



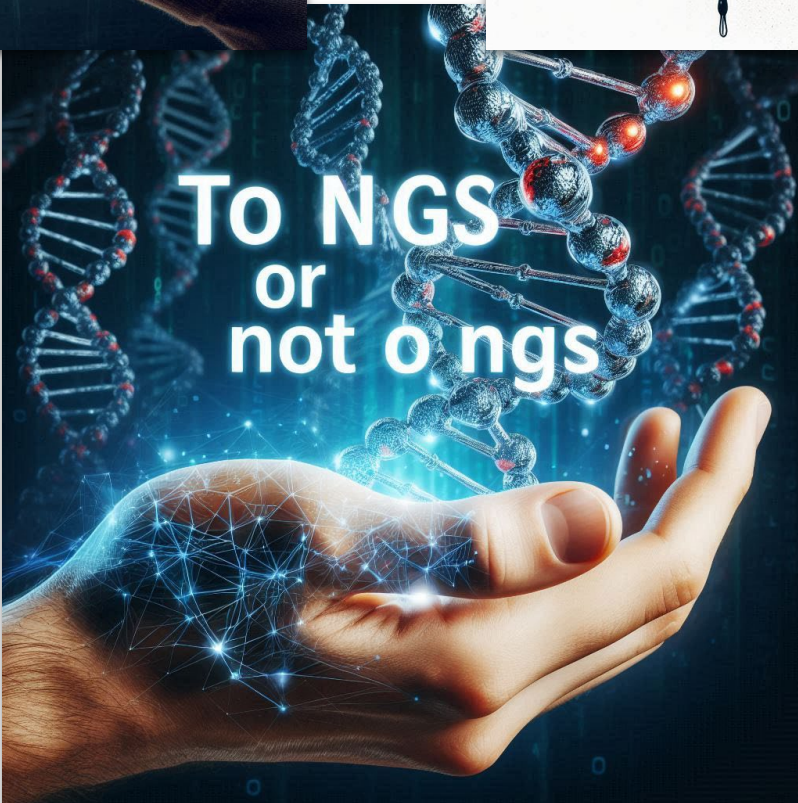
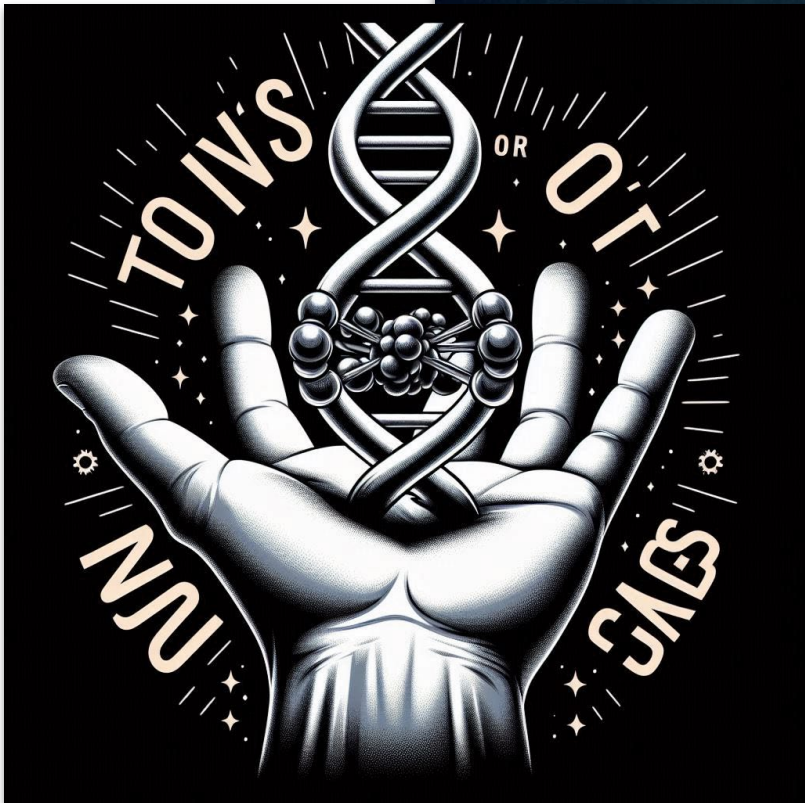
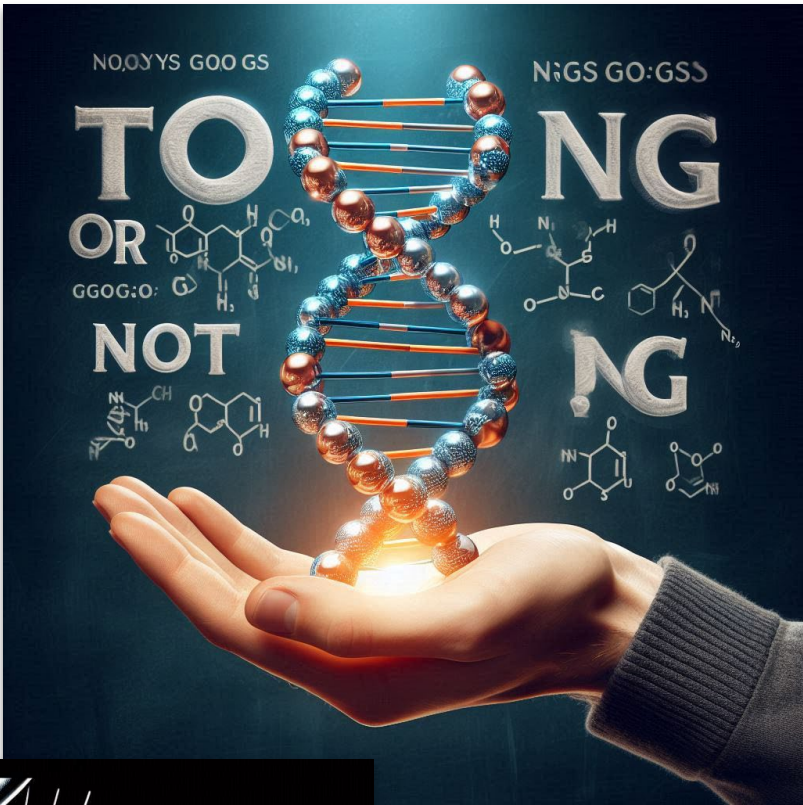
To NGS or Not to NGS

