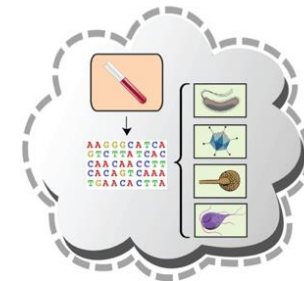




University of California
San Francisco



Metagenomic Sequencing Approaches for Diagnosis and Genomic Characterization of Blood-borne Infections

Charles Chiu, MD / PhD

Associate Professor, Department of Laboratory Medicine and Medicine / Infectious Diseases

Director, UCSF-Abbott Viral Diagnostics and Discovery Center

Associate Director, UCSF Clinical Microbiology Laboratory

University of California, San Francisco

Surveillance and Screening of Blood-Borne Pathogens

2018 IPFA /PEI 25th International Workshop

Major Diagnostic Challenges in Infectious Diseases



Pneumonia

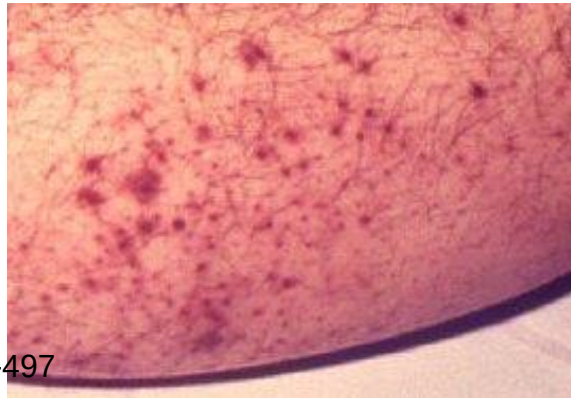
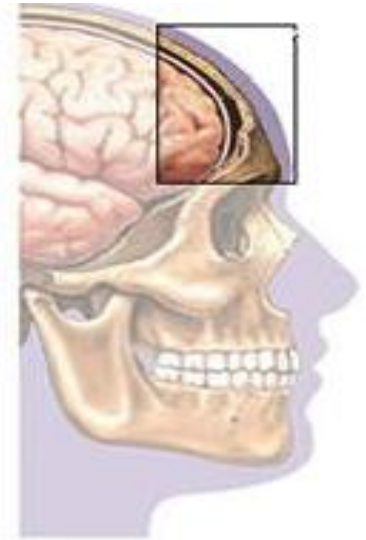
15 – 62% unknown cause

- Van Gageldonk-Lafeber, (2005) *CID* 41:490-497
- Louie, et al., (2005) *CID* 41:822-828
- Ewig, et al. (2002) *Eur Respir J* 20:1254-1262
- Jain, et al., (2015) *NEJM* 373(5)

Meningitis / Encephalitis

40 – 60% unknown cause

- Glaser, et al., (2006) *CID* 43:1565-1577
- Vora, et al., (2010) *Neurology* 82:443-451



Fever / Sepsis

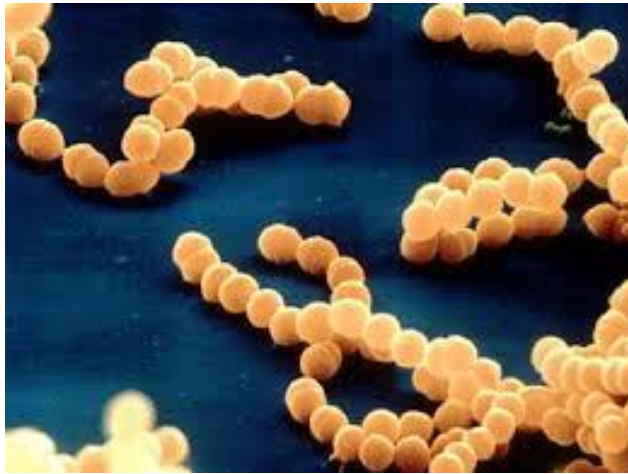
~20% unknown cause

- Eber, et al. (2010) *Arch Intern Med* 170:347-353
- Vincent, et al. (2015) *Critical Care Med* 43(11).

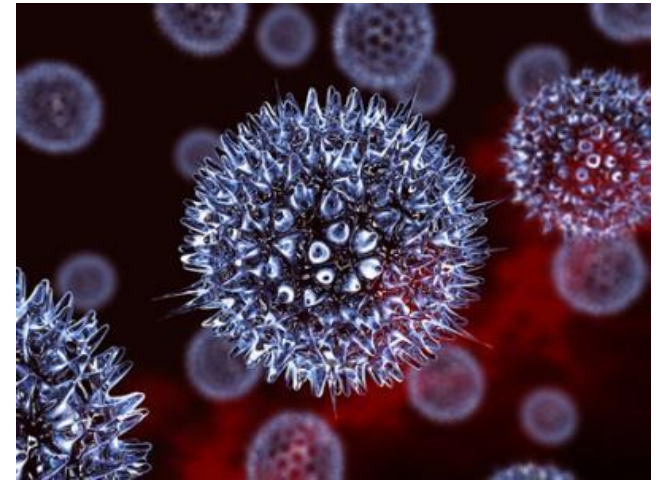
Failure to obtain a timely diagnosis leads to delayed / inappropriate therapy, increased mortality, and excess healthcare costs

All Microbes can be Uniquely Identified by mNGS

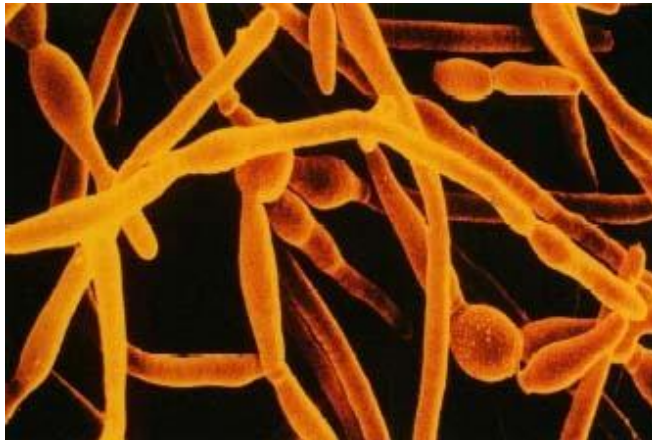
Bacteria



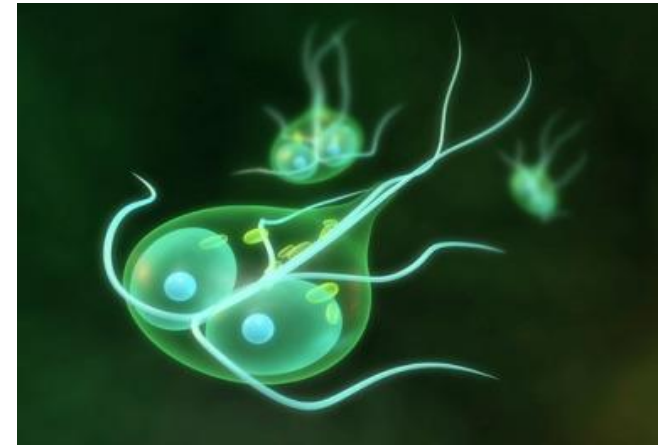
Viruses



Fungi

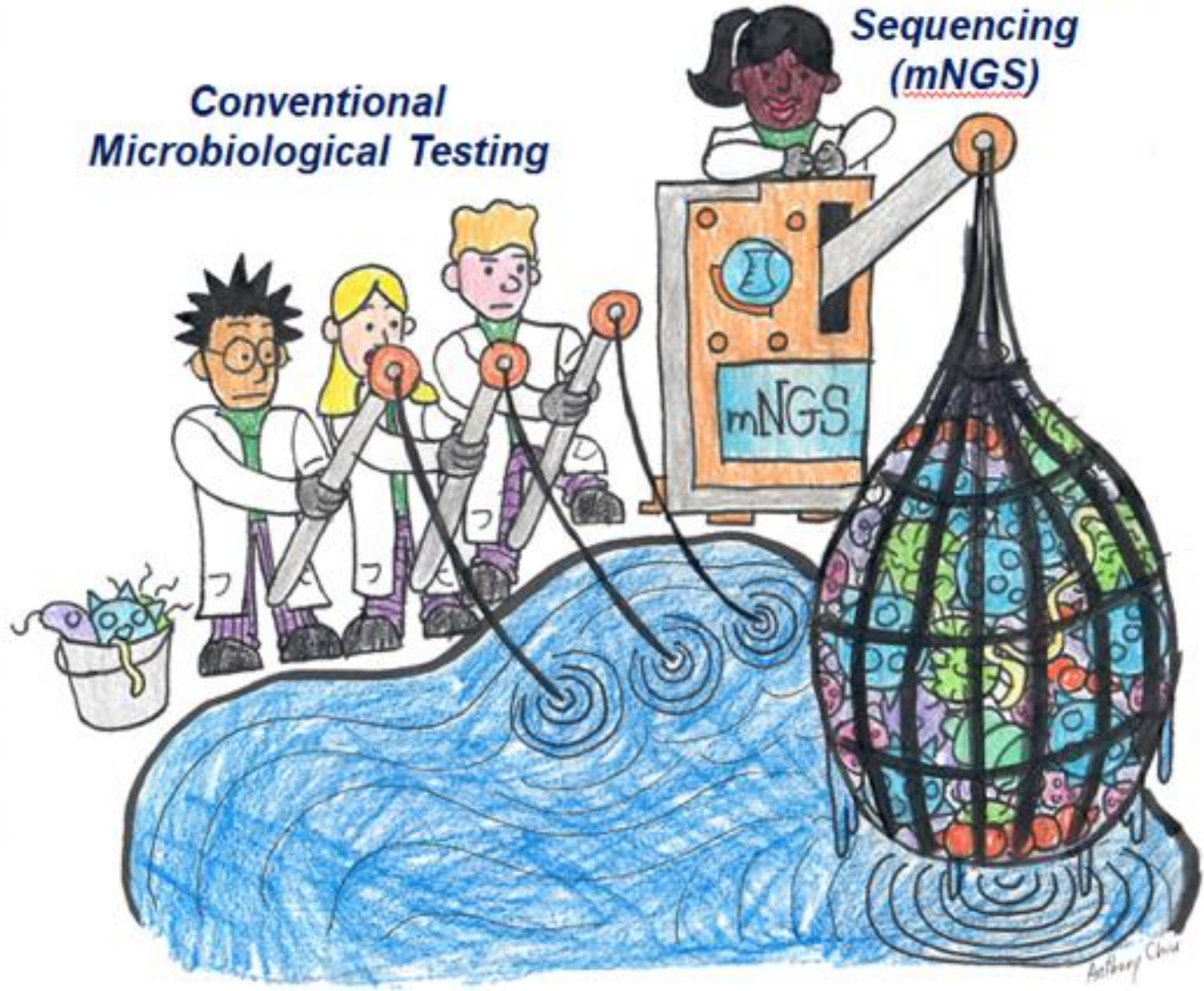


Parasites



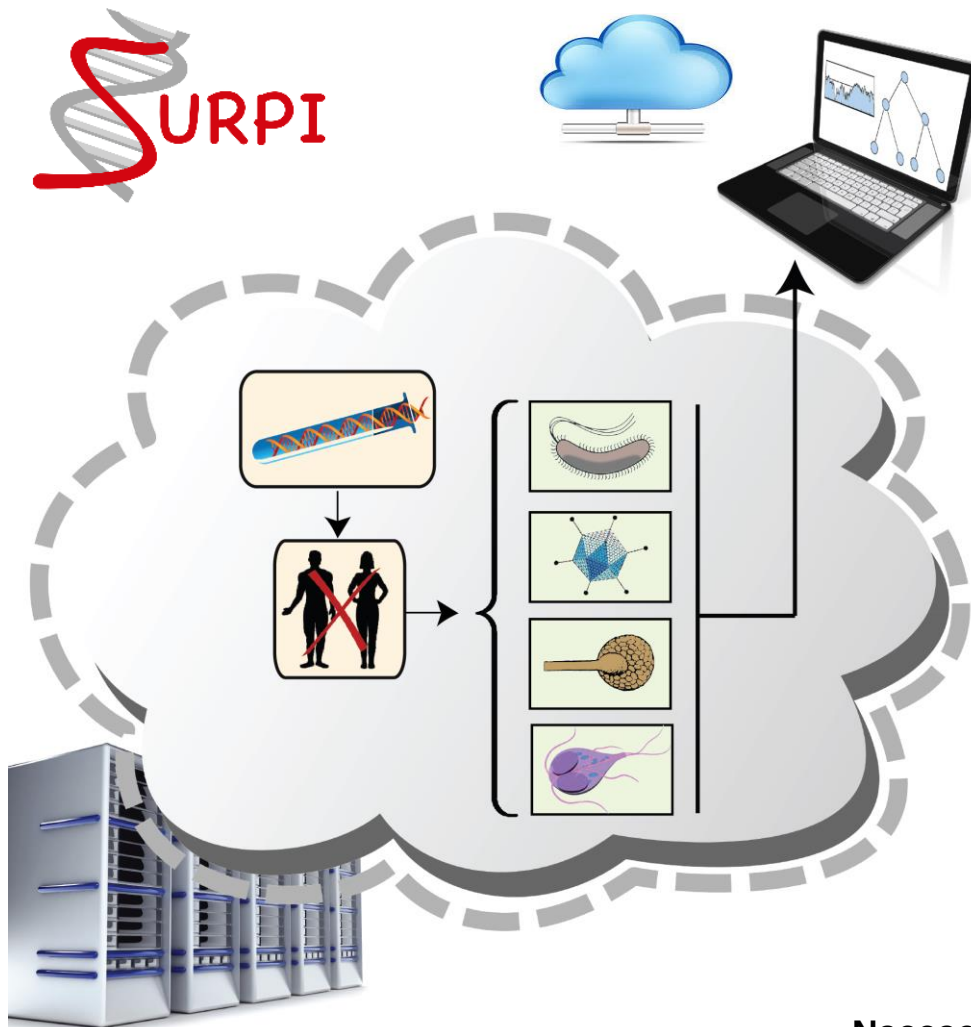
**Conventional
Microbiological Testing**

