

Genomics goes mobile



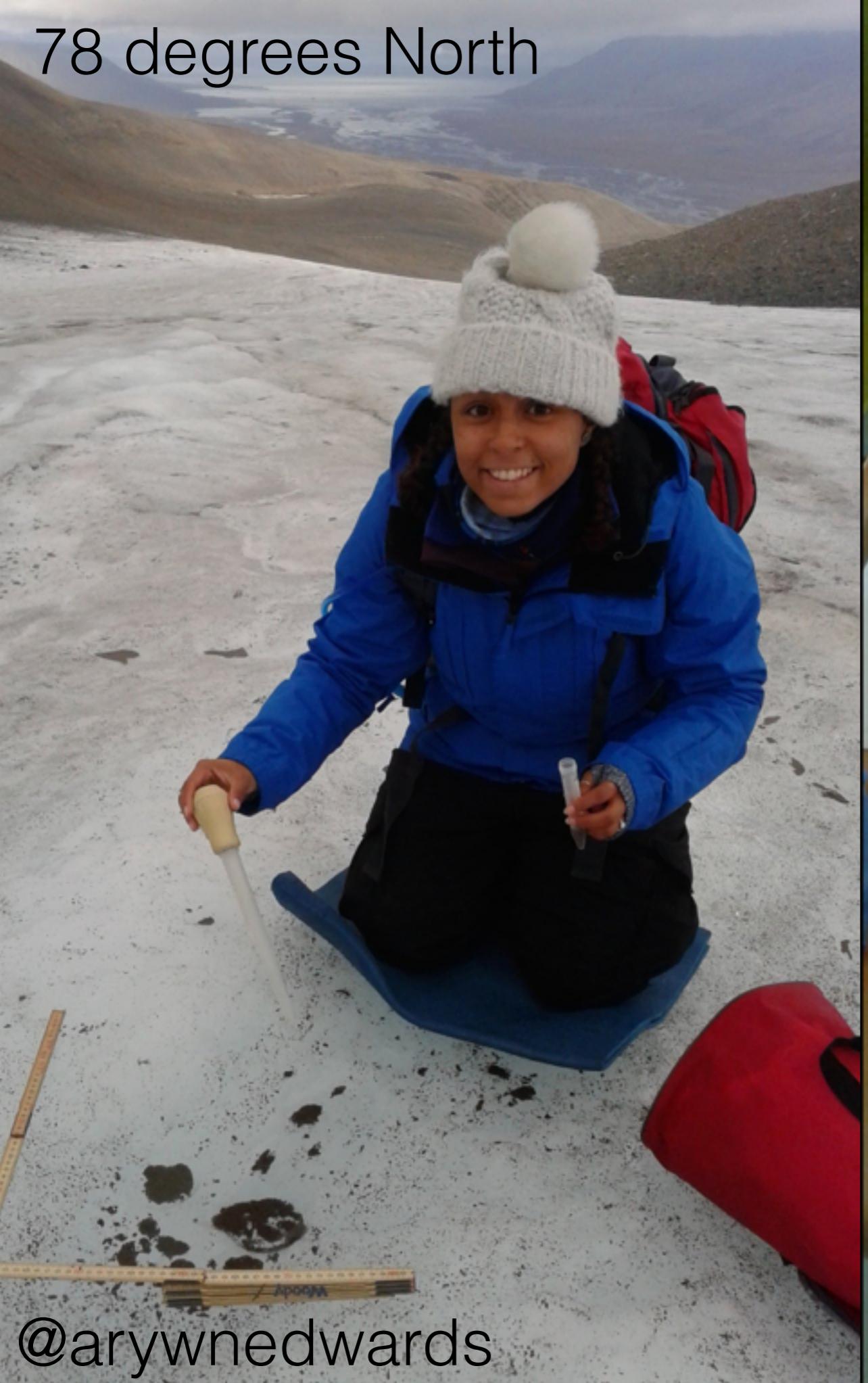
Trains, planes and
autochthonous transmission of
Zika in Brazil

