32	30	37	47	47	48
Roche_454	0	0	3	3	8	7	6	6	6	2	2	1	1	0
sanger	0	2	0	4	1	4	1	2	2	2	2	1	0	1
Ion Torrent	0	0	0	0	0	0	0	3	2	4	1	2	4	2
Published sequences	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Not available	1	0	0	1	0	1	0	0	0	0	0	0	1	0

379 publications 62% with de novo assembly

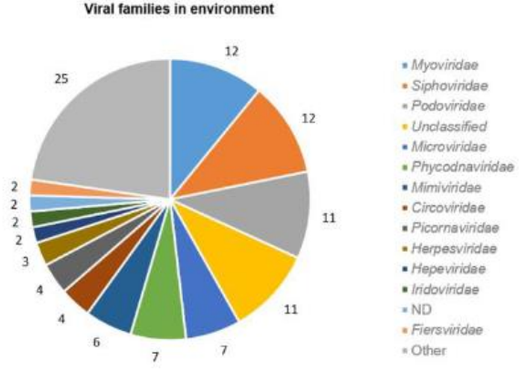


	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
animal	1	2	2	7	4	16	20	23	29	28	31	39	40	38
plant	0	0	1	1	1	2	0	2	3	4	6	5	5	6
environment	0	1	0	0	5	2	4	4	10	6	6	8	9	7

RNA viruses predominant



DNA viruses predominant



681 novel viruses were identified

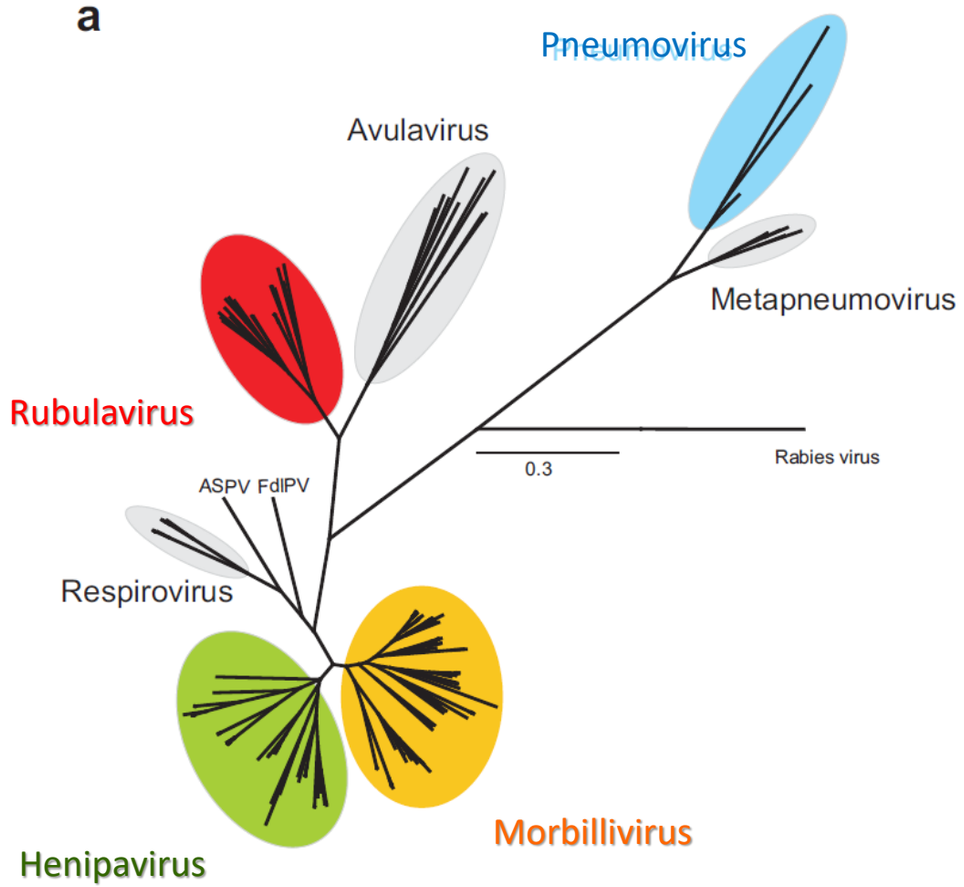
- 290 with DNA genome
- 348 with RNA genome
- 43 unclassified

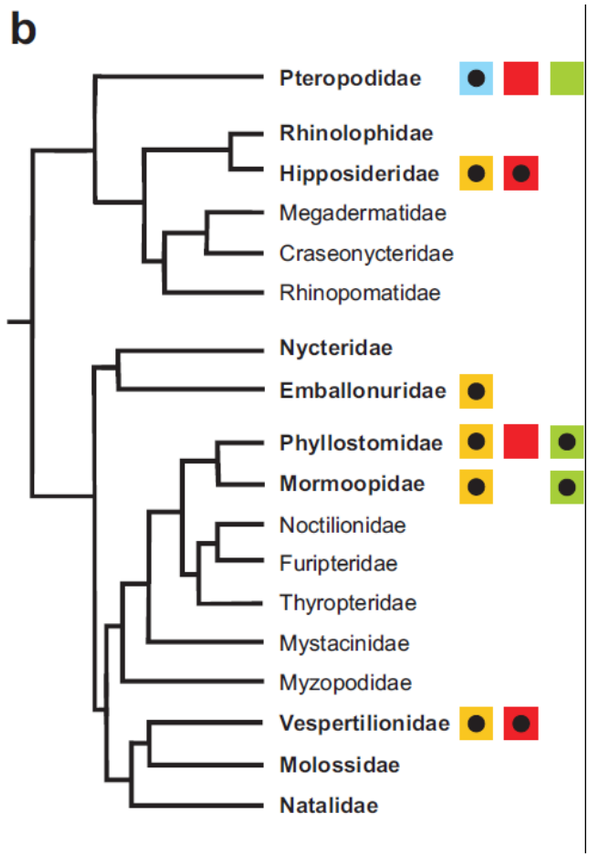
- 516 in animal samples
- 110 in environmental samples
- 55 in plant samples

Bassi, C et al. Novel Virus Identification through Metagenomics: A Systematic Review. *Life* **2022** doi: 10.3390/life12122048

