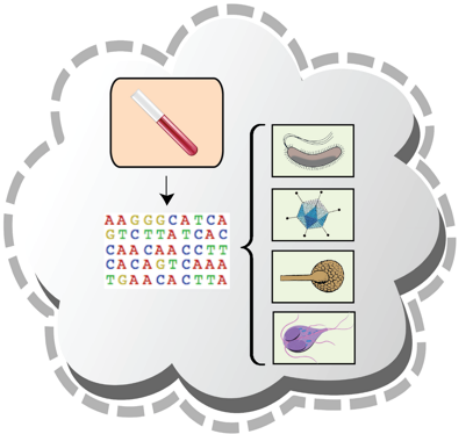
University of California
San Francisco



Next-Generation Sequencing for Diagnosis of Infections

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Associate Director, UCSF Clinical Microbiology Laboratory

Disclosures

- Abbott Diagnostics (research support for pathogen discovery)
- Rubicon Genomics (scientific advisory board)
- Karius, Inc. (scientific advisory board)

Summary of Talk

1. Metagenomic next-generation sequencing (mNGS) for infectious disease diagnosis, including the SURPI bioinformatics pipeline

2. Clinical case studies of mNGS

3. The California Initiative to Advance Precision Medicine (CIAPM) "Precision Diagnosis of Acute Infectious Diseases" project

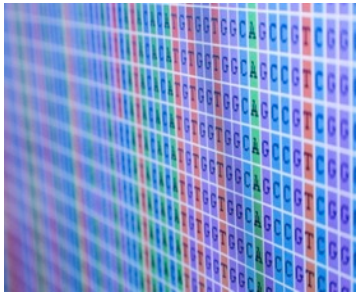
1. Other applications of NGS (point-of-care nanopore sequencing, transcriptomics)

Precision Diagnosis with Genomic Testing can Impact Clinical Decision-Making in Infectious Diseases

California Initiative to Advance Precision Medicine

CLIA Laboratory

Metagenomic NGS



Bioinformatics

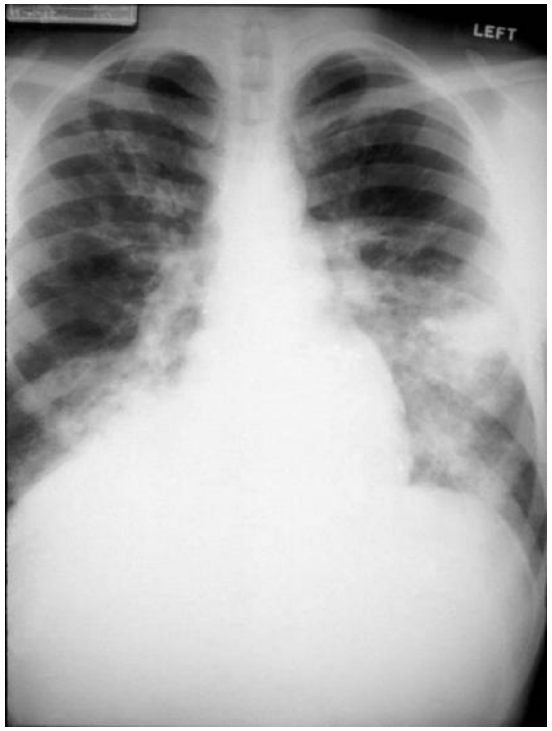
Agent ID

Cost-effective and actionable information for early treatment

Lower healthcare costs
Improved patient outcomes

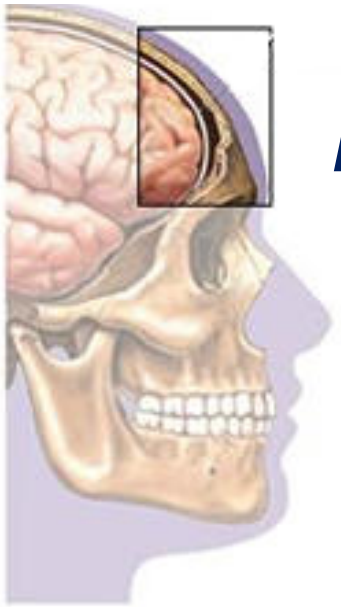
Turnaround time: hours – days (versus days – weeks)

Targeted Infectious Diseases in Hospitalized Patients



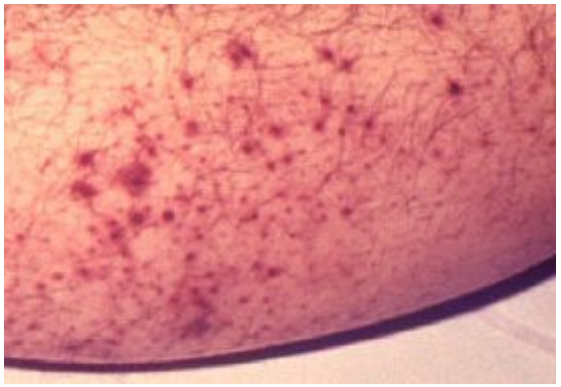
Pneumonia

15 – 25%
unknown cause



Meningitis / Encephalitis

50 – 70%
unknown cause



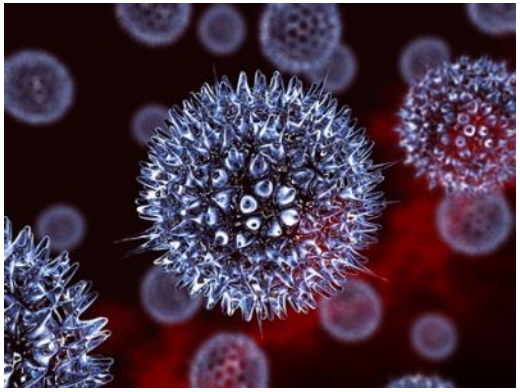
Fever / sepsis

~20 – 30% unknown cause

Nearly All Microbes can be Uniquely Identified by "Shotgun" Nucleic Acid Sequencing

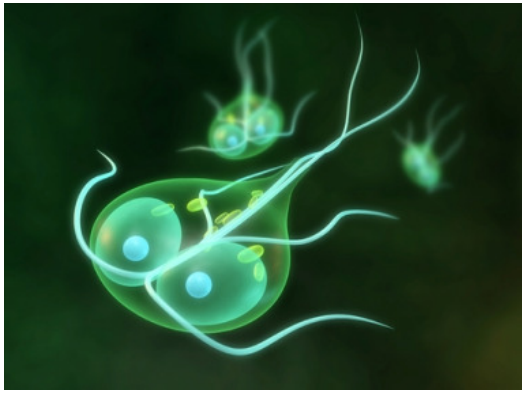
Bacteria

Viruses

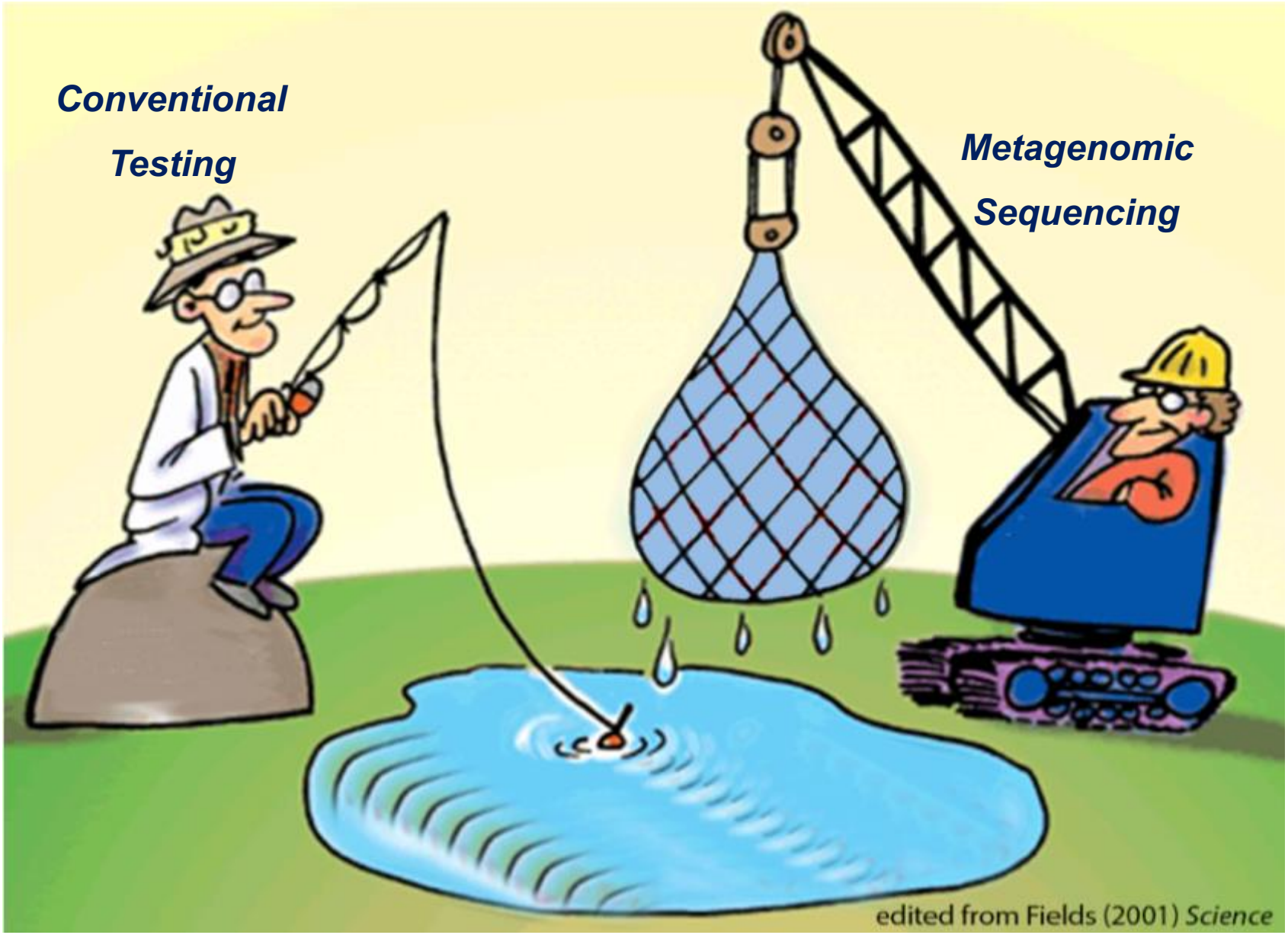


Fungi

Parasites



Metagenomic Sequencing – Casting a Wide Net

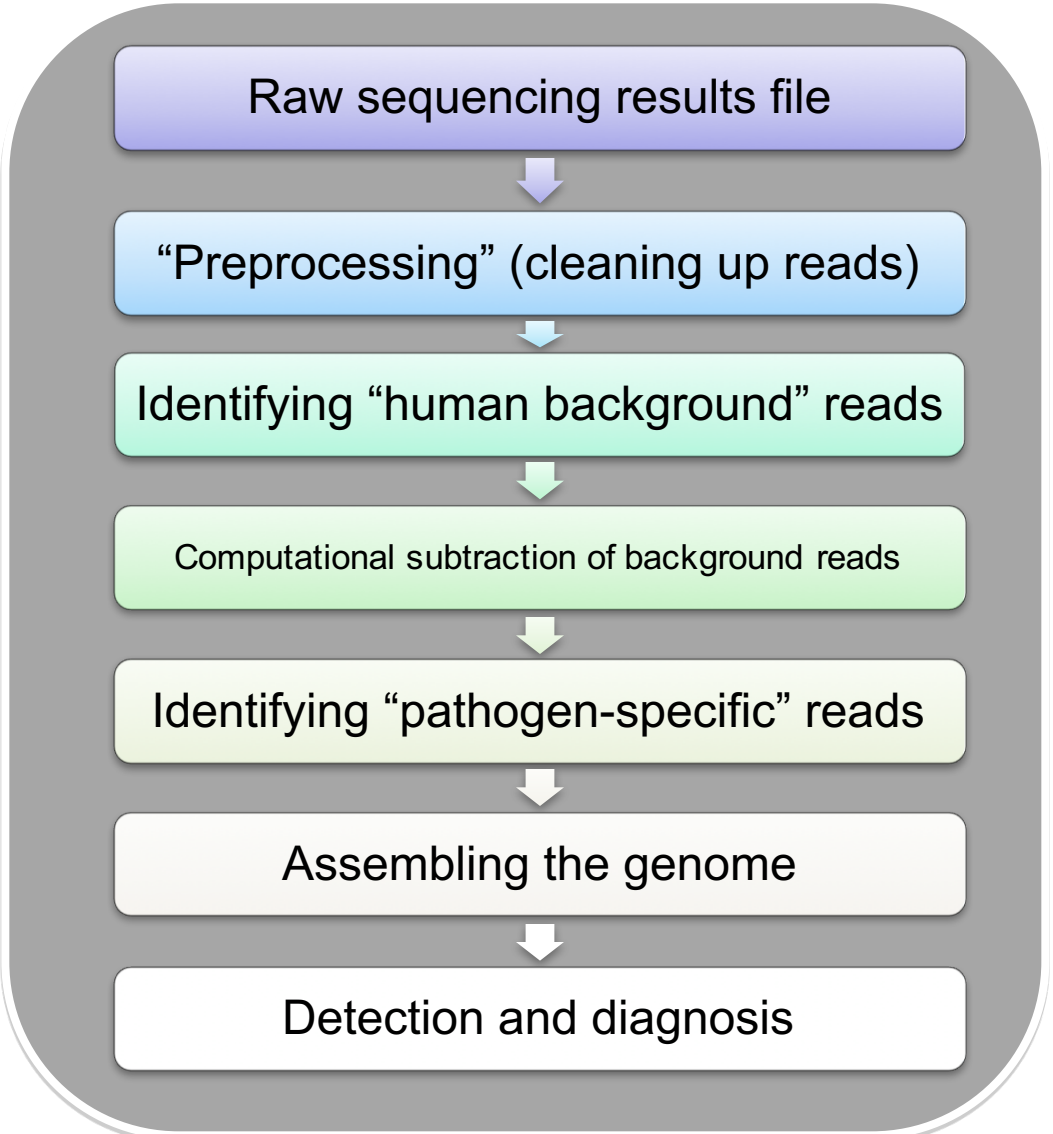


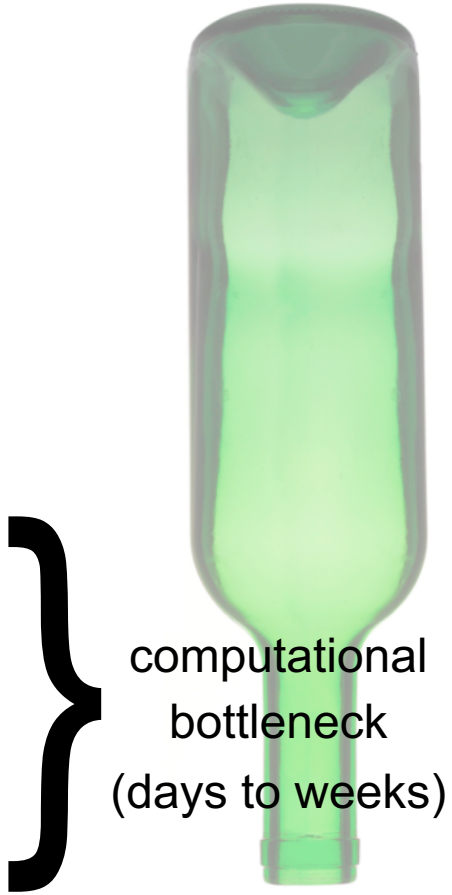
Metagenomic Next-Generation Sequencing (mNGS) for Infectious Disease Diagnosis