@aphilosof



porecamp.github.io

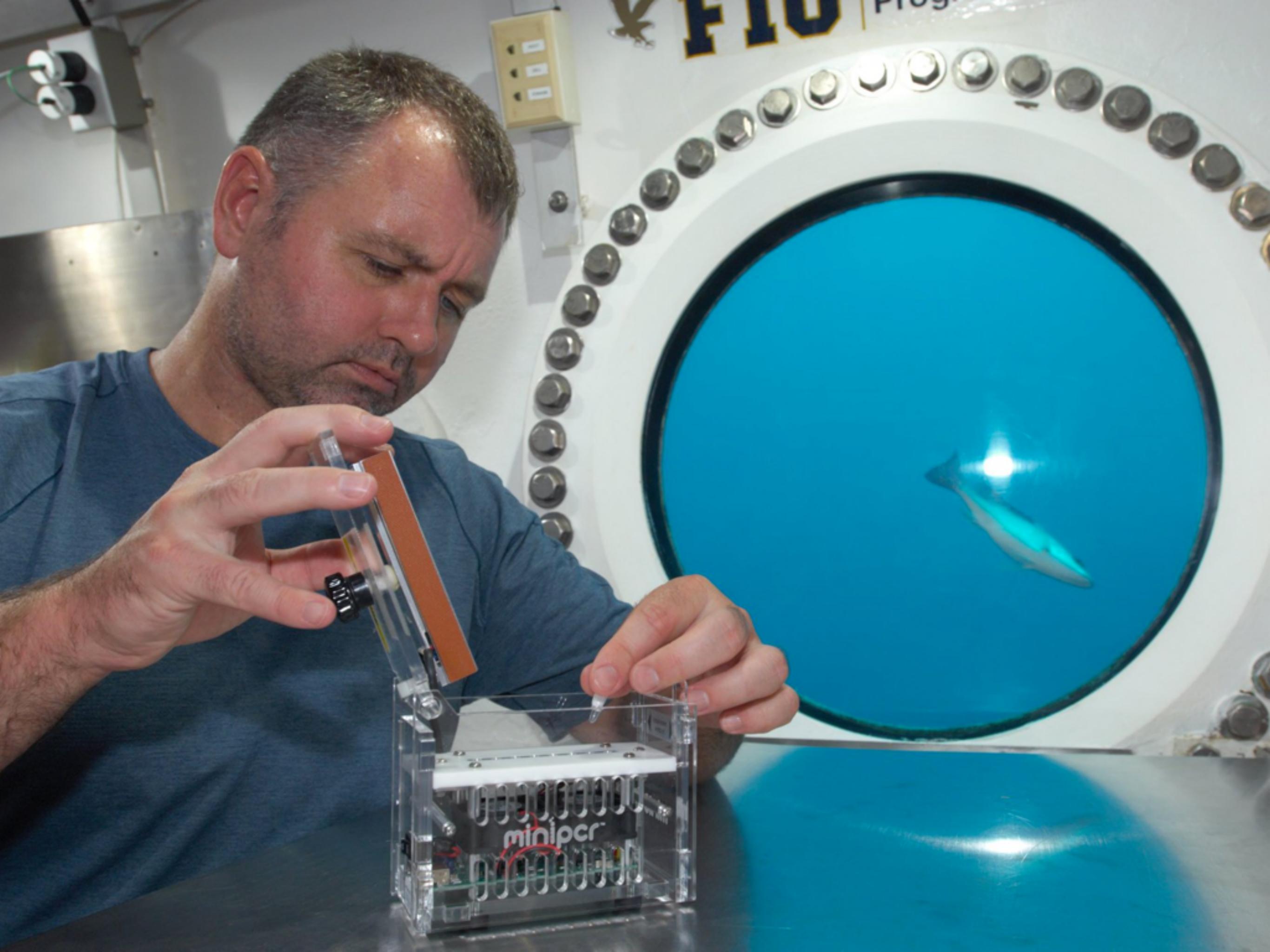
78 degrees North



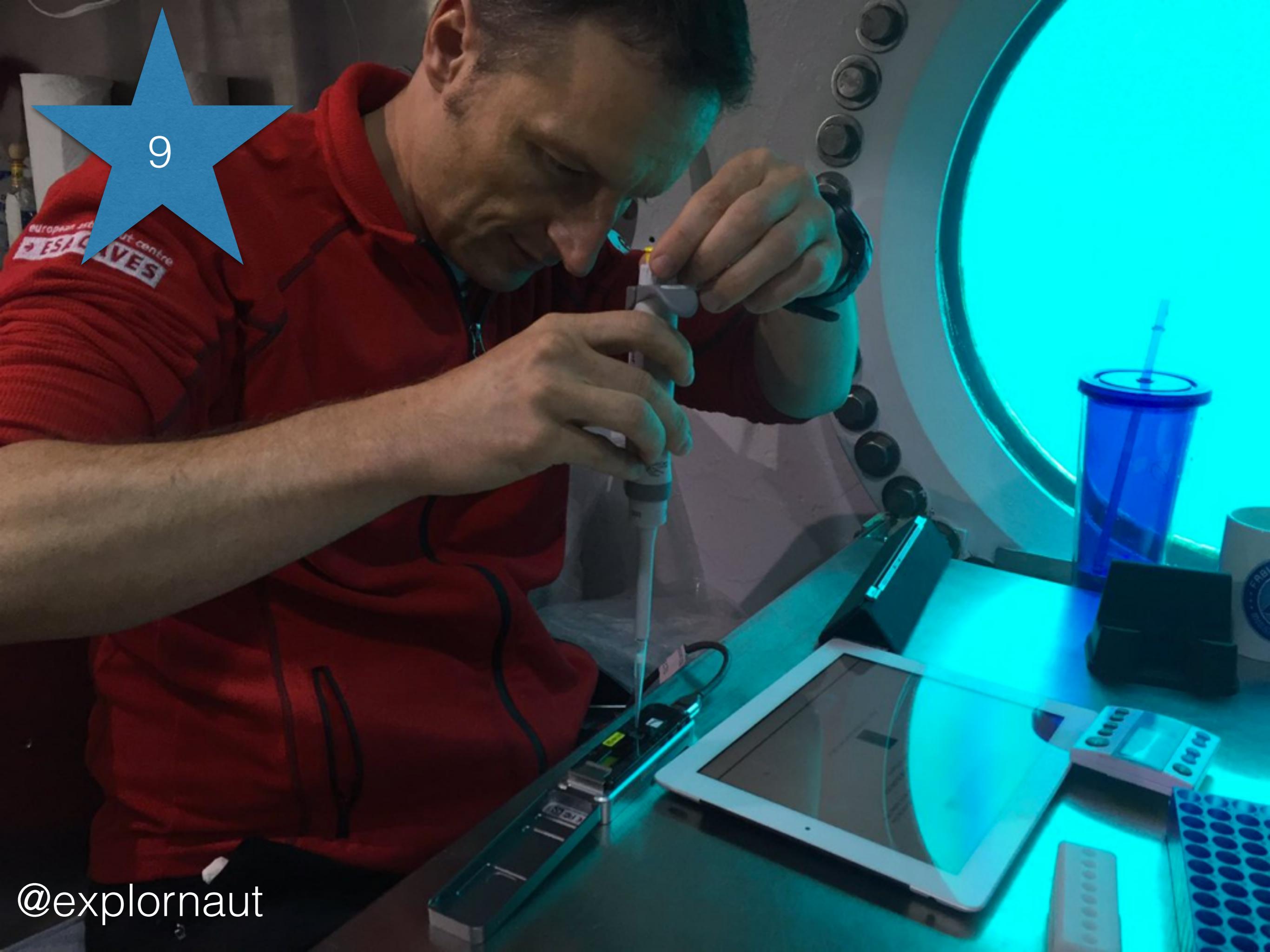
@arywnedwards



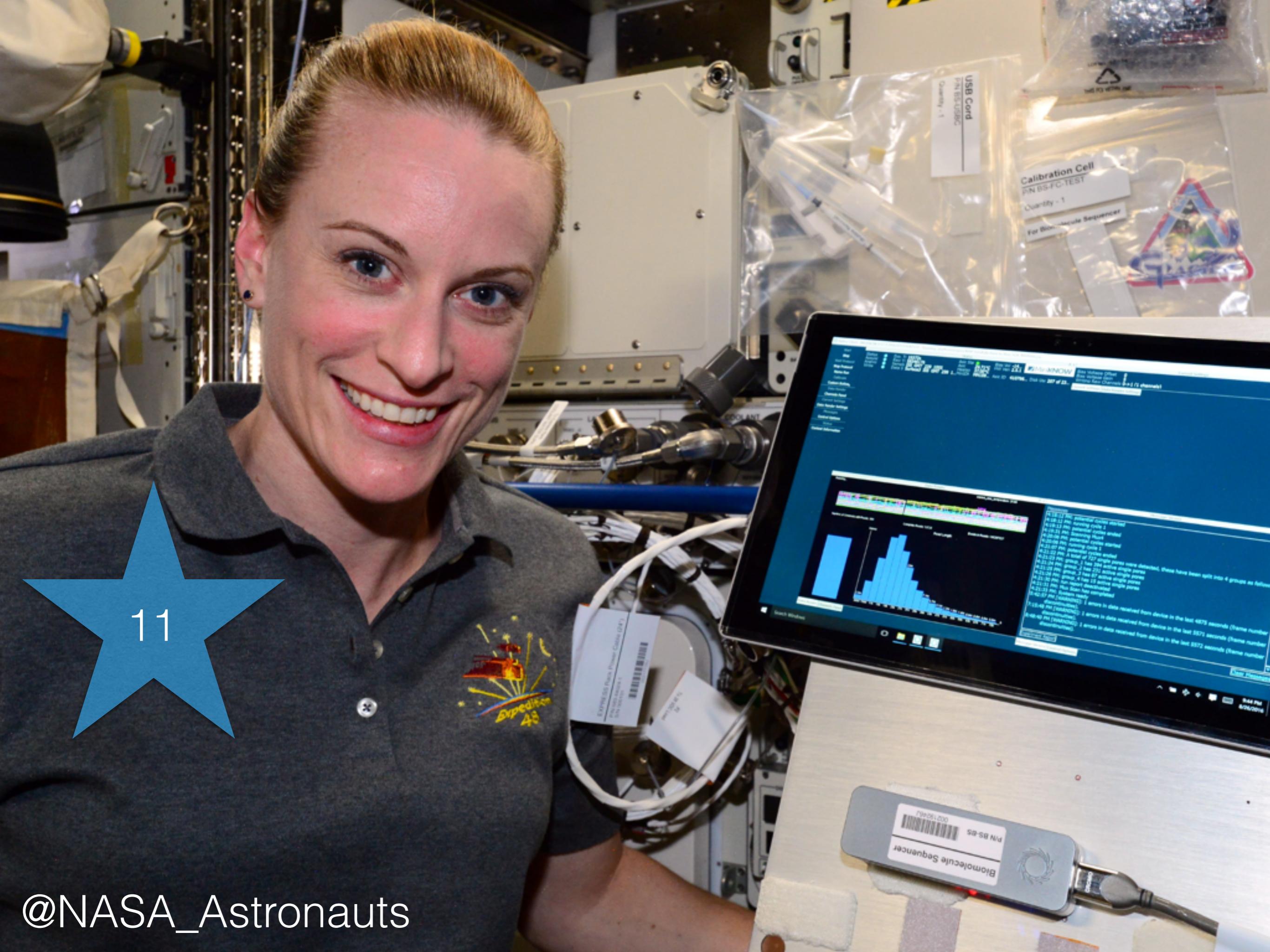
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9



@explornaut



@NASA_Astronauts

Evolution and epidemiology of Zika virus

Microcephaly and birth defects

ZiBRA project: present and future

Genetic diversity of Zika virus (ZIKV)

- ▶ Enveloped, icosahedral
- ▶ +ssRNA nonsegmented genome (11Kb)
- ▶ Transmitted by *Aedes aegypti* (*Culex*?)
- ▶ Identified in 1947 in Uganda
- ▶ Typically caused mild febrile illness
- ▶ 16 known cases until 2007
- ▶ Two distinct phylogenetic clades:
 - *African genotype*

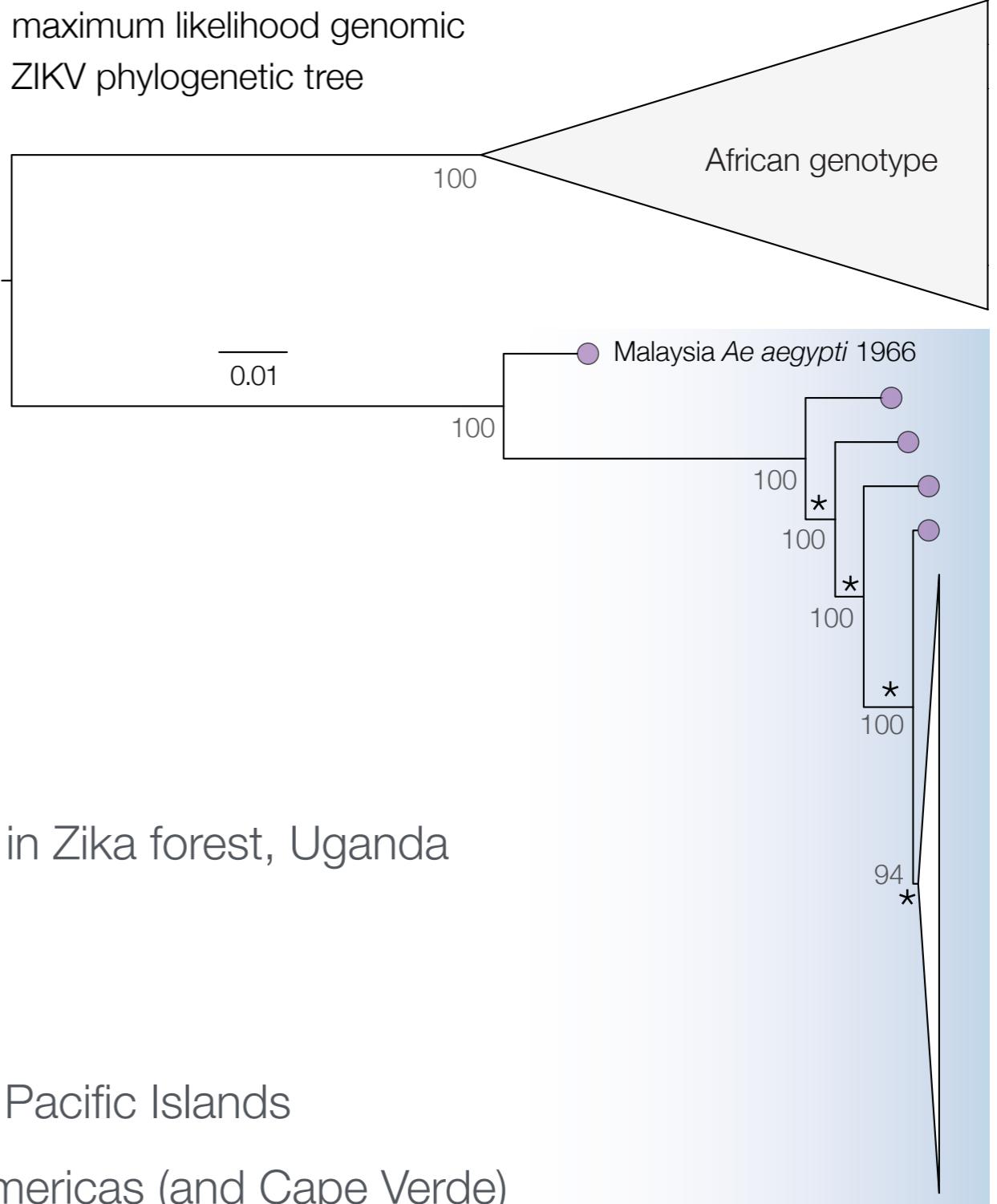
Isolated in 1947 from sentinel monkeys in Zika forest, Uganda

- *Asian genotype*

Isolated in 1966 in Malaysia

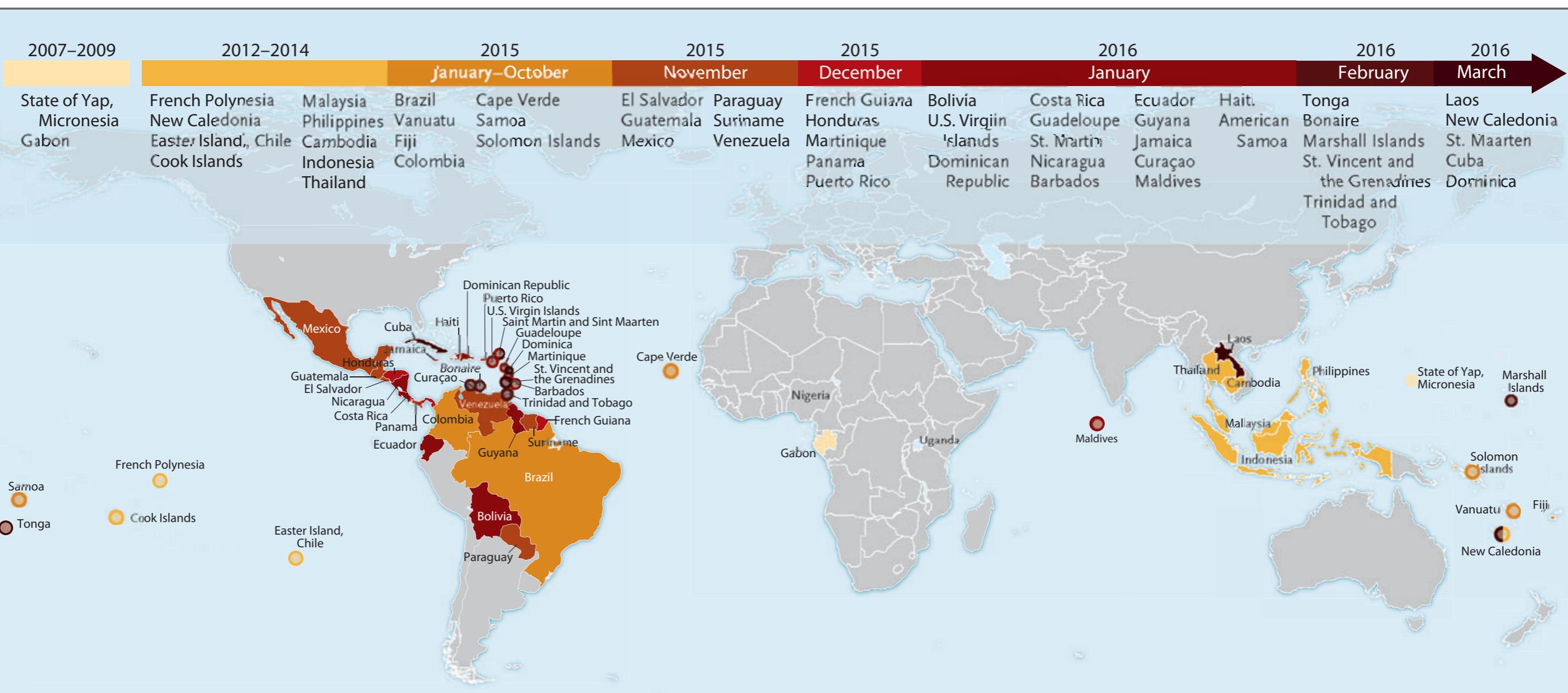
Spread throughout Southeast Asia and Pacific Islands

Causing the ongoing epidemic in the Americas (and Cape Verde)