Bats from all evolutionary stem lineages carry paramyxoviruses

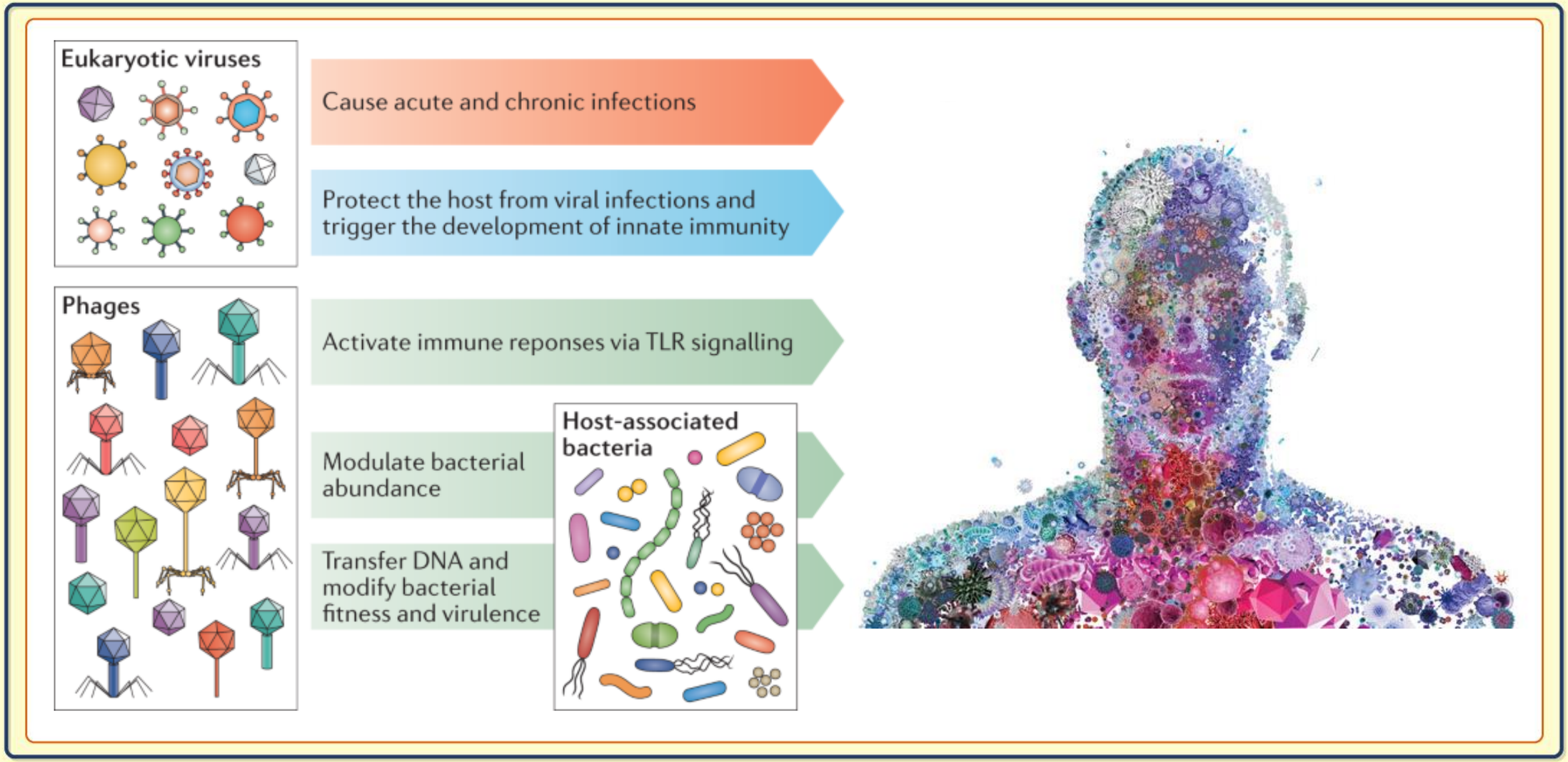
- Products of random cDNA amplification were sequenced (454 Roche).
- 66 new paramyxoviruses in a worldwide sample of 119 bat and rodent species





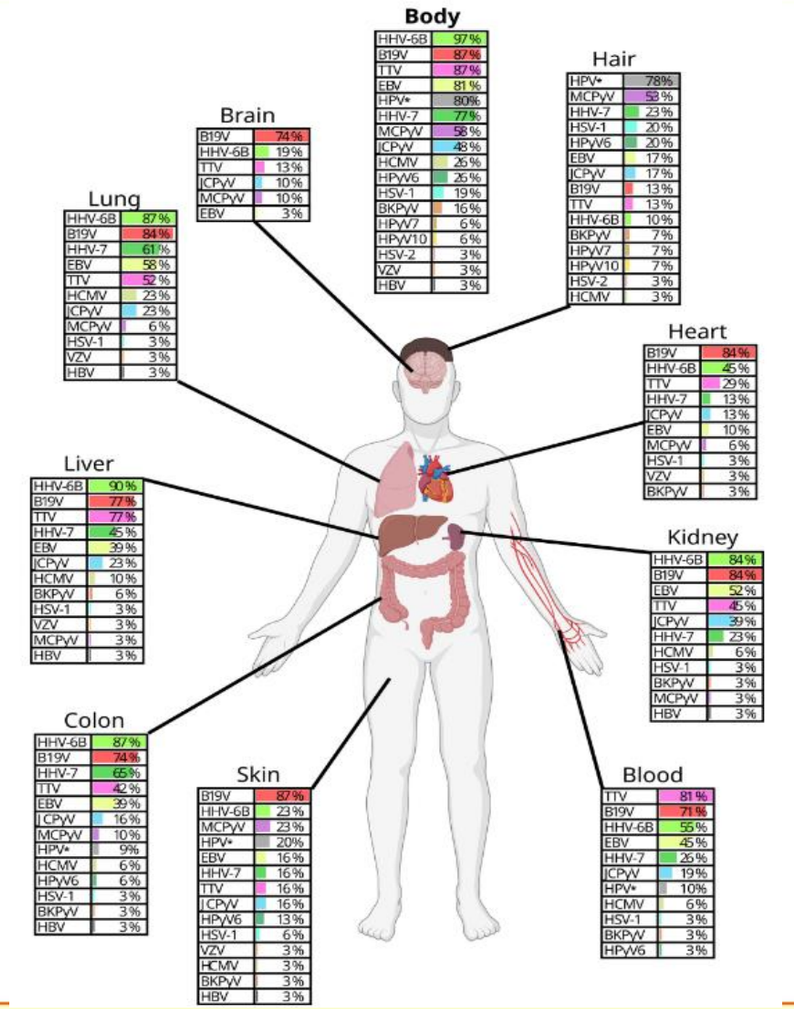
Drexler JF, et al. Bats host major mammalian paramyxoviruses. Nat Commun. 2012 doi: 10.1038/ncomms1796

Human virome: relevant component of human microbiome



Liang, G., Bushman, F.D. The human virome: assembly, composition and host interactions. *Nat Rev Microbiol* **19,** 514–527 (2021). https://doi.org/10.1038/s41579-021-00536-5

The «normal» DNA virome (enrichment of DNA samples)



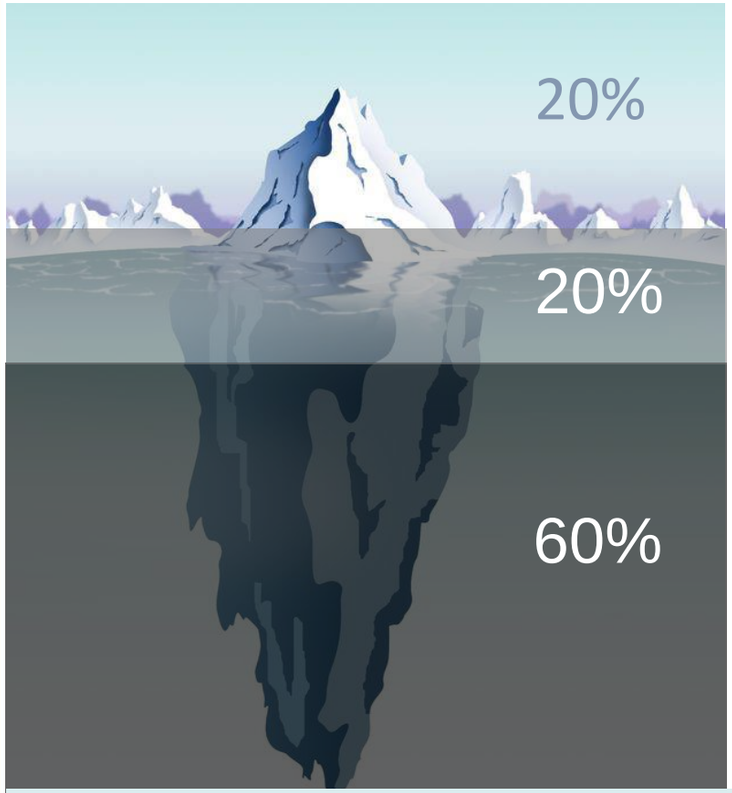
- Tissues from deceased persons
- Capture of viral DNA

