Microseek: an innovative approach to unraveling clinical metagenomic secrets

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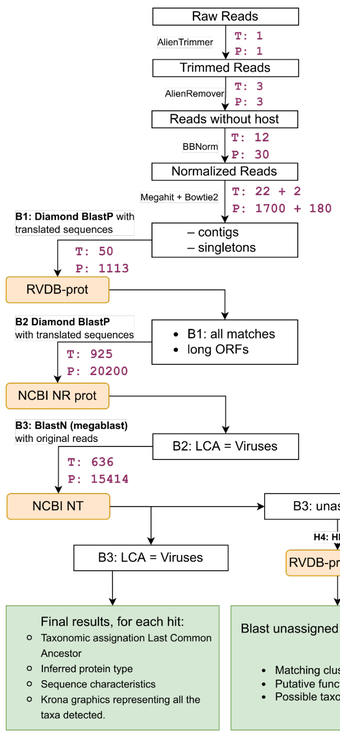
BACKGROUND

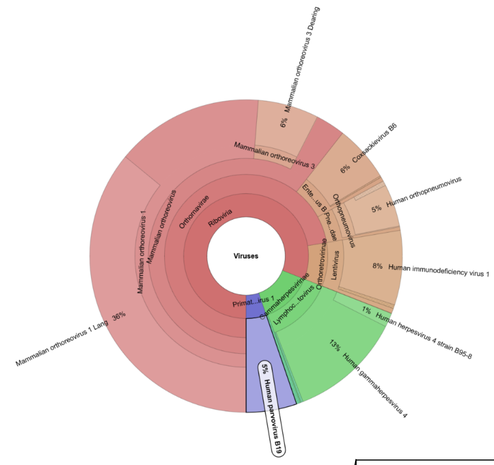
We present Microseek, a pipeline for virus identification and discovery, integrated with RVDB-prot, a comprehensive, curated and regularly updated database of viral proteins.

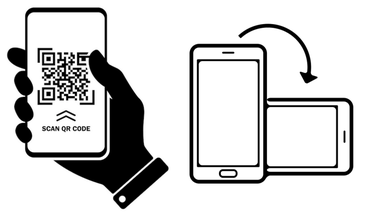
METHODS

Microseek analyses metagenomic Next Generation Sequencing (mNGS) raw data by performing quality steps, de novo assembly, and by scoring the Lowest Common Ancestor (LCA) from translated reads and contigs. Microseek runs on a local computer. The outcome of the pipeline is displayed through a user-friendly and dynamic graphical interface.

The pipeline & its user-friendly interface







LIVE DEMO
SCAN ME!

Figure 1. Microseek pipeline steps and output examples. A/ Step details of the pipeline. For each operation, software and database names are indicated. The times on the right represent the cumulated duration of each step (in minutes), respectively for the Tissues (T) and Plasma (P) datasets and corresponding to spiked experiments at d1. For instance, for Plasma dataset, B1 step would take 1113 minutes if it was run with a single CPU as a unique chunk. B/ Example of a result browser webpage. The Parvovirus B19 taxon was selected from the Krona chart as an example. C/ Resulting list of hits of the Tissues dataset after filtering on Human Parvovirus B19 with e-value ≤10^{-3}, showing information such as the existence of contigs (C), e-value, %ID, length of the sequence and protein names. Information boxes associated to each hit give the nt and aa sequences and allow direct access to the corresponding NCBI entries.

Pérot, P.; Bigot, T.; Temmam, S.; Regnault, B.; Eloit, M. Microseek: A Protein-Based Metagenomic Pipeline for Virus Diagnostic and Discovery. *Viruses* 2022

RESULTS

Drawing from two representative mNGS datasets obtained from human tissue and plasma specimens, we present its performance. In-silico spikes of known viral sequences, as well as spikes of fabricated Neopneumovirus viral sequences generated with varying evolutionary distances from known members of the Pneumoviridae family, were employed. Results were compared to Chan Zuckerberg ID (CZ ID), a benchmark cloud-based mNGS pipeline.

