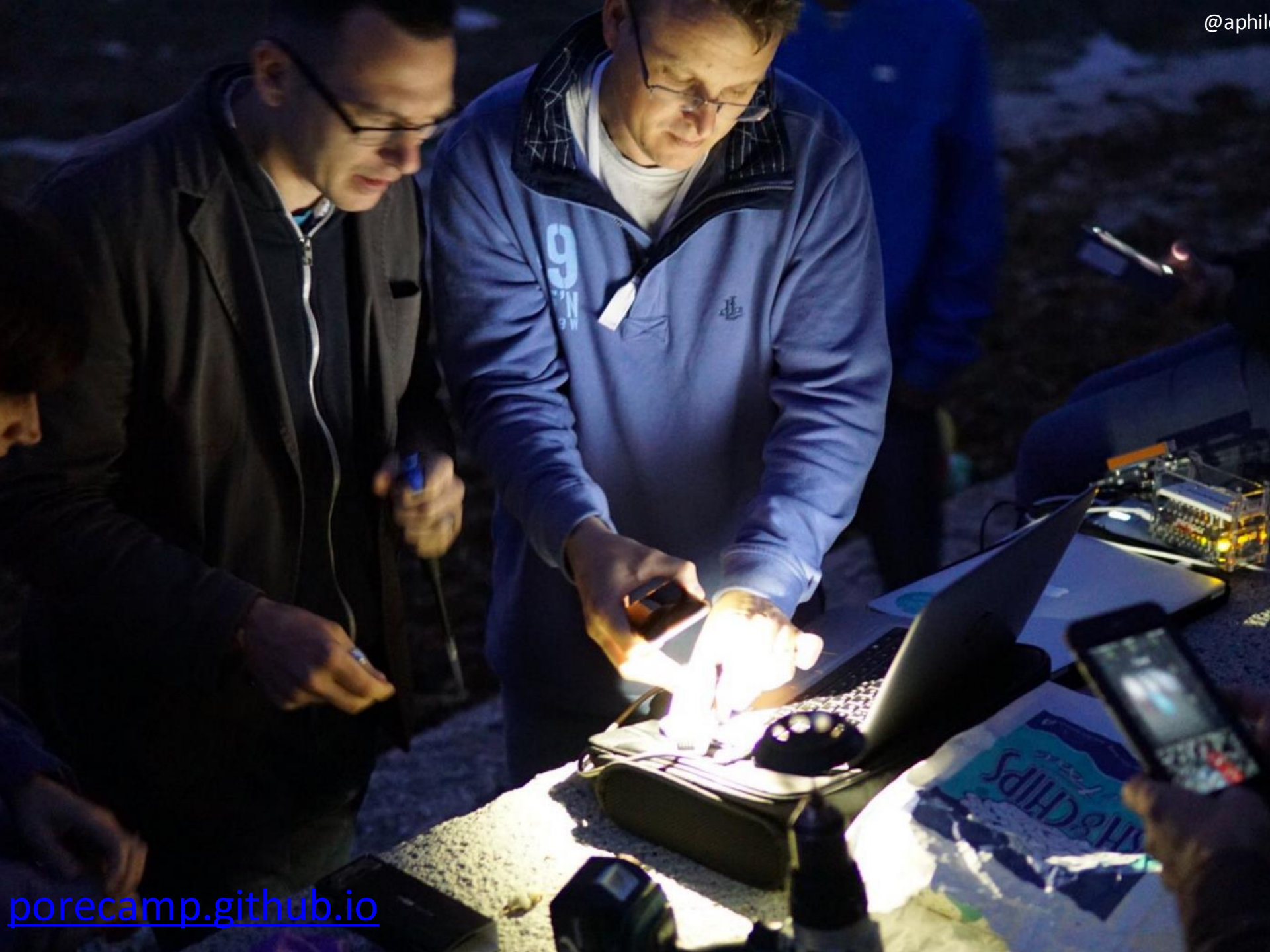


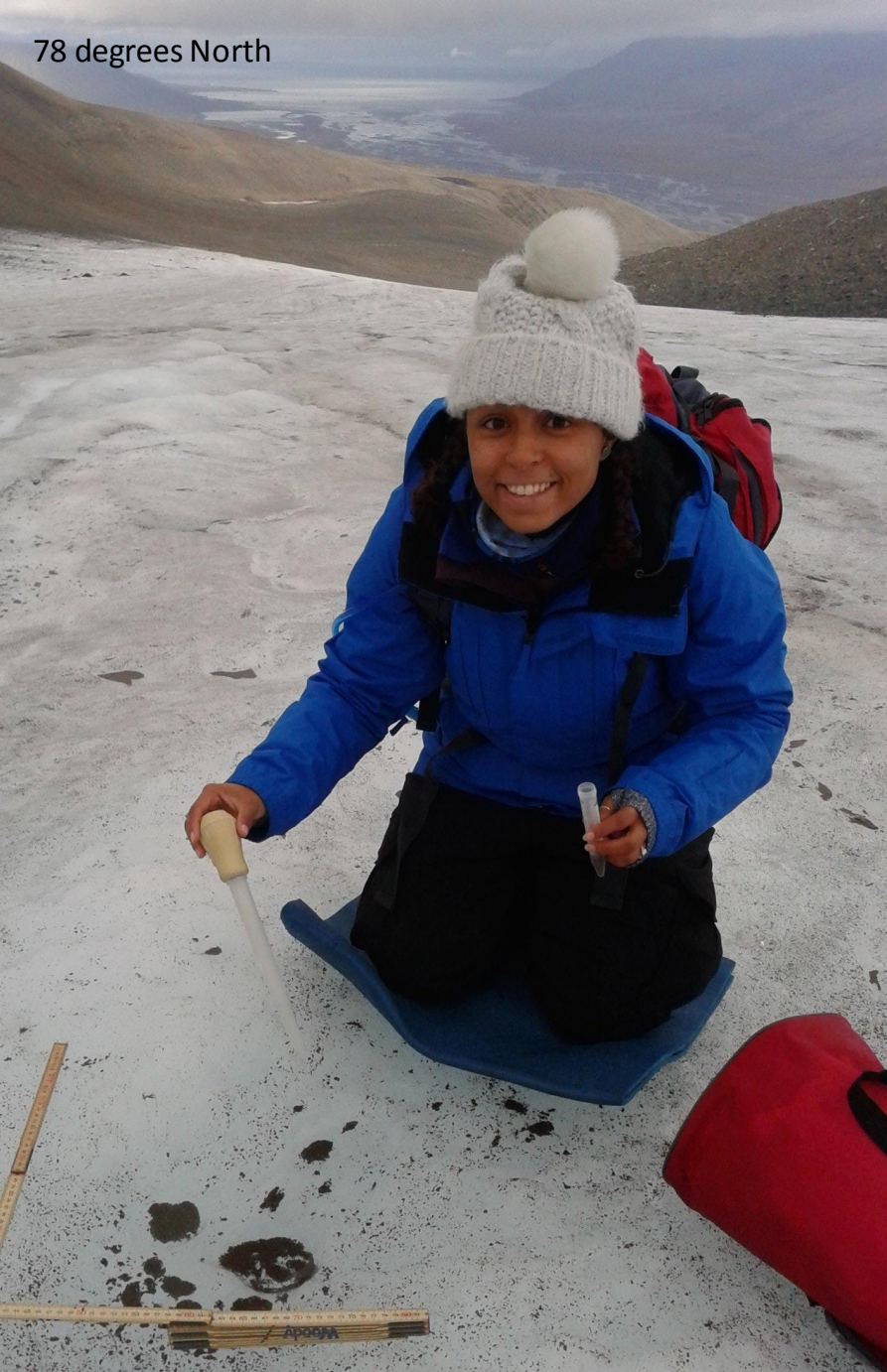
How to deploy a