**Metagenomic
Next-Generation
Sequencing
(mNGS)**



The SURPI Bioinformatics Pipeline

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



- *Directly addresses computational analysis bottleneck*
- *SURPI+ (clinical version) – automated analysis*



Precision Diagnosis of a Mysterious Infection

3 hospitalizations over 4
months

44 days in the ICU

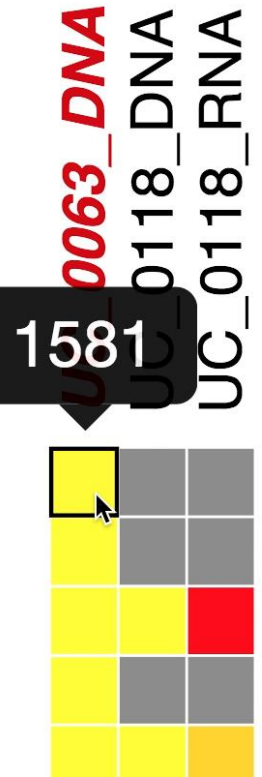
>100 inconclusive tests

Brain biopsy and induced
coma

(Wilson, et al., 2014, *New England Journal of Medicine*;
photo courtesy of the Osborn family)

Leptospira santarosai

Leptospira borgpetersenii
unclassified
Leptospira interrogans
Propionibacterium acnes



The Precision Diagnosis of Acute Infectious Diseases (PDAID) Study

Meningitis/Encephalitis



7 hospitals in CA and nationwide
Enroll/consent patients
204 total completed study
CSF collected
Clinical chart review

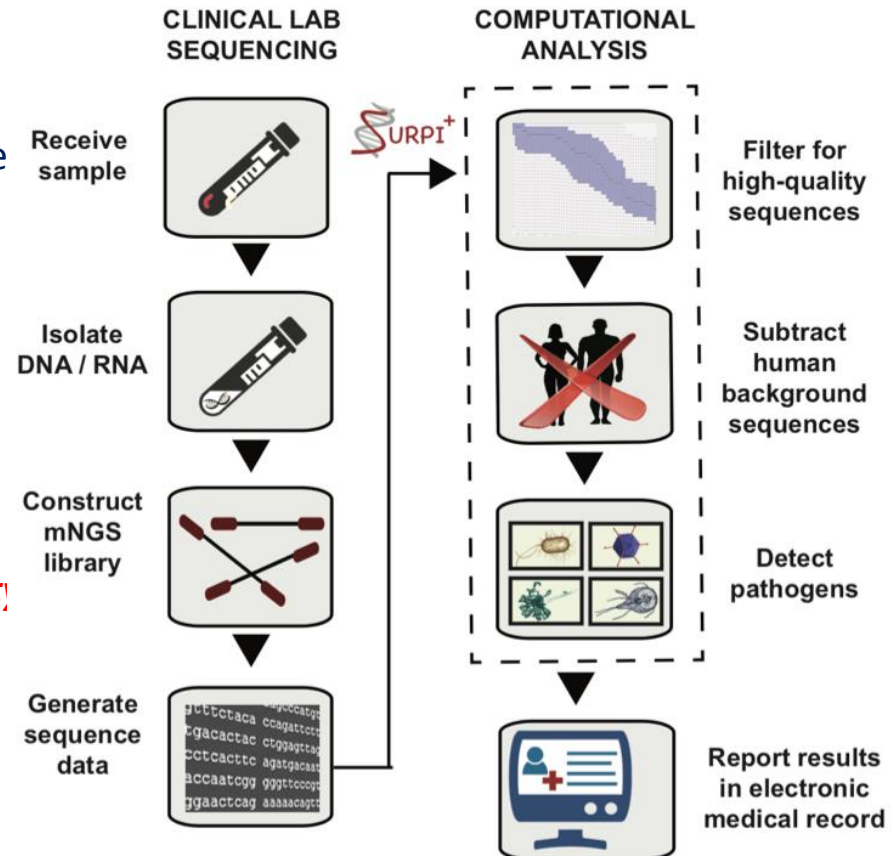
mNGS assay validated in CLIA lab
86% analytic sensitivity, 98% specificity

Clinical report in
patient EMR

APeX



Clinical microbial
sequencing board



Miller, et al., 2018 (manuscript under review);
Wilson, et al., 2018 (manuscript under review)

58 y/o immunosuppressed woman with fever, headache, nausea/vomiting

- History of idiopathic pulmonary fibrosis status post bilateral lung transplant in 2011, multiple sclerosis, on chronic immunosuppression
- Admitted to hospital in October 2016 with 8 days of fever, headache (“worst in my life”), nausea/vomiting, neck stiffness, and photophobia
- Neurological symptoms: admitted to “5 years” of word-finding difficulty and slurred speech, 1 year of dizziness / falls, and 1 month of leg weakness; also had first-time seizure in March 2016
- Resident of Orange County; no sick contacts; travel to mountains in Utah in August 2016, Caribbean in 2010, and throughout Europe decades ago
- Fever to 38.3°C, pancytopenic, transaminitis (negative for hepatitis A,B, and C); MRI – white matter intensities related to MS
- Started on empiric antimicrobials: IV vancomycin, ceftazidime, acyclovir, and voriconazole

- Lumbar puncture done, showing a lymphocytic pleocytosis

- WBC 10, 88% lymphocytes, protein 29, glucose 48

Microbiology (non-HEV)

Blood

CMV DNA quantitative PCR negative
 Cryptococcal Ag negative
 EBV PCR detected <10
 HSV-1/2 PCR negative
 Fungal culture negative
 Bacterial culture negative
Toxoplasma gondii DNA PCR negative
 MTB Quantiferon-Gold assay negative
 Adenovirus DNA PCR negative
 Parvovirus B19 DNA PCR negative
 West Nile IgG / IgM negative
 Rickettsia RMSF and typhus IgG / IgM negative
 Varicella zoster DNA PCR negative
Coccidioides IgG and IgM EIA negative
 Hepatitis A Ab total negative
 Hepatitis A Ab IgM negative

CSF

Cryptococcal Ag negative
 Enterovirus PCR negative
 Fungal culture negative
 Bacterial Gram stain and culture negative
 VZV PCR negative
 HSV-1/2 PCR negative
 CMV DNA quantitative PCR negative

Nasopharyngeal swab

Respiratory Virus Panel (RVP) PCR negative

MRI brain: Stable periventricular and subcortical and juxtacortical T2/FLAIR white matter intensities w/T1 hypointensity

Abdominal ultrasound:

Normal liver size, homogeneous in echogenicity.
 Normal spleen size. No ascites. Normally distended gallbladder containing sludge, without stones.

Imaging

Blood

Hepatitis A IgM negative
 Hepatitis B core IgM negative
 Hepatitis B surface antigen negative
 Hepatitis C Antibody negative
 HBV DNA PCR negative
 Cryptococcal antigen negative
 HIV 4th generation Ag/Ab negative
 JCV Ab 2.31 [>0.40 positive]
 RPR negative
 Aspergillus antigen EIA negative
Coccidioides IgG/IgM EIA negative
Toxoplasma gondii DNA PCR negative

Peritoneal fluid

Bacterial gram stain and culture negative

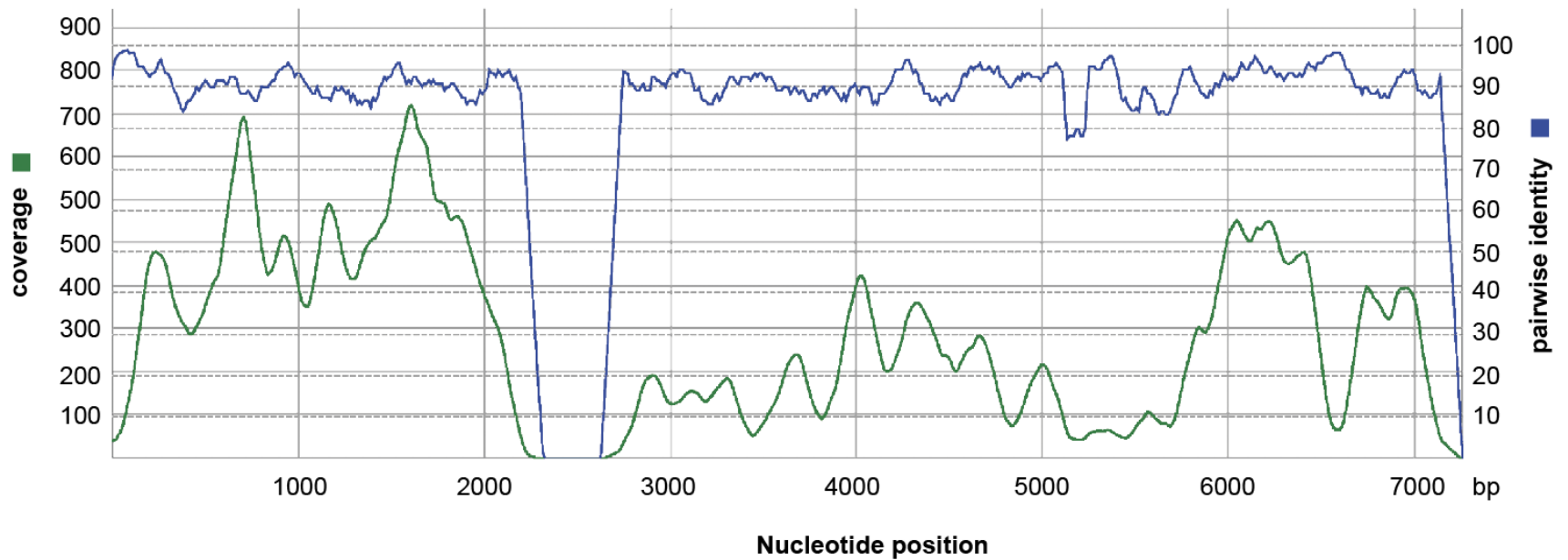
MRI brain: Unchanged

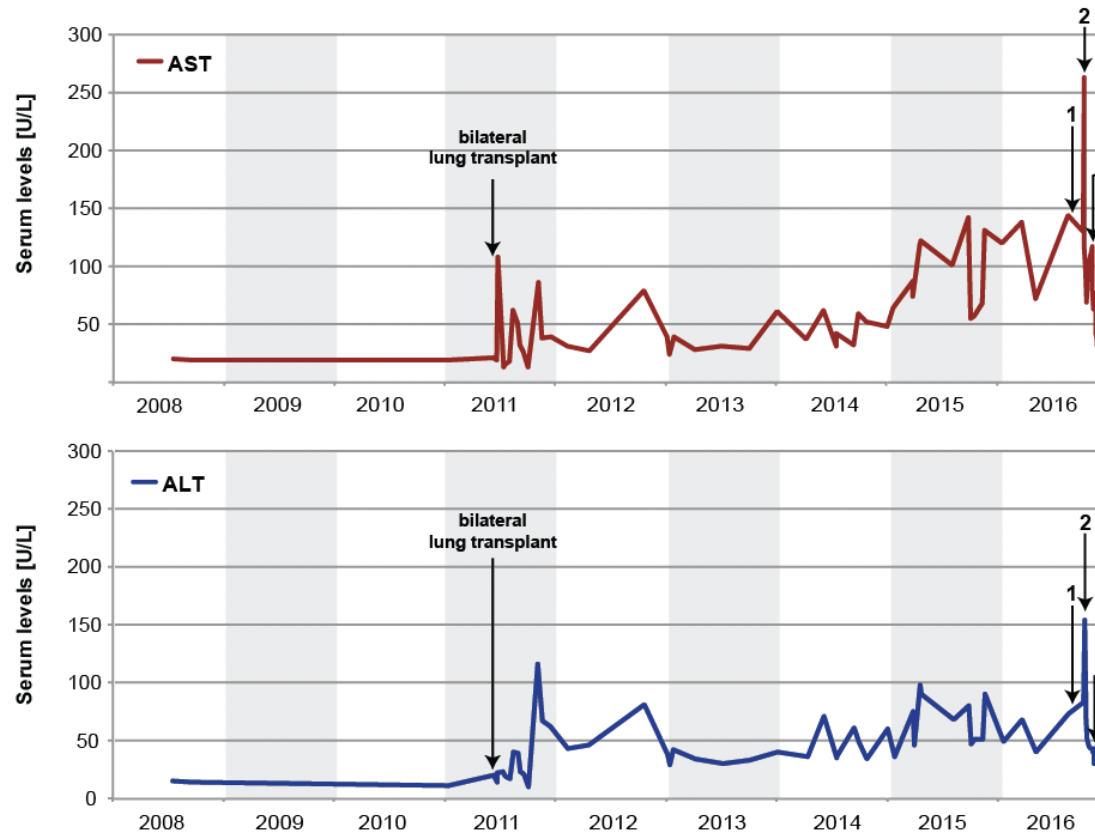
Abdominal ultrasound: Cirrhotic appearing liver without focal lesion, patent hepatic vessels, small volume ascites. Spleen upper normal in size.