Faria et al. Science 2016

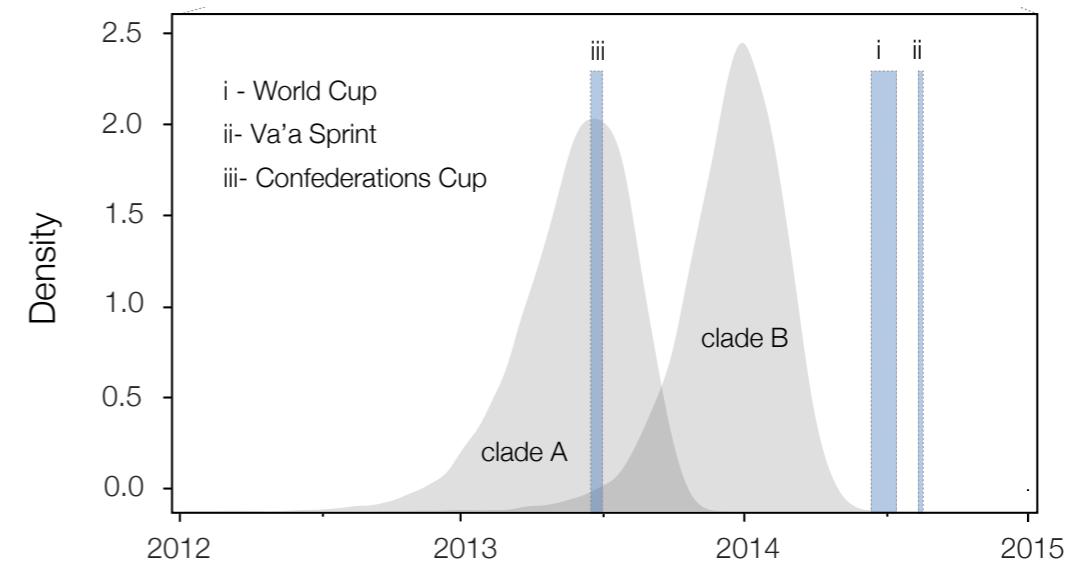
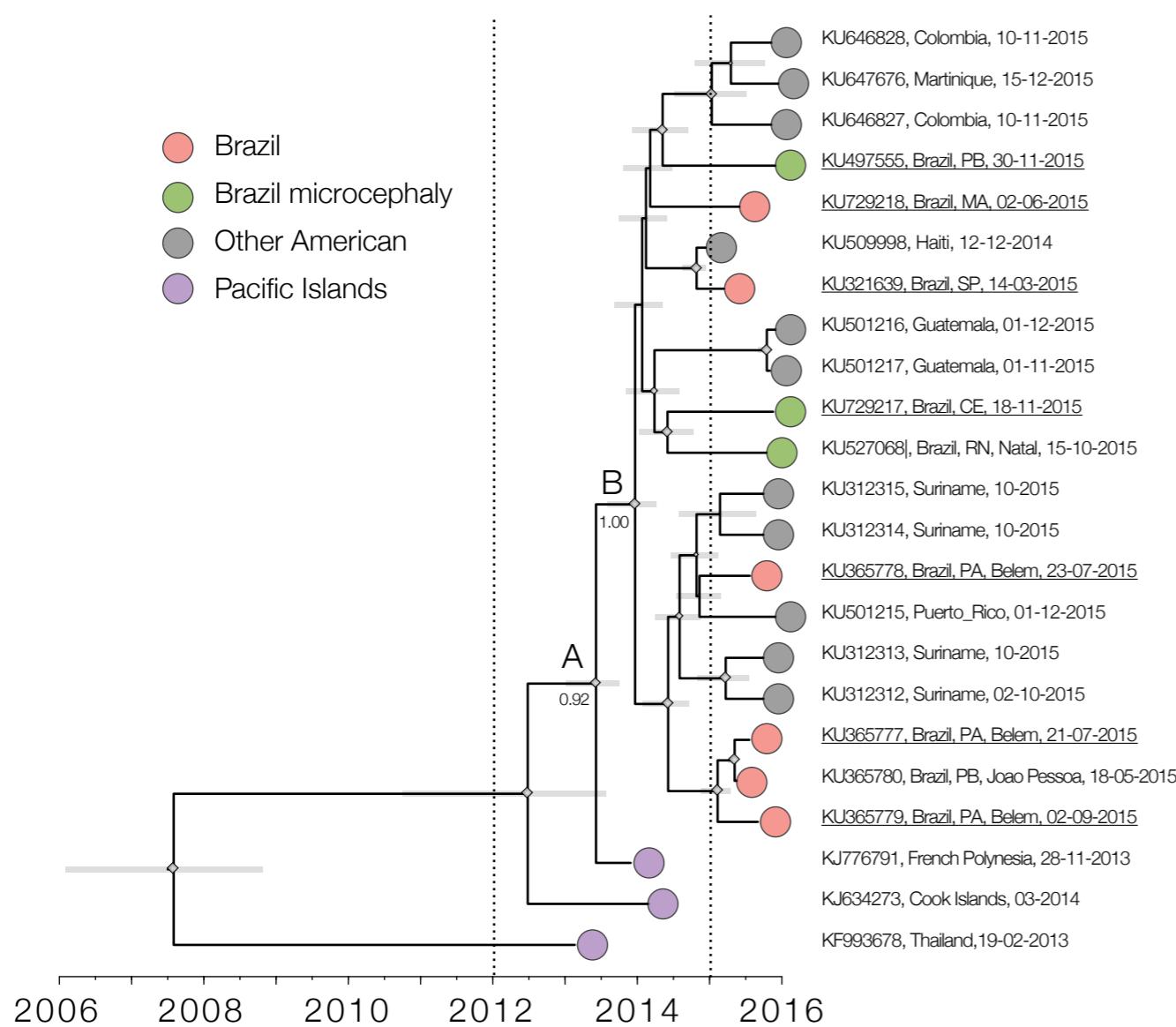
Timeline of Zika virus



Petersen et al., NEJM 2016

Zika virus was introduced in Brazil before April 2014

*ZIKV Asian genotype
molecular clock phylogeny*

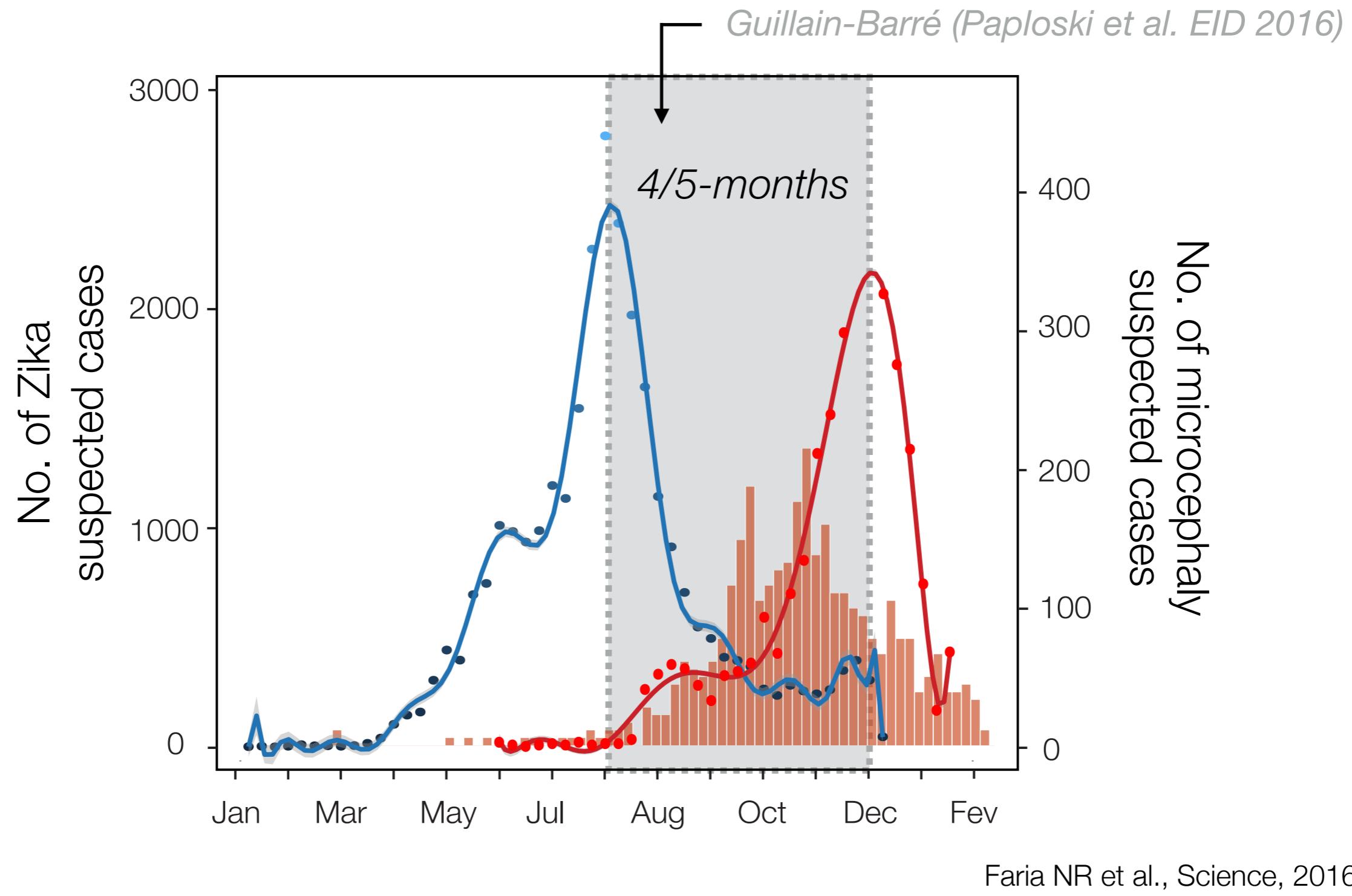


Zika virus has a high rate of evolutionary change (approx. 10^{-3} s/s/y)

Faria NR et al., Science, 2016



Temporal and spatial association between Zika virus and microcephaly notified cases



Unsolved questions

How did Zika virus spread so quickly compared to dengue?

Does its rapid spread involve other vectors (eg. Culex?)

When will the Zika virus epidemic “stop” in Brazil?

Impact of population-level immunity/cross-protection with other flaviviruses

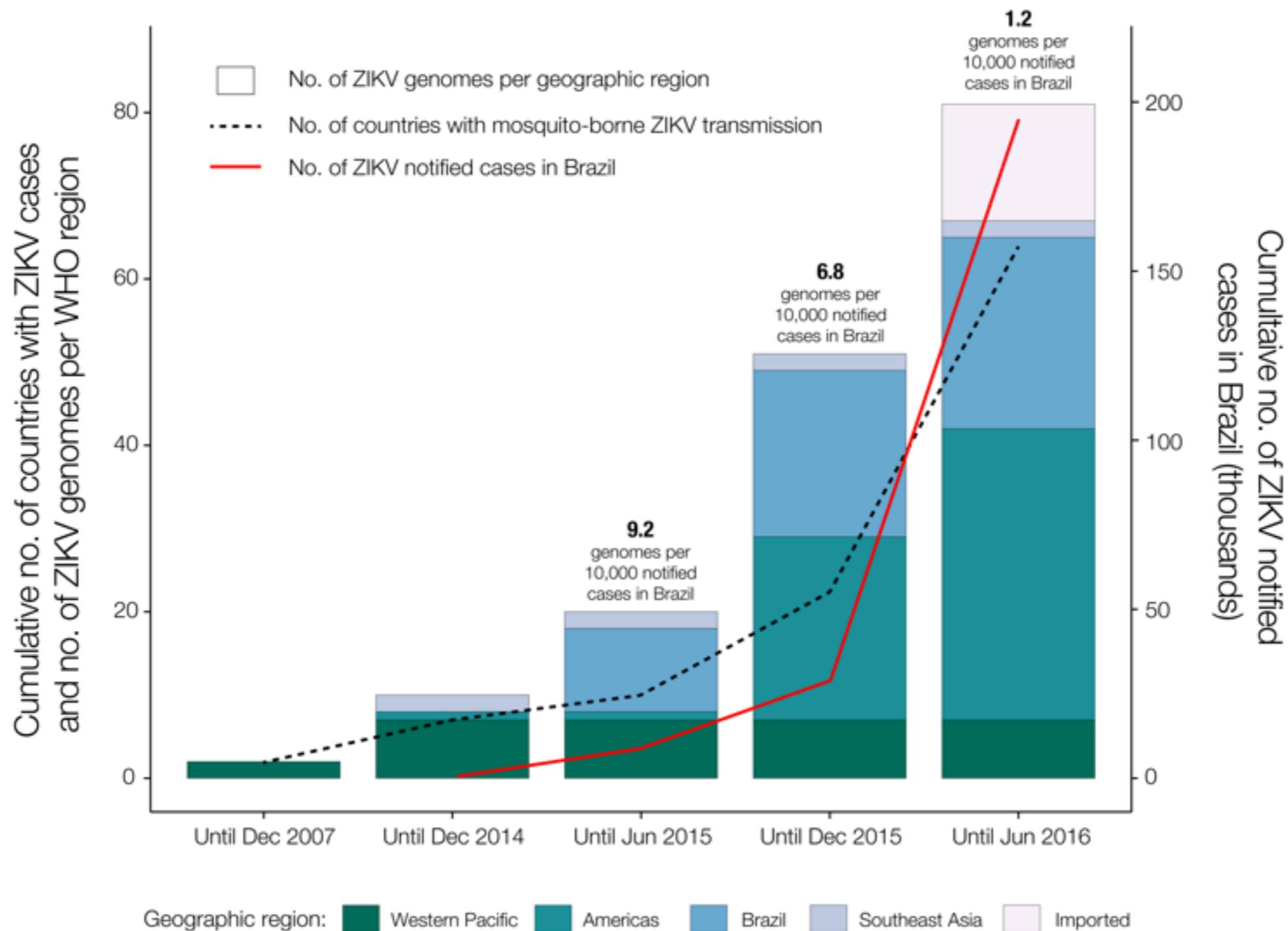
What drives the spread of ZIKV in the country?