- **Metagenomic sequencing = shotgun, random sequencing of all of the RNA and DNA in a clinical sample**
- **"Unbiased" approach to detect and reconstruct the genomic sequence of nearly any and all pathogens including viruses, bacteria, fungi, and parasites**
- **Feasible due to vast increases in sequencing capacity with current instruments**

HiSeq 2500 (Illumina, Inc.)

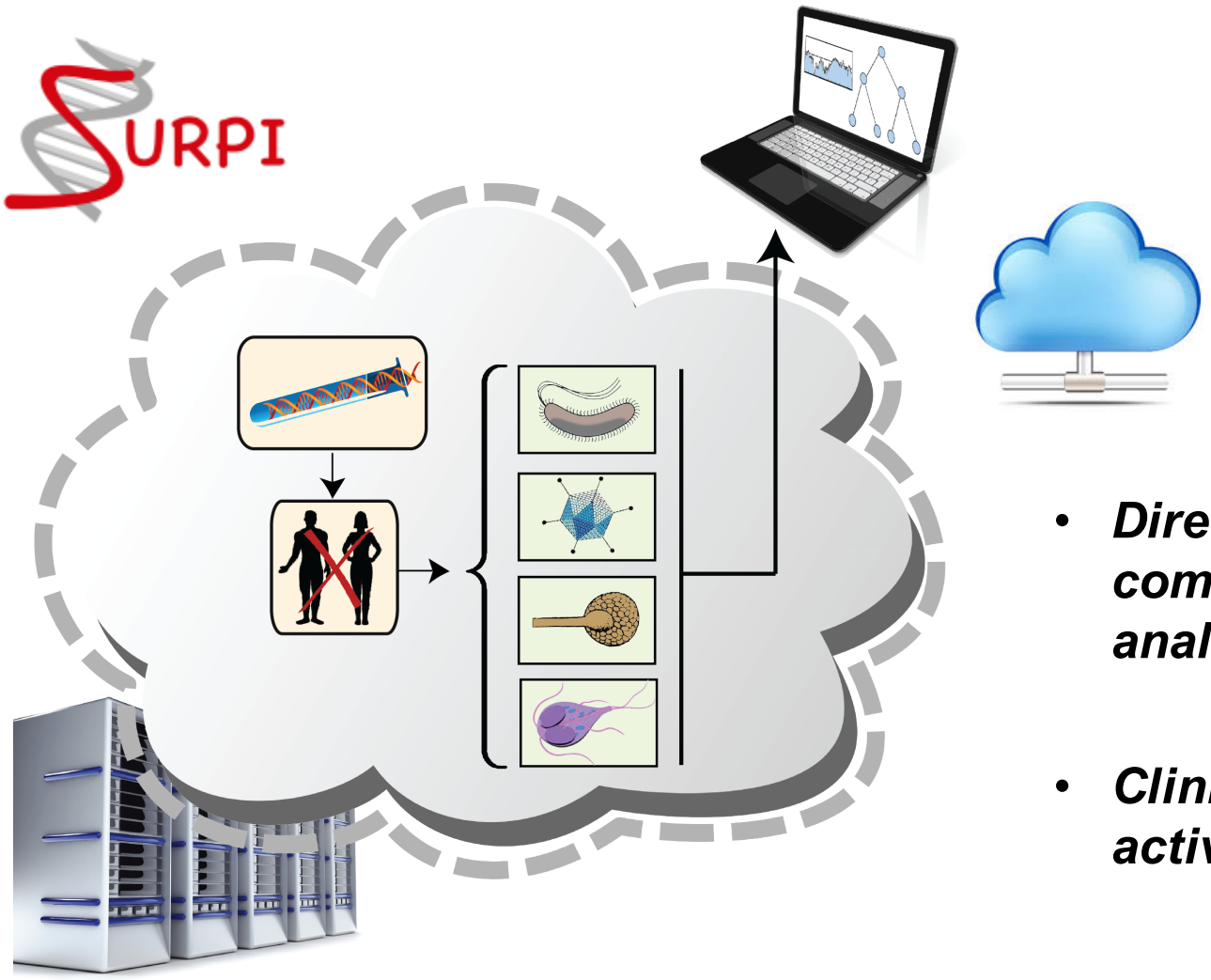
Identifying Pathogens by their Specific DNA Sequence





The SURPI Bioinformatics Pipeline

"Sequence-based ultra-rapid pathogen identification" (minutes – hours)



- ***Directly addresses computational analysis bottleneck***
- ***Clinical version, in active development***

(Naccache, *et al.*, 2014, *Genome Research 24(7):*1180-1192)

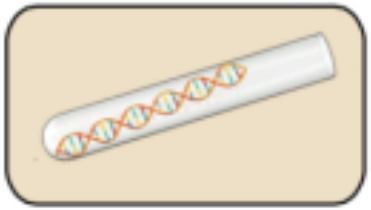
CLIA
(Clinical Laboratory Improvement Amendments)

- CLIA = federal regulatory framework for clinical laboratory testing
  - Took effect Sept. 1992
  - CLIA laboratories are accreditated (certified) by inspection agency (e.g. CAP)
- Sets minimum standards under which all clinical laboratories operate
- Requires validation and quality assurance for all laboratory tests used in clinical care

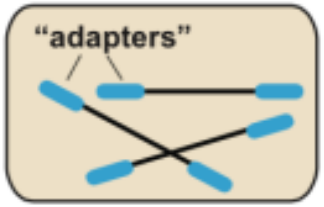
Validation of the metagenomic NGS assay in a CLIA laboratory is essential for clinical implementation

mNGS Clinical Workflow in a CLIA Laboratory

Clinical sample

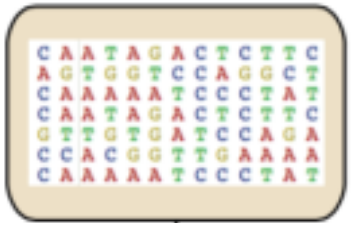


Nucleic acid extraction

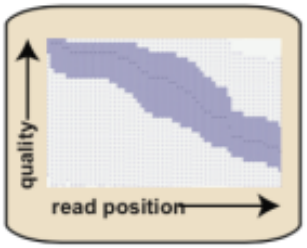


cDNA/DNA library preparation

mNGS

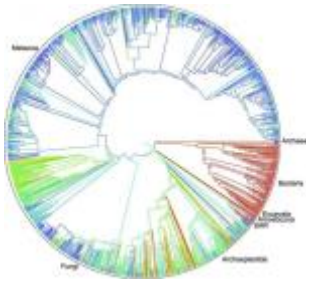


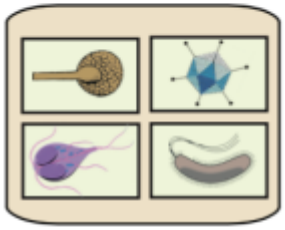
Host depletion



Adapter, quality and low-complexity trimming

Host subtraction

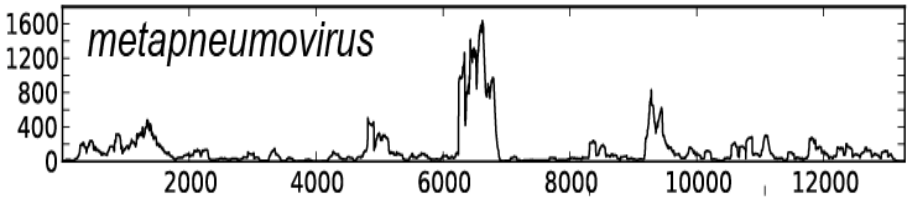


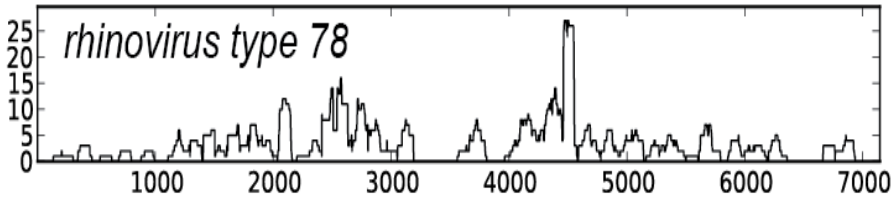


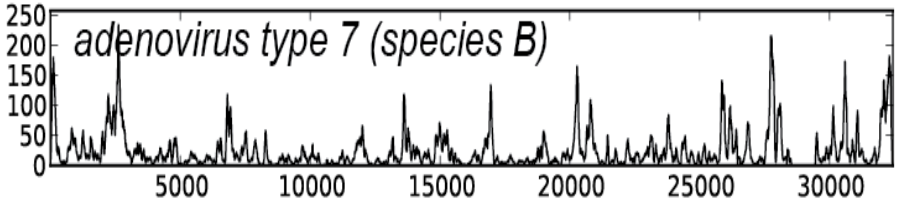
Alignment against GenBank

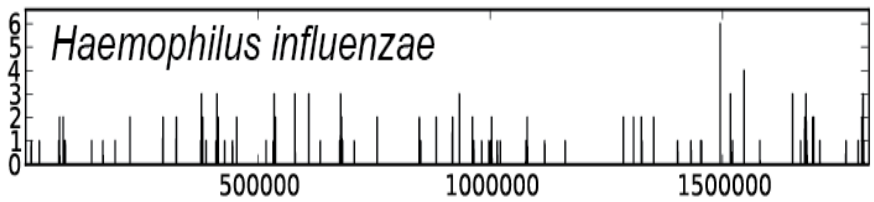
SURPI (Sequence Based Ultra-Rapid Pathogen Identification) Pipeline

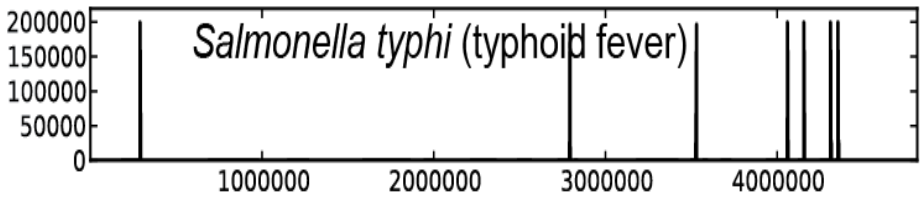
Same mNGS Approach Can Diagnose a Range of Acute Febrile Illnesses

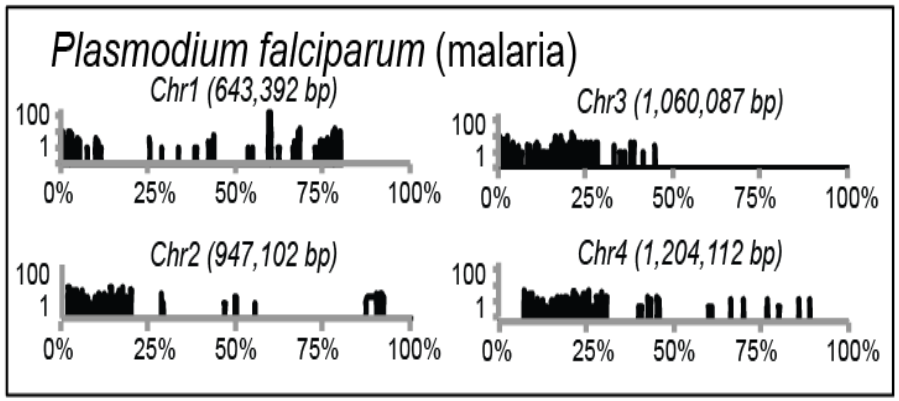






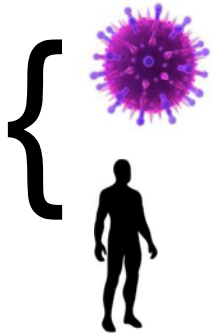






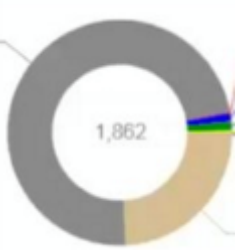
SURPI+ (Clinical Version)