Family	Types
Herpesviridae	Types 1-8
Papillomaviridae	Types 2, 6, 11, 16, 18, 31, 45
Polyomaviridae	Types 1-13
Parvoviridae	B19V, bocavirus 1-4, cutavirus
Anelloviridae	Torque Teno virus
Hepadnaviridae	Hepatitis B virus
Poxviridae	Variola major and minor

- Library built on captured DNA fragments
- Library sequencing
- Virus identification
- Most prevalent (>80%): **HHV-6, B19, TTV, EBV, HPV, HHV-7**

Pyöriä L et al. Nucleic Acids Research 2023 doi: 10.1093/nar/gkad199

A large part of the virome is not known

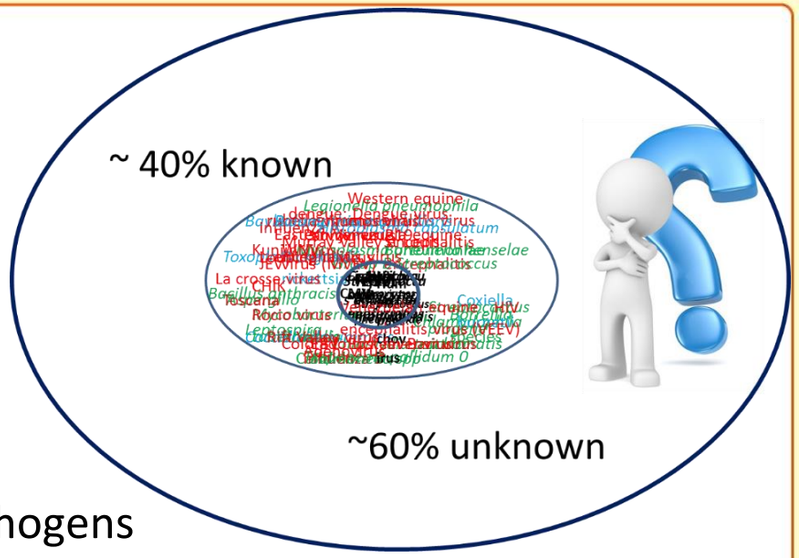


"Known virome"

"Grey virome"

"Dark virome"

…At least 500.000 mammal viruses are waiting to be discovered...



What about:

- ✓ Emerging pathogens
- ✓ Agents that are pathogenic only in the presence of individual risk factors
- ✓ Known agents with unknown or not suspected pathogenic potential
- ✓ Pathogens related to epidemic situations/seasonality
- ✓ Unknown agents

Presentation outline

NGS applications to virology

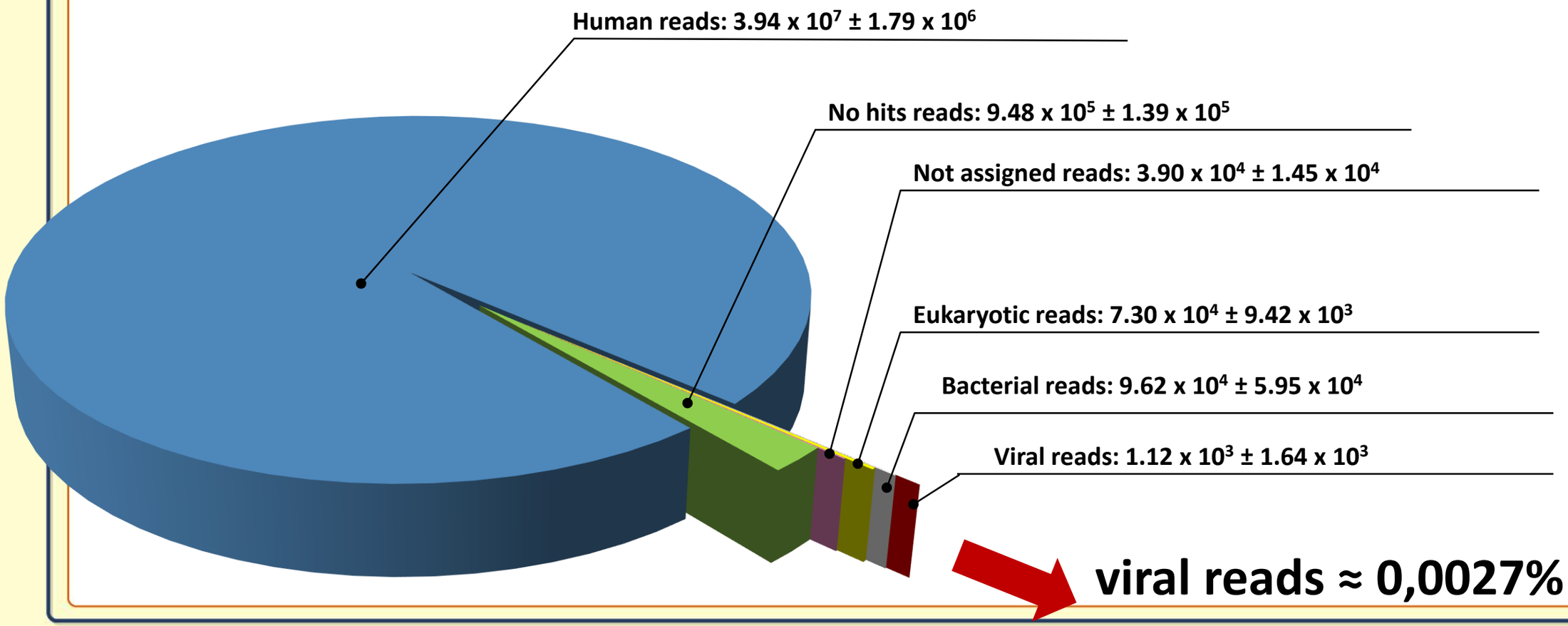
- Metagenomics
- NGS challenges; potential bias; standardization; ethical issues

Studying the Human Virome: challenges

- ✓ **Challenge 1:** Absence of a universal viral sequence
- ✓ **Challenge 2:** Pre-treatment (nuclease treatment, filtration, …)
- ✓ **Challenge 3:** Low quantity entities, low-biomass samples
- ✓ **Challenge 4:** RNA and DNA from the host and bacteria
- ✓ **Challenge 5:** Contaminats and the “Kit-ome”
- ✓ **Challenge 6:** Bioinformatic analysis

Results from the Virology Lab, INMI: 44 CSF analyzed by metagenomics

Average reads per sample: $4.05 \times 10^7 \pm 1.76 \times 10^6$ (95% CI)