Contribution of human mobility, population density, vector abundance

Are any pathogen genetic factors responsible for microcephaly?

Or is it simply a function of rapid spread in naive population?

Genome sequencing of the ZIKV epidemic in Brazil is currently very limited



Evolution and epidemiology of Zika virus
Microcephaly and birth defects
ZiBRA project: present and future

MRC

Medical
Research
Council

zibraproject.org



ZIKA IN BRAZIL REAL TIME ANALYSIS

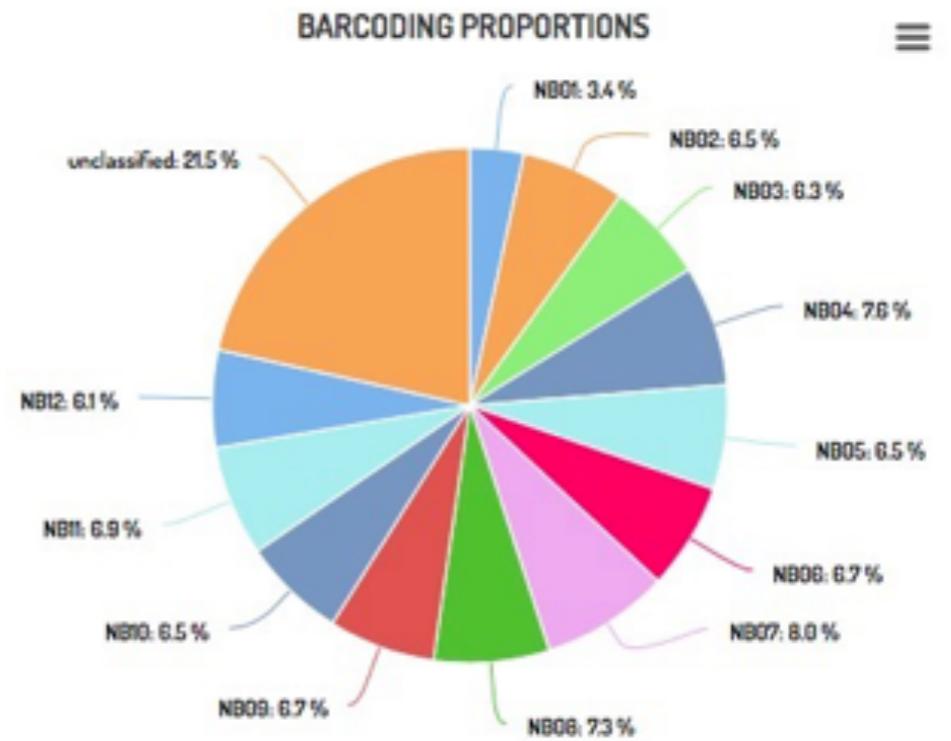


Project Goals

- ▶ 1000km road trip through affected regions of NE Brazil
- ▶ Sequence 750 genomes ...
- ▶ at a cost of <£50/genome - achieved through multiplexing
- ▶ Collect mosquitos at same locations
- ▶ Detect Zika and speciate mosquitos through RT and sequencing

Many differences to Ebola project

- ▶ Barcoding (native barcoding)
- ▶ Multiplex PCR scheme (35 amplicons in 4 pools)
- ▶ R9
- ▶ Volume not speed
- ▶ Mobile laboratory



What's new with R9?

Local basecalling!

Choose between nanonet and MinKNOW

Faster speeds

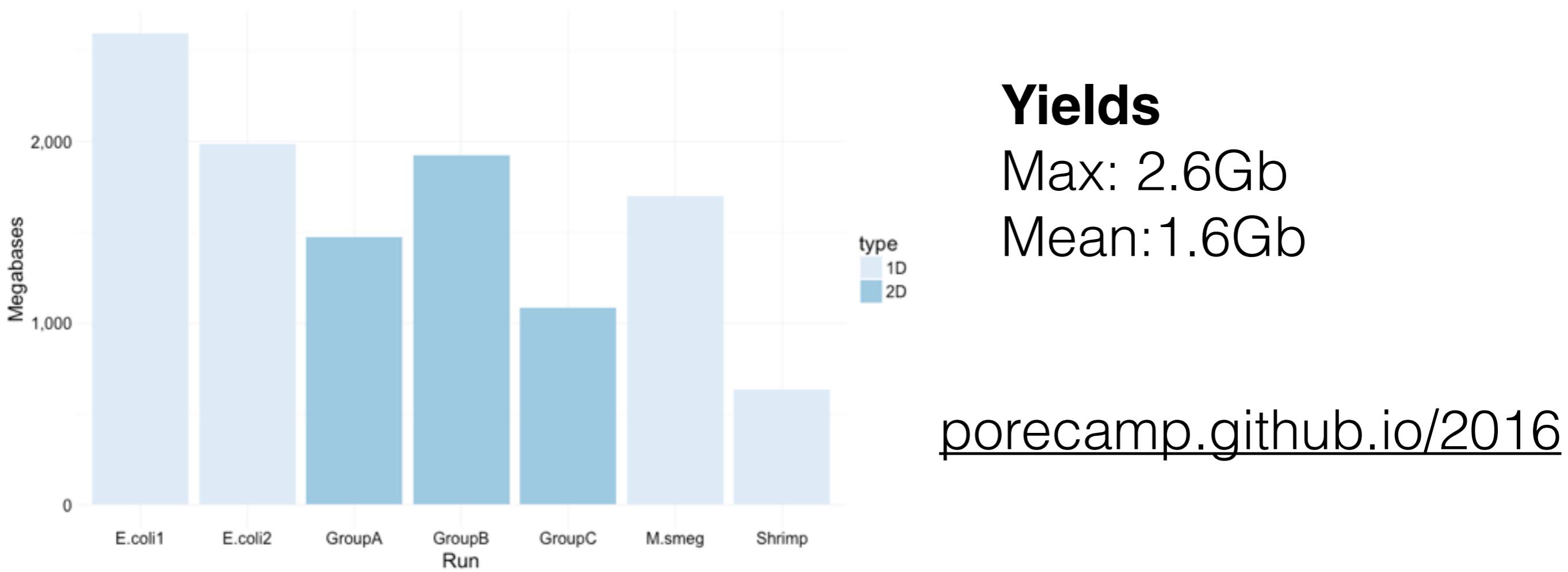
250bps versus 70bps - higher throughput

New pore

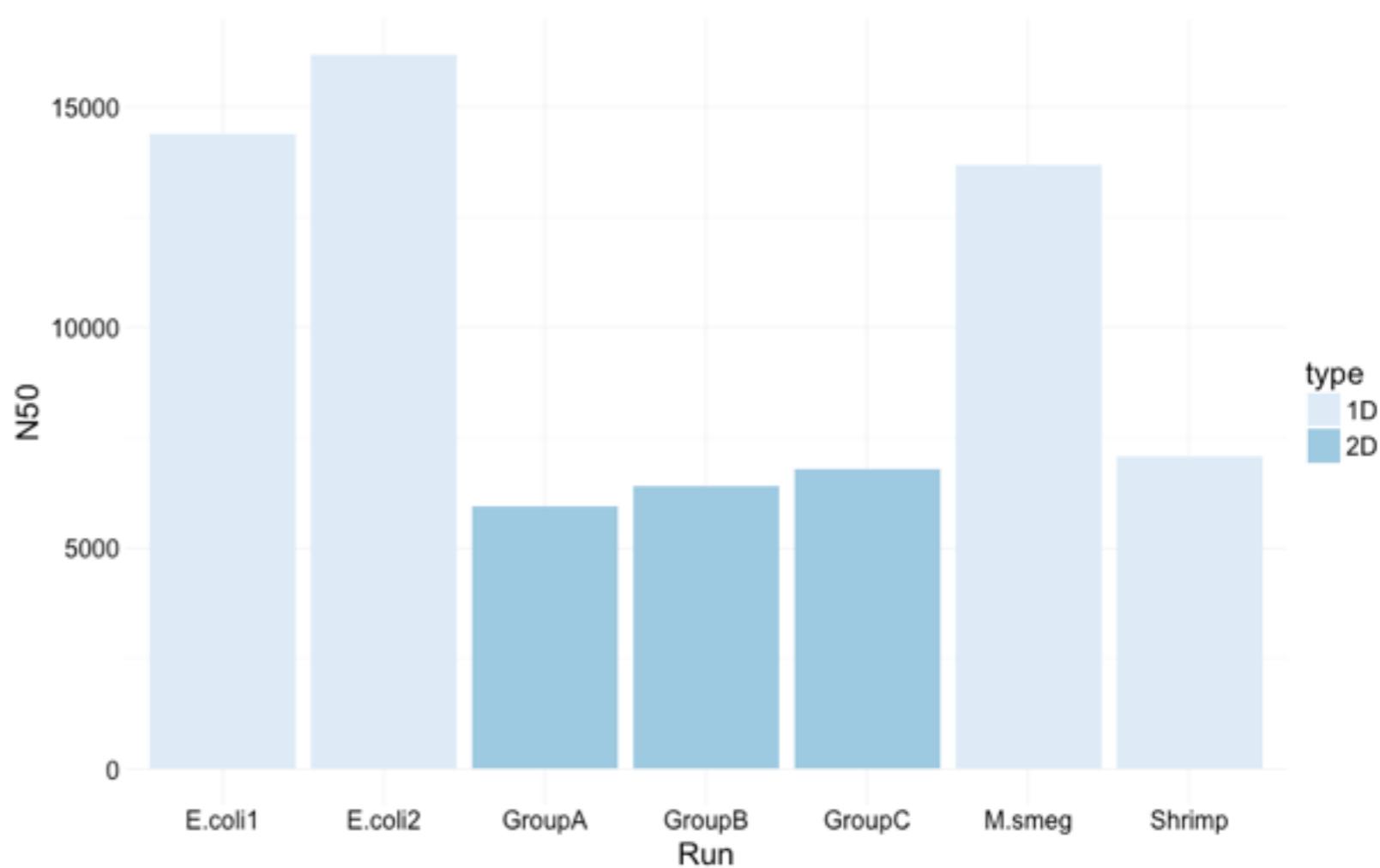
E. coli derived CsgG - gives much better (1D) accuracy