Liver ultrasound elastography: shear wave liver stiffness 2.1m/sec consistent with METAVIR score of F3-F4

Hepatitis E virus

mapped to GenBank AB089824, 7,262 bp (Hepatitis E virus genomic RNA, complete genome, isolate: HE-JA10)
assembly 930% complete, 90.2% average pairwise identity





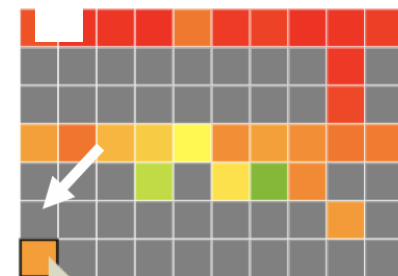
- Patient treated with ribavirin and is clinically improved
- This is a likely case of donor-transmitted HEV (positive anti-HEV antibody testing of donor's serum)

15 y/o child from Massachusetts with meningoencephalitis

Lentivirus	Human immunodeficiency virus 1
Pegivirus	GB virus C
Gammaretrovirus	Murine leukemia virus
Molluscipoxvirus	Molluscum contagiosum virus
Lentivirus	*
Flavivirus	Powassan virus
Varicellovirus	Human herpesvirus 3
Gammaretrovirus	*
-	Caribou feces-associated gemycircularvirus
-	Human papillomavirus type 174
Betapapillomavirus	Betapapillomavirus 1
Mastadenovirus	Human mastadenovirus C
Cypovirus	Dendrolimus cypovirus XY-2009a

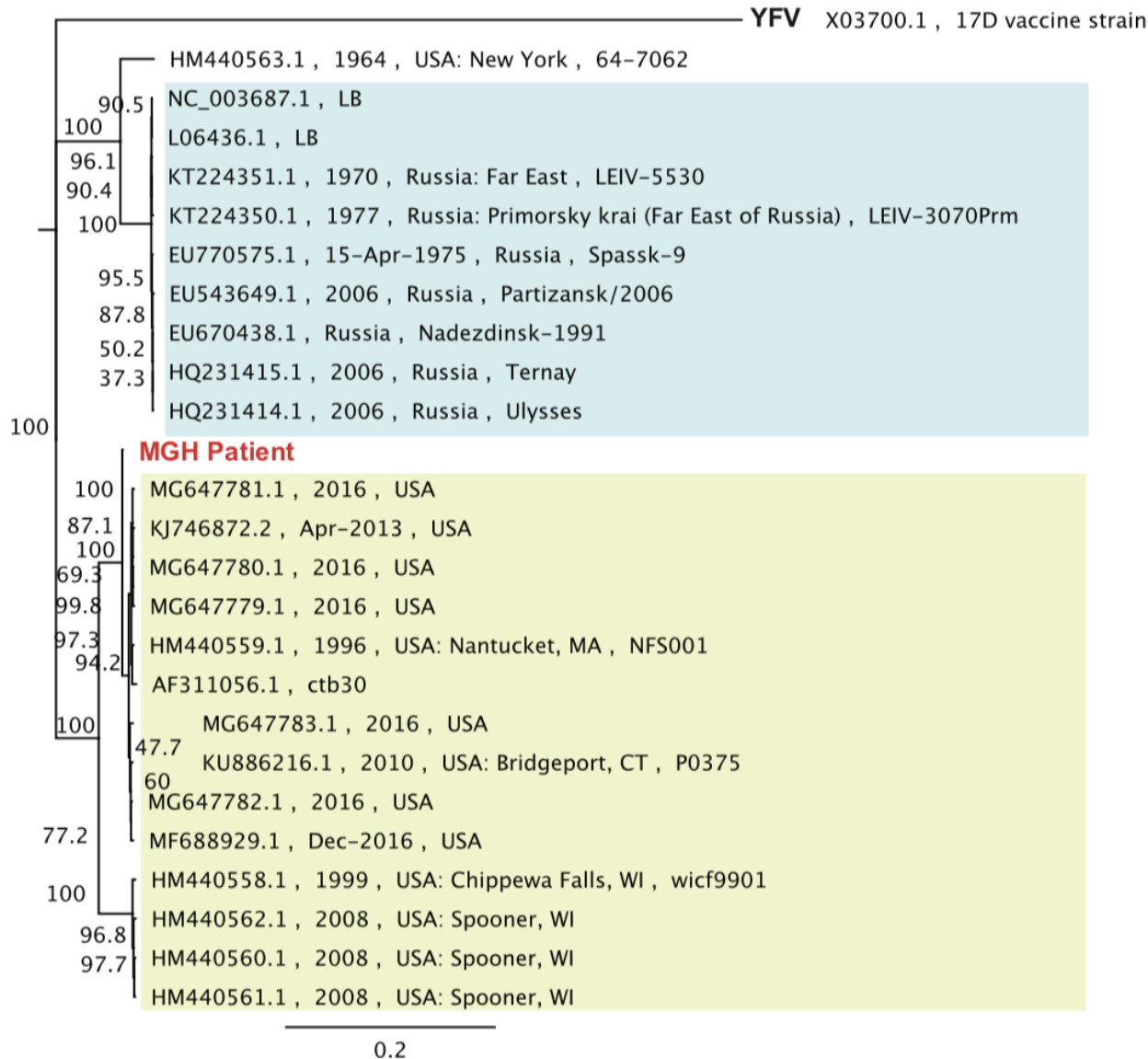


.....C_1799_1809
C_1800_1810
 MNC_1801_1811
 MNC_1802_1812
 MNC_1803_1813
 MNC_1804_1814
 MNC_1805_1815
 MNC_1806_1816
 MNC_1807_1817
 MNC_1808_1818

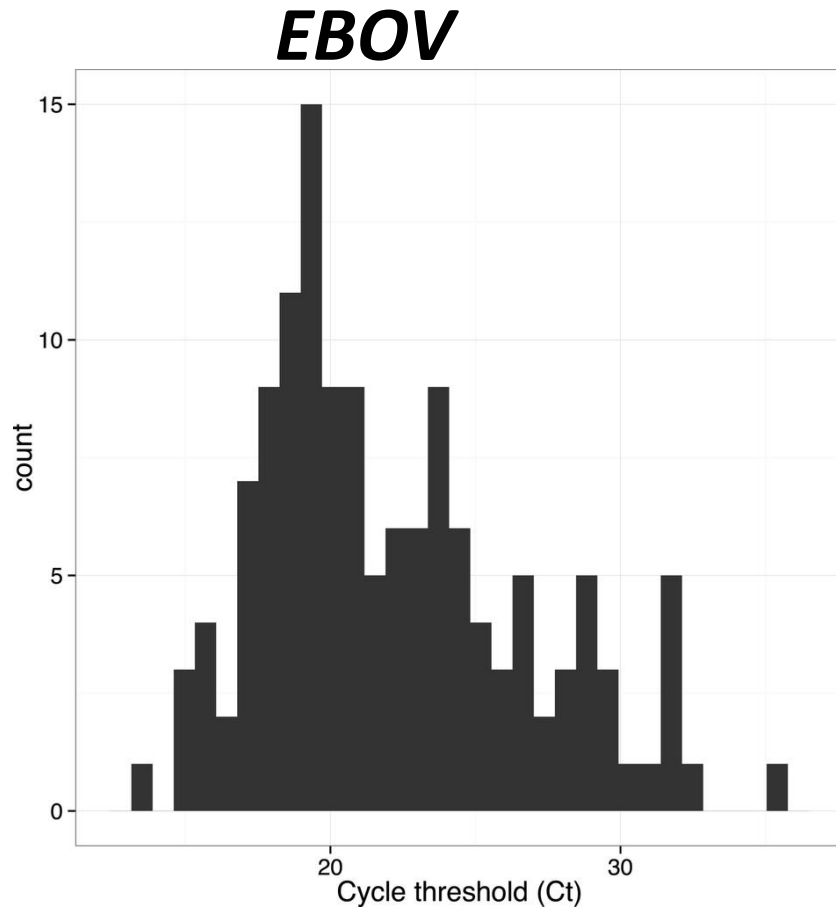


Analytical, RNA prep		
cell reads	88	100.0%
sample reads	847	10.4%
species reads	88	100.0%
total reads	22667	0.4%
Blast NCBI nt		

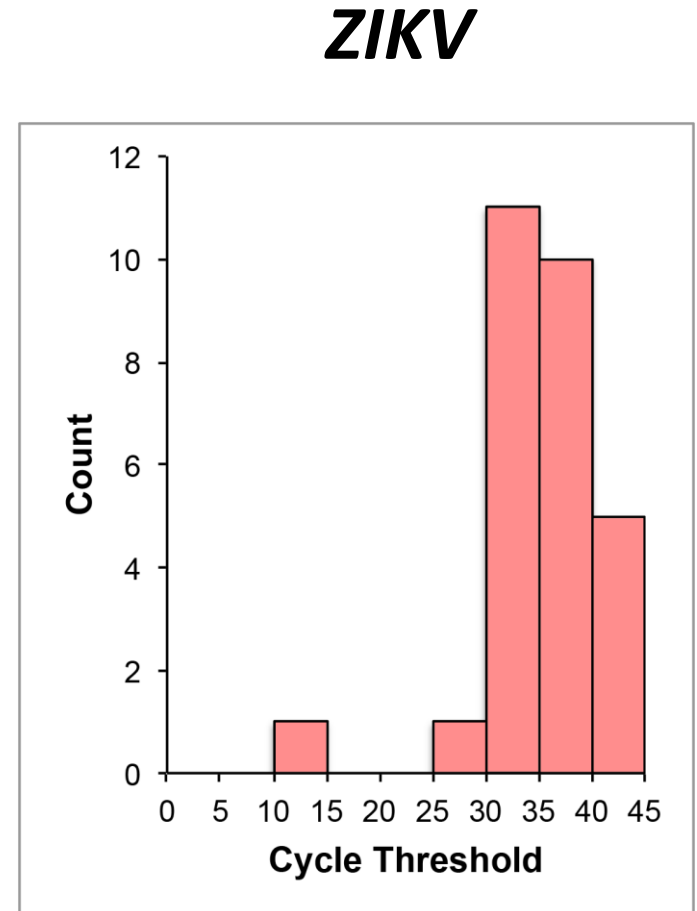
Phylogeny of tickborne Powassan virus



Enhancing Metagenomic Detection Sensitivity



(Quick, et al., 2016, *Nature* 530:228-232)



(Naccache, Thézé, et al., 2016, *EID*, 22:10)

set of reference genomes

multiple sequence alignment

HIV (3,571 genomes)

ZIKV (44 genomes)

partition aligned genomes into overlapping 250-nt segments

select forward and reverse spiked 13-nt primers

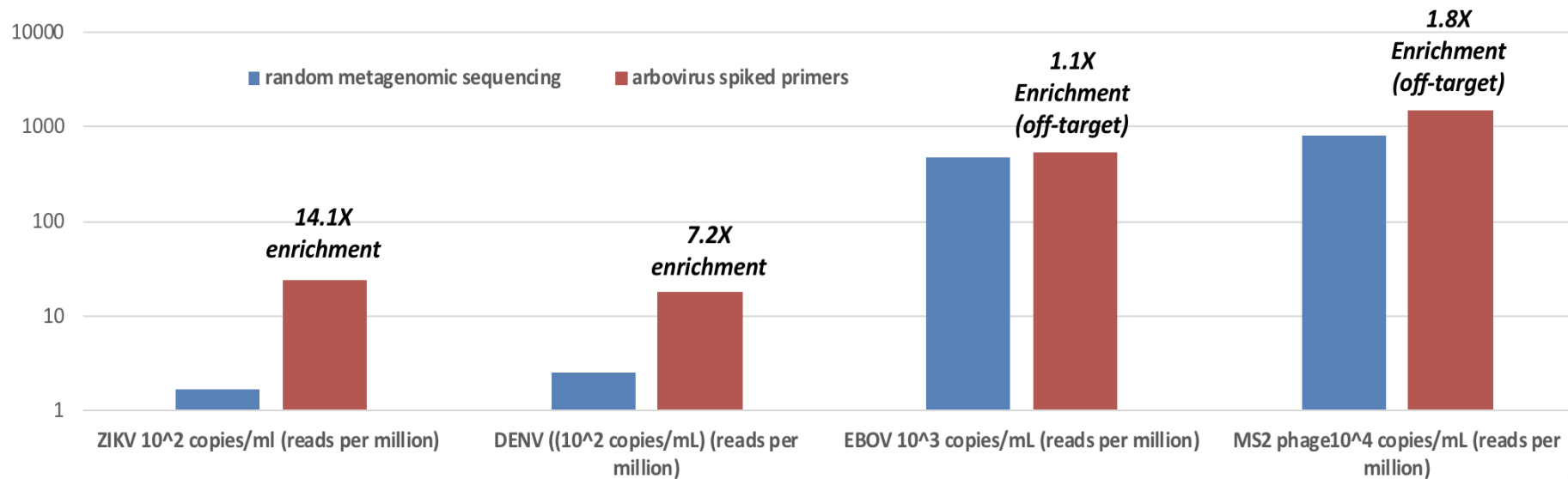
- filter primers by Tm (<2 SD from mean)
- remove self-dimers / cross-dimers
- remove homopolymer repeats (≥5 nt)

generate consensus sequence and partition into 250-nt segments

select forward and reverse spiked 13-nt primers

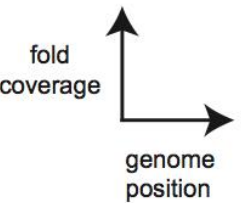
spiked 13-nt primer

MSSP (Metagenomic Sequencing with Spiked Primers) Enrichment

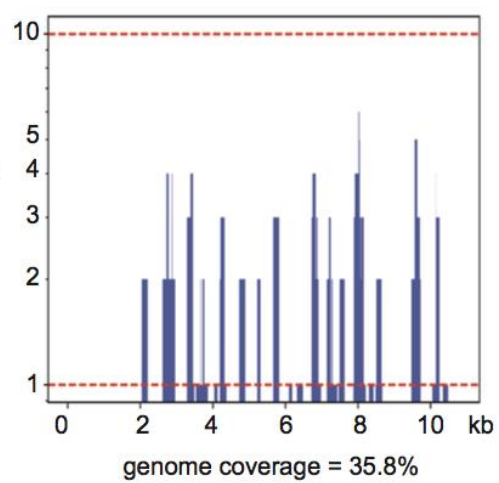


Thézé, et al., 2018, Cell Host & Microbe, in press

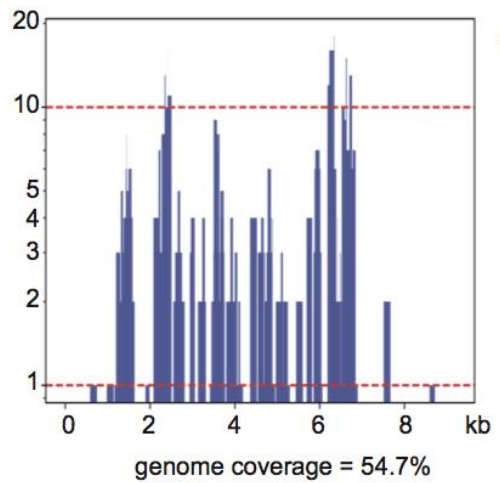
Deng, et al., 2017, manuscript in preparation