Successful Clinical Application of mNGS for Infectious Disease Diagnostics

	Case(s)	mNGS Results and Specimen Types	Confirmatory Testing	Diagnosis
Central nervous systems infections	42-year-old man with chronic lymphocytic leukemia presented with bilateral hearing loss. He developed neurological deterioration.	1612 of 134068968 (0.0012%) reads **from brain tissue** RNA aligned to **astrovirus**, **no** astrovirus reads were obtained from the 6658656 reads **from CSF**.	RT-PCR for astrovirus from the brain biopsy	Neuroinvasive astrovirus infection
	34-year-old Australian man with X-linked agammaglobulinemia suffering from 3 years of meningoencephalitis that defied an etiologic disease despite extensive conventional testing, including brain biopsy.	5 of 25069677 (0.00002%) and 2 of 13661871 (0.00001%) reads of the CSF and brain biopsy aligned to **Cache Valley virus.**	CVV RT-PCR of the brain biopsy and immunohistochemistry straining of the FFPE brain tissue	Chronic viral meningoencephalitis: Cache Valley virus
Bloodstream infections	Serum samples from 15 patients with known Zika virus infections in Brazil.	13 of 15 samples were positive for **Zika virus** by mNGS ranging from 2 to 281099 reads per sample (0.0004%–4.1% of total reads). Five samples were also positive for **Chikungunya virus.**	All 15 samples were positive for Zika virus RT-PCR. Two of 5 samples were confirmed positive for Chikungunya virus by nested RT-PCR.	Confirmation of Zika virus infection and discovery of coinfection with Chikungunya virus in 2 patients.
	Three patients in central Africa presenting with acute hemorrhagic fever.	Sequencing of the third patient's serum yielded 0.029% of reads with nucleotide or protein homology to a **novel rhabdovirus.**	Confirmatory PCR showed viral titers of 1.09×10^6 RNA copies/mL.	Novel rhabdovirus: Bas-Congo virus

Simmer PJ et al. Clinical Infectious Diseases 2018 doi: 19.1093/cid/cix881

Simmer PJ et al. Clinical Infectious Diseases, 2018, https://doi.org/10.1093/cid/cix881

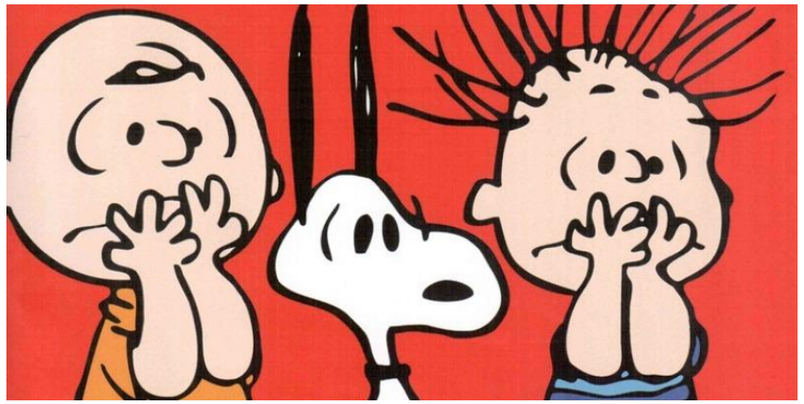
Successful Clinical Application of mNGS for Infectious Disease Diagnostics

	Case(s)	mNGS Results and Specimen Types	Confirmatory Testing	Diagnosis
Respiratory tract infections	A series of 22 hematopoietic stem cell transplant recipients with acute respiratory illnesses. mNGS was applied to study both the microbial composition and host response of BAL fluid specimens	mNGS identified previously unrecognized pathogens for which standard testing was negative (human coronavirus 229E, human rhinovirus A, *Corynebacterium propinquum*, and *Streptococcus mitis*)	6/22 confirmed by standard testing 6/22 negative by standard testing but confirmed mNGS findings by independent PCR testing. 10/22 mNGS identified microbes of uncertain or unlikely pathogenicity that were not confirmed by standard testing nor independent PCR.	mNGS confirmed the diagnosis of acute respiratory illness in 6 patients mNGS identified 6 previously unrecognized pathogens of acute respiratory illness.
Ocular infections	Intraocular fluid samples were obtained from subject with bilateral chronic uveitis with unknown etiology.	585 of 1648220 (0.41%) reads of aqueous humor and 10 of 12111540 (0.01%) reads from vitreous fluid aligning to **rubella virus.**	The rubella virus uveitis was confirmed by RT-PCR of the aqueous fluid.	A new diagnosis of chronic rubella virus uveitis