1. Filtering for misannotations in GenBank



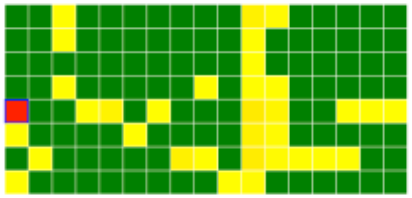
1. metadata tagging

2. taxonomic classification



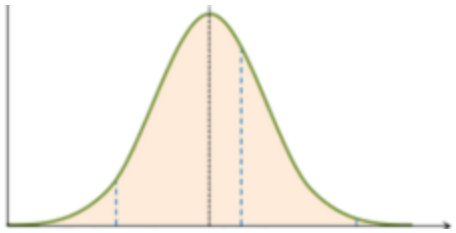
3. pipeline optimization

4. visualization (SURPI*viz*)

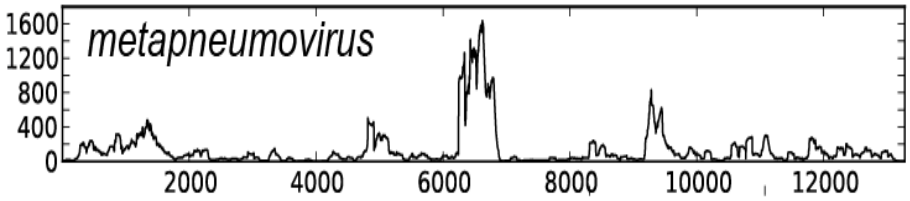


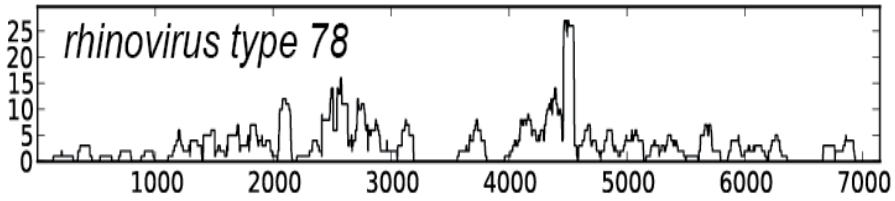
5. server and cloud implementation

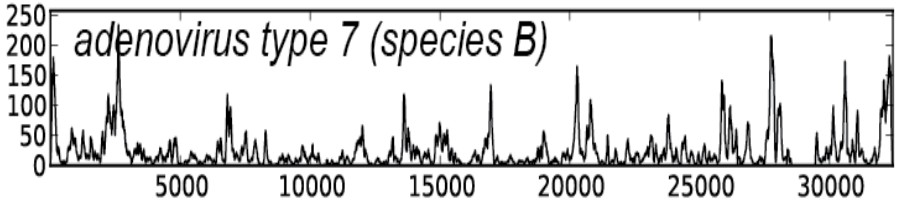
6. metrics for normalization of results

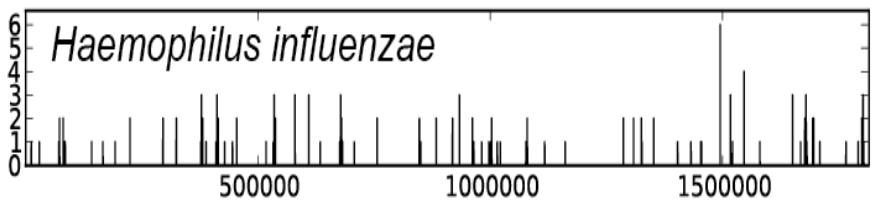


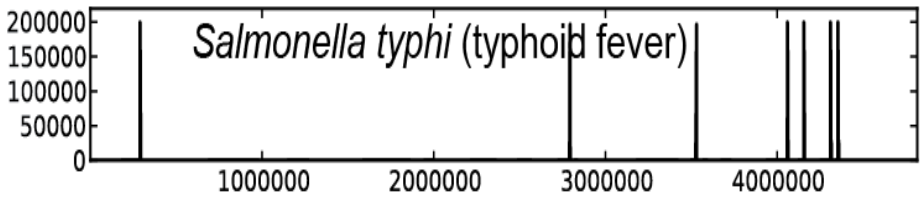
Same NGS Approach Can Diagnose a Range of Acute Febrile Illnesses

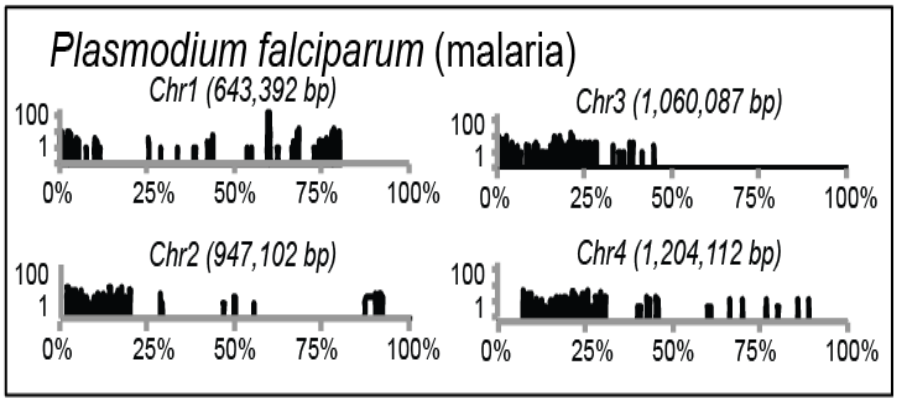












Neuroleptospirosis Diagnosed by Metagenomic NGS (mNGS)

3 hospitalizations over 4 months

44 days in the ICU

>100 inconclusive tests

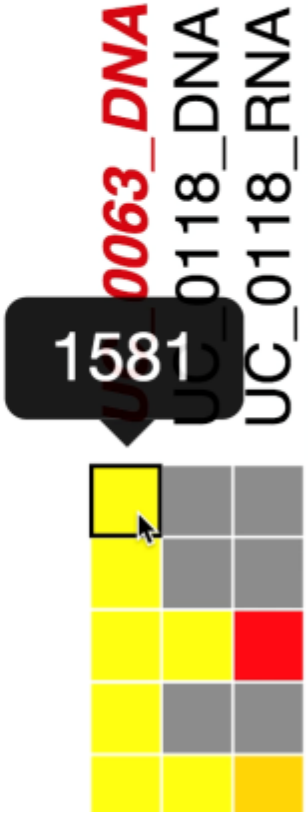
3 empiric treatments with no effect

Brain biopsy and induced coma

Cured 2 weeks after NGS dx with appropriate treatment

(Wilson, et al., 2015, *New England Journal of Medicine*; photo courtesy of the Osborn family)

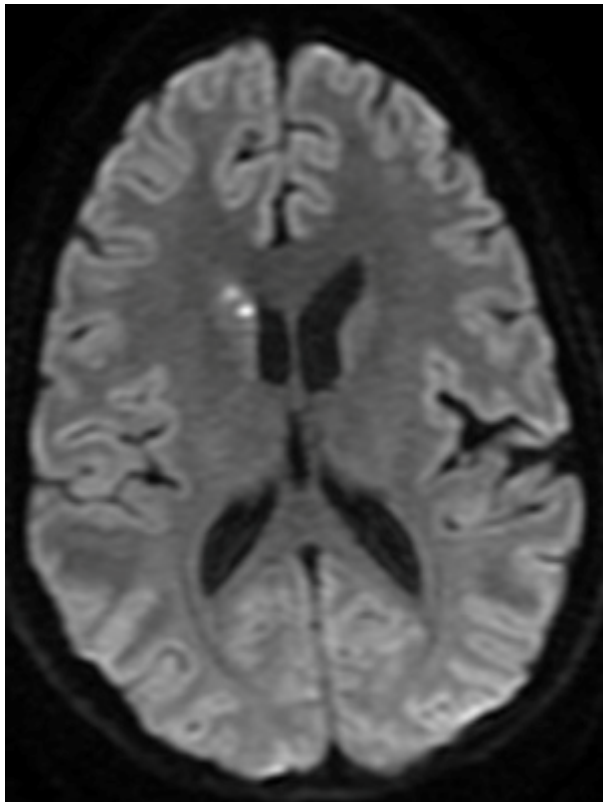
SURPIviz Contact SURPI Runs



Leptospira santarosai
Leptospira borgpetersenii
unclassified
Leptospira interrogans
Propionibacterium acnes

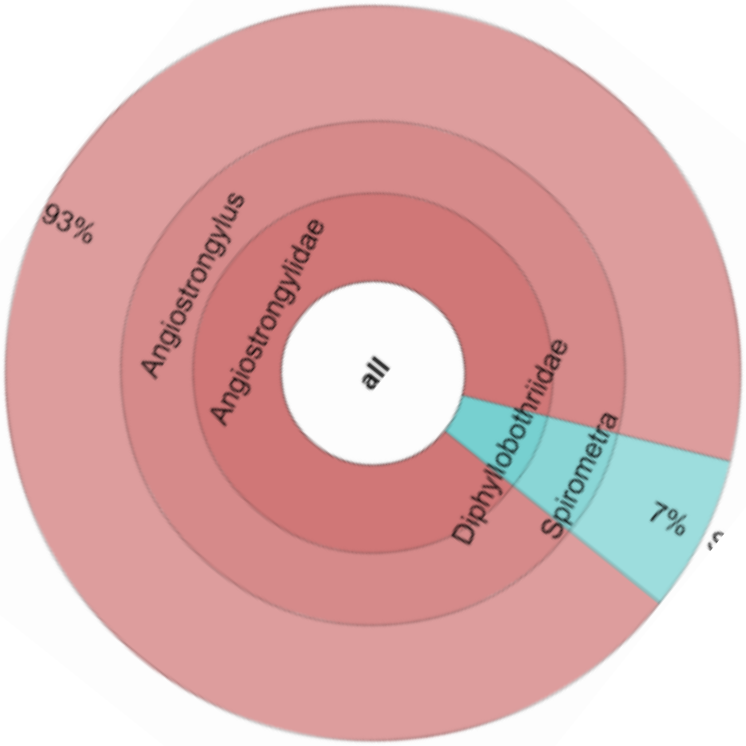
A Case of Fever and Eosinophilia

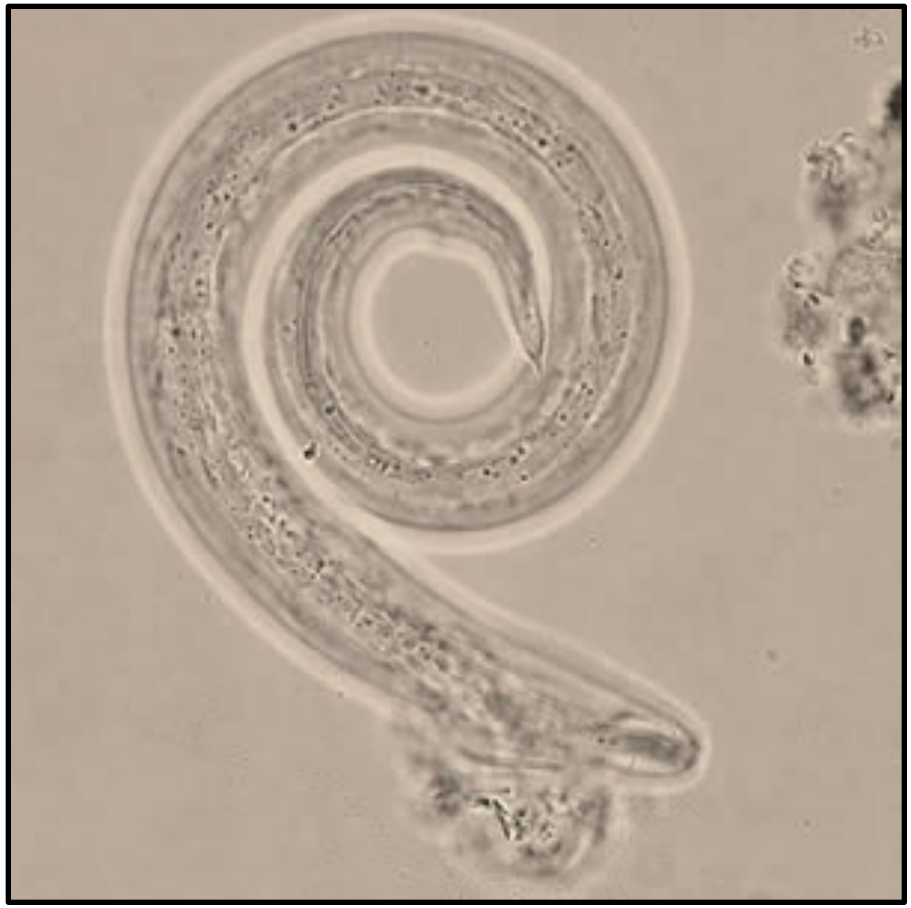
- 35 y/o female, travel to Fiji for honeymoon
- Itchy skin rash, joint pains, fever / chills
- Progresses to severe headache, blurry vision, diplopia (double vision), paresthesias ("pins-and-needles")
- Cerebrospinal fluid with 280 white blood cells, 30% eosinophils
- Infectious disease workup negative (>40 tests)
- Treated empirically with steroids for presumed organism, but still symptomatic after 2 weeks