portable lab for the surveillance of emerging infectious diseases

Josh Quick

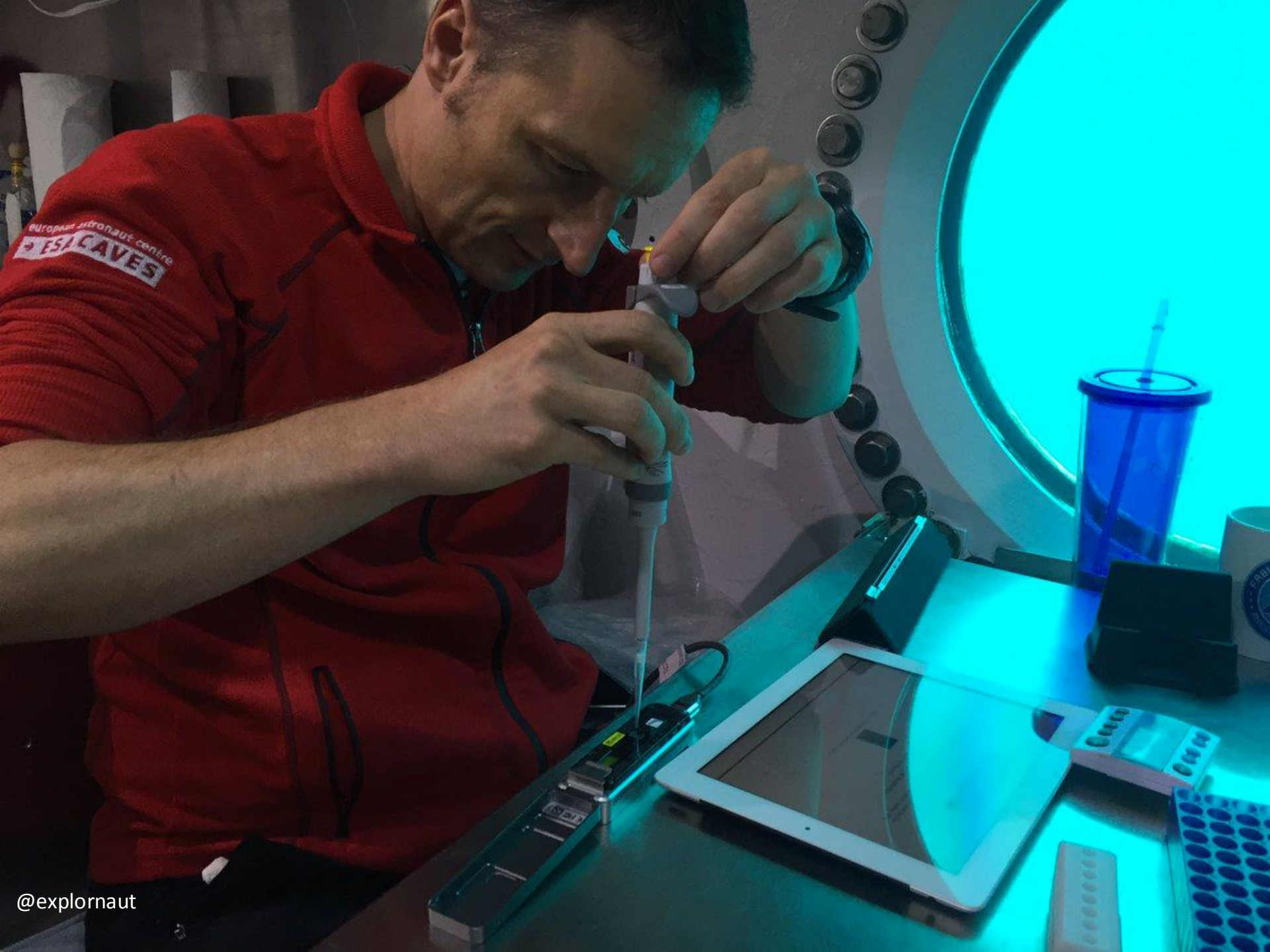