Next Step for Next-Generation Sequencing

Preconference Workshop, ID Lab Con March 24, 2025

Dr. Kelly F. Oakeson

To NGS or Not to NGS



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There is an uptick of MRSA in a NICU, and Infection Prevention at a hospital has inquired about sequencing to further understand the dynamics and if an outbreak is occurring

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State epidemiologists are investigating measles cases and have learned that differentiation of vaccine strain and clinical strain of measles can have an impact on quarantine and recommendations for community

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An individual is presenting with neurological symptoms in a hospital and everything has been ruled out, ID doc wants to explore metagenomics to examine potential infectious reasons for symptoms

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Case:

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475 of 3,063,784 (0.016%) reads from the CSF specimen mapped to the *Leptospiraceae* family with the closest matched genome of *Leptospira borgpetersenii*.

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Confirmatory Test:

PCR and serologic testing at the CDC subsequently confirmed the presence of *Leptospira santarosai* infection

Outcome:

Treated with a course of penicillin G and discharged after 1 month with mild neurologic deficits.

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The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing

Michael R. Wilson, M.D., Samia N. Naccache, Ph.D., Erik Samayoa, B.S., C.L.S., Mark Biagtan, M.D., Hiba Bashir, M.D., Guixia Yu, B.S., Shahriar M. Salamat, M.D., Ph.D., Sneha Somasekar, B.S., Scot Federman, B.A., Steve Miller, M.D., Ph.D., Robert Sokolic, M.D., Elizabeth Garabedian, R.N., M.S.L.S., Fabio Candotti, M.D., Rebecca H. Buckley, M.D., Kurt D. Reed, M.D., Teresa L. Meyer, R.N., M.S., Christine M. Seroogy, M.D., Renee Galloway, M.P.H., Sheryl L. Henderson, M.D., Ph.D., James E. Gern, M.D., Joseph L. DeRisi, Ph.D., and Charles Y. Chiu, M.D., Ph.D.

SUMMARY

A 14-year-old boy with severe combined immunodeficiency presented three times to a medical facility over a period of 4 months with fever and headache that progressed to hydrocephalus and status epilepticus necessitating a medically induced coma. Diagnostic workup including brain biopsy was unrevealing. Unbiased next-generation sequencing of the cerebrospinal fluid identified 475 of 3,063,784 sequence reads (0.016%) corresponding to leptospira infection. Clinical assays for leptospirosis were negative. Targeted antimicrobial agents were administered, and the patient was discharged home 32 days later with a status close to his premorbid condition. Polymerase-chain-reaction (PCR) and serologic testing at the Centers for Disease Control and Prevention (CDC) subsequently confirmed evidence of *Leptospira santarosai* infection.

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mNGS Results:

277 of 23,638,587 (0.0012%) reads from CSF DNA aligned to the *Brucella* genus, with most aligning to *B. melitensis*, no *Brucella* reads were found in the 9,161,626 CSF RNA reads.

Confirmatory Test:

Brucella PCR and serologies confirmed Chronic neurobrucellosis

Outcome:

Treated with a course of doxycycline and rifampin. Two weeks after starting therapy she reported that her symptoms fully resolved.

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Case:

An 11-year-old girl from Mexico presented with 4 weeks of headache, back pain, nausea, and emesis. CSF was found to be positive for EBV and HHV7. She was treated with acyclovir and discharged. Two weeks later, she presented with back pain and worsening headache. She was rehospitalized with a diagnosis of tuberculosis.

mNGS Results:

277 of 23,638,587 (0.0012%) reads from CSF DNA aligned to the *Brucella* genus, with most aligning to *B. melitensis*, no *Brucella* reads were found in the 9,161,626 CSF RNA reads.

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Journal of the Pediatric Infectious Diseases Society

BRIEF REPORT

Neurobrucellosis: Unexpected Answer From Metagenomic Next-Generation Sequencing

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Jeffrey M. Bender¹, Erik Samayoa^{3,5}, Elizabeth Pham^{3,5}, Guixia Yu^{3,5},
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Charles Y. Chiu³⁻⁵

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Clinical Infectious Diseases

REVIEW ARTICLE



Understanding the Promises and Hurdles of Metagenomic Next-Generation Sequencing as a Diagnostic Tool for Infectious Diseases

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Agnostic metagenomic next-generation sequencing (mNGS) has emerged as a promising single, universal pathogen detection method for infectious disease diagnostics. This methodology allows for identification and genomic characterization of bacteria, fungi, parasites, and viruses without the need for a priori knowledge of a specific pathogen directly from clinical specimens. Although there are increasing reports of mNGS successes, several hurdles need to be addressed, such as differentiation of colonization from infection, extraneous sources of nucleic acid, method standardization, and data storage, protection, analysis, and interpretation. As more commercial and clinical microbiology laboratories develop mNGS assays, it is important for treating practitioners to understand both the power and limitations of this method as a diagnostic tool for infectious diseases.

Keywords. metagenomics; diagnosis; next-generation sequencing; bioinformatics.

Thank you!