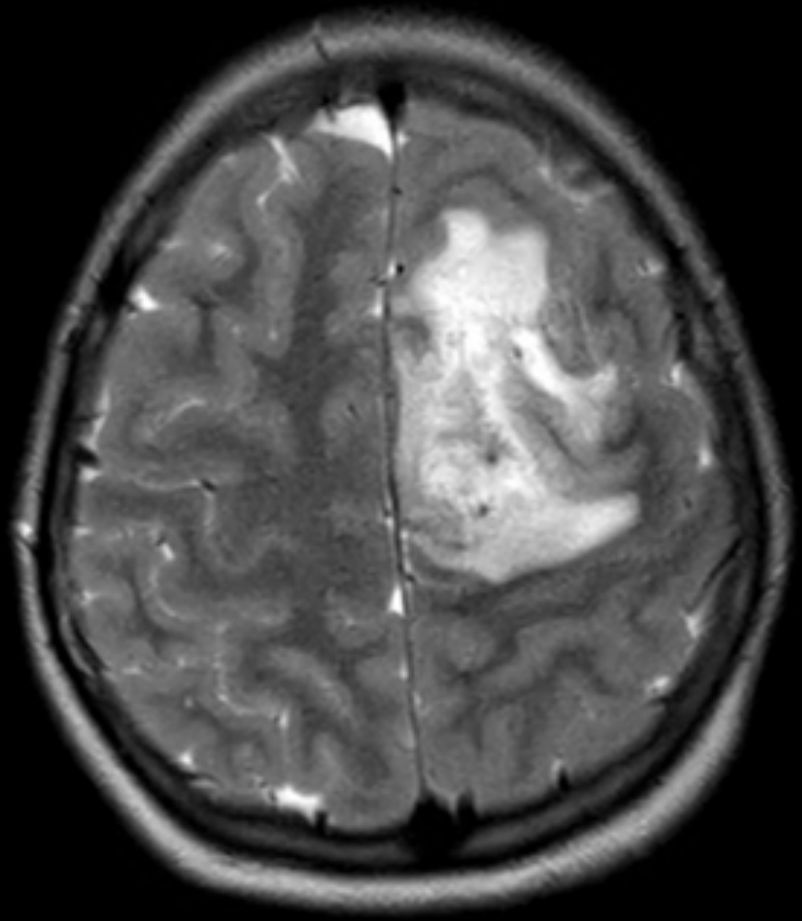
(Naccache, et al., 2016, manuscript in preparation)

mNGS Dx: Eosinophilic Meningitis from *Angiostrongylus cantonensis*, the rat lungworm





Laboratory confirmation by the US CDC

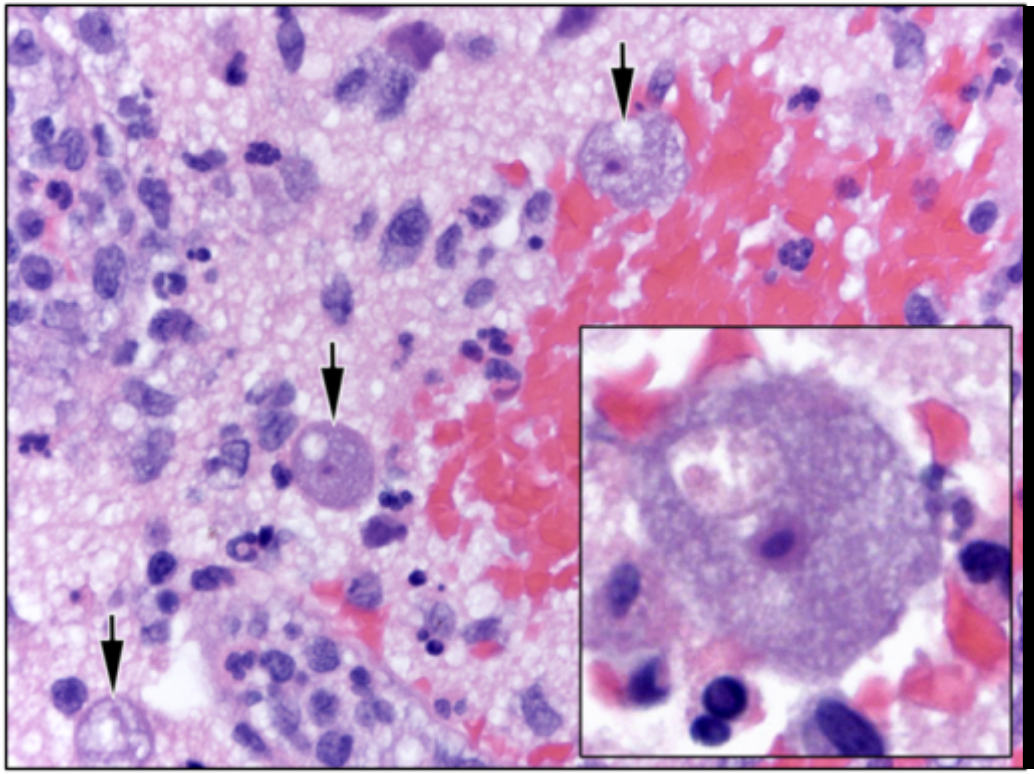


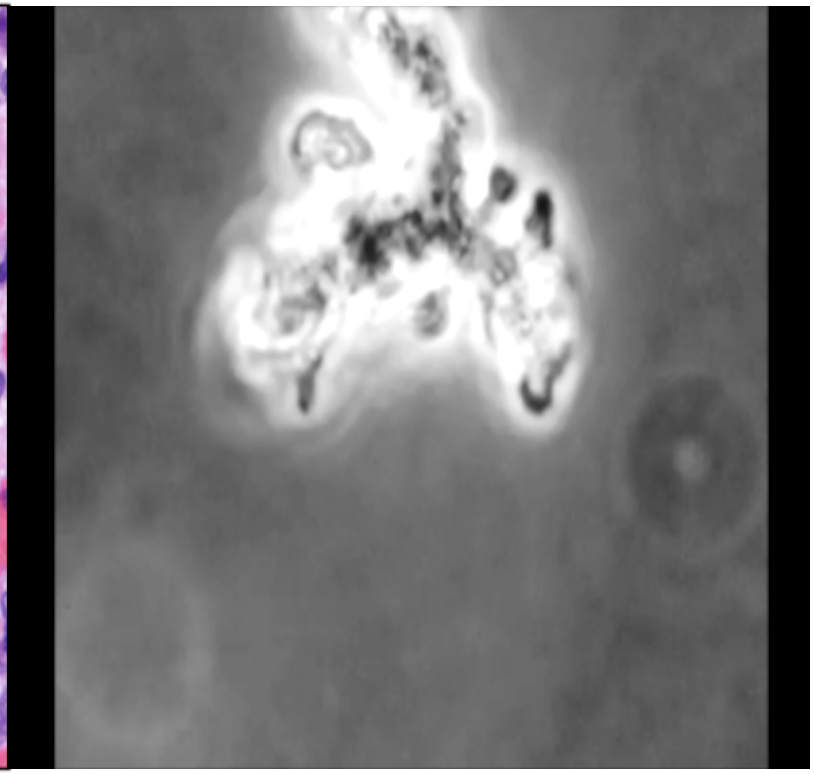
A Case of Hemorrhagic Encephalitis

- 15 year-old girl with type 1 diabetes
- 7 days of headache, vomiting, arm weakness, and confusion
- Elevated WBC count in CSF (pleocytosis); hemorrhagic frontal / occipital lobe brain lesions on MRI
- Extensive diagnostic testing negative
- "Classic" presentation for hemorrhagic form of ADEM → given IV steroids x 5 days

(Greninger, et al., 2015, *Genome* Medicine, 7:113)

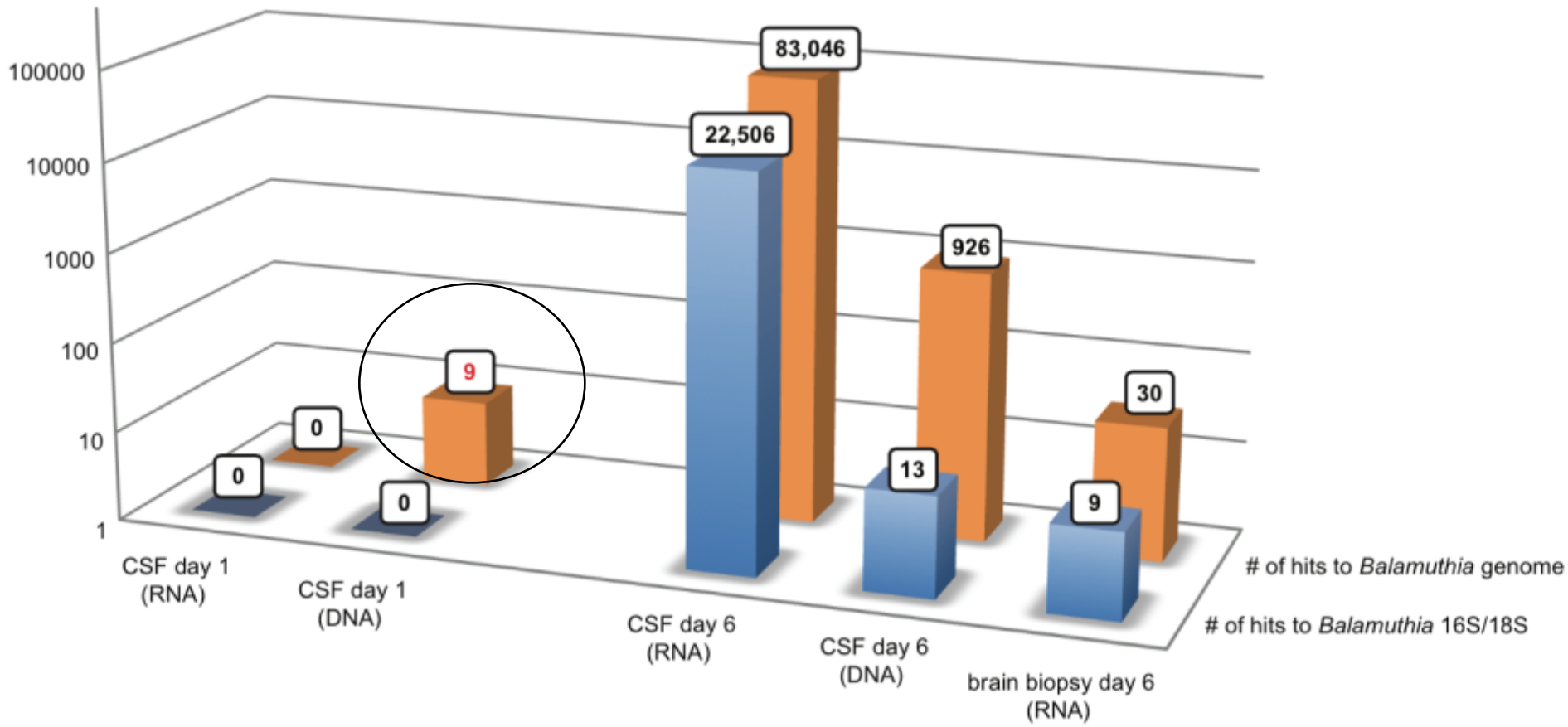
mNGS Dx: *Balamuthia mandrillaris* encephalitis





- By SURPI, 6,540 sequences out of 2,813,691 (0.23%) from *Balamuthia mandrillaris*
- Brain biopsy pathology from HD 6 → hemorrhagic necrotizing process with numerous amoebae
- Despite combination amoebicidal therapy, she developed intracranial hypertension, cardiac arrest, and died

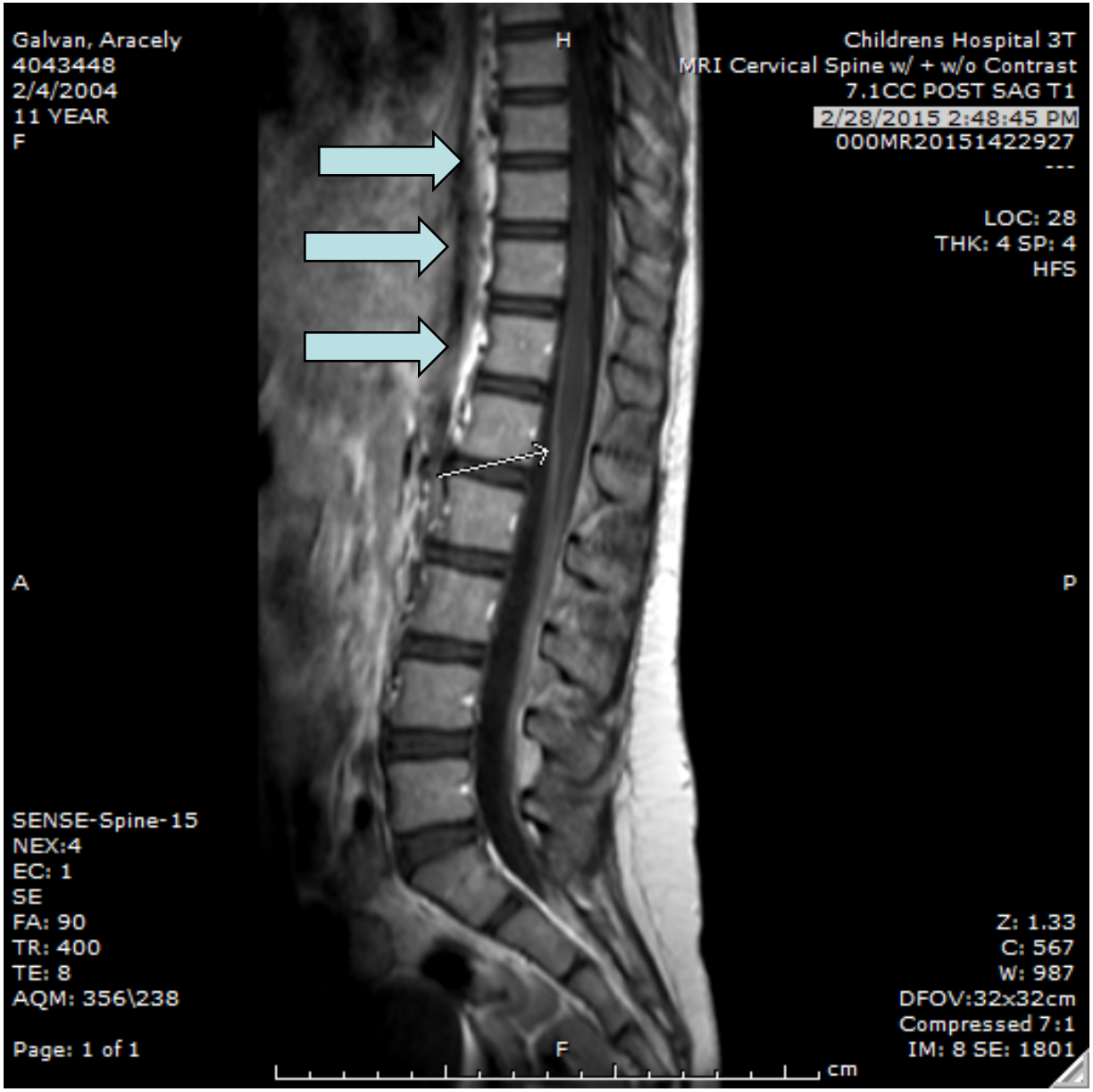
Could We Have Made the Diagnosis Earlier?