1D Rapid chemistry

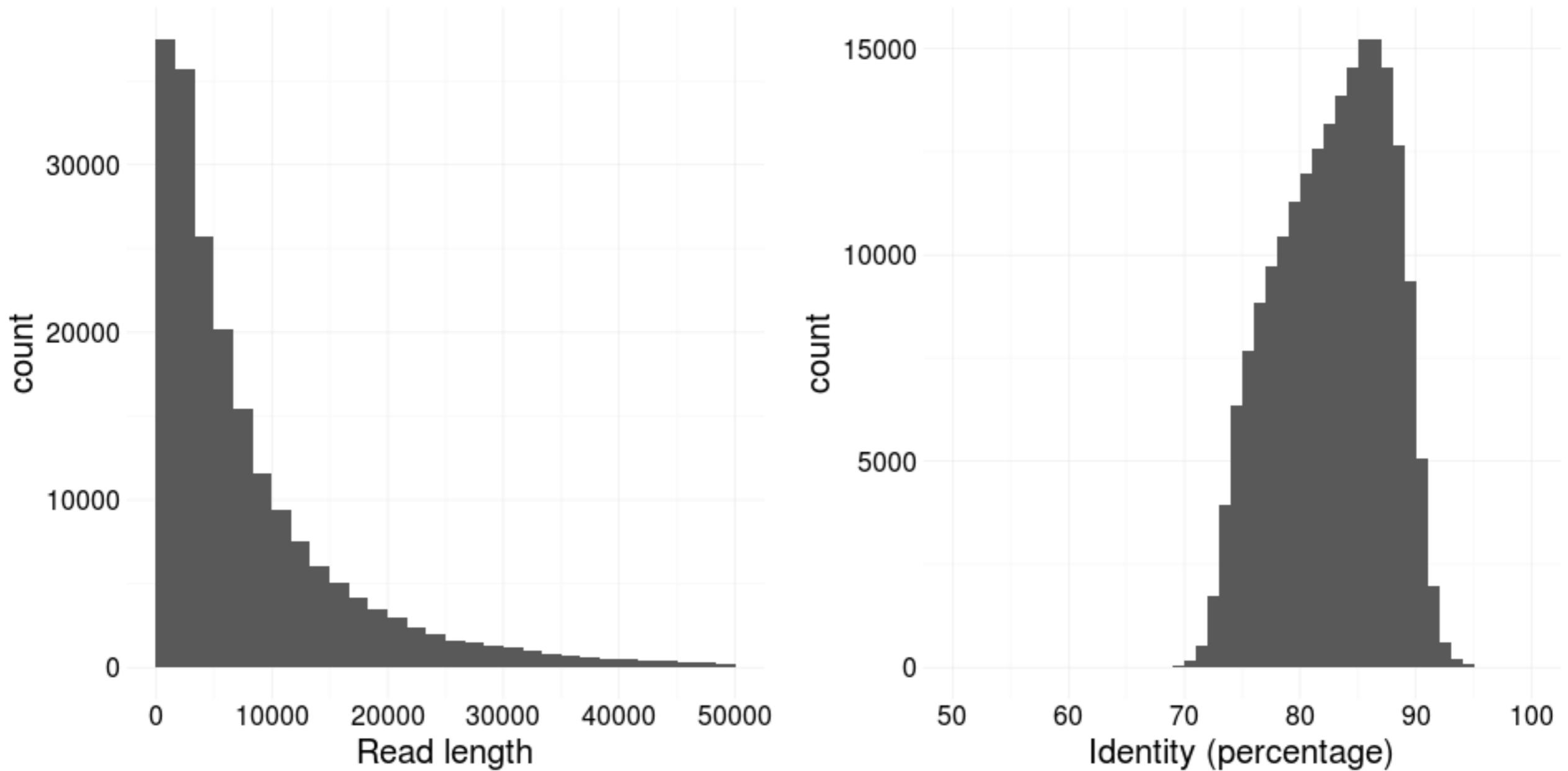
5-10 minute library preparation; very long reads