Simmer PJ et al. Clinical Infectious Diseases 2018 doi: 19.1093/cid/cix881

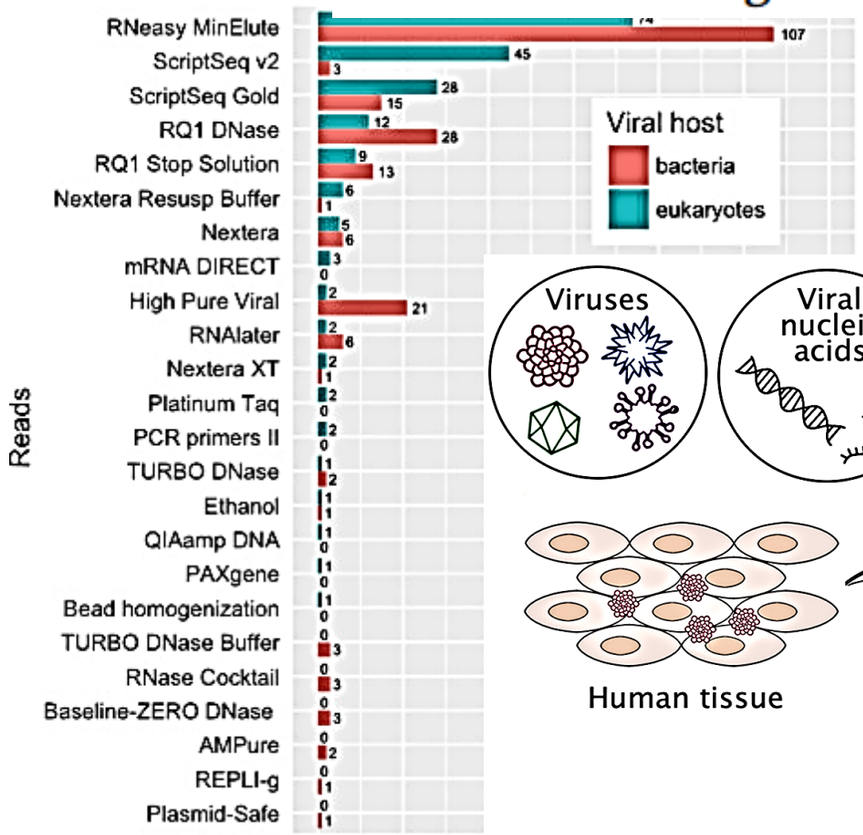
Challenge 5

Contaminants

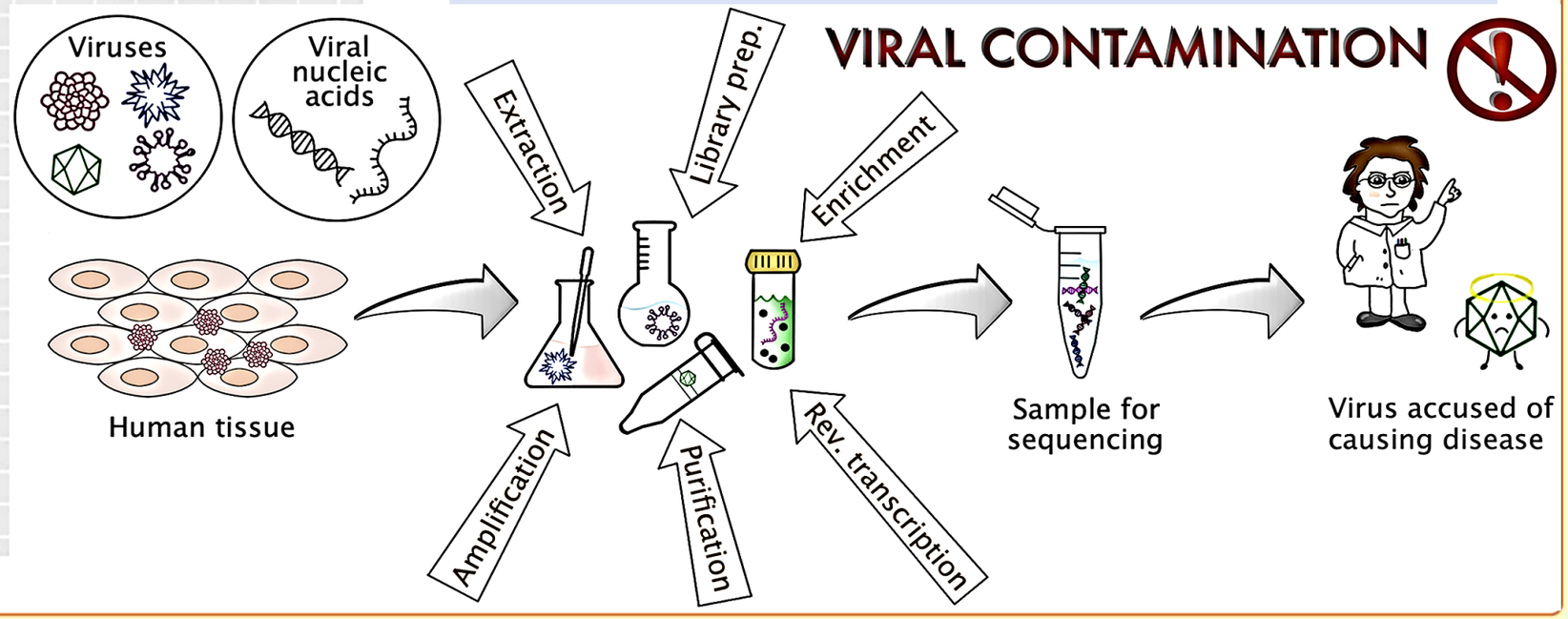


The “Kit-ome”: Contaminants in high-throughput sequencing

Contaminating viral sequences in high-throughput sequencing viromics: a linkage study of 700 sequencing libraries



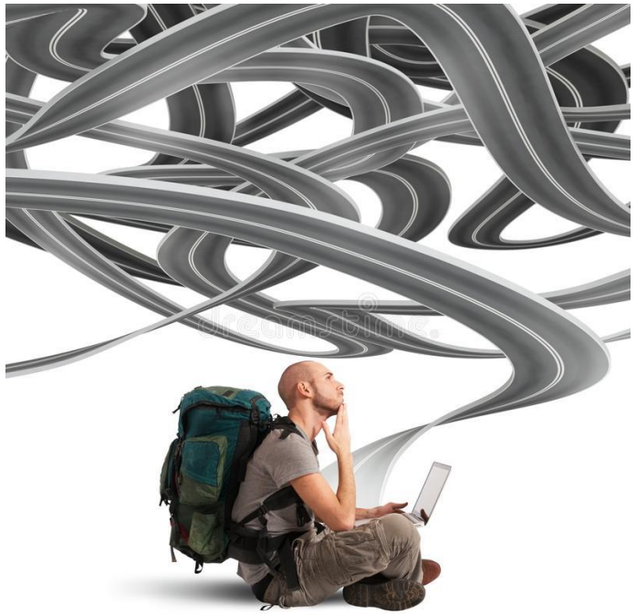
>65% of all viral sequences identified were within viral clusters linked to the use of laboratory components.



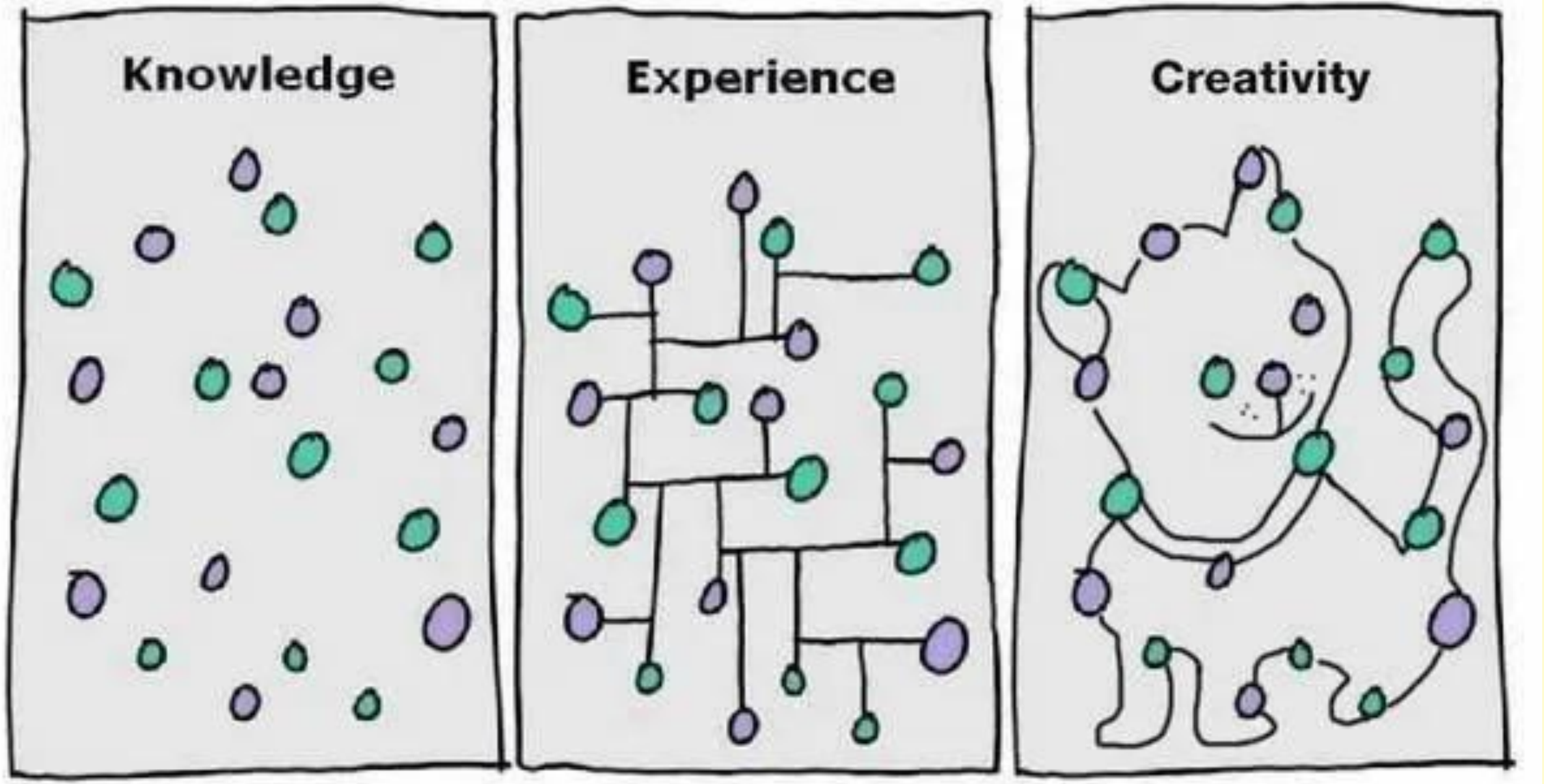
Asplund M et al Clin Microbiol Infect. 2019 doi: 10.1016/j.cmi.2019.04.028