Completeness of Reference Databases is Critically Important!

A Case of Headache and Back Pain

- 11 y/o female from Mexico
- 1 month prior to admission developed headache, nausea, abnormal jerking movements of fingers, hands, and legs (polyminimyoclonus)
- Pets: dog and turtle. H/o *Salmonella* infection 3 months ago (fever but no diarrhea)
- Father and sister with remote history of pulmonary TB
- CSF: 183 WBC (100% mono), glucose 25, protein 270
- EBV and HHV-7 detected in CSF
- She was treated with acyclovir, then ganciclovir.
- 2 weeks after discharge, she developed back pain and worsening HA
- Started empirically on 4-drug TB therapy



MRI spine:

leptomeningeal enhancement along the conus medullaris involving the cauda equina without underlying signal abnormality of the spinal cord

”...these findings represent an infectious or inflammatory process...”

A Case of Headache and Back Pain

- Laboratory testing negative for enteroviral PCR, HSV PCR, HHV-6 PCR, bacterial cx, fungal stain, fungal culture, *M. Tb* PCR x 3, AFB smear, mycobacterial culture, PPD, IGRA, HIV, RPR, crypto Ag, histo urinary Ag, cysticercosis Ab, CSF cocci ID/CF, *Brucella* IgM EIA (IgG EIA positive), malignant cells
- Poor response to 4-drug therapy – concern for drug-resistant TB:
  - ethambutol was changed to ethionamide
  - levofloxacin added to RIPE
- She improved substantially on 5-drug TB therapy and was discharged on RIPE + levofloxacin
- 1 month later, re-admitted with back pain, fatigue, and episodes of shaking of her extremities
- Repeat testing of CSF negative – what to do now?

Before Normalization...

SPECIES	GENUS	FAMILY	NTC	POS	124_134	123_133
*	*	Enterobacteriaceae	246,369	21,342	2,350	52,116
Escherichia coli	Escherichia	Enterobacteriaceae	170,966	11,959	1,601	35,144
Propionibacterium acnes	Propionibacterium	Propionibacteriaceae	126,533	5,329	1,158	43,477
*	*	*	41,826	3,194	457	8,593
*	Brucella	Brucellaceae	0	0	251	0
Enterococcus faecium	Enterococcus	Enterococcaceae	526	96	85	26
Enterococcus casseliflavus	Enterococcus	Enterococcaceae	371	54	79	11
Pseudomonas sp. TKP	Pseudomonas	Pseudomonadaceae	941	205	69	56
*	Escherichia	Enterobacteriaceae	3,865	210	35	744
Pseudomonas stutzeri	Pseudomonas	Pseudomonadaceae	1,094	40	32	36

After Normalization...

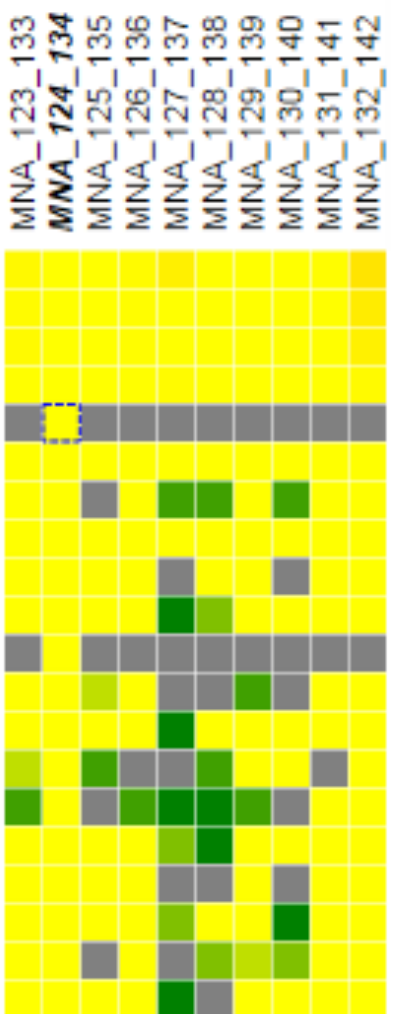
SPECIES	GENUS	FAMILY	NTC	POS	124_134	123_133
*	*	Enterobacteriaceae	1	0	0	0
Escherichia coli	Escherichia	Enterobacteriaceae	1	0	0	0
Propionibacterium acnes	Propionibacterium	Propionibacteriaceae	1	0	0	0
*	*	*	1	0	0	0
*	Brucella	Brucellaceae	0	0	16	
Enterococcus faecium	Enterococcus	Enterococcaceae	1	0	0	0
Enterococcus casseliflavus	Enterococcus	Enterococcaceae	1	0	0	0
Pseudomonas sp. TKP	Pseudomonas	Pseudomonadaceae	1	0	0	0
*	Escherichia	Enterobacteriaceae	1	0	0	0
Pseudomonas stutzeri	Pseudomonas	Pseudomonadaceae	1	0	0	0
Brucella melitensis	Brucella	Brucellaceae	0	0	2	
Xanthomonas campestris	Xanthomonas	Xanthomonadaceae	1	0	0	0
*	Burkholderia	Burkholderiaceae	1	0	0	0

Before Normalization...

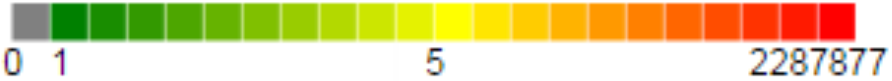
Accession: All

Prep: ☑ DNA ☐ RNA

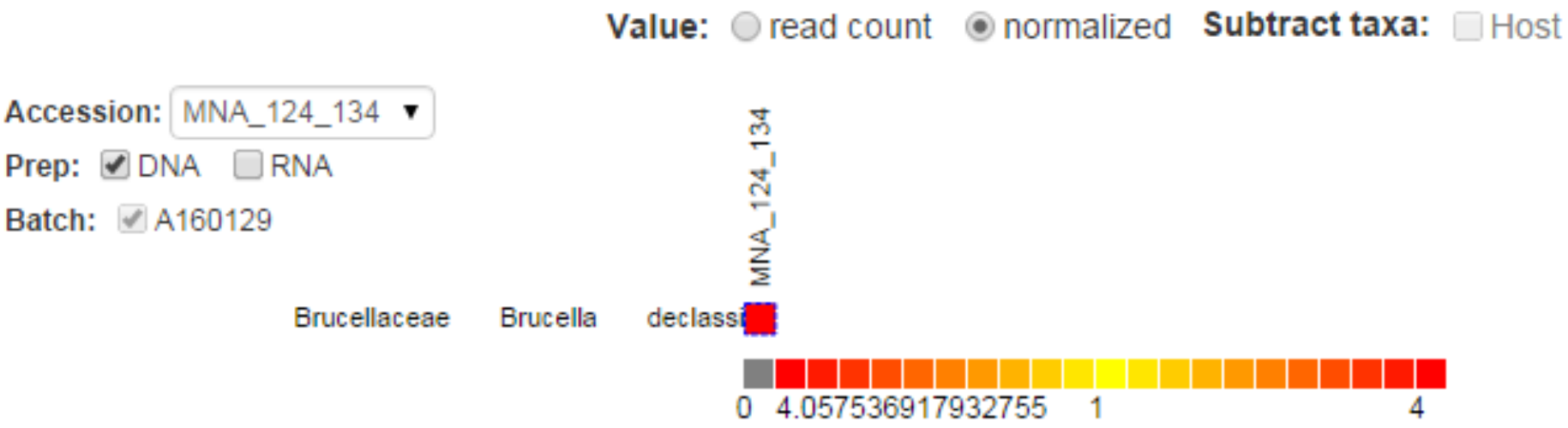
Batch: ☑ A160129



Enterobacteriaceae	declassified	declassified
Enterobacteriaceae	Escherichia	Escherichia coli
Propionibacteriaceae	Propionibacterium	Propionibacterium acnes
declassified	declassified	declassified
Brucellaceae	Brucella	declassified
Enterococcaceae	Enterococcus	Enterococcus faecium
Enterococcaceae	Enterococcus	Enterococcus casseliflavus
Pseudomonadaceae	Pseudomonas	Pseudomonas sp. TKP
Enterobacteriaceae	Escherichia	declassified
Pseudomonadaceae	Pseudomonas	Pseudomonas stutzeri
Brucellaceae	Brucella	Brucella melitensis
Xanthomonadaceae	Xanthomonas	Xanthomonas campestris
Burkholderiaceae	Burkholderia	declassified
Corynebacteriaceae	Corynebacterium	Corynebacterium glutamicum
Pasteurellaceae	Haemophilus	Haemophilus influenzae
Staphylococcaceae	Staphylococcus	Staphylococcus epidermidis
Streptococcaceae	Streptococcus	Streptococcus salivarius
Pseudomonadaceae	Pseudomonas	declassified
Burkholderiaceae	Burkholderia	Burkholderia cepacia
Propionibacteriaceae	Propionibacterium	declassified



After Normalization...



Brucellosis

- IgG ELISA (+)
- Brucellosis and TB
  - Overlapping clinical features
- A false-positive *Brucella* IgG ELISA in active TB patients was reported. Int J Gen Med (2011), 4:207-210
- A false-positive *Brucella* IgM EIA was reported. CDC recommends that all positive results obtained by rapid serologic assays be confirmed with *Brucella*-specific agglutination testing MMWR (2008), 57(22):603-605
- Spinal brucellosis has variable clinical picture; lumbar region most common with abscess, myelitis, nerve root contrast enhancement Int J Infect Dis (2008), 12(6):573-577

Brucella agglutinins 1:128 (borderline pos) → pt treated with doxycycline and rifampin x 6 weeks with complete response

UCSF University of California San Francisco

UCLA

Berkeley UNIVERSITY OF CALIFORNIA

UCDAVIS UNIVERSITY OF CALIFORNIA

CIAPM Project:
Precision Diagnosis of Acute Infectious Diseases

syapse

California Department of PublicHealth

Google Genomics

DNAnexus

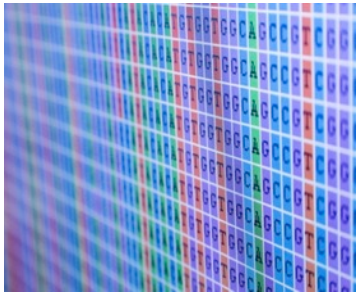
Quest Diagnostics®

Precision Diagnosis with Genomic Testing can Impact Clinical Decision-Making in Infectious Diseases