Microseek *versus* CZ ID

Detection of known viruses

Table 1: Six known human viruses representing six groups of the Baltimore classification were selected as input reference sequences for spiking experiments. ssDNA: Human parvovirus B19; dsDNA: EBV; ssRNA+: Coxsackievirus B6; ssRNA-: Human respiratory syncytial virus A (HRSV-A); dsRNA: Mammalian orthoreovirus type 1; and retrovirus: Human immunodeficiency virus type 1 (HIV-1).

ssDNA	Parvovirus B19
dsDNA	Epstein–Barr virus
ssRNA+	Coxsackievirus B6
ssRNA-	Respiratory syncytial virus
dsRNA	Reovirus 1 (10 segments)
Retrovirus	HIV-1

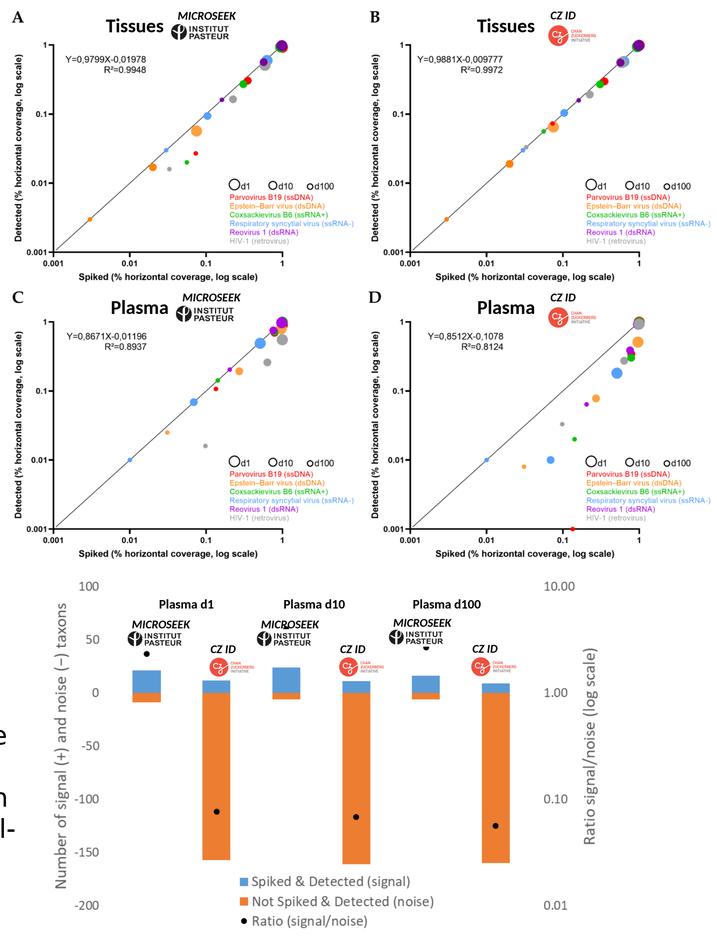
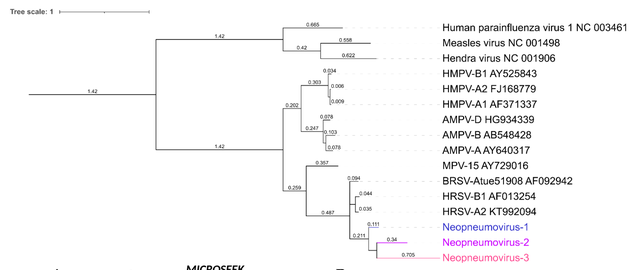


Figure 3. Detection of six known viruses (spiked vs. detected). Scatter plots of spiked (X axis) vs. detected (Y axis) percentage horizontal coverage for the 6 known viruses of the study, for the Tissues (**A**,**B**) and Plasma (**C**,**D**) experiments. Each virus is depicted by 3 points, corresponding to the d1 (large circles), d10 (intermediate circles) and d100 (small circles) experiments, and is associated with a specific color (red: Parvovirus B19; orange: Epstein-Barr virus; green: Coxsackievirus B6; blue: HRSV; purple: Reovirus-1; grey: HIV-1). The identity line (Y = X) is represented in black.

Figure 5. Signal-to-noise ratio in the Plasma experiments (known viruses). Histograms below the table represent the signal (blue bars with counts above zero on the primary axis) vs. noise (orange bars with counts below zero on the primary axis) and associated signal-to-noise ratios (black dots on the secondary axis).