Challenge 6

Bioinfomatic analysis



The bioinformatic analysis



Bioinformatic analysis: the importance of the pipeline

Contents lists available at ScienceDirect

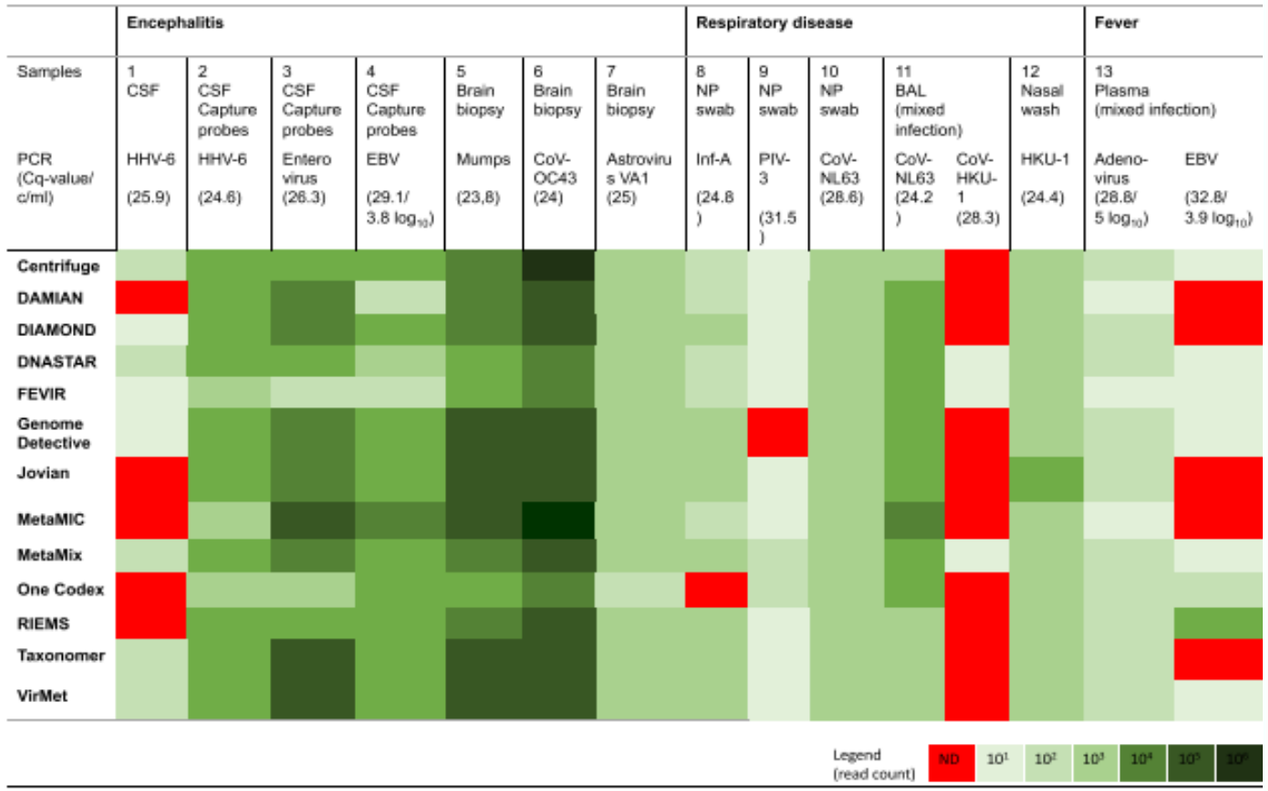
Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Benchmark of thirteen bioinformatic pipelines for metagenomic virus diagnostics using datasets from clinical samples

A benchmark of metagenomic pipelines currently used in clinical virology laboratories, initiated by the European Society for Clinical Virology Network on NGS

- Metagenomic datasets from 13 clinical samples (with encephalitis or viral respiratory infections characterized by PCR) were selected.
- The **datasets** were analyzed with 13 different pipelines used in virological diagnostic laboratories.
- Viral pathogens **with high loads** were detected by all pipelines
- Low abundance pathogens and mixed infections were **only detected by 3/13**



De Vries JJC et a. J Clin Virol 2021 doi: 10.1016/j.jcv.2021.104908.

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Benchmark of thirteen bioinformatic pipelines for metagenomic virus diagnostics using datasets from clinical samples

A benchmark of metagenomic pipelines currently us
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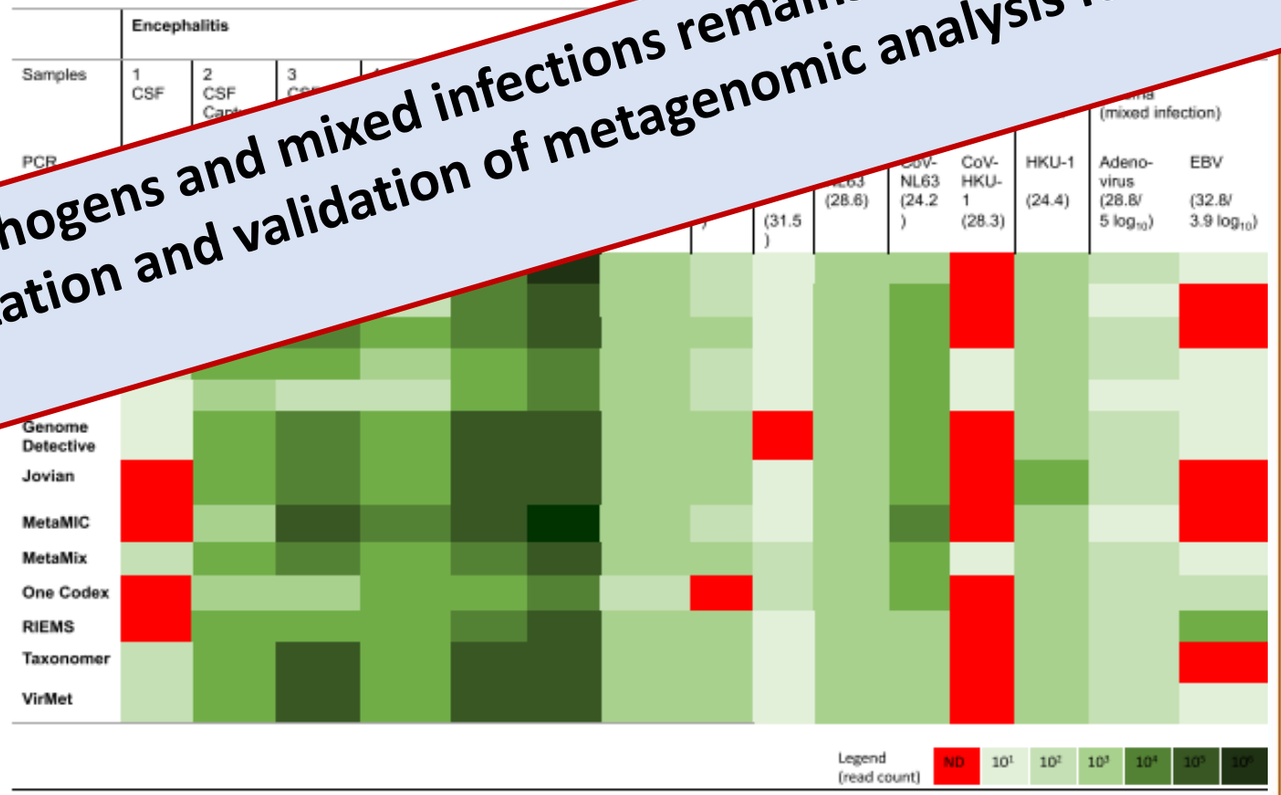
European Society for Clinical Virol