California Initiative to Advance Precision Medicine

CLIA Laboratory

Metagenomic NGS



Bioinformatics

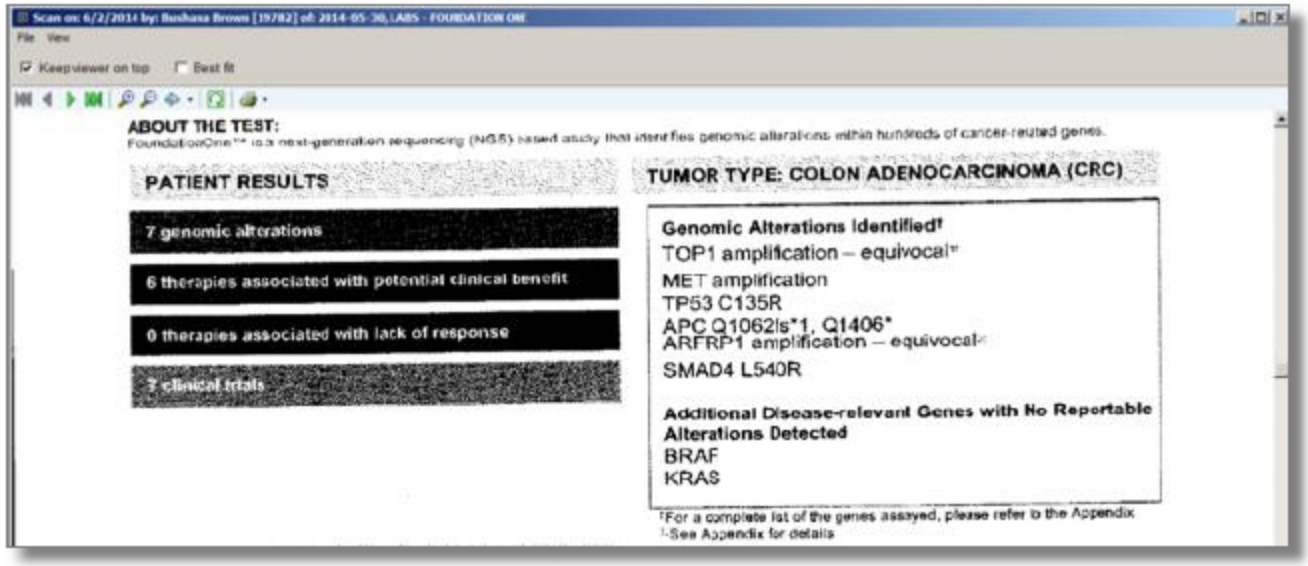
Agent ID

Cost-effective and actionable information for early treatment

Lower healthcare costs
Improved patient outcomes

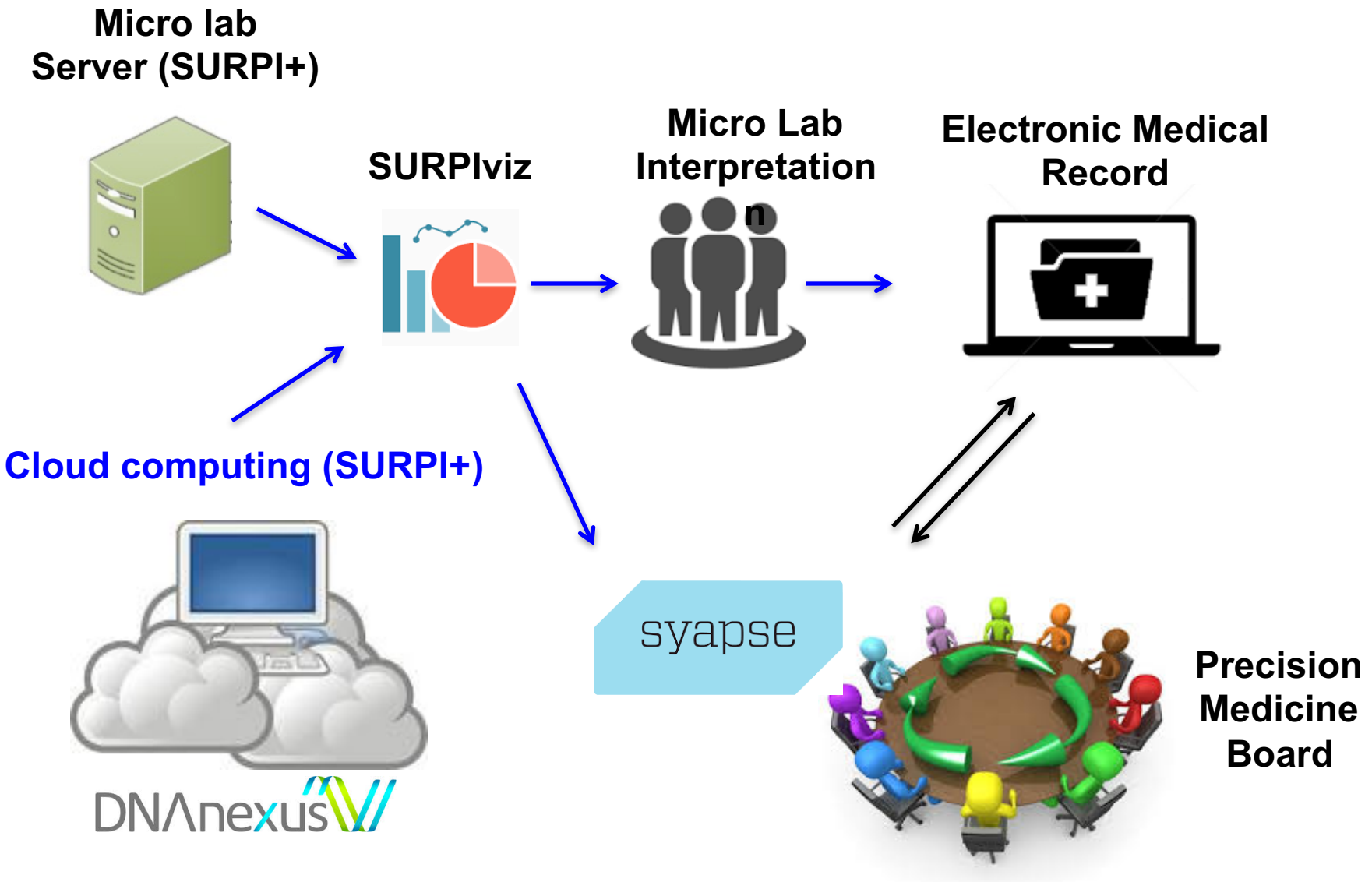
Turnaround time: hours – days (versus days – weeks)

How Laboratory Data Currently Gets into the EMR



This is the standard for data obtained from outside labs!

The Lab-Physician Interface



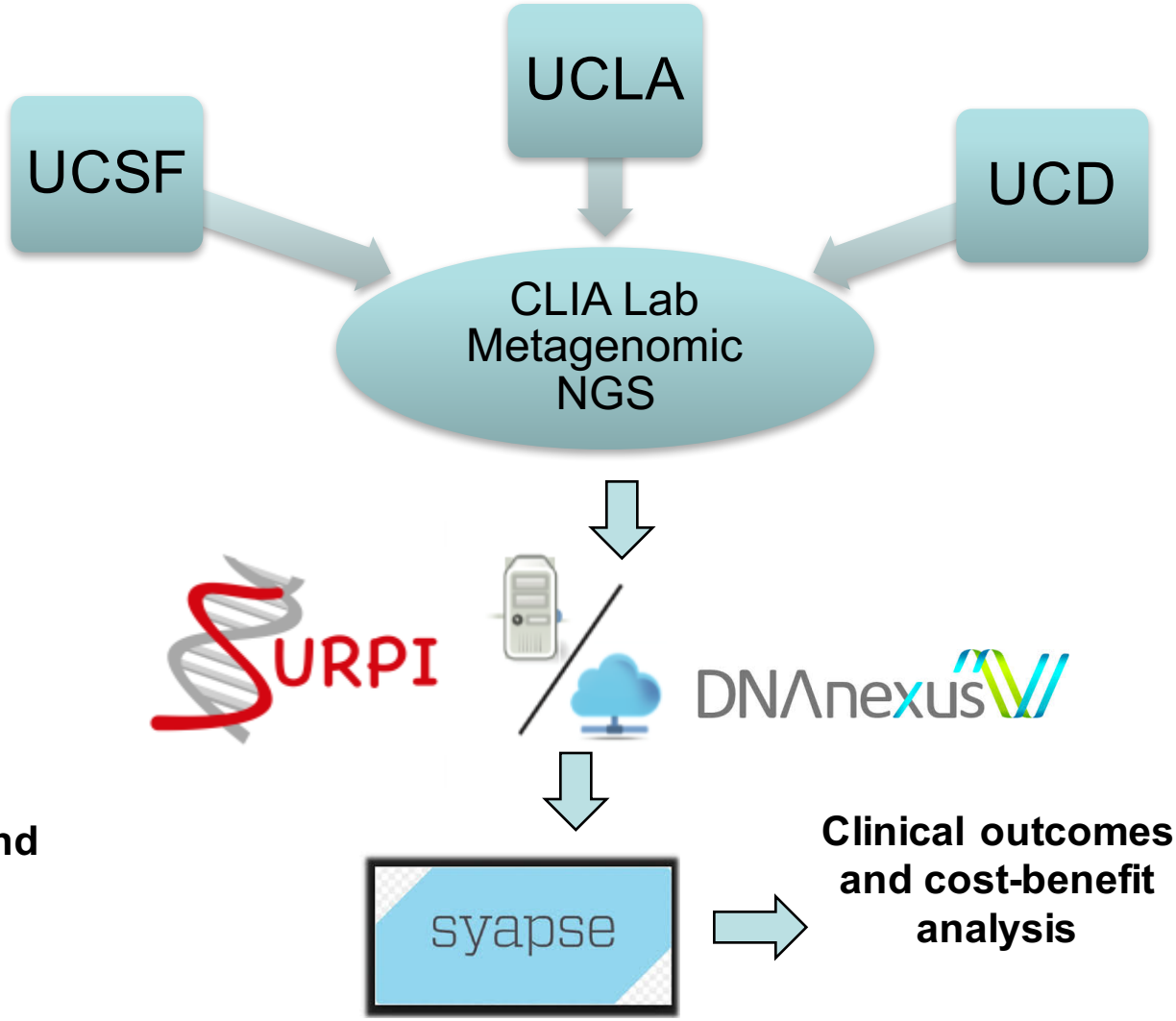
Prospective Clinical Study Comparing mNGS to Conventional Testing (n=300)

Patient enrollment (n=300) and sample / metadata collection

Running CLIA-validated NGS assay

Cloud-/server-based SURPI NGS bioinformatics analysis

Results reporting in EMR and visualization in Syapse interface



Clinical outcomes and cost-benefit analysis

Demo project will set the stage for future deployment in clinical labs

Projected Timeline

CLIA laboratory validation

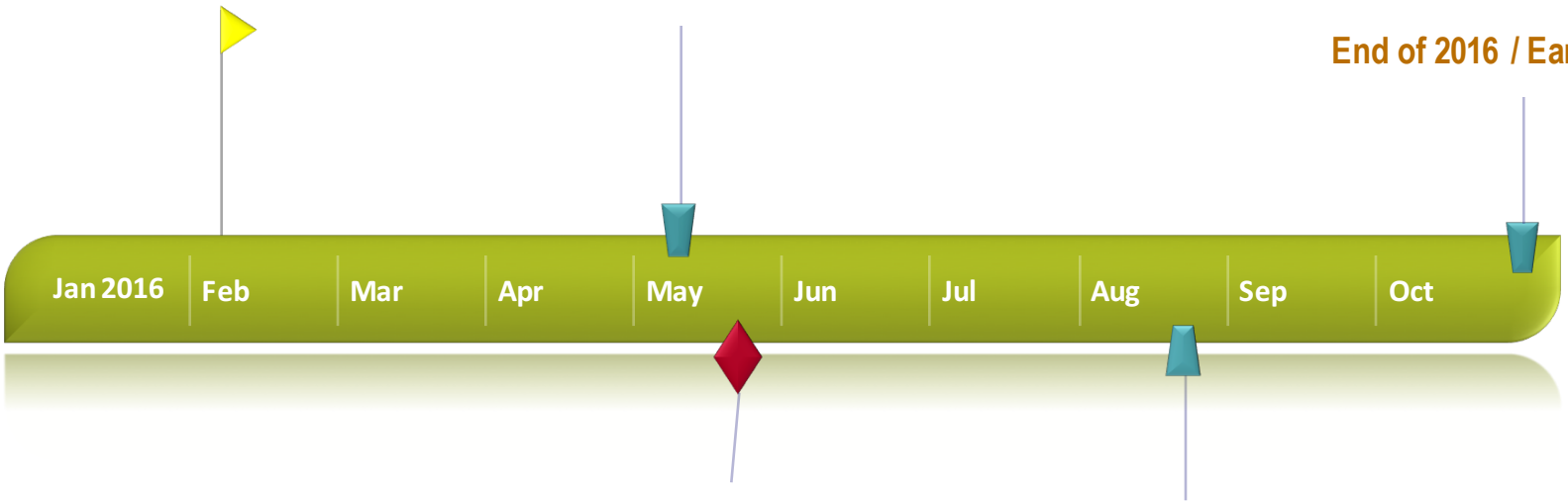
August 2015 - present

Clinical CSF mNGS assay launch

May, 2016

Clinical BAL mNGS assay launch (pneumonia)

End of 2016 / Early 2017



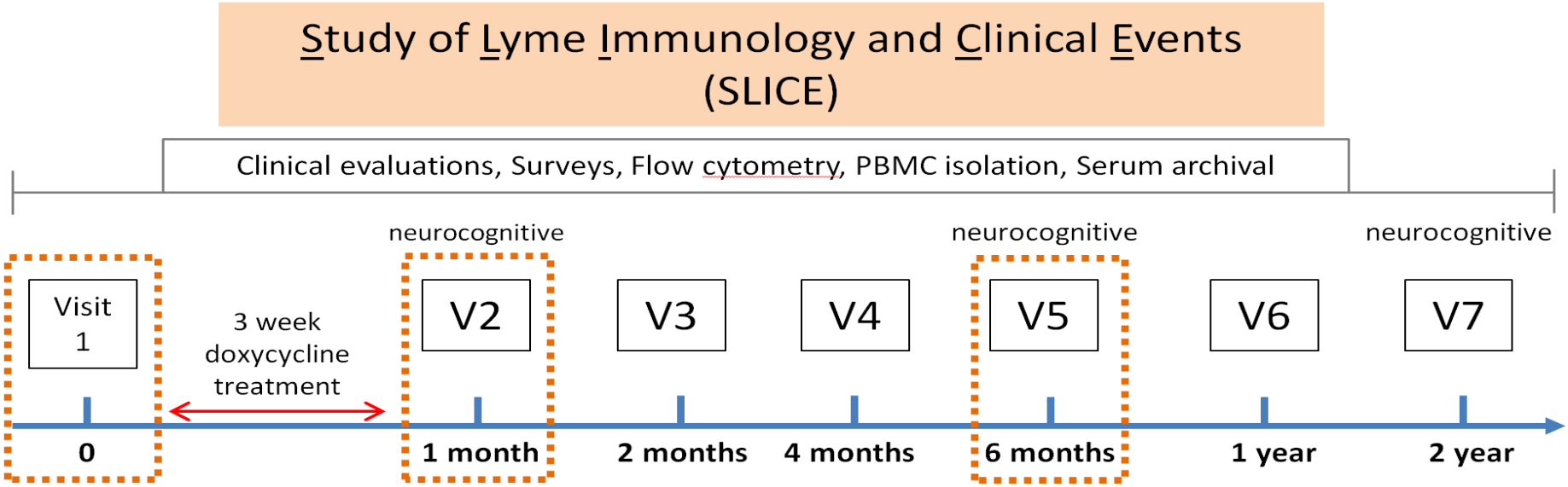
May, 2016

UC CIAPM prospective study launch

August, 2016

Clinical plasma mNGS assay launch (fever, sepsis)

Lyme Transcriptome Profiling
(Identification of Diagnostic Host Biomarkers for Lyme)