Detection of novel viruses



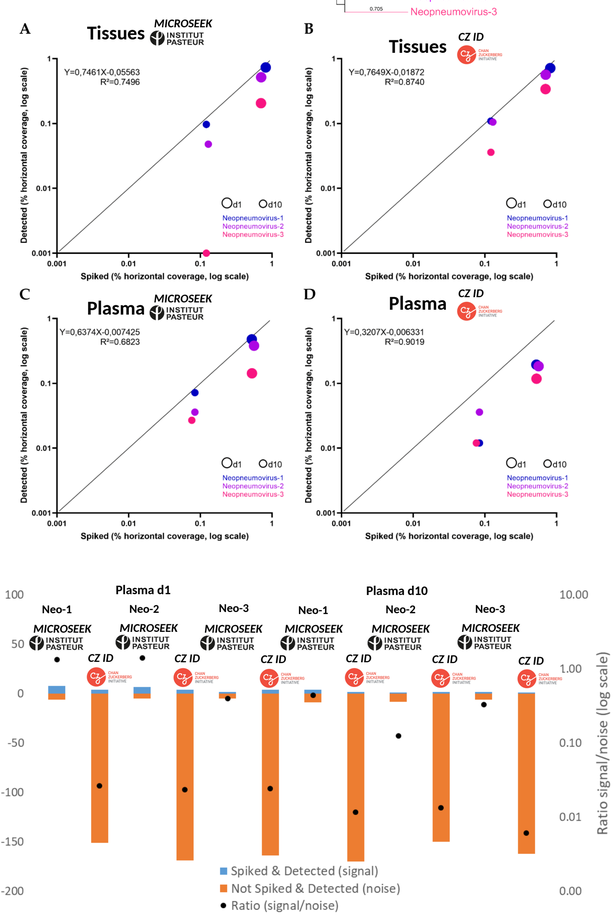


Figure 2. Phylogeny of Neopneumoviruses. Protein sequences of the polymerases were aligned with MAFFT v7.450, and the phylogeny was reconstructed with IQtree 2.0.6 with model of sub-stitution JTT+F+G4. AMPV: Avian metapneumovirus; HMPV: Human metapneumovirus; MPV: Murine orthopneumovirus; BRSV: Bovine orthopneumovirus; HSRV: Human respiratory syncytial virus.

Figure 4. Detection of three Neopneumoviruses (spiked vs. detected). Scatter plots of spiked (X axis) vs. detected (Y axis) percentage horizontal coverage for the 3 fake Neopneumoviruses of the study, for the Tissues (**A**,**B**) and Plasma (**C**,**D**) experiments. Each virus is depicted by 2 points, corresponding to the d1 (large circles) and d10 (intermediate circles) experiments, and is associated with a specific color (blue: Neopneumovirus-1; purple: Neopneumovirus-2; pink: Neopneumovirus-3). The identity line (Y = X) is represented in black.

Figure 6. Signal-to-noise ratio in the Plasma experiments (novel viruses). Histograms below the table represent the signal (blue bars with counts above zero on the primary axis) vs. noise (orange bars with counts below zero on the primary axis) and associated signal-to-noise ratios (black dots on the secondary axis).

Clinical Infectious Diseases
MAJOR ARTICLE
Identification of Umbre Orthobunyavirus as a Novel Zoonotic Virus Responsible for Lethal Encephalitis in 2 French Patients with Hypogammaglobulinemia

Contents lists available at ScienceDirect
Infection, Genetics and Evolution
journal homepage: www.elsevier.com/locate/meegid
Short communication
Whole genome sequencing and phylogenetic characterization of a novel bat-associated picornavirus-like virus with an unusual genome organization

nature
Article | Published: 16 February 2022
Bat coronaviruses related to SARS-CoV-2 and infectious for human cells

Insights into the Host Range, Genetic Diversity, and Geographical Distribution of Jingmenviruses

Clinical Infectious Diseases
MAJOR ARTICLE
First Case of Lethal Encephalitis in Western Europe Due to European Bat Lyssavirus Type 1

ORIGINAL ARTICLE
The virome of *Rhipicephalus*, *Dermacentor* and *Haemaphysalis* ticks from Eastern Romania includes novel viruses with potential relevance for public health

frontiers in Microbiology
ORIGINAL RESEARCH
Monitoring Silent Spillovers Before Emergence: A Pilot Study at the Tick/Human Interface in Thailand

Microbiology Resource Announcements
Six Nearly Complete Genome Segments of a Novel Reovirus Identified in Laotian Batflies

CONCLUSION

Microseek reliably identifies known viral sequences and performs well for the detection of distant pseudoviral sequences, especially in complex samples such as in human plasma, while minimizing non-relevant hits.