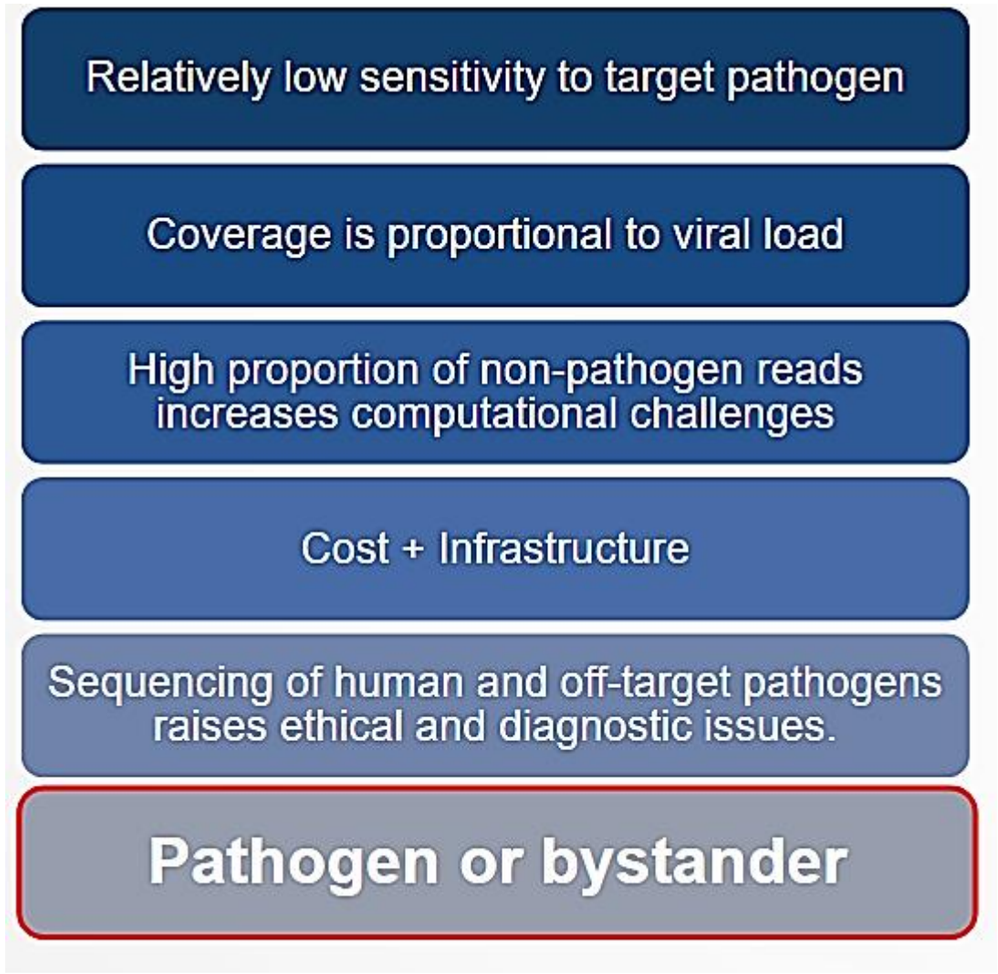
Detection of low abundant viral pathogens and mixed infections remains a challenge, implicating the need for standardization and validation of metagenomic analysis for clinical diagnostic use

- Metagenomic datasets from 13 clinical samples (with encephalitis or viral respiratory infections characterize
PCR) were selected.
- The **datas**
with high loads were
ted by all pipelines
- Low abundance pathogens and mixed infections were **only detected by 3/13**



De Vries JJC et a. J Clin Virol 2021 doi: 10.1016/j.jcv.2021.104908.

A top challenge: clinical interpretation of NGS data



We need to redefine what is "normal" for interpretation of disease associations and diagnostics in clinical virology

Ethical considerations

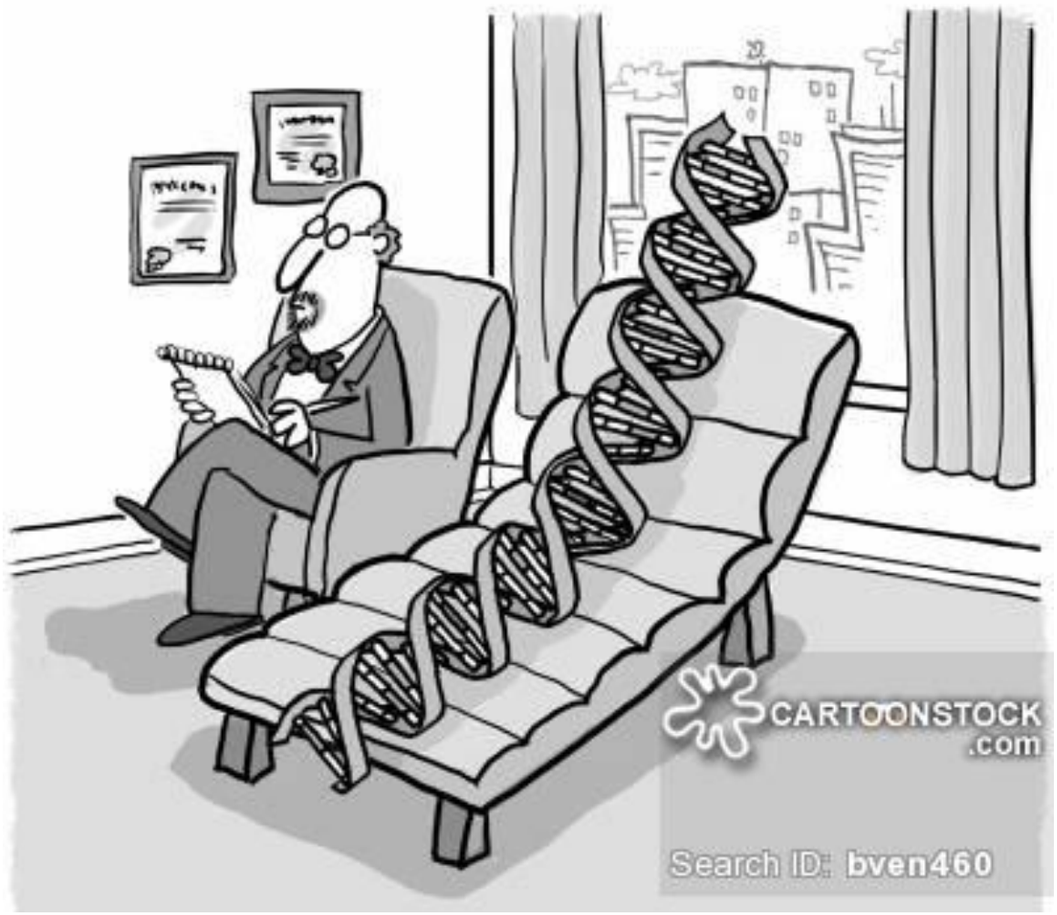
- When an assay is launched for clinical use, medically important, putatively important and unimportant findings have to be considered
- Along with accumulating data from research, **currently irrelevant findings may become relevant** in the future, e.g. if a new disease association is established or if a new drug is aunched on the market
- Thus, **storing all sequence information** for future use may be justified, however subject to (inter)national legislation
- With a potent method such as mNGS, **incidental microbiological findings** are expected. The clinician has to be aware of such a possibility and has to be prepared to explain the impact of such findings to the patient
- Sequence reads of human host background has to be considered as well, as they contain even more **sensitive information** (e.g. biological sex, hereditary characters/defects, identity of parents…..)