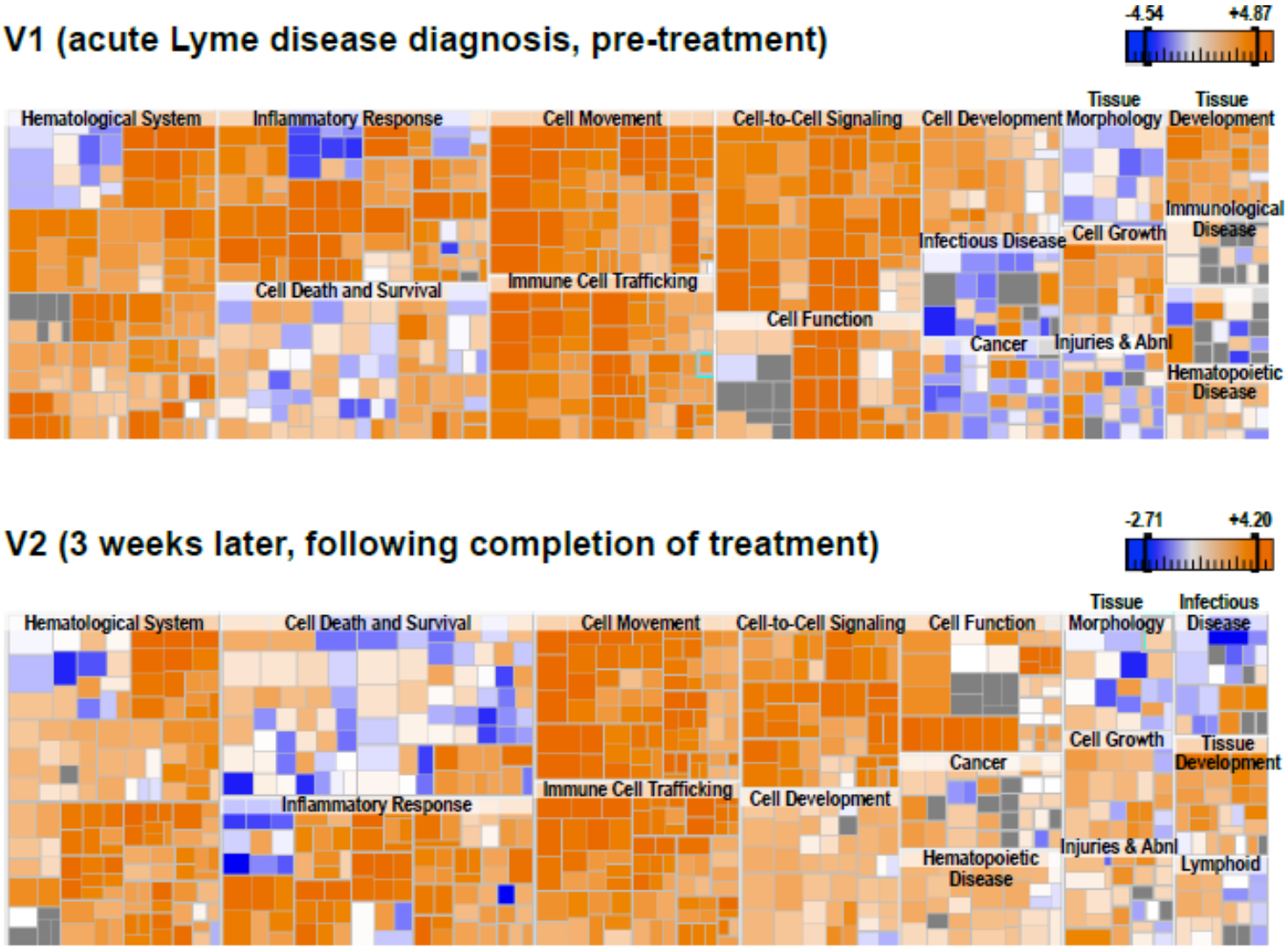


29 patients at 3 time points (V1, V2 and V5)

13 healthy controls

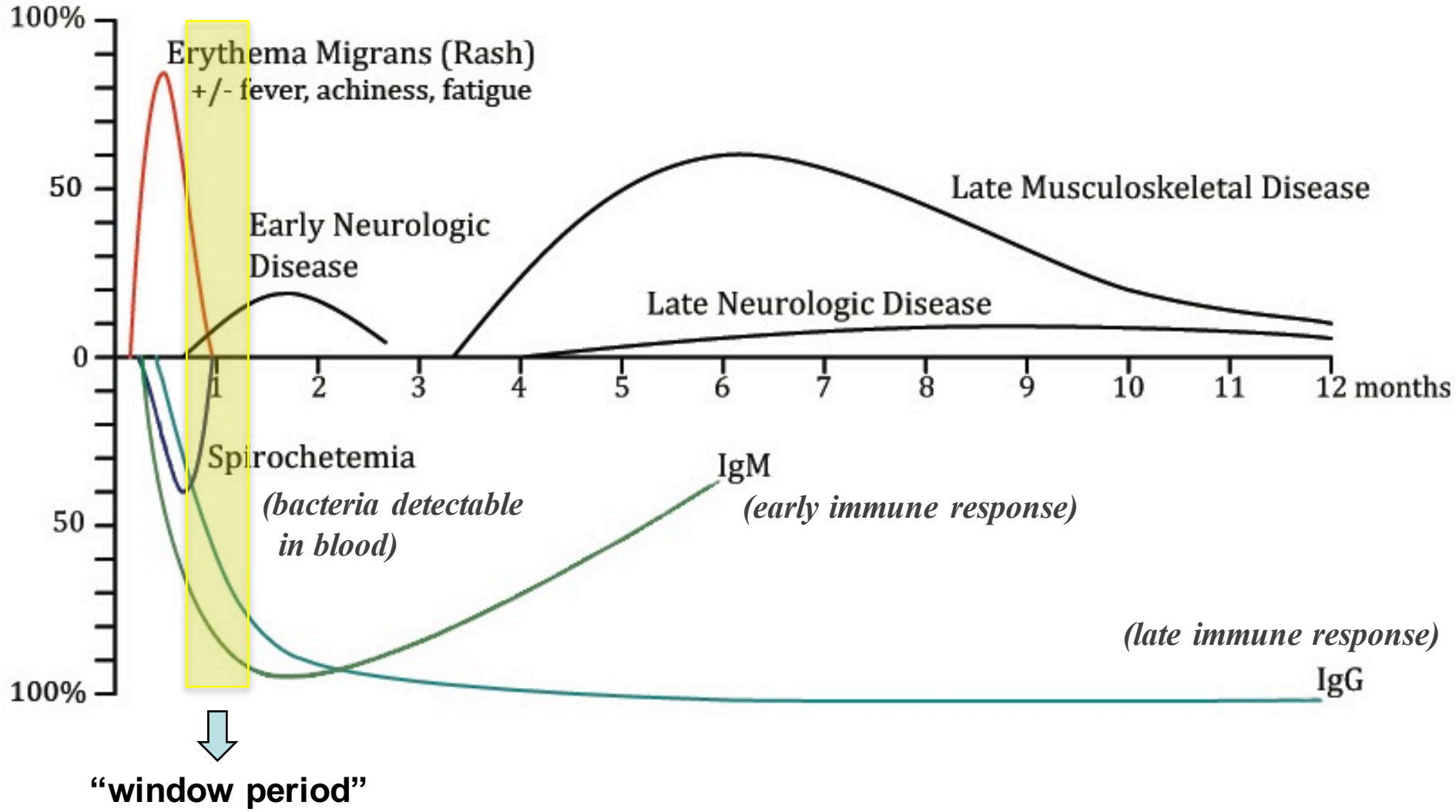
(Bouquet, et al., 2016, *mBio* 7(1):e00100-16)

Identification of a Host Biomarker Signature for Lyme Disease Diagnosis – From 30,000 to 60 Genes

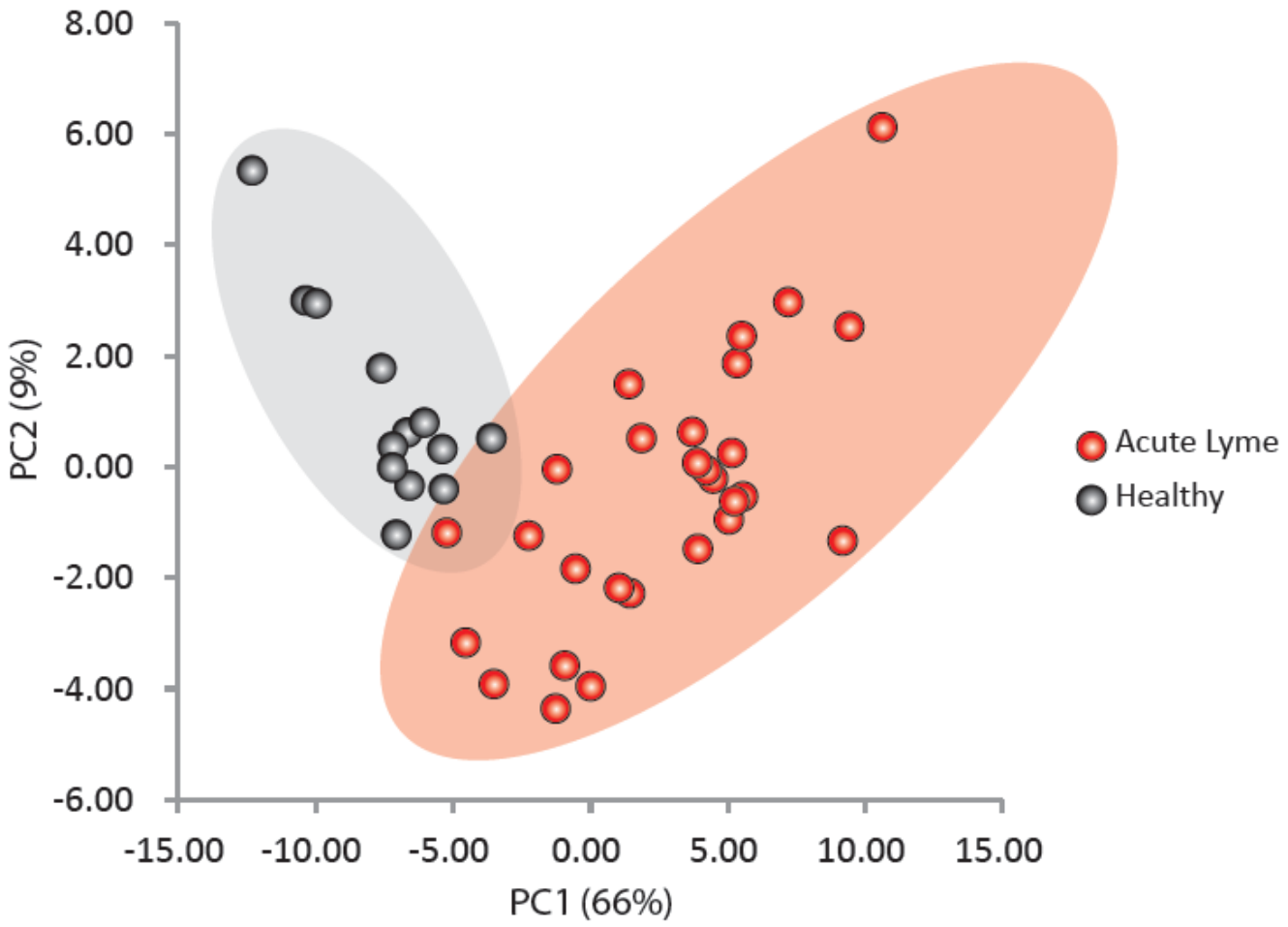


(Bouquet, et al., 2016, *mBio* 7(1):e00100-16)

Early Diagnosis and Treatment is Key to Preventing Later Complications



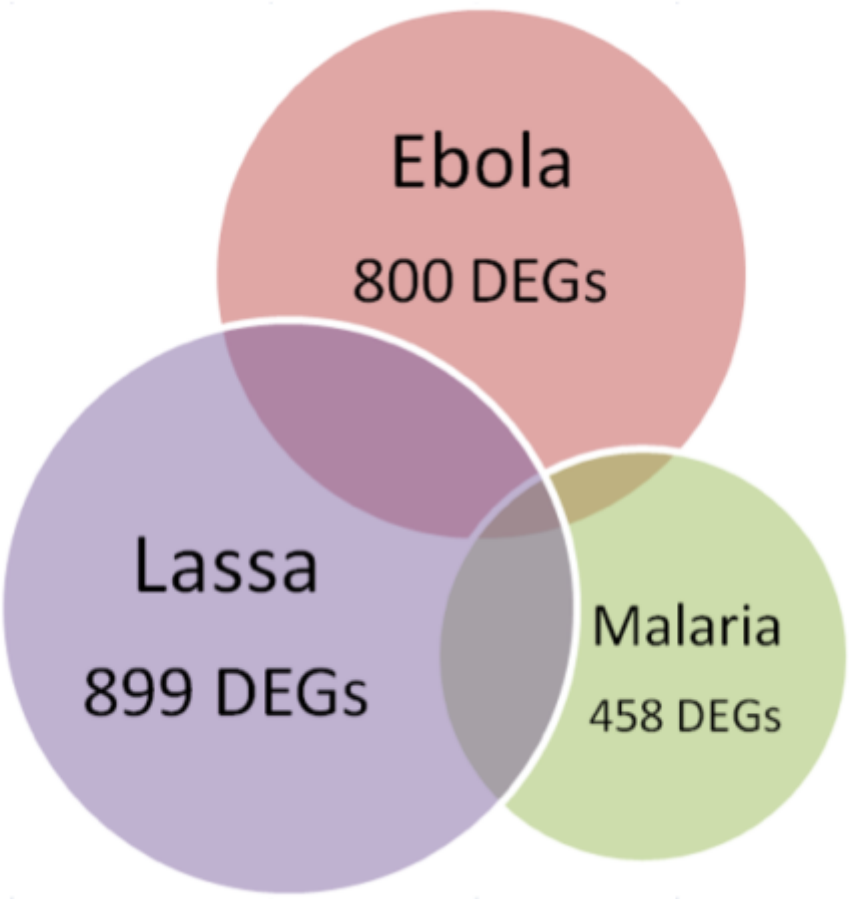
Diagnosis of Lyme Disease using Human Host Biomarkers