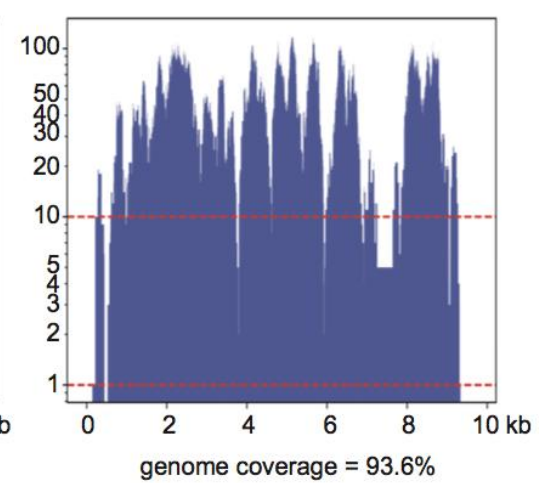
ZIKV (100 copies/mL)



**HIV (1000 copies/mL),
unenriched**

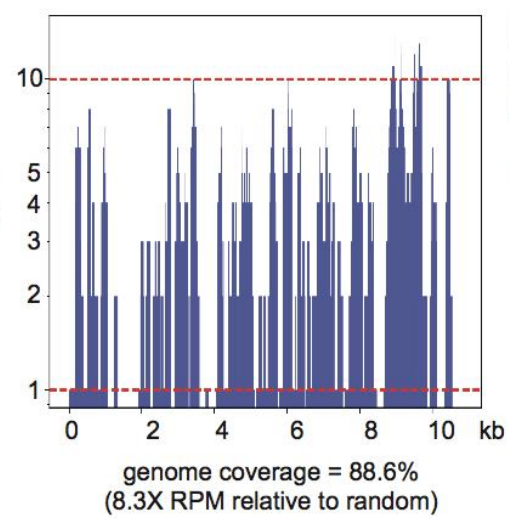
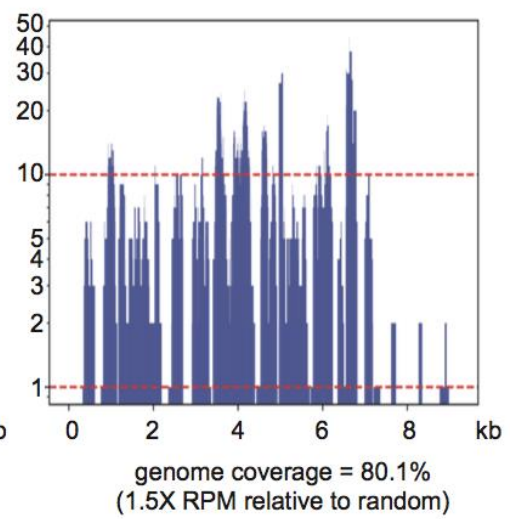
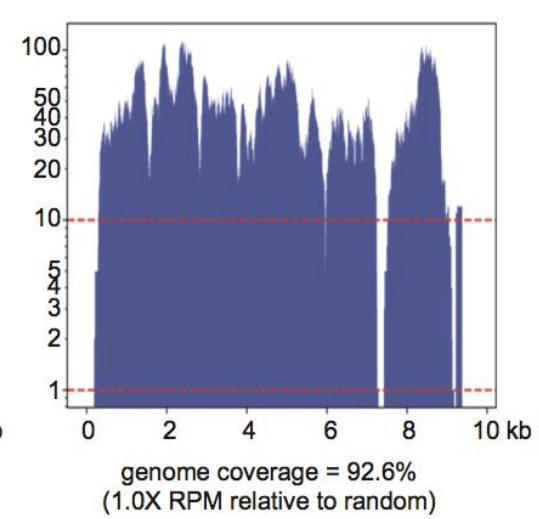


**HCV (10,000 copies/mL),
unenriched**



**Random Primers
Only**

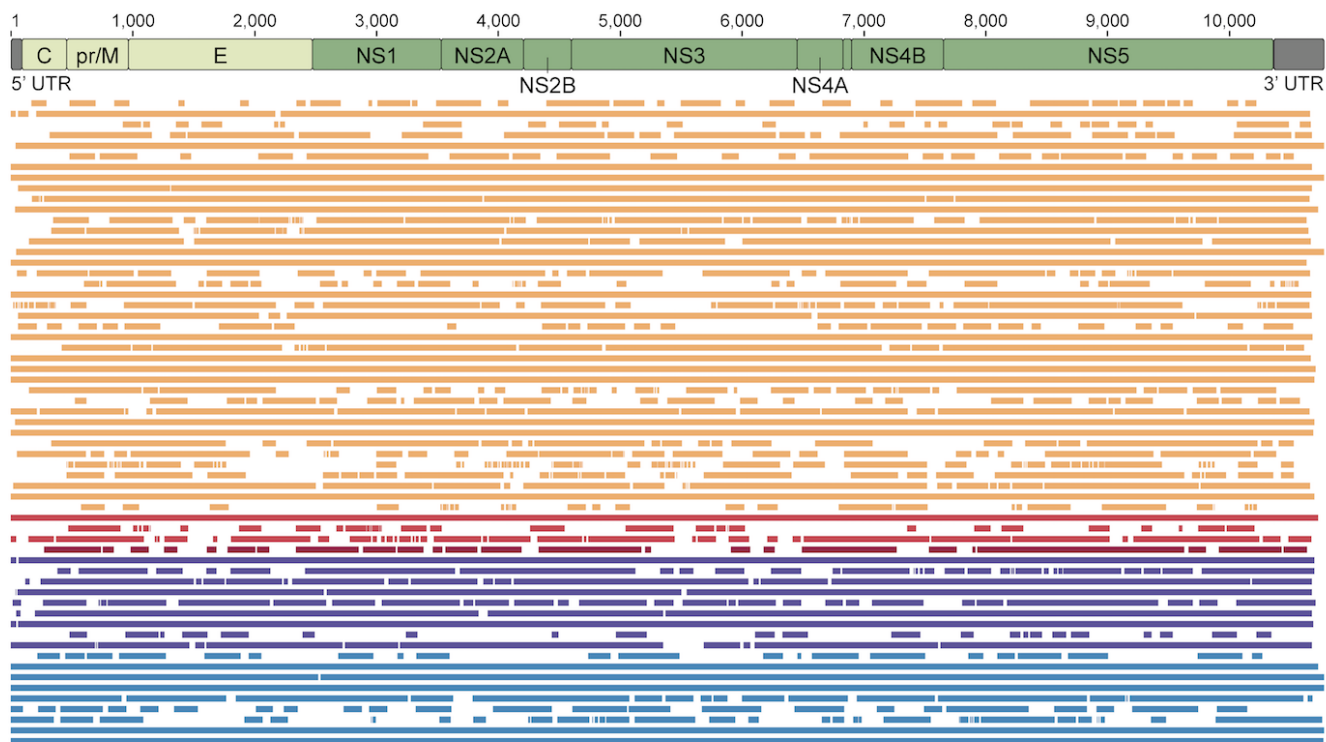
**ZIKV Reverse +
Random Primers
(5:1 Ratio)**