N50
Max: 16.1kb
Mean (1D): 12.8kb
Mean (2D): 6.3kb



R9 Rapid Kit Results: 1D reads







ZiBRA management team

team1

Database
RNA extractions

team2

RT-PCR detections
Virus sequencing

team3

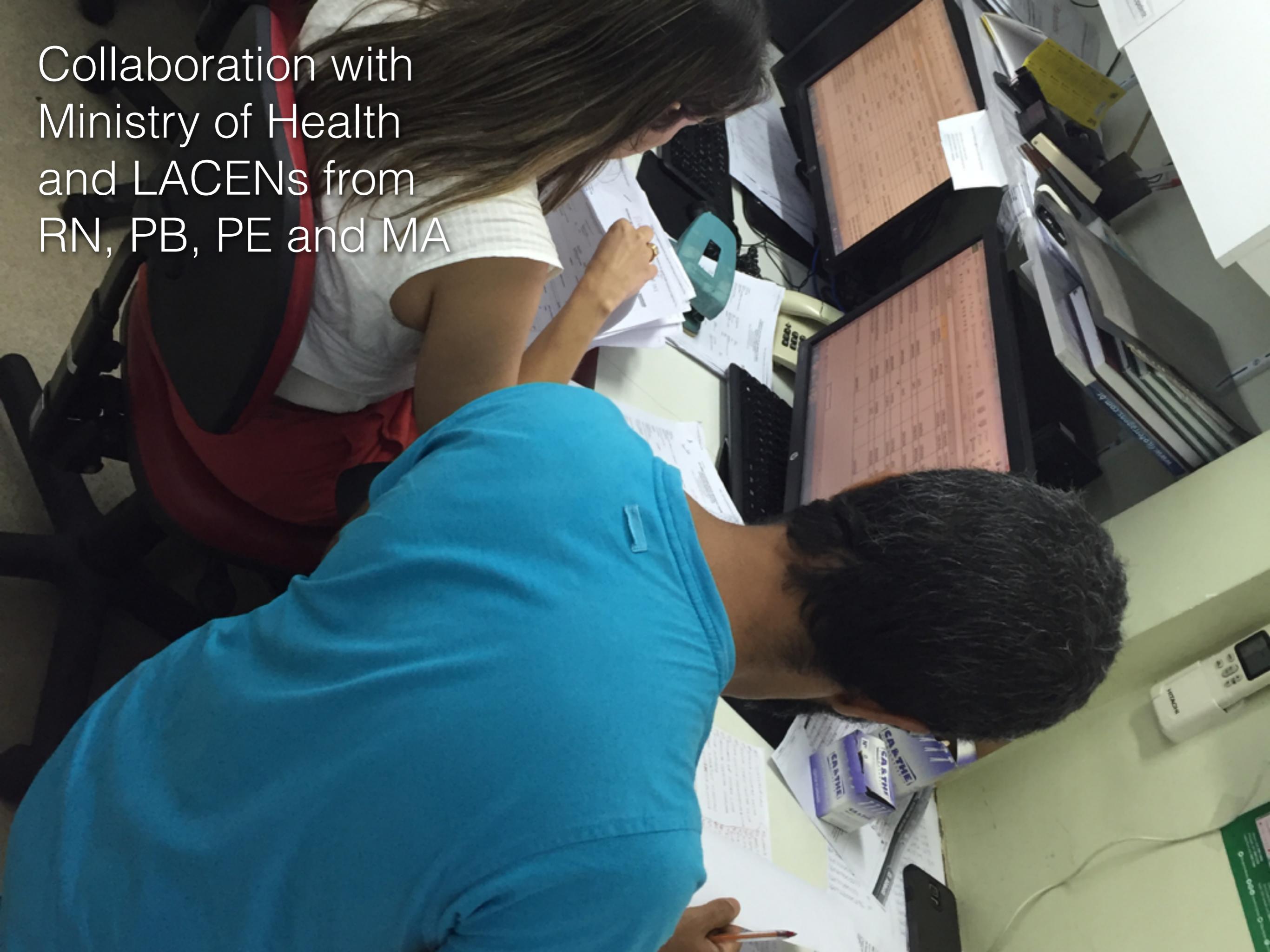
Mosquito capture
and identification

team4

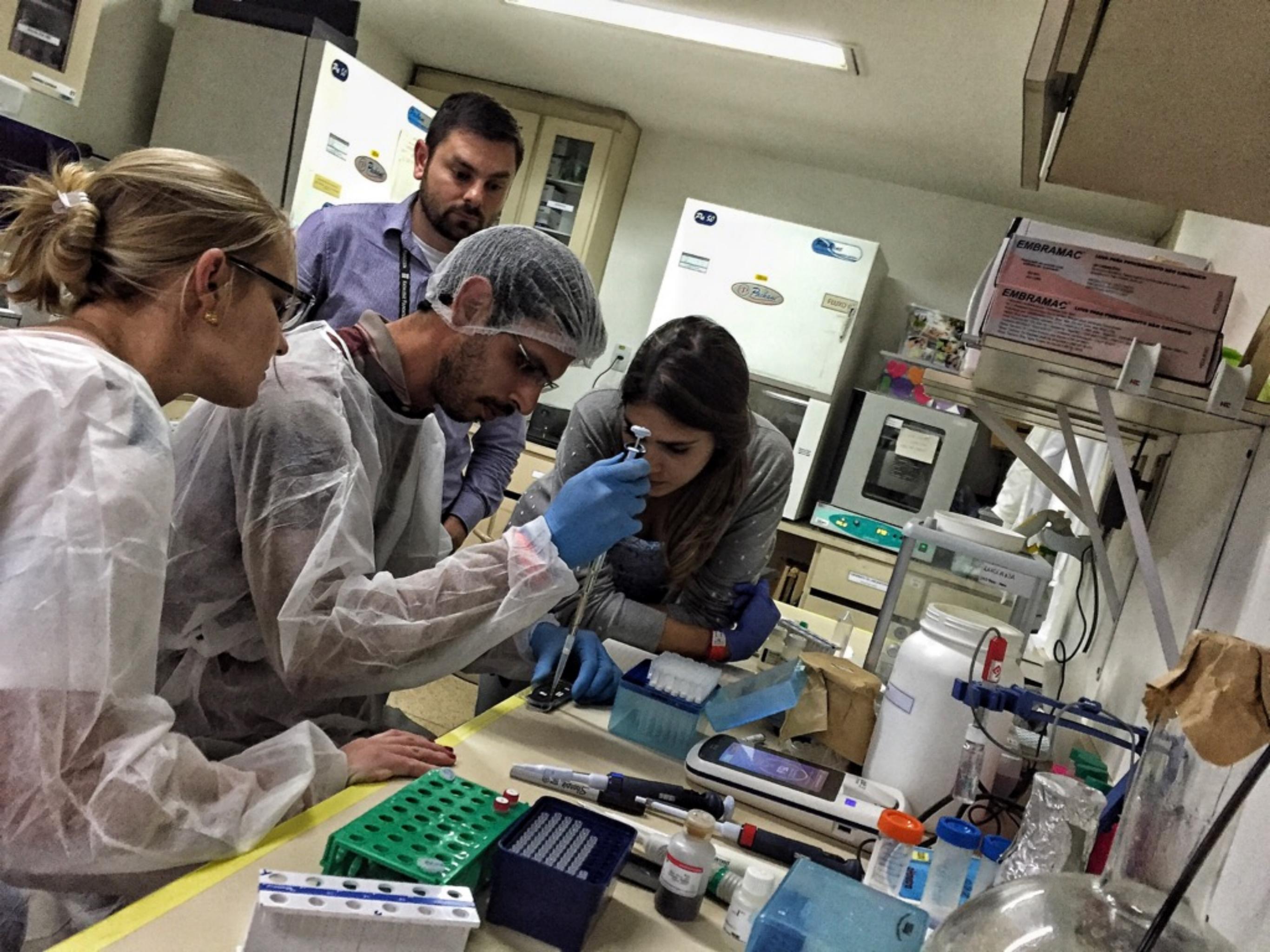
IT and analyses
Media coverage

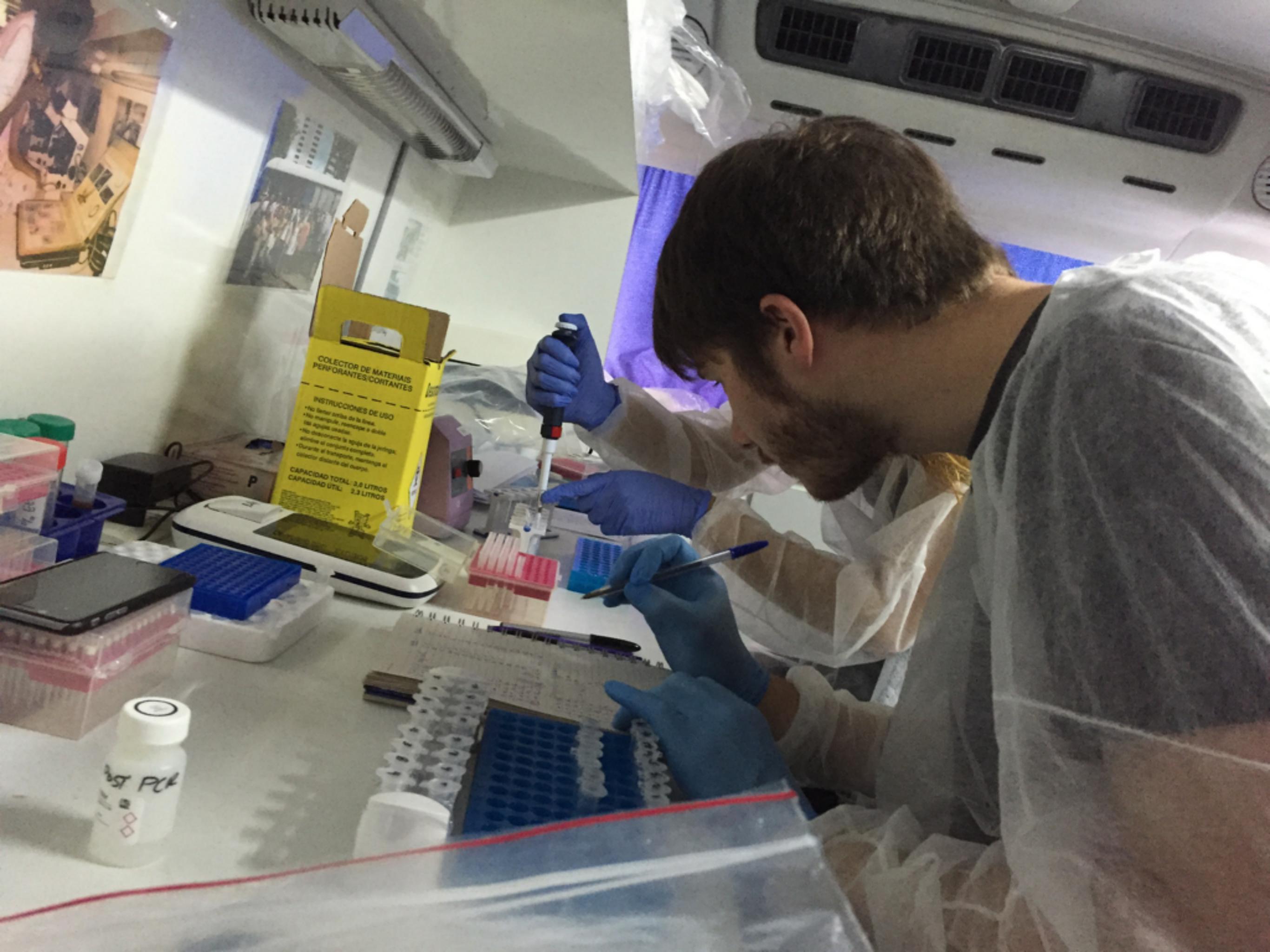


Collaboration with
Ministry of Health
and LACENs from
RN, PB, PE and MA









COLECTOR DE MATERIAIS
PERFORANTES/CORTANTES

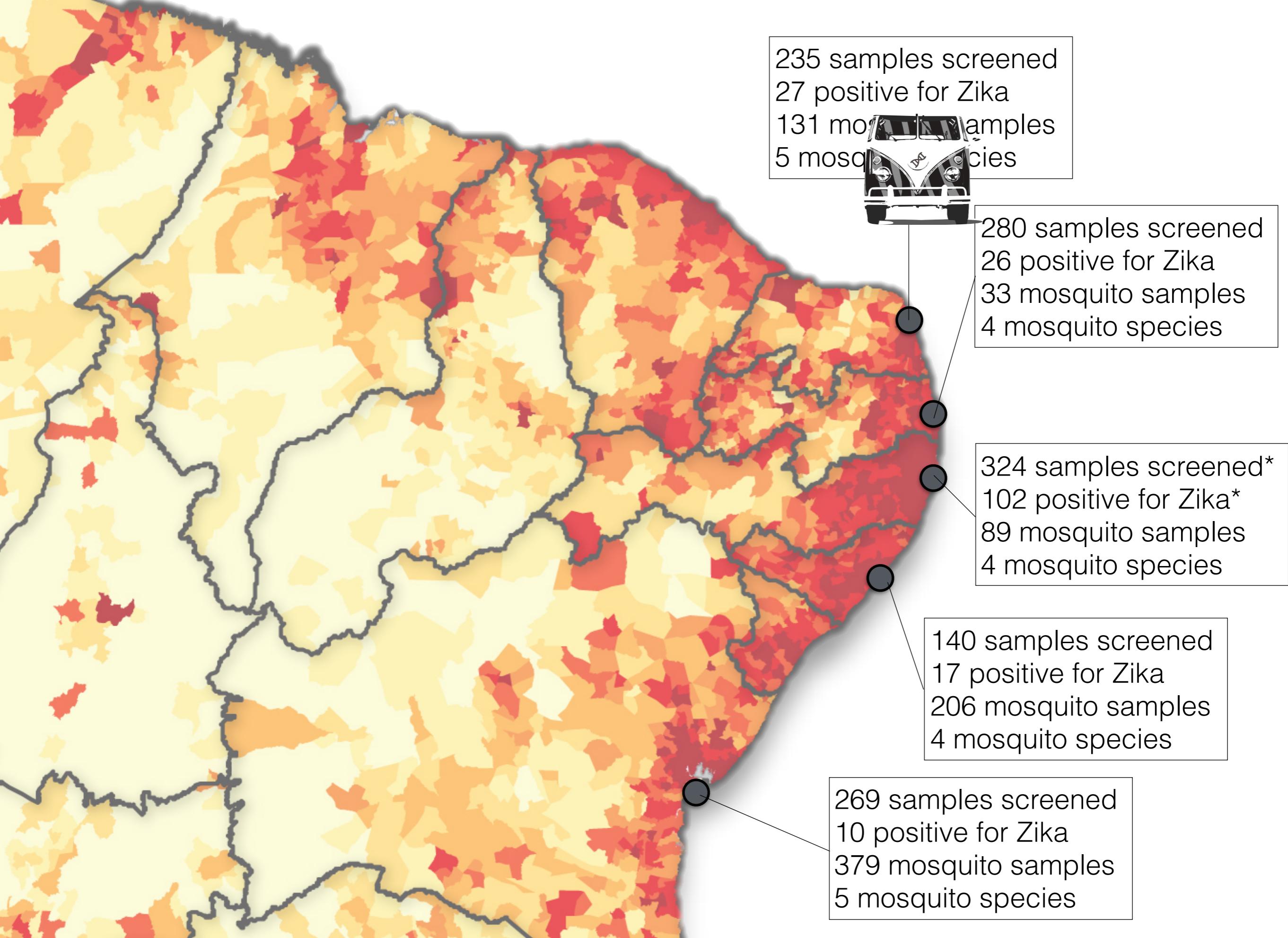
INSTRUCCIONES DE USO

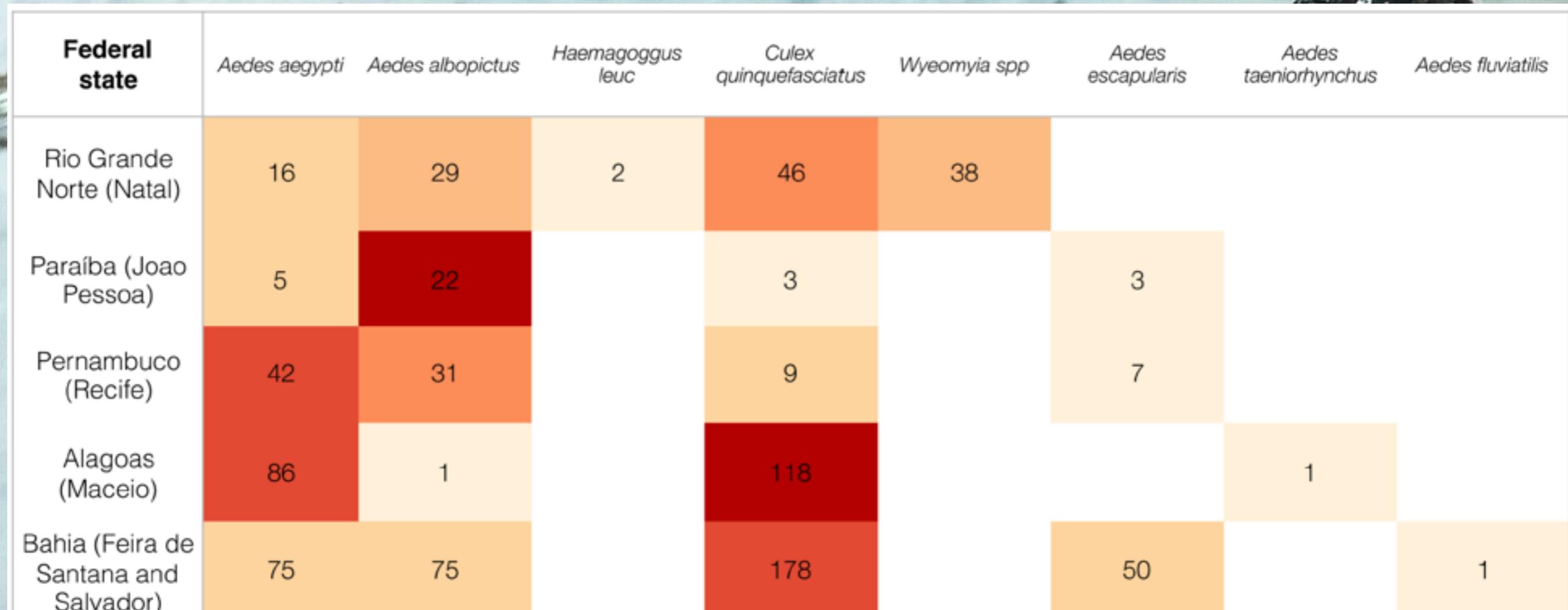
- No llenar más de la línea.
- No manipule, remueva o doble las agujas usadas.
- No desconecte la aguja de la jeringa.
- Elimine el conjunto completo.

CAPACIDAD TOTAL: 3,0 LITROS
CAPACIDAD UTIL: 2,3 LITROS









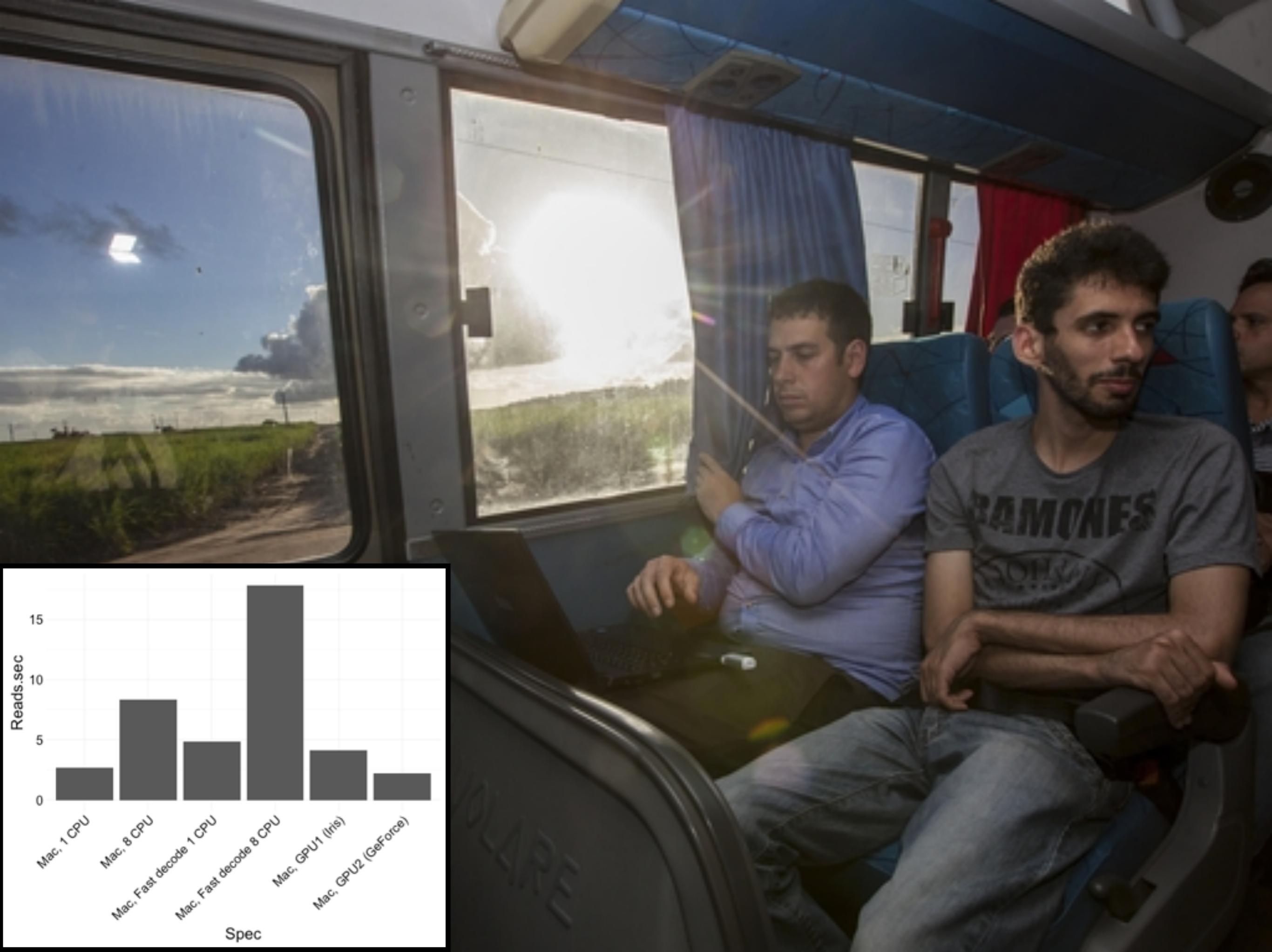
Absent 1 10 20 30 40 50 % of total population