University of Birmingham



78 degrees North



@arywnedwards





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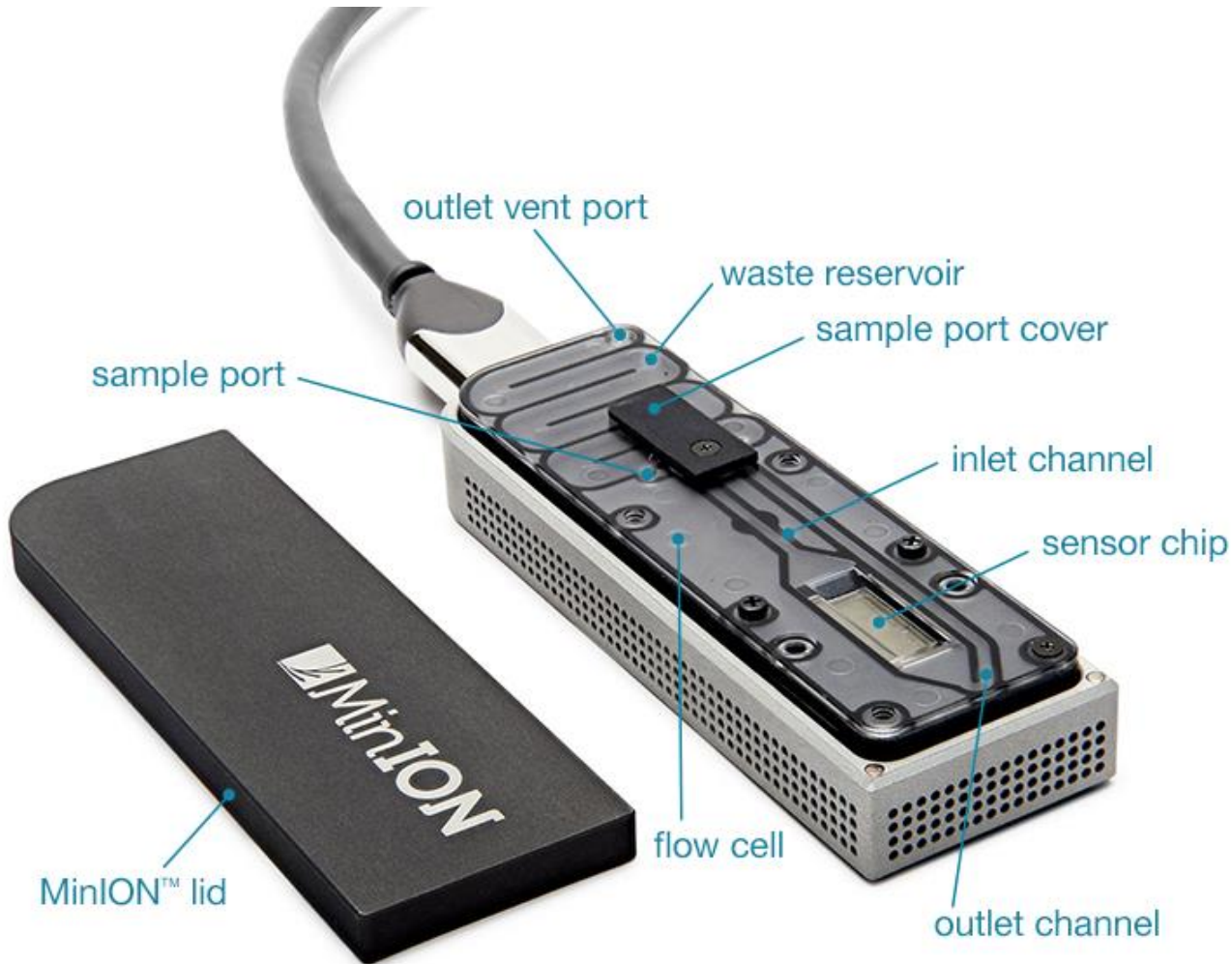
THE NORTH FACE