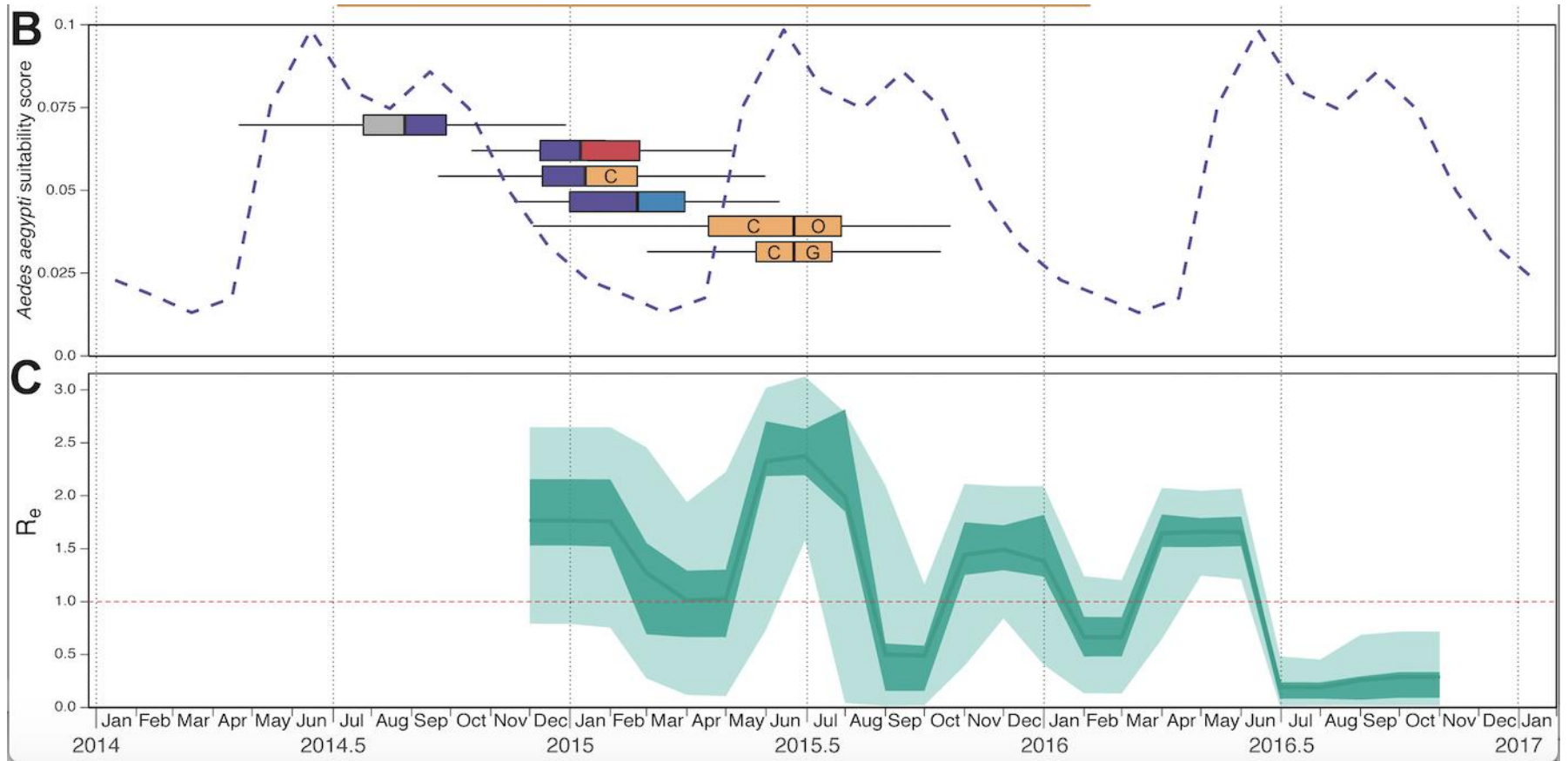


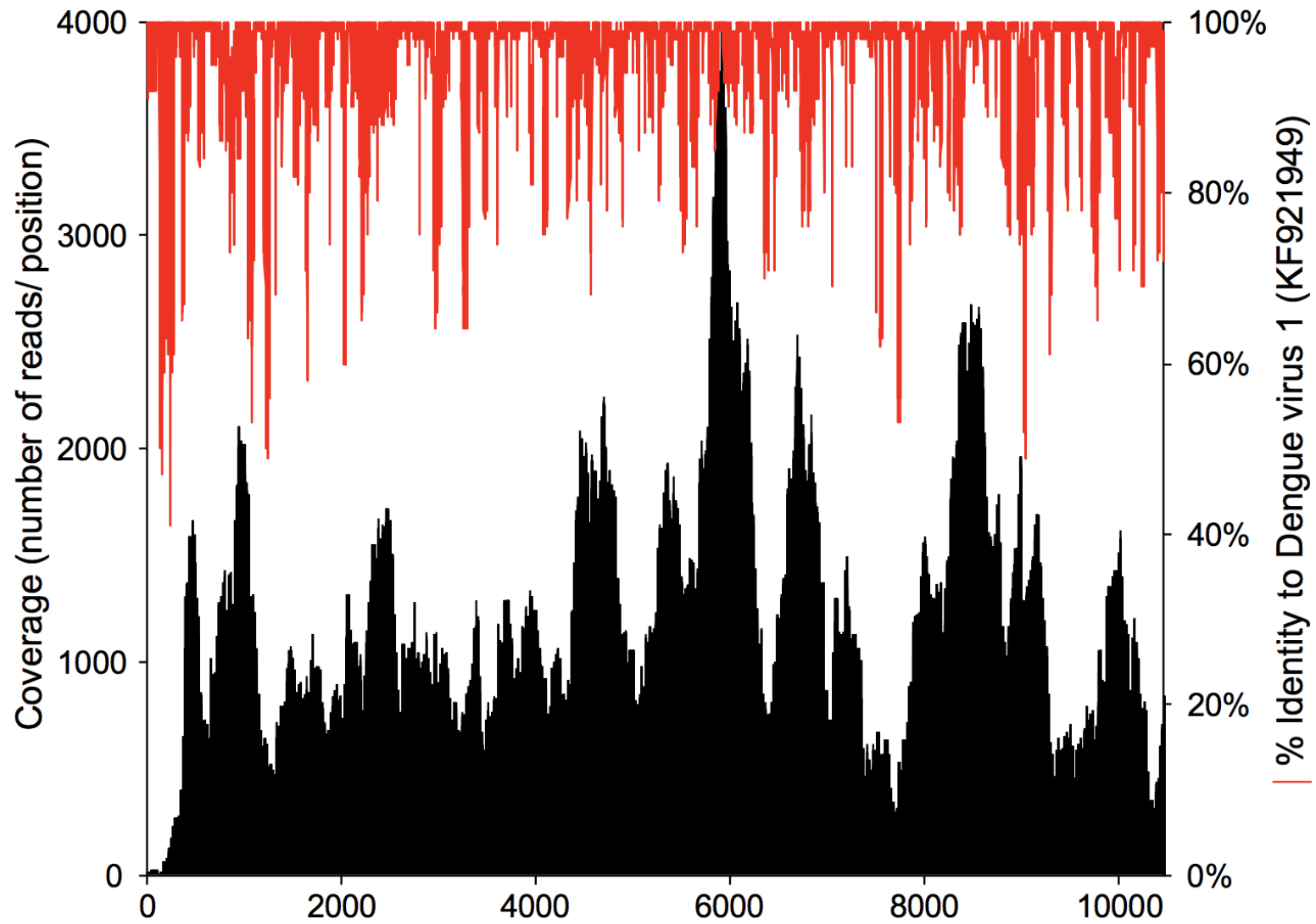
Julien Thézé and Oliver Pybus
University of Oxford, UK



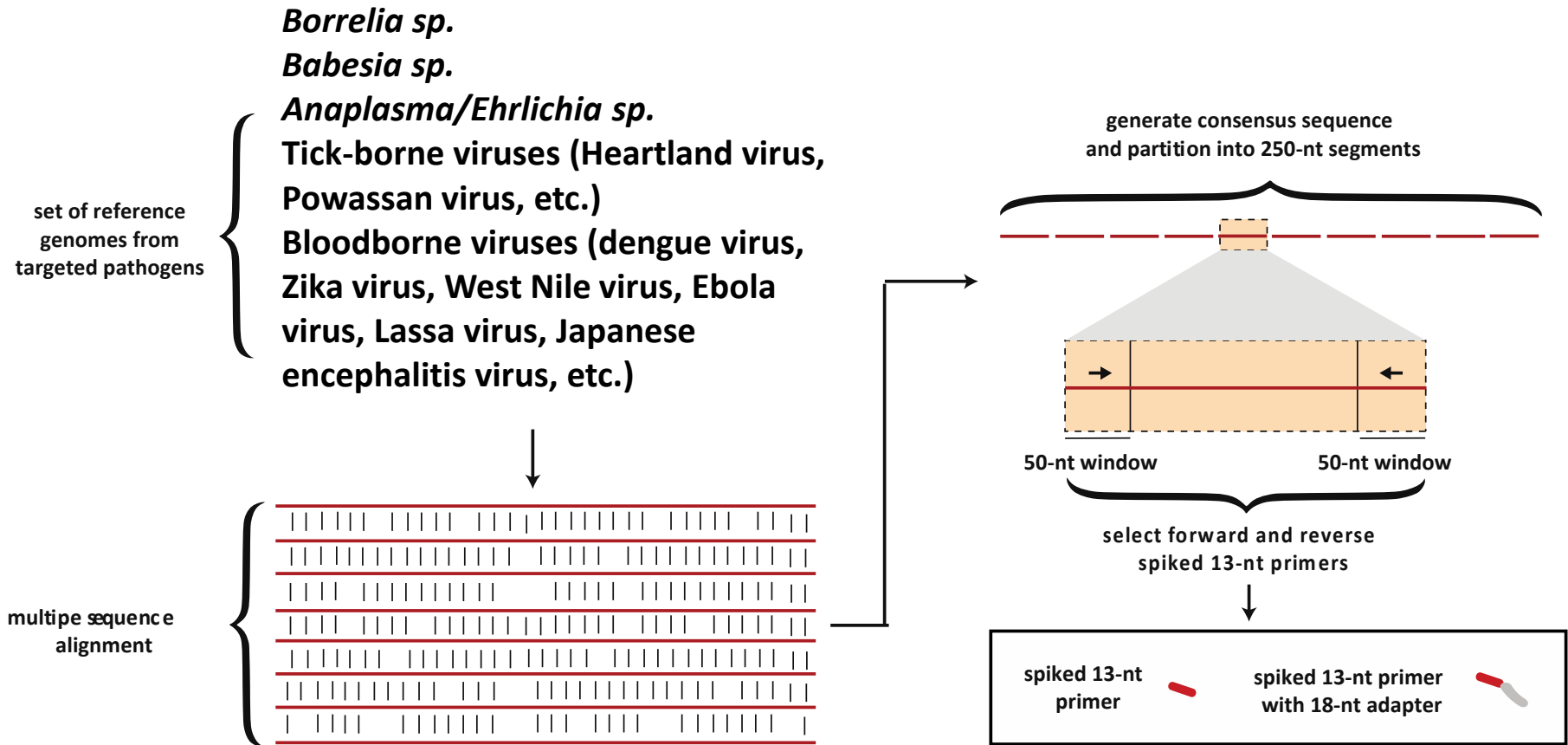


- Mexico
- Belize
- Guatemala
- El Salvador
- Honduras
- Nicaragua
- Costa Rica
- Panama



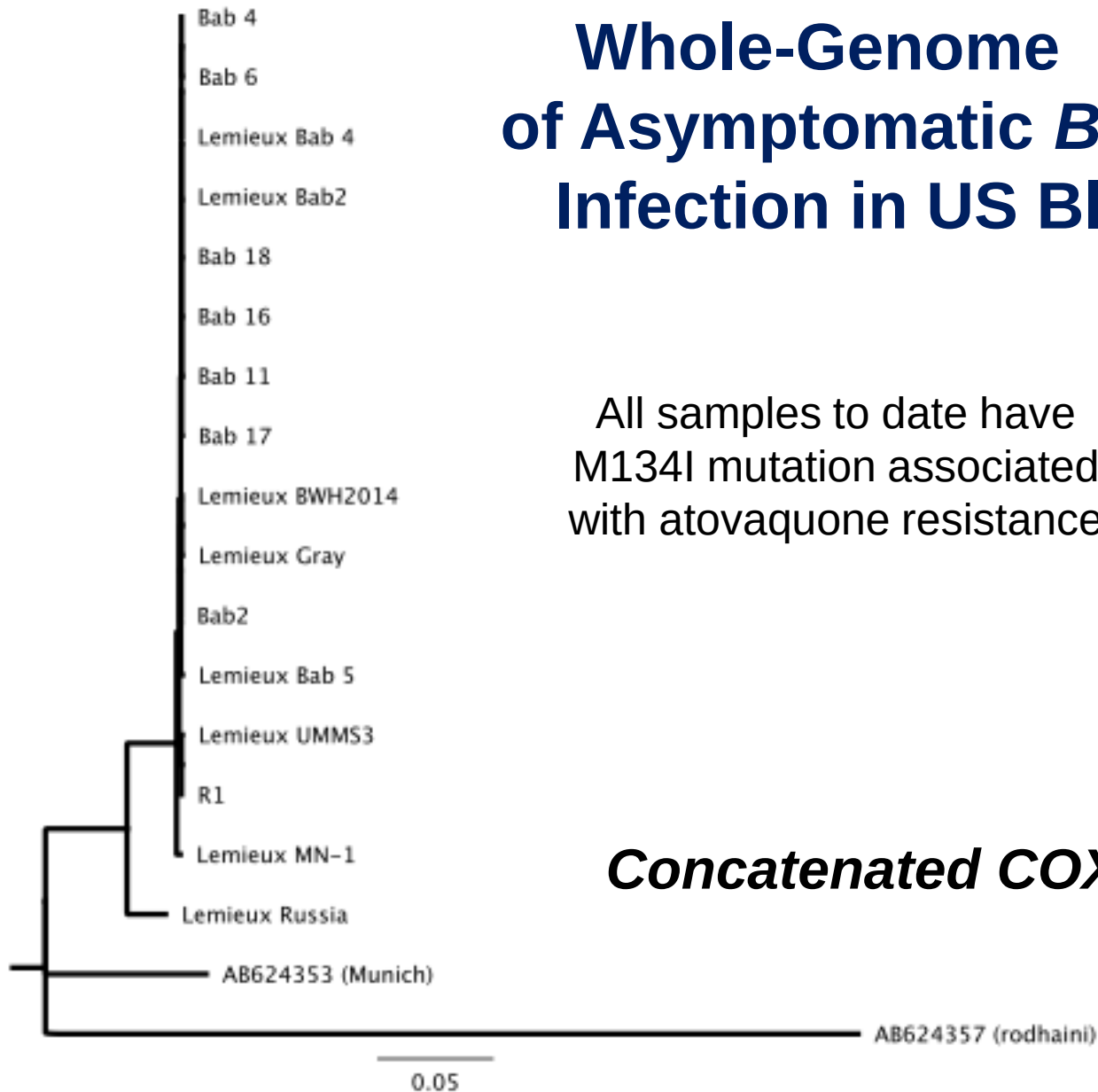


BloodSeq Panel



Whole-Genome Sequencing of Asymptomatic *Babesia microti* Infection in US Blood Donors

All samples to date have
M134I mutation associated
with atovaquone resistance



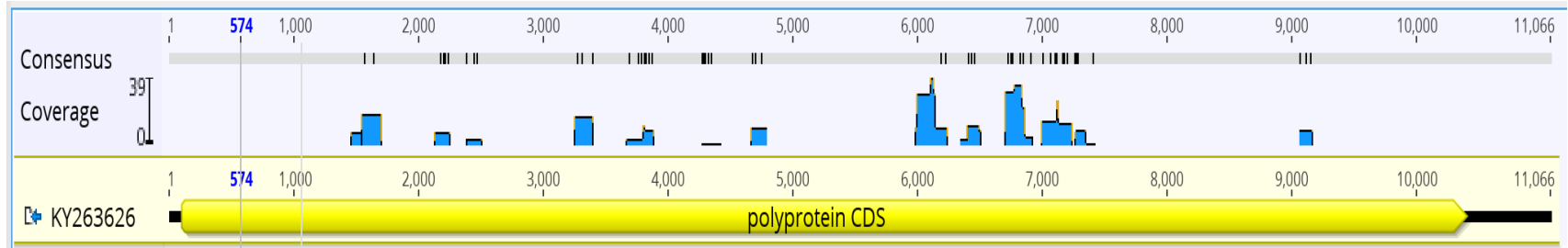
Usutu Virus Detection in an African Blood Sample using HIV Primers

Sequencing Stats		
Read Type	Run1	Run2
Raw Data	19,969,548	690,200,472
Human immunodeficiency virus 1	69374	2384992
Usutu virus	201	6502
% Usutu virus	0.001%	0.001%

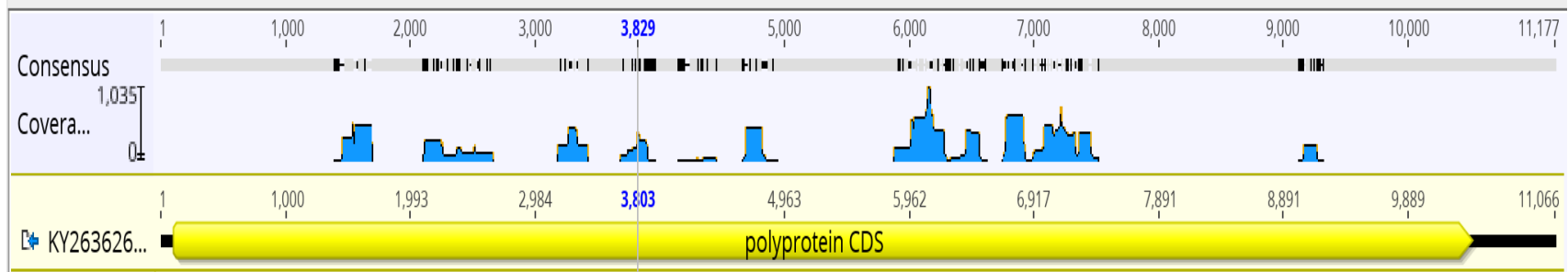
Collaboration with Mary Rodgers and John Hackett, Abbott Laboratories

Usutu Virus Detection using HIV Primers

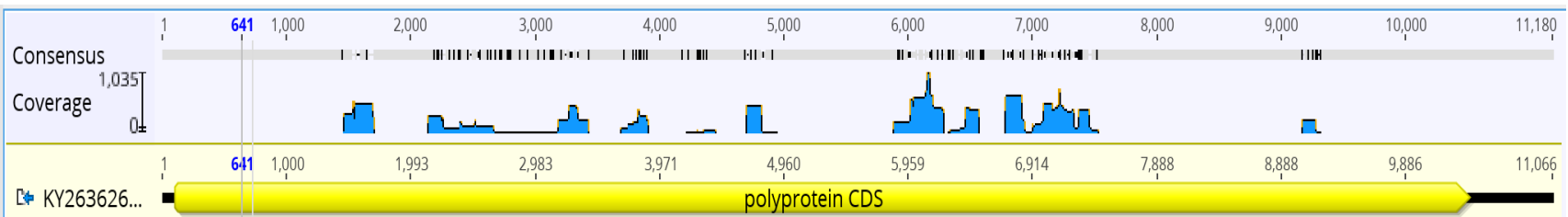
Original sequencing (20%)



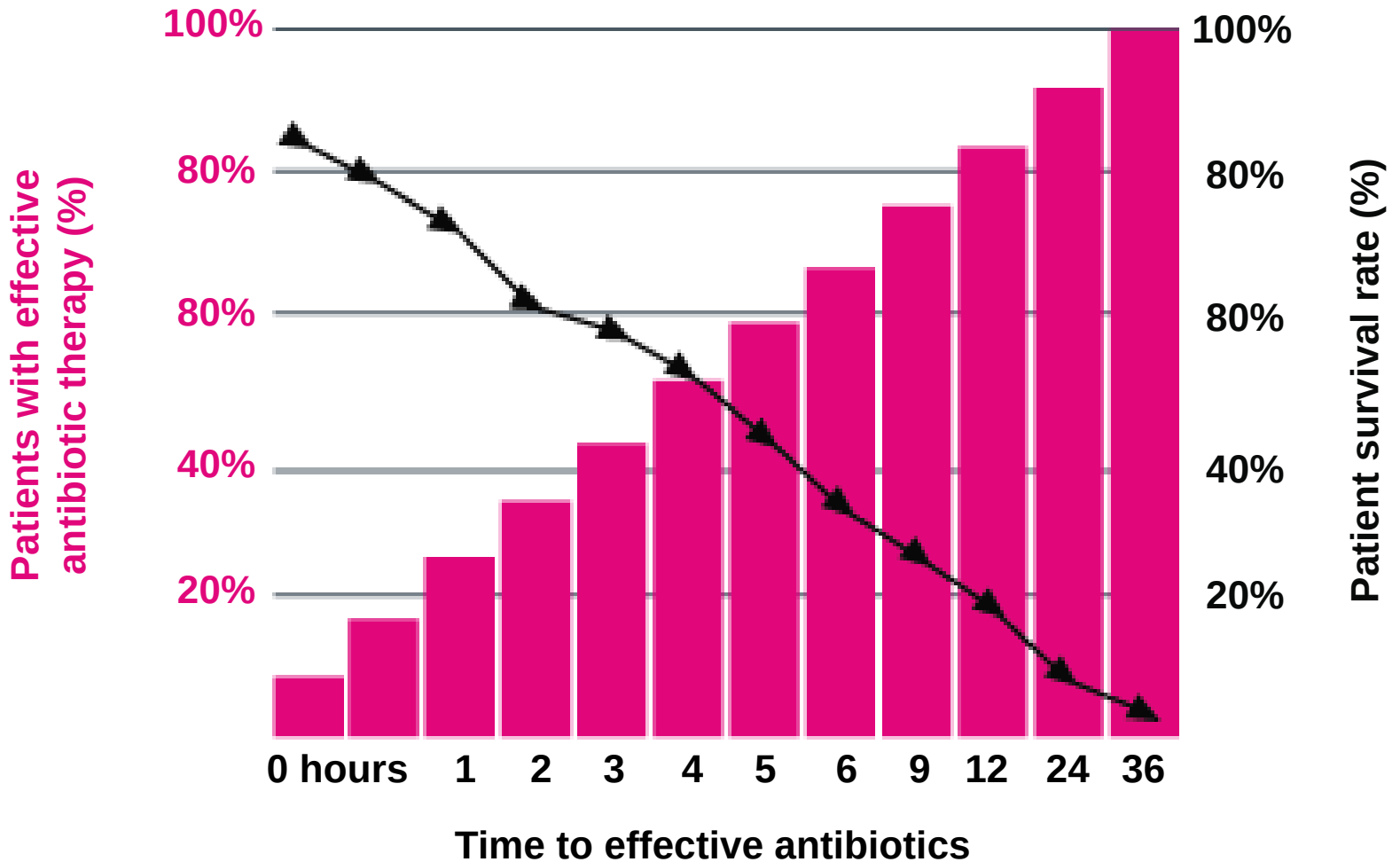
Deeper sequencing (29%)



With Sanger sequencing (34%)

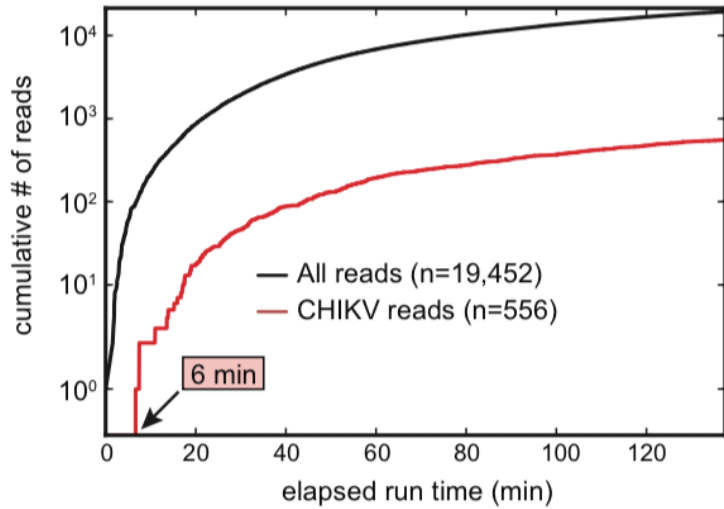


Metagenomics for Diagnosis of Sepsis in Point-of-Care Settings



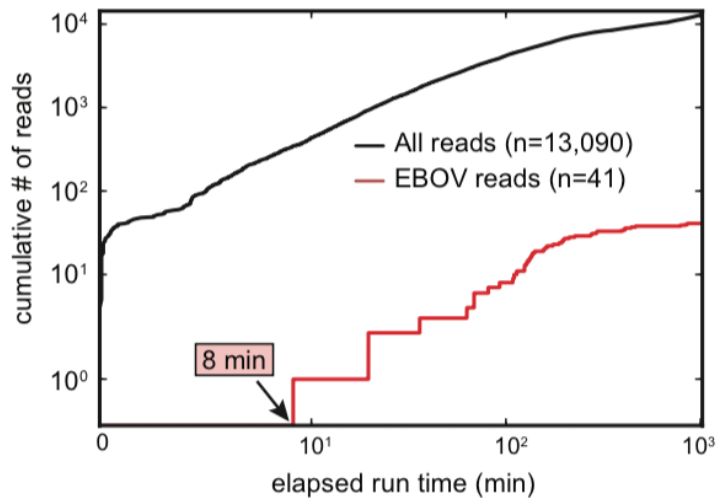
Nanopore Sequencing – Sample-to-Answer Diagnosis in <3 hr

Chik1 (9.1×10^7 copies/mL)



*Chikungunya
Virus*

Ebola1 (1×10^7 copies/mL)



*Ebola
Virus*



Differential Diagnosis of Tropical Febrile Illness

BACTERIAL

- Rickettsioses
- Bacillary Dysentery
- Plague
- Meningococemia
- Typhoid fever
- Other bacterial septicemia
- Leptospirosis
- Ehrlichiosis
- Tuberculosis
- Bartonellosis
- Brucellosis

VIRAL

- Arboviral infections
- Viral hepatitis
- Enterovirus
- Measles
- Rubella
- Acute Retroviral Syndrome (HIV)
- Epstein-Barr virus
- Parvovirus
- Roseola virus
- Filovirus infection (Ebola, Marburg)
- Lassa virus
- bunyaviruses

OTHER

- Malaria / Babesiosis
- Amebiasis
- Visceral leishmaniasis
- Acute schistosomiasis
- Filarial fever
- Trypanosomiasis