NGS offers significant advantages for the application to clinical virology

- NGS application to virology emerged about 10 years ago
- Since then, enormous progress
- However, several issues remain to be addressed:
  - ✓ optimal sample processing (enrichment, RNA vs DNA, shotgun, amplicon,…)
  - ✓ bioinformatics and data mining
  - ✓ contamination
  - ✓ **clinical interpretation (pathogen vs bystander)**
- Progress needed for standardized products/tools/assay design and data analysis
- Incidental findings
- Nevertheless, progress is running faster than anticipated

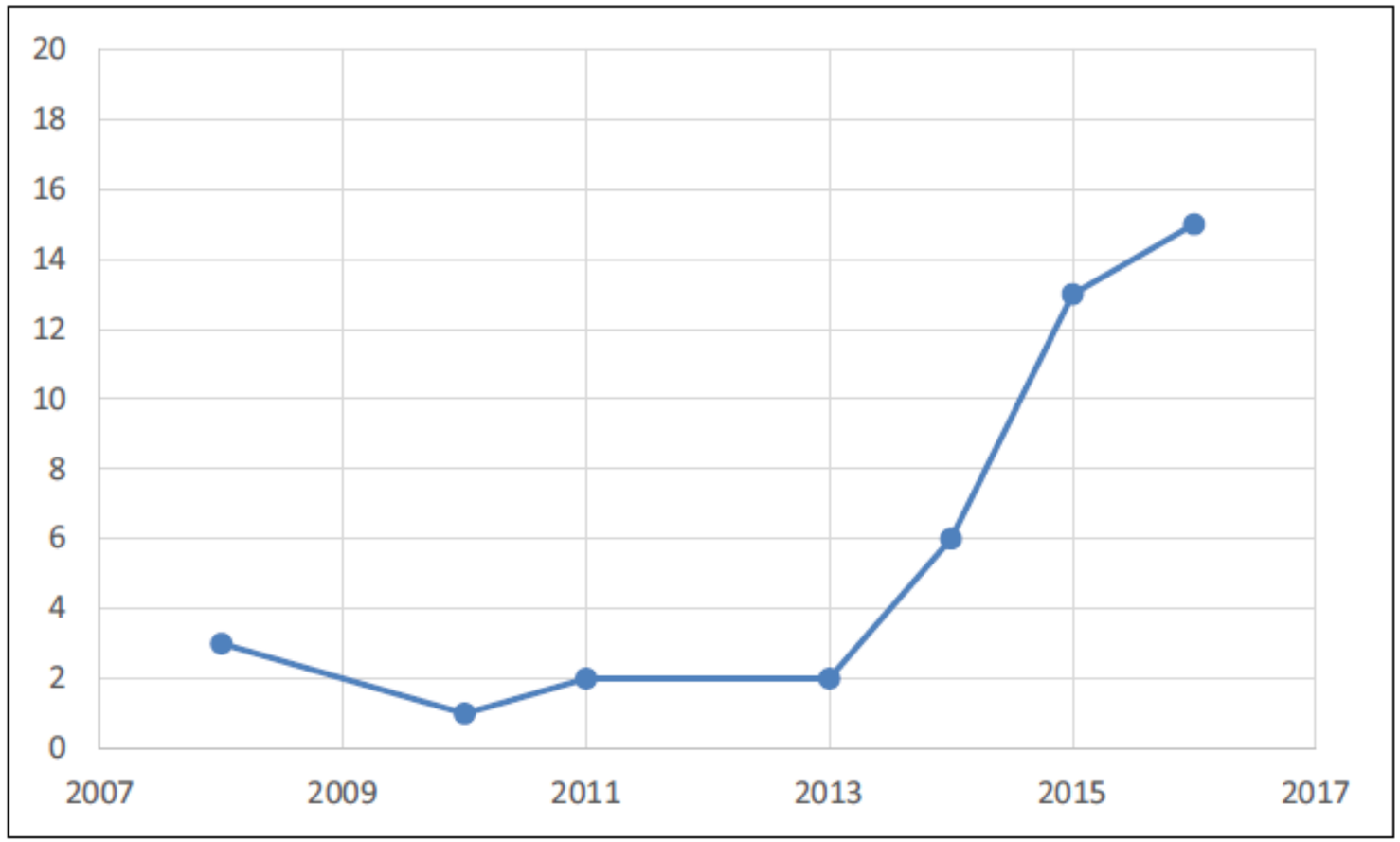
- Work in progress

Review of NGS Data “The truth is rarely pure and never simple.” Oscar Wilde



Special thanks to Barbara Bartolini
National Institute for Infectious Diseases
«L. Spallanzani» Rome

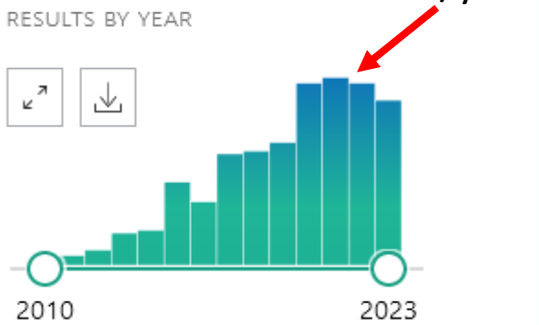
Trend of publications of Encephalitis Cases involving NGS



PubMed®

394 results

~60/year



Search terms:
Encephalitis and Next-Generation Sequencing
By Nov 5 2023

Brown JR et al. J of Inf 2018 doi: 10.1016/j.jinf.2017.12.014