59 human transcripts → 96% sensitivity / 100% specificity in distinguishing Lyme patients from controls

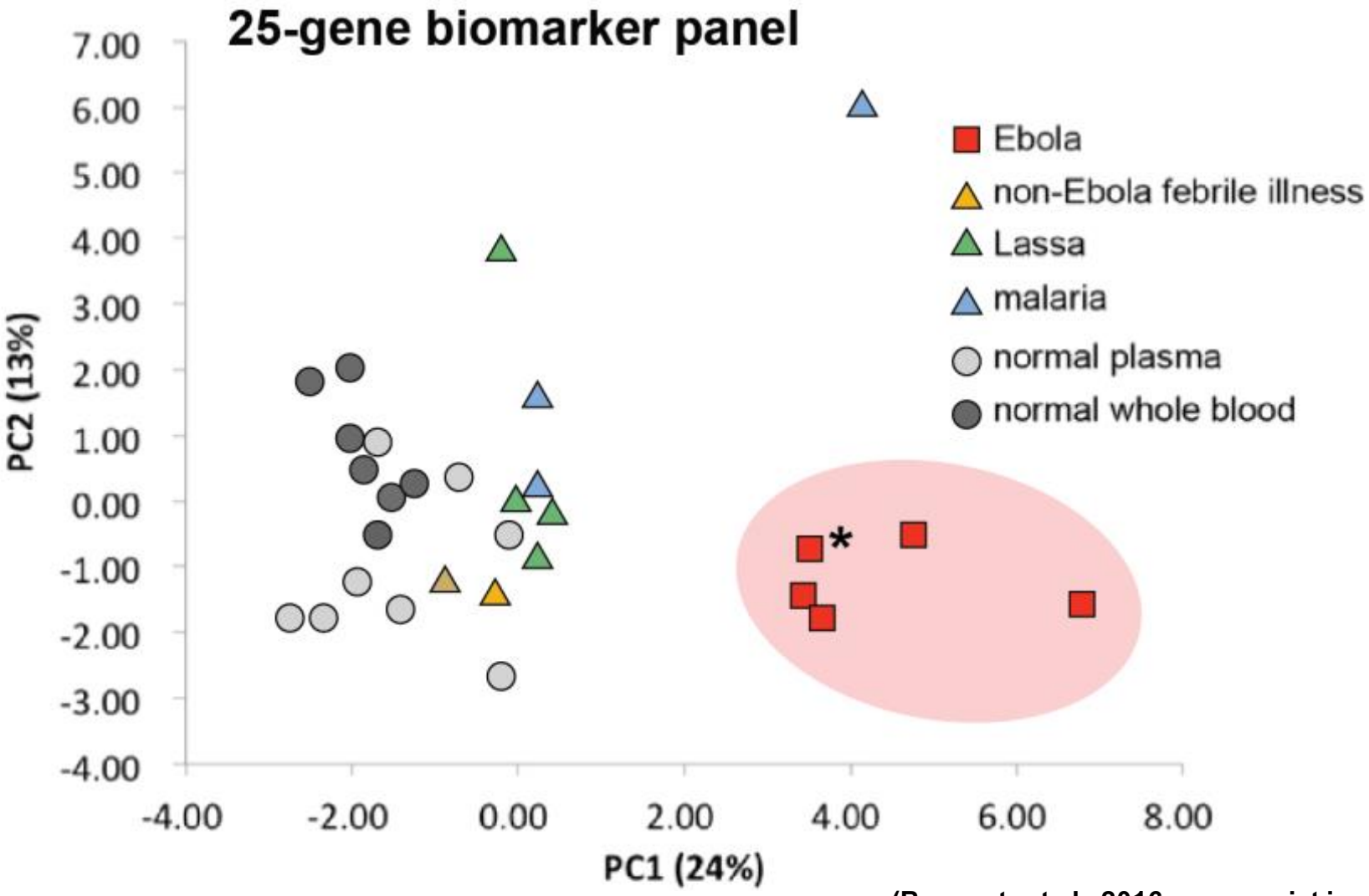
NGS-Based Human Transcriptome Profiling For Diagnosis of Ebola Virus Infection

Training set - 8 EBOV whole blood, 4 Lassa virus plasma, 3 malaria plasma, 8 healthy whole blood and 8 healthy plasma



(Bouquet, *et al.*, 2015, manuscript in preparation)

Differential Diagnosis of Acute Hemorrhagic Fever by Gene Expression Profiling



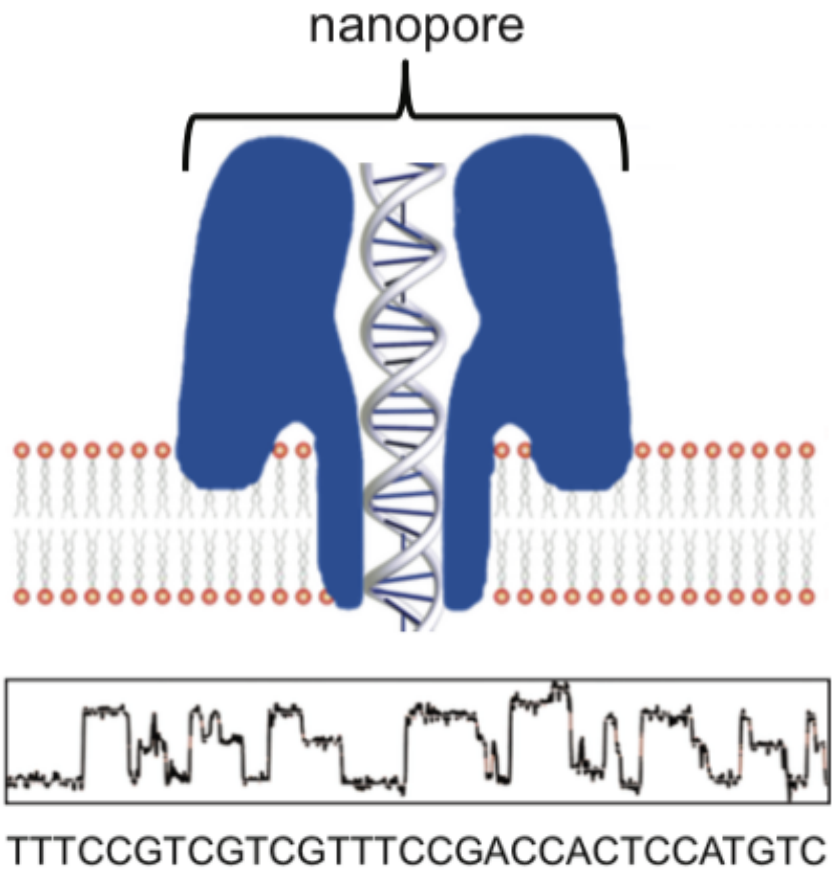
(Bouquet, et al., 2016, manuscript in preparation)

Febrile Outbreak Surveillance with Nanopore Sequencing

MinION (Oxford Nanopore Technologies)

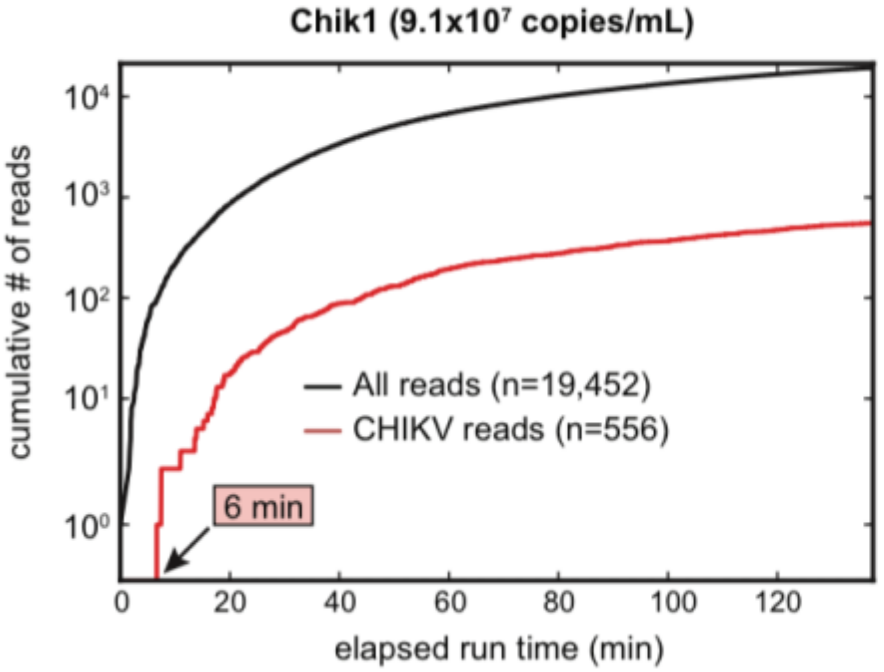
Nanopore Sequencing for Real-Time Pathogen Identification

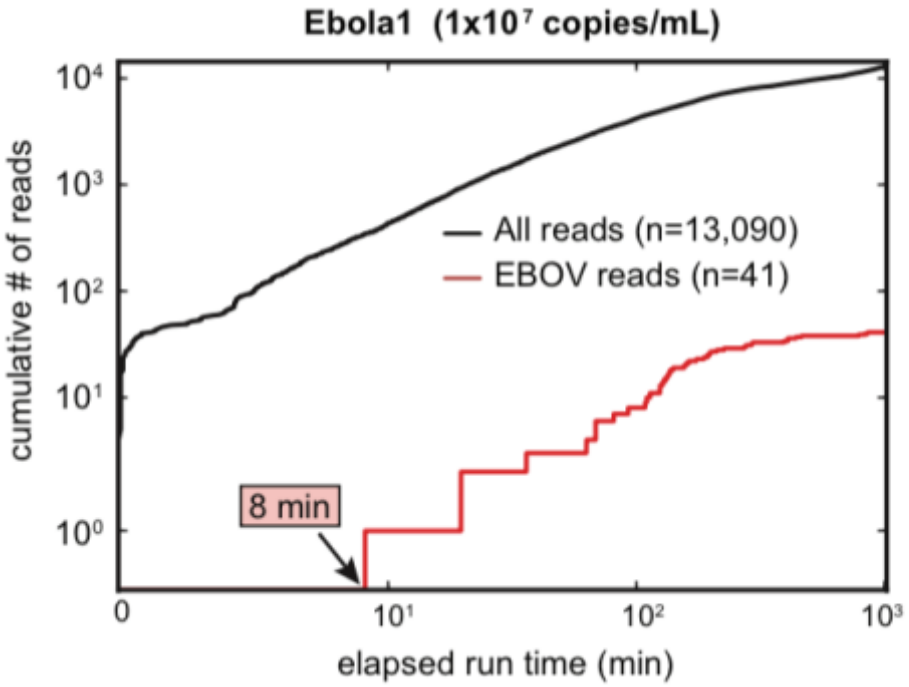
MinION (Oxford Nanopore Technologies)



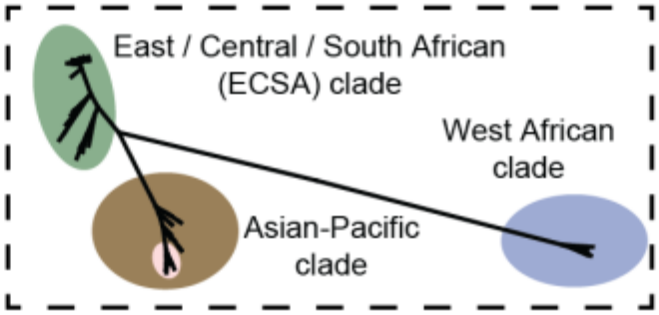
(Greninger, et al., 2015, *Genome Medicine* 7:99)

Viral Reads Detected <8 Min into Sequencing Run





Tracking Emergence of *Chikungunya virus* in Puerto Rico by Nanopore Sequencing



Indonesia [1985] (HM045797)
Indonesia (Bali) [2013] (KM623291)
Indonesia [2007] (FJ807897)
Micronesia (Yap State) [2013] (KJ451623)
British Virgin Islands [2014] (KJ451624)*
Martinique [2013]
St. Martin [2013] (H20235)
St. Martin [2013] (H20236)
Puerto Rico strain PR-S6 [2014]
(MiSeq)
(MinION)
100
100
100
100

(Chiu, et al., 2015, *Emerging Infectious Diseases;* Greninger, et al., 2015, *Genome Medicine*)

mNGS surveillance of Zika virus

Sample #	# Raw reads	# Zika reads	mNGS	PCR
1	1,486,130	63	+	+
2	1,449,221	87	+	+
3	11,546	2	+	+
4	1,321,572	9	+	+
5	1,114,194	24	+	+
6	1,179,486	11	+	ND
7	6,903,397	419,115	+	+
*8	1,094,355	83	+	+
9	743,266	1,072	+	+
10	1,031,160	16	+	ND
11	933,134	125	+	+
12	1,727,181	33	+	+
13	1,180,561	2	+	ND
14	1,744,295	0	ND	+
15	1,353,875	11	+	ND

*co-infection with chikungunya virus

(Sardi, et al., 2016, submitted)

Summary

- Emerging genomic technologies, especially metagenomic next-generation sequencing, are promising for precision diagnosis of infectious diseases

- New bioinformatics pipelines such as SURPI greatly improve computational turnaround times and make data analysis accessible to clinical and public health laboratories

- Many regulatory and technical challenges remain, but next-generation sequencing technologies have evolved to where we can now implement them in clinical medicine and public health

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