Protocol Optimization on the MinION Nanopore Sequencer



Wayne Deng, PhD

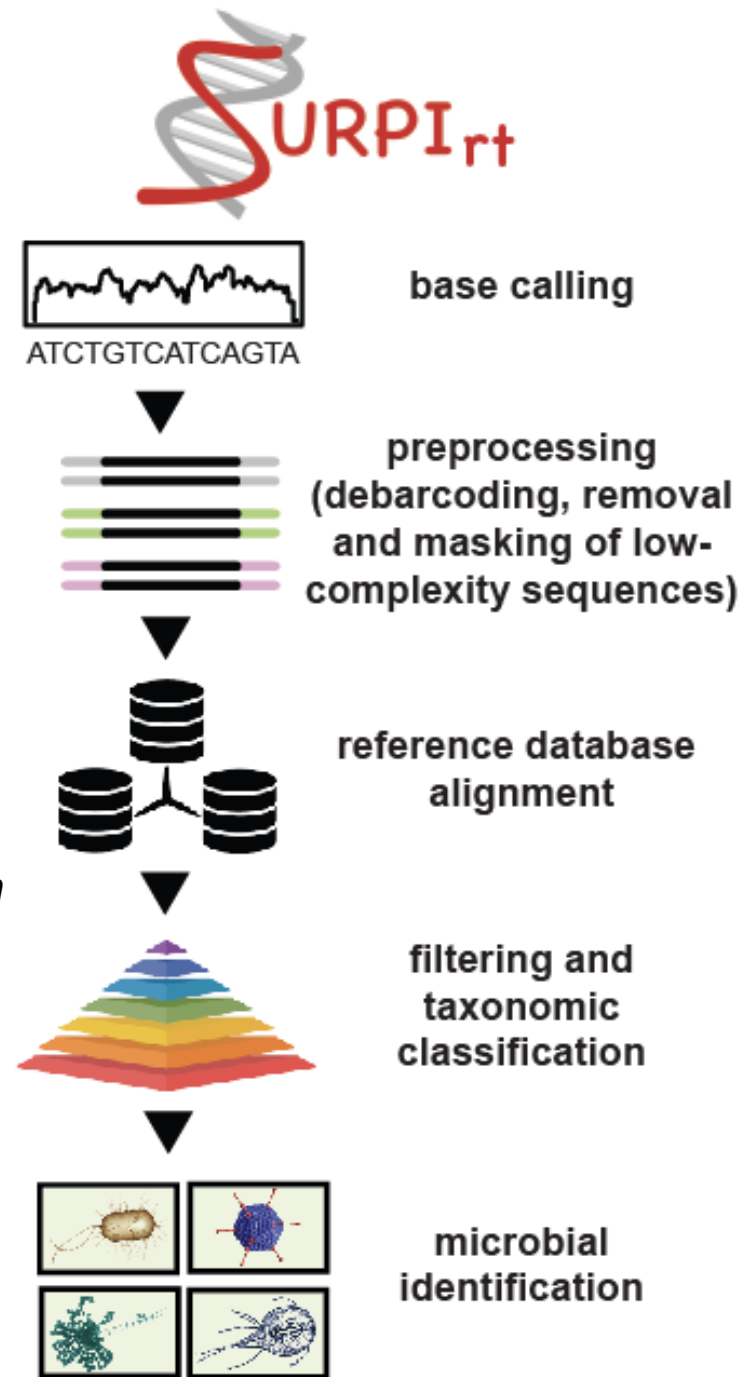


Dianna Ng, MD

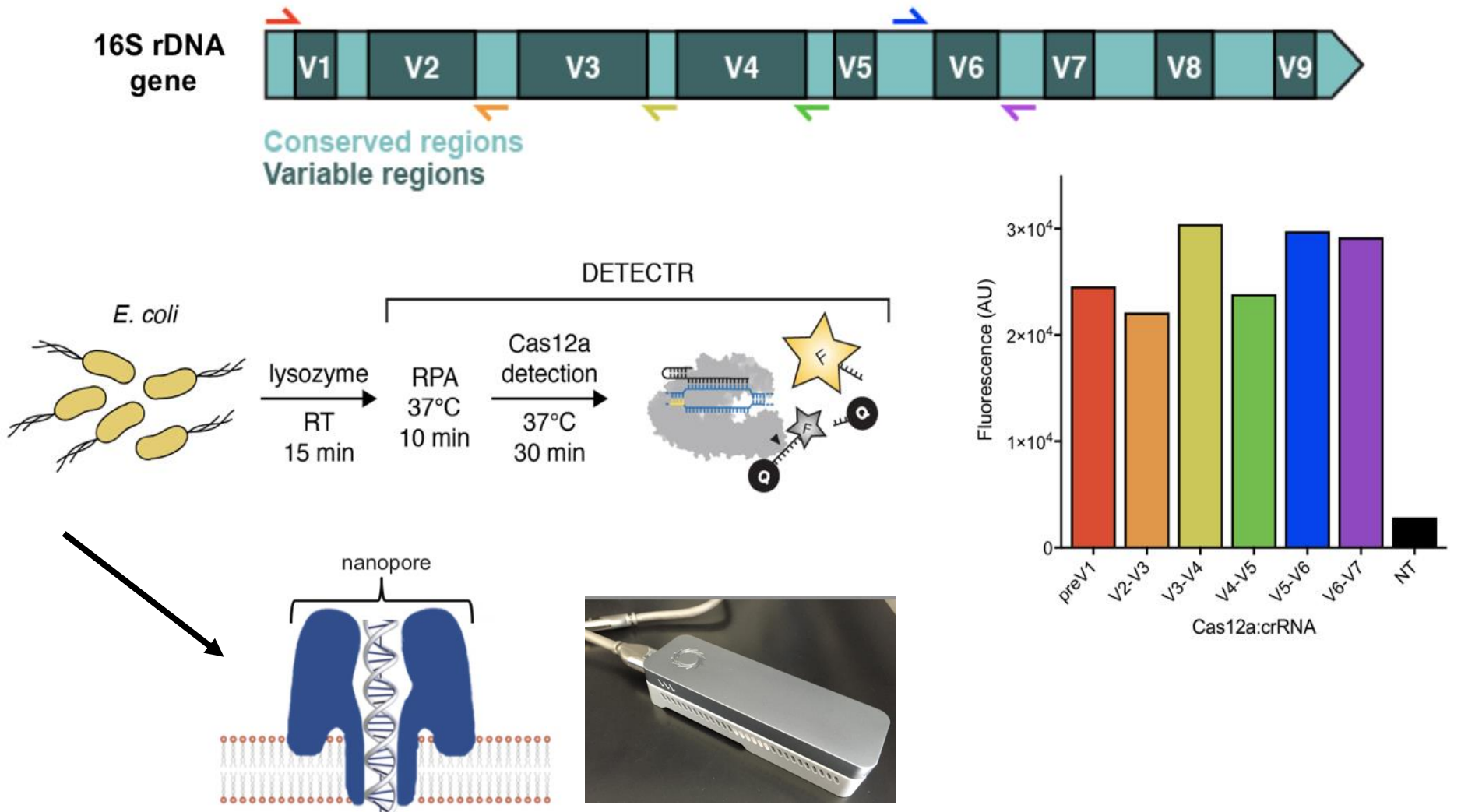


Scot Federman, BA

*Can analyze 1 million
reads in 10 minutes
on a laptop*



Pan-Bacterial Detection in <30 Min by CRISPR-CAS12



Real-Time Pathogen Detection in Infected Body Fluids from Patients

Sample ID	Nanopore Findings	Pathogen Reads	Total # of Reads	Real-time Detection	Nanopore RPM	HiSeq RPM
JDL052* (BAL)	<i>Streptococcus pneumoniae</i>	5,816	400,000	2 min (320/50,000)	13,819	22,663
132 (plasma)	<i>Haemophilus influenzae</i>	7	240,000	2 hr (7 reads)	28	69
133 (BAL)	<i>Haemophilus influenzae</i>	436	280,000	2 min (25/4,000)	2,718	11,096
81S (joint fluid)	<i>Staphylococcus aureus</i>	140	107,600	2 min (1/4,000)	1,300	2,792
78S* (pleural fluid) **	<i>Staphylococcus lugdunensis</i>	24	60,000	10 min (8/20,000)	400	3,737

*blinded sample

**90 minutes sample-to-result (direct sequencing)

Real-Time Pathogen Detection in Infected Body Fluids from Patients

Sample ID	Nanopore Findings	Pathogen Reads	Total # of Reads	Real-time Detection	Nanopore RPM	HiSeq RPM
69S* (BAL)	<i>Streptococcus pneumoniae</i>	8	600,000	30min (3 reads)	13.3	209
83* (abscess fluid)	<i>Staphylococcus aureus</i>	5	3 million	>2hr	1.7	?
87* (abdominal fluid)	<i>Candida glabrata</i>	10	1.98 million	>2hr	5.1	12.9
89* (perihepatic fluid)	<i>Candida glabrata</i>	9	1 million	>2hr	9	82.9
NP15* (plasma, HCV 10 ² copies/mL)	HCV	9	200,000	30 min	45	—
NP28* (plasma, DENV 10 ³ copies/mL)	DENV	39	66,454	20 min	586	—

***blinded sample**

Nanopore Sequencing of Clinical Samples

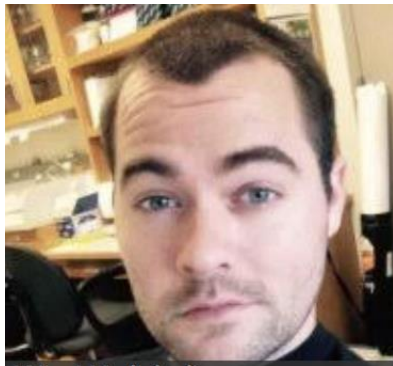
Sample ID	Nanopore Findings	Pathogen Reads	Total # of Reads	Real-time Detection
BM1 (plasma, 40 parasites/ μ l)	<i>Babesia microti</i>	31	40,000	20 min
PF1 (plasma, 10 parasites/ μ l)	<i>Plasmodium falciparum</i>	15	50,000	20 min
Mex1 (plasma, 10^3 copies/mL)	DENV	7	50,000	20 min
Mex2 (nasal swab, 10^4 copies/mL)	Influenza B	4	50,000	20 min
DRC1 (nasal swab)	Influenza A	2	40,000	20 min

INRB (Institut National de Recherche Biomedicale)

Anne Rimoin and UCLA-DRC Team



**Anne Rimoin,
PhD**



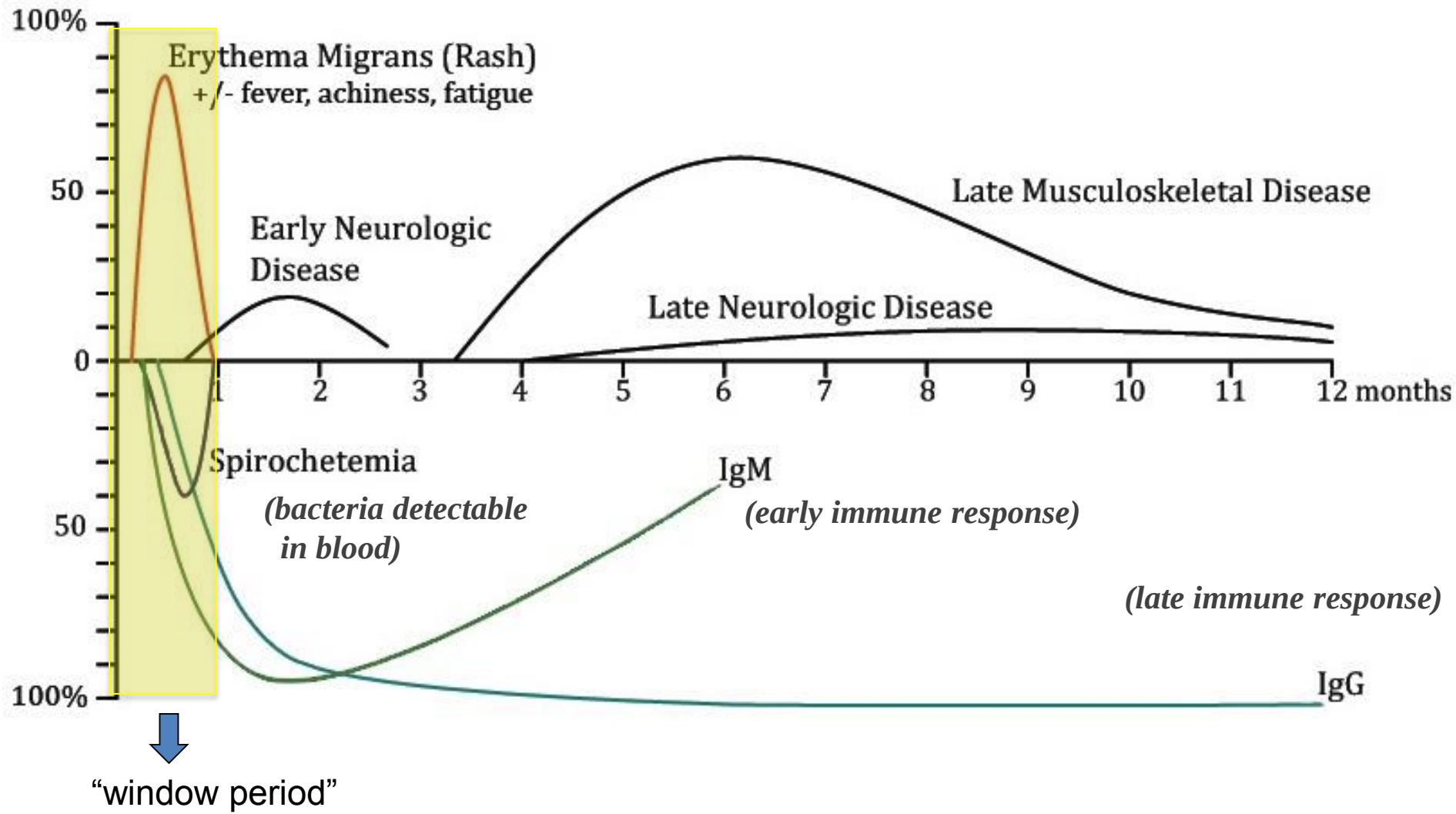
**Matt Bramble,
PhD**



**Russell
Williams, PhD**

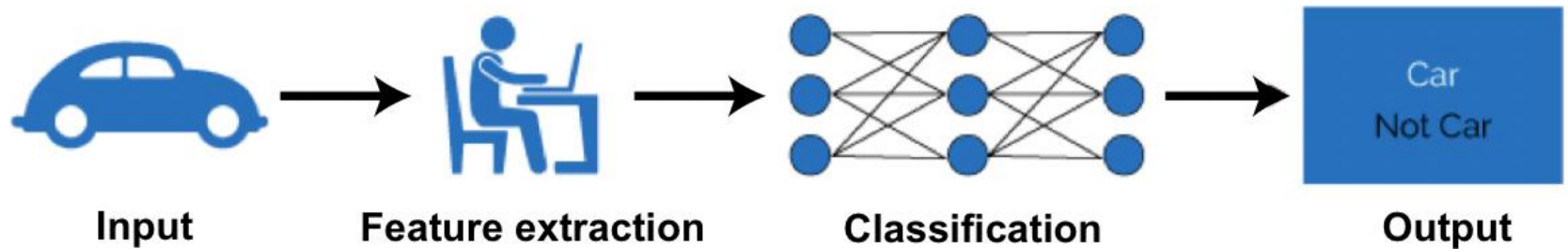


Identification of Bloodborne Infections by Host Response Analysis

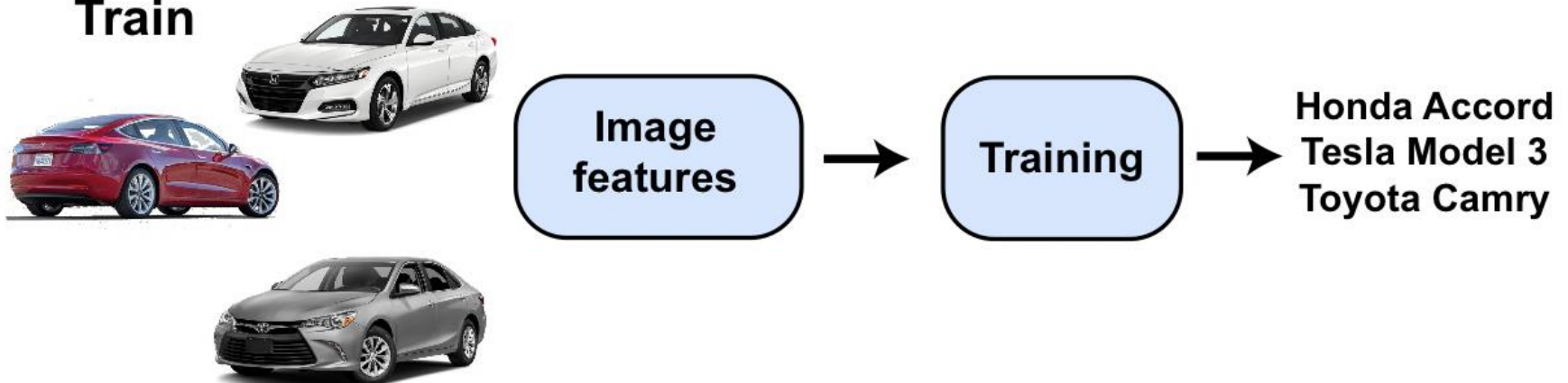


Antibody testing misses the window period for diagnosing early Lyme disease

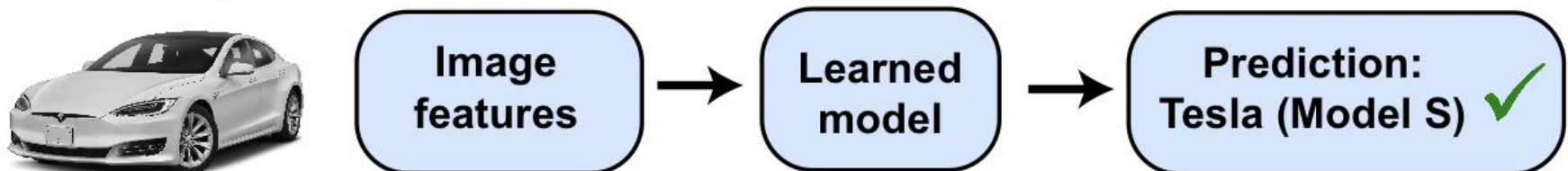
Machine Learning of RNA-Seq Data to Predict Causes of Infection



Train

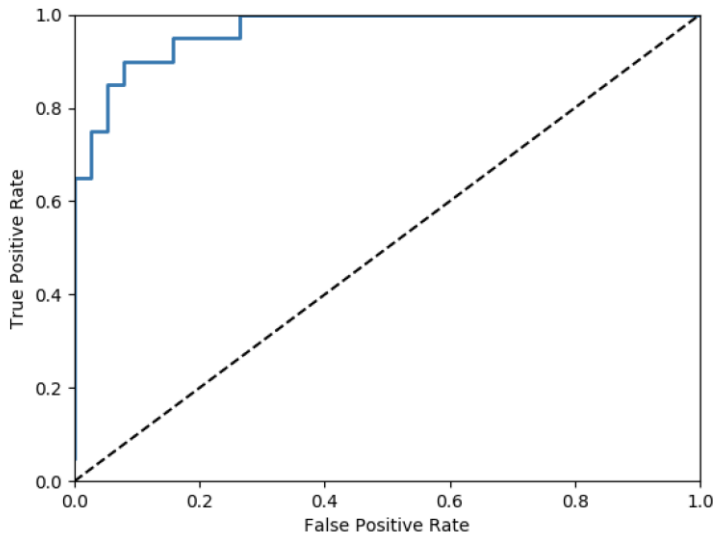


Deploy



Machine Learning-Based Analyses of Host Response

90% accuracy in discriminating bacterial from viral infection from CSF (preliminary analysis)



OR

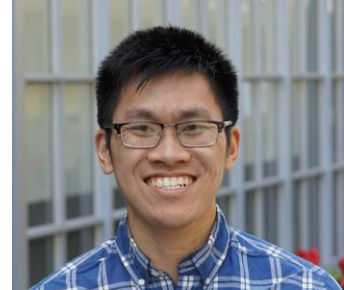


- Training set: 25 bacterial positive cases, 48 viral positive cases
- Test set: 6 bacterial positive cases, 9 viral positive cases

	CV	RSME	Score	CV	RSME	STD	CV	Accuracy
Linear SVM		0.221034		0.226150			0.900000	
Logistic Regression		0.250920		0.265278			0.866667	
Polynomial SVM		0.323491		0.220651			0.846667	
Stochastic Gradient Descent		0.241841		0.248018			0.840000	
AdaBoost		0.274579		0.290871			0.806667	



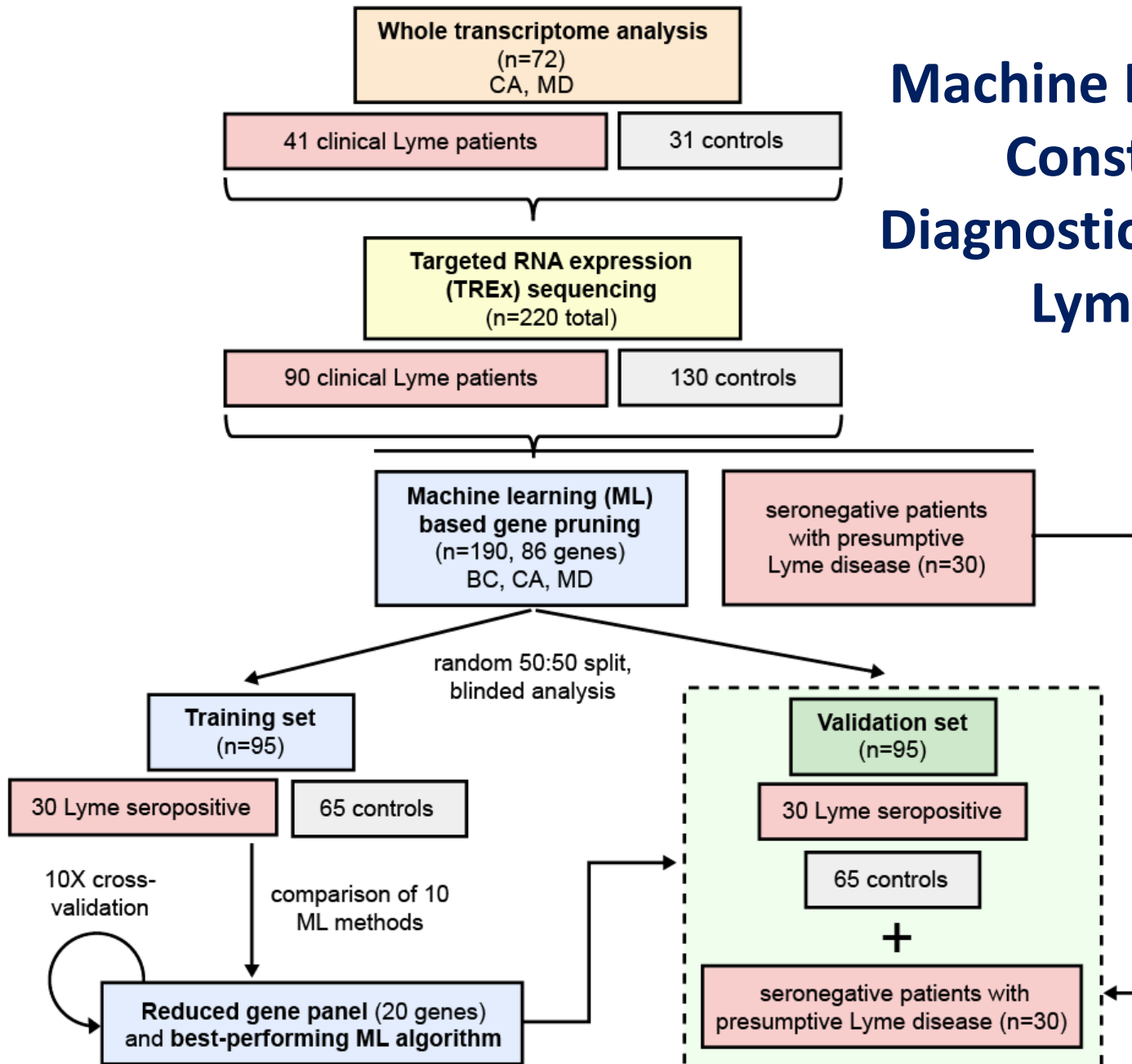
Aditi Paranjpe, MS



Tony Li, MS

(with Matt Massie and Anthony Joseph, UC Berkeley)

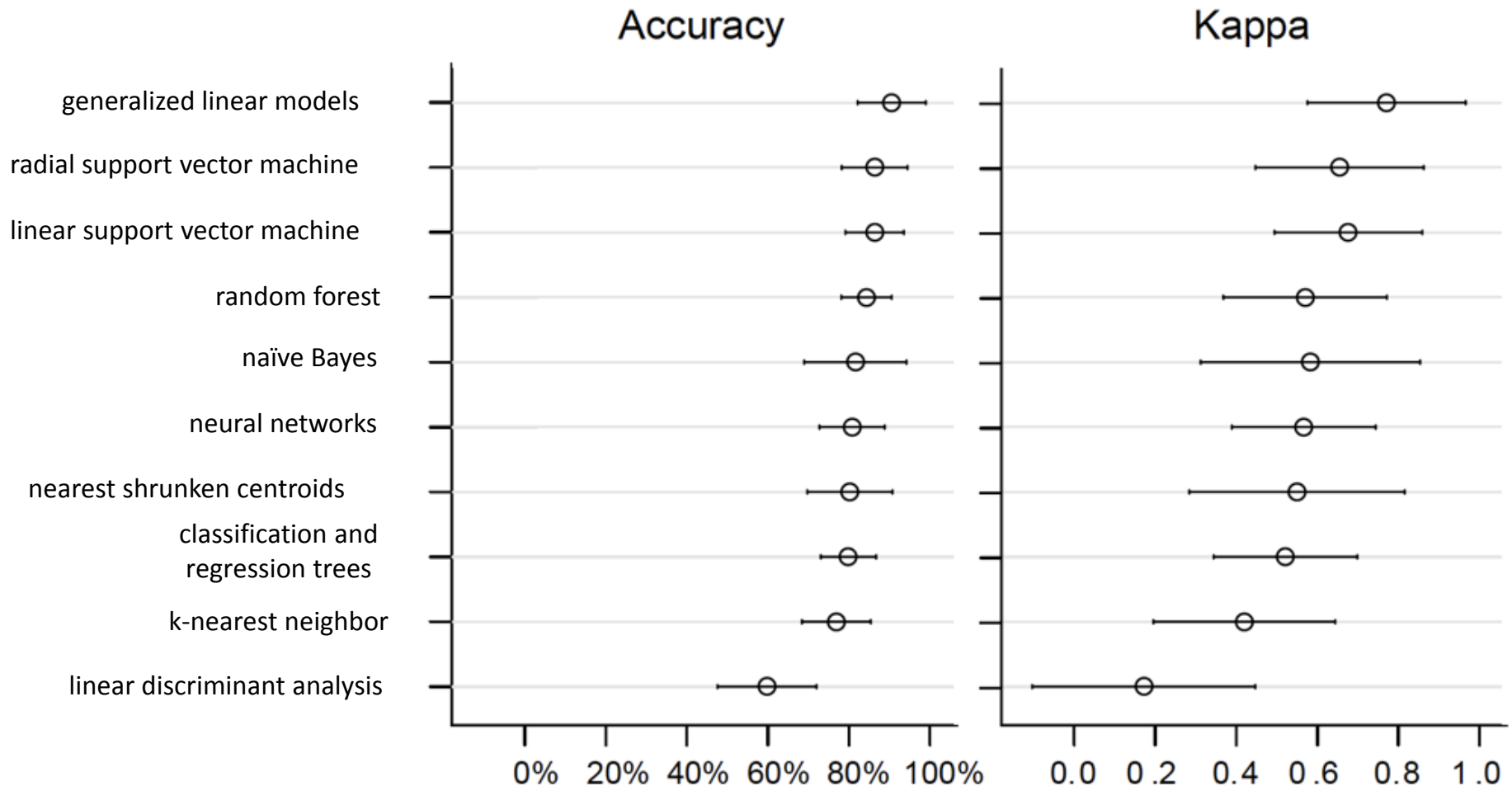
Machine Learning-Based Construction of Diagnostic Panel for Early Lyme Disease



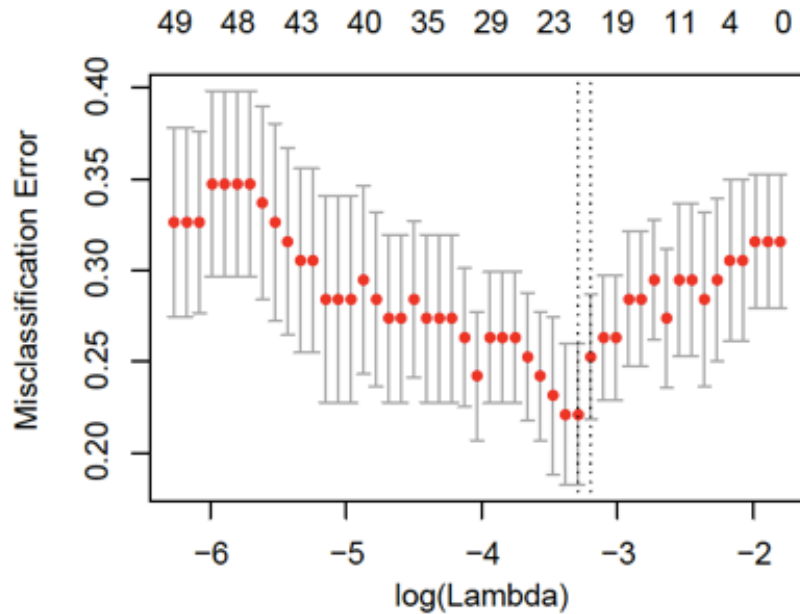
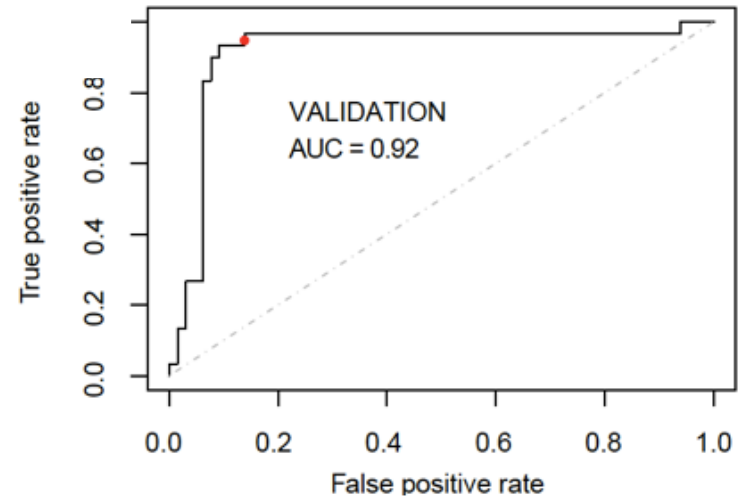
Jerome Bouquet, PhD

(Bouquet, et al., 2018, under review)

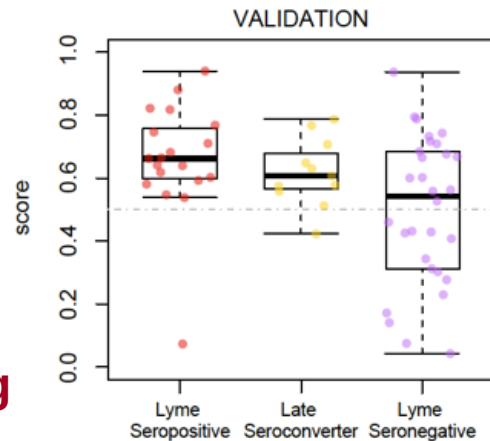
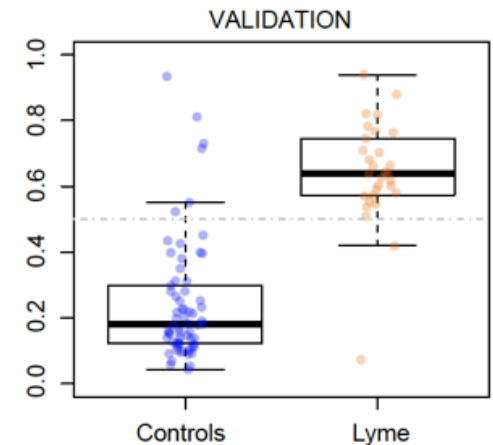
Accuracy and Kappa Statistics for 10 Different Machine Learning Methods



20-Gene Lyme Classifier Panel for Diagnosis of Early Lyme Disease



- Best validation set results
 - **94% sensitivity**
 - **93% specificity (seropositive)**
 - **91% accuracy (including seronegative patients)**



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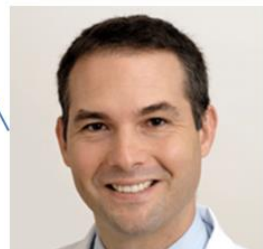
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