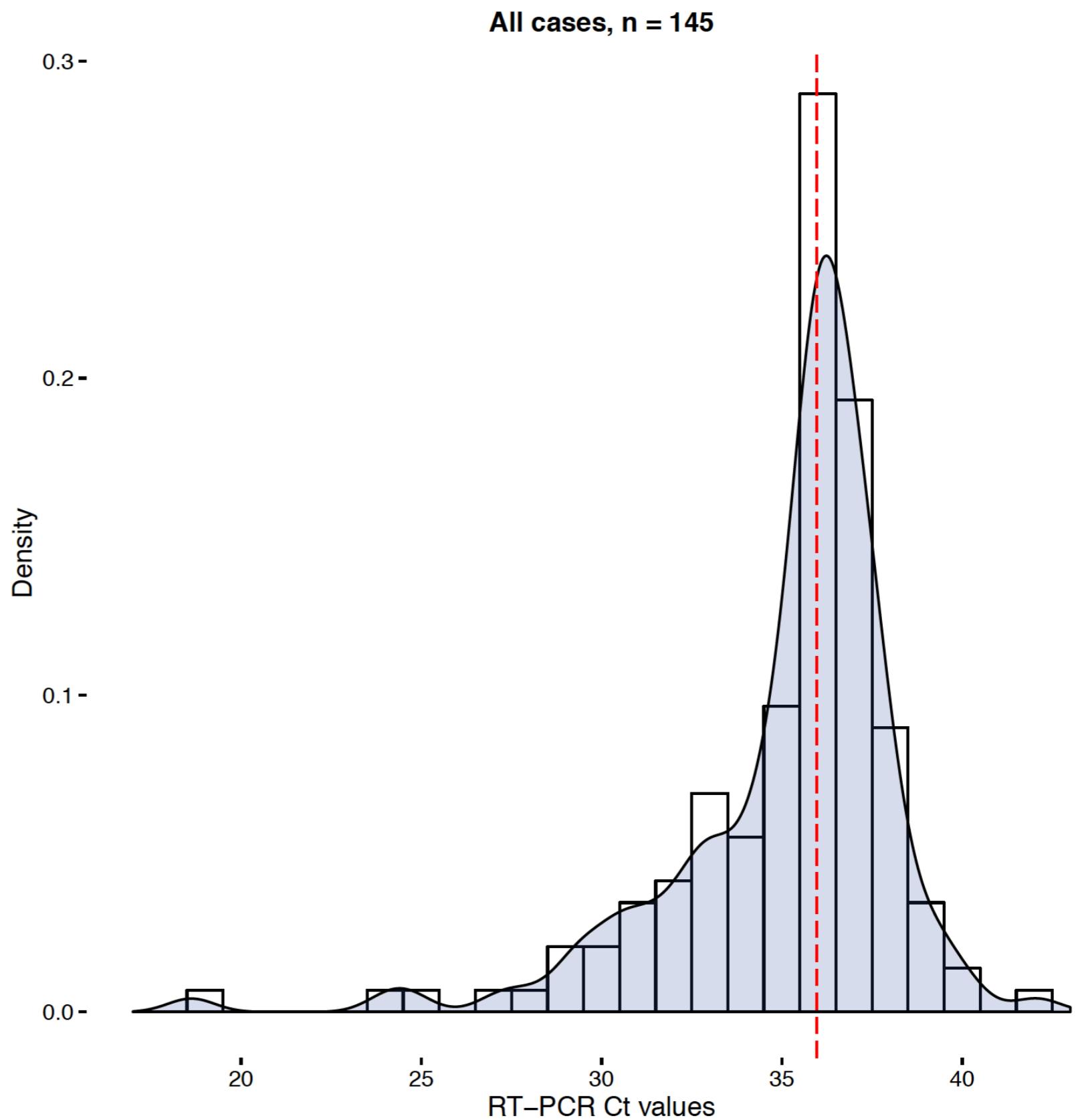
Aedes aegypti captured in
Salvador, Bahia
ZiBRA | 16 June 2016

~55 libraries sequenced in 1 week over 5 runs

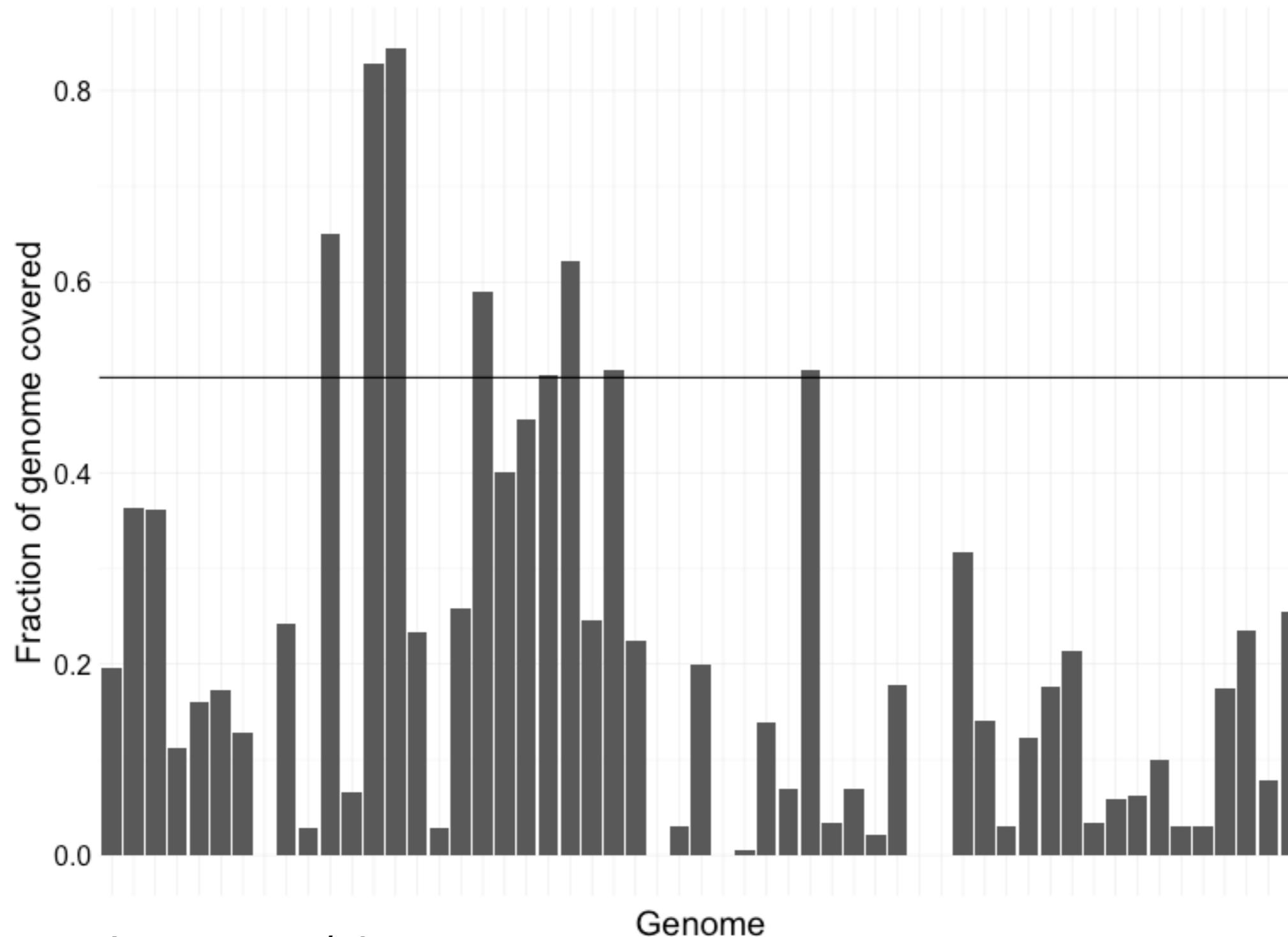




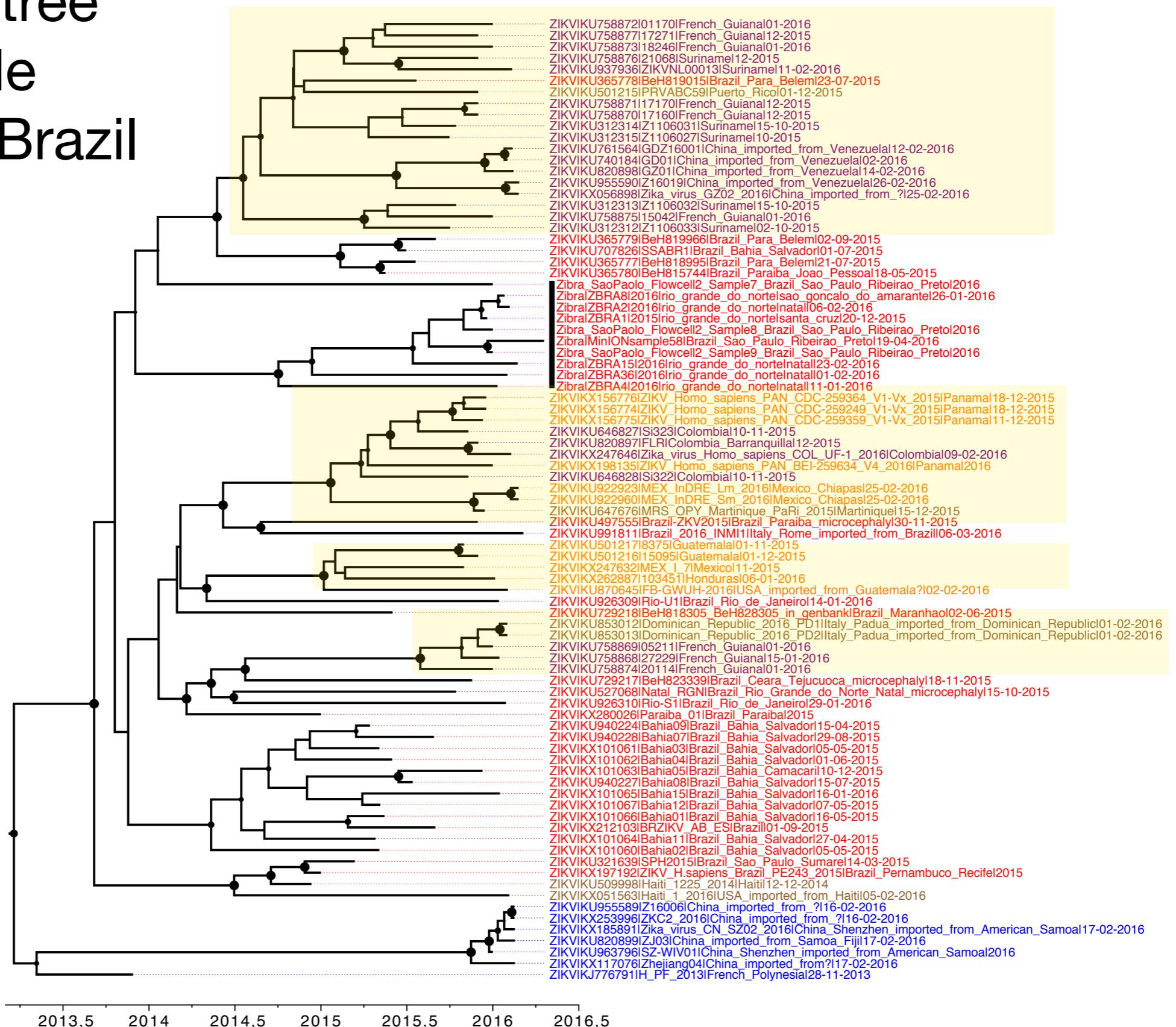
Technical challenges:
Zika Ct values are
very low



Just eight genomes have >50% coverage



Phylogenetic tree shows multiple exports from Brazil



Zika virus pilot

By Karthik Gangavarapu | April 5, 2016 | Data

We recently received plasma samples from Zika virus (ZIKV) patients in Colombia. We performed QC on the samples and unfortunately very few had detectable levels of ZIKV by qPCR. We extracted RNA from two of these patient samples (Z184 and Z186), as well as a positive control (seed stock of the Malaysian strain P6-740 passaged once on BHK-21 cells) kindly provided by Nathan Grubaugh and Greg Ebel at [Colorado State](#).

Samples

- Z184, 42 year old female with fever, rash, joint pain, myalgia, eye pain, and cephalgia. Symptom onset December, 2015 [2 ZIKV reads]
- Z186, 33 year old male with fever, rash, joint pain, myalgia, eye pain, and cephalgia. Symptom onset December, 2015 [33 ZIKV reads]
- P6-740, positive control of Malaysian strain P6-740 passaged once on BHK-21 cells [20,729 ZIKV reads]

<http://andersen-lab.com/zika-virus-pilot/>

PRIMAL SCHEME

Scheme name

Email

Fasta

No file chosen

One or more viral reference genomes in FASTA format

Amplicon length

Overlap

 Generate my scheme!

Want to try it out but no genomes to hand?

[Download rabies.fasta](#)

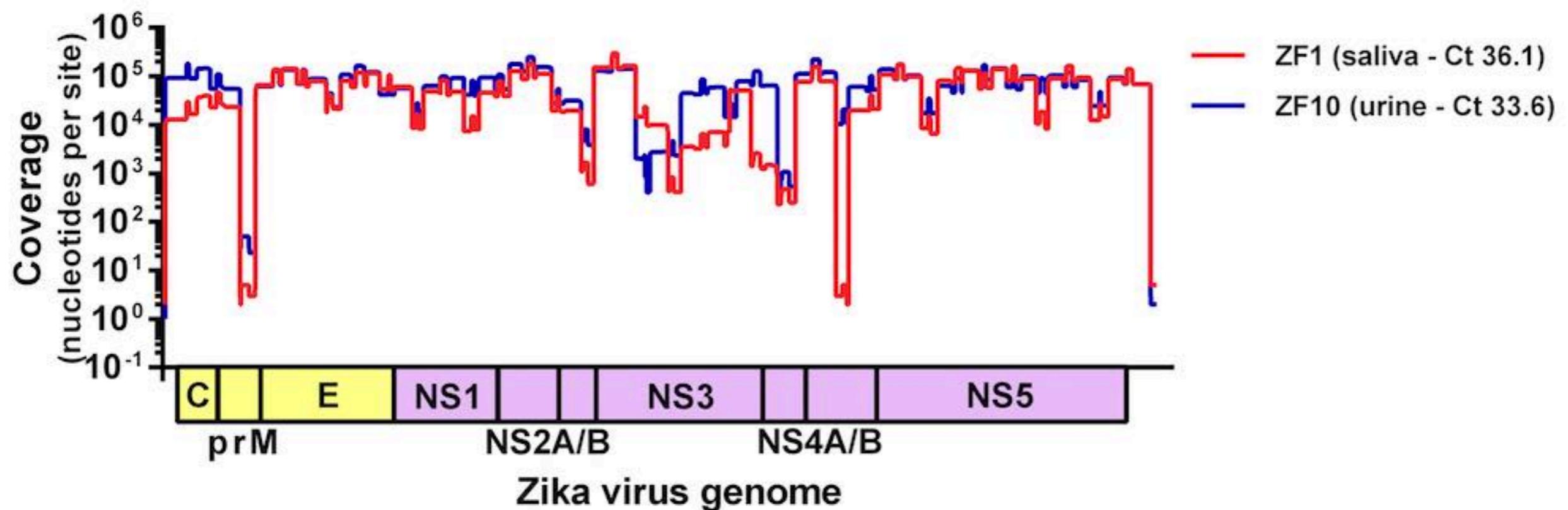
Primer Table

Region No.	Pool	Left Primer Name	Left Primer Sequence
1	1	400_1_LEFT_3	AGCAGAAAGTGTCAAGTTGAAAGCA
2	2	400_2_LEFT_1	AGCAATGCAGTTCTTGAGGGG
3	1	400_3_LEFT_4	GAGCAAATATCAGGACAAAACACCG
4	2	400_4_LEFT_2	ATGAAGACTGTTCAAGGGCTGGT
5	1	400_5_LEFT_0	GGGTCAAGTCAGATCCCTAAATGC
6	2	400_6_LEFT_4	AACCAGAAGTCCAGAAGCTGTCT
7	1	400_7_LEFT_4	GATCTTCAATCCGAGTGCT
8	2	400_8_LEFT_1	TAGCCTCCTGTTCCAGTCGTAC
9	1	400_9_LEFT_3	GAAAGCCAATCCTCGAAAGCCA
10	2	400_10_LEFT_0	ACGGATCCAAACTCCCCCTAAG
11	1	400_11_LEFT_0	AAAAACCCCTCCTGTGTCAGC
12	2	400_12_LEFT_0	ATTAGCTTGTCAAGGAGCCCCA

Version 2.0 protocol now in use in USA and UK



Ecuadorean sample Ct 26.7 sequenced at Public Health England on MinION



Two Florida samples Ct 33.6 and 36.1 sequenced in the Andersen Lab

Conclusions

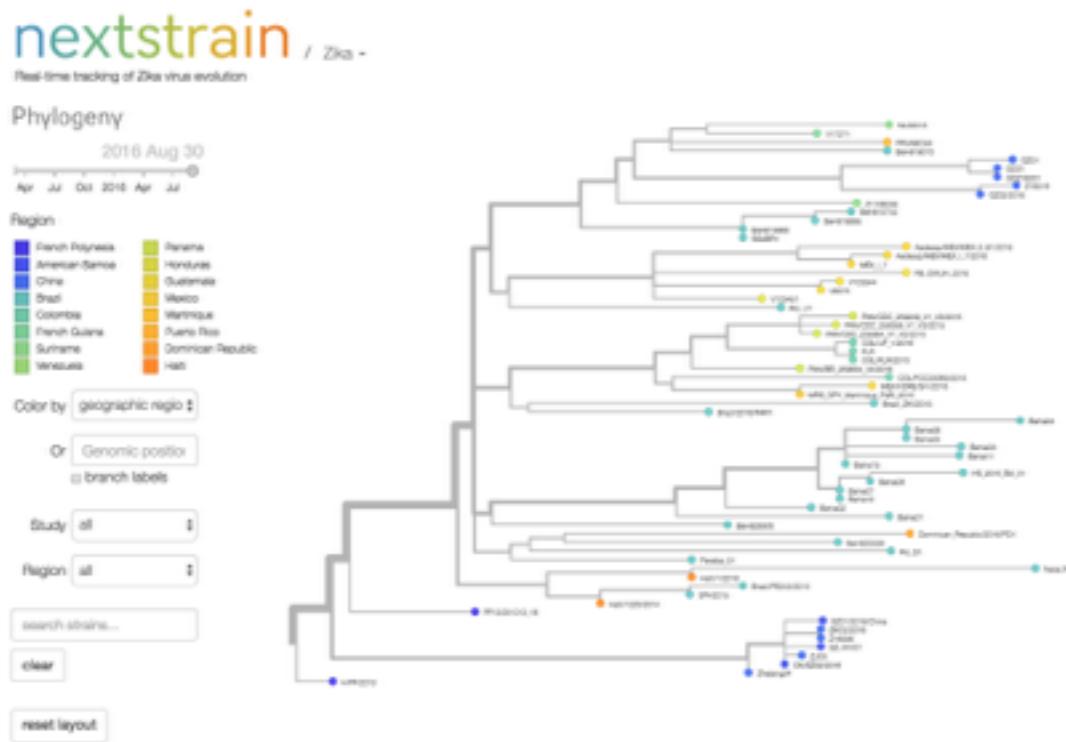
Mobile sequencing is here

But sample preparation lags behind our ability to sequence

**Many technical and logistical challenges
sequencing Zika in Brazil**

But real-time genomic surveillance coming online now

nextstrain.org/zika



'Nobody's looking': why US Zika outbreak could be bigger than we know

Zika tests are complicated, time-consuming and expensive, leading scientists to believe states at risk for the disease may already have undetected cases



A Miami-Dade county mosquito inspector sprays around homes in the Wynwood area of Miami. Local mosquitos have infected 15 people with the Zika virus. Photograph: ddp USA/Rex/Shutterstock

On the road team (1)

Nicholas Loman, Institute of Microbiology and Infection, University of Birmingham

Luiz Alcantara, Fundação Oswaldo Cruz (FIOCRUZ), Salvador, Bahia, Brazil

Nuno Rodrigues Faria, University of Oxford, United Kingdom

Marcio Nunes, Instituto Evandro Chagas, Ministry of Health, Belém, Brazil

Josh Quick, Institute of Microbiology and Infection, University of Birmingham

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Trevor Bedford, Fred Hutchinson Cancer Research Center, United States

Bruna Nascimento, Instituto Evandro Chagas, Ministry of Health, Belém, Brazil

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Magliones (director of the LACEN Maceio)

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Constancia Ayres (coordinator of the Department of Entomology, FioCruz Recife)

Manoel Barral Neto (director FioCruz Bahia)



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Themis Rocha de Souza, Laboratório Central do Rio Grande do Norte, Universidade Potiguar

Joao Felipe Bezerra, Universidade federal do RN, Faculdade Natalense de Ensino e Cultura

Claudio Maerovich, Diretor do Departamento de Doenças Transmissíveis, Ministry of Health

Giovanini Evelim Coelho, Programa Nacional de Controle da Dengue, Brazilian Ministry of Health

Mariana Verotti, Coordenadora de Laboratórios, Brazilian Ministry of Health

Wanderson Kleber de Oliveira, Brazilian Ministry of Health

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Matt Loose, University of Nottingham, Nottingham, United Kingdom

Paul Klenerman, University of Oxford, United Kingdom

Steve Pullan, Public Health England, Porton Down, United Kingdom

Jared Simpson, Ontario Institute for Cancer Research, Toronto, Canada

Pedro Vasconcelos, Instituto Evandro Chagas, Ministry of Health, Belém, Brazil

Charles Chiu, University of California San Francisco, United States

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Julio Rosa Croda, FioCruz Mato Grosso do Sul, Cuiabá

Silvana Beutinger Marchioro, Universidade Federal da Grande Dourados - UFGD, Dourados

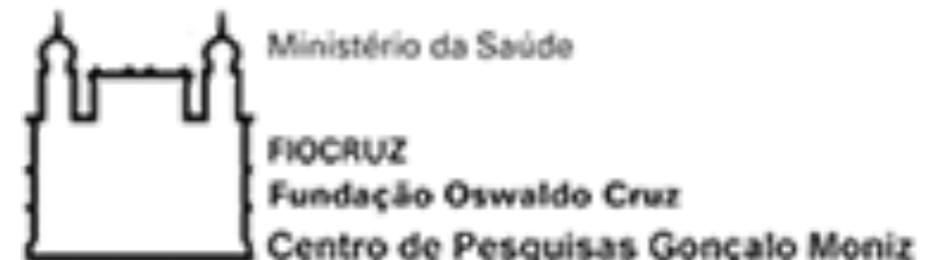
Rosemary Dokos, Oxford Nanopore Technologies

Oliver Hartwell, Oxford Nanopore Technologies

Richard Ronan, Oxford Nanopore Technologies

<http://zibraproject.github.io/>

<https://twitter.com/zibraproject>



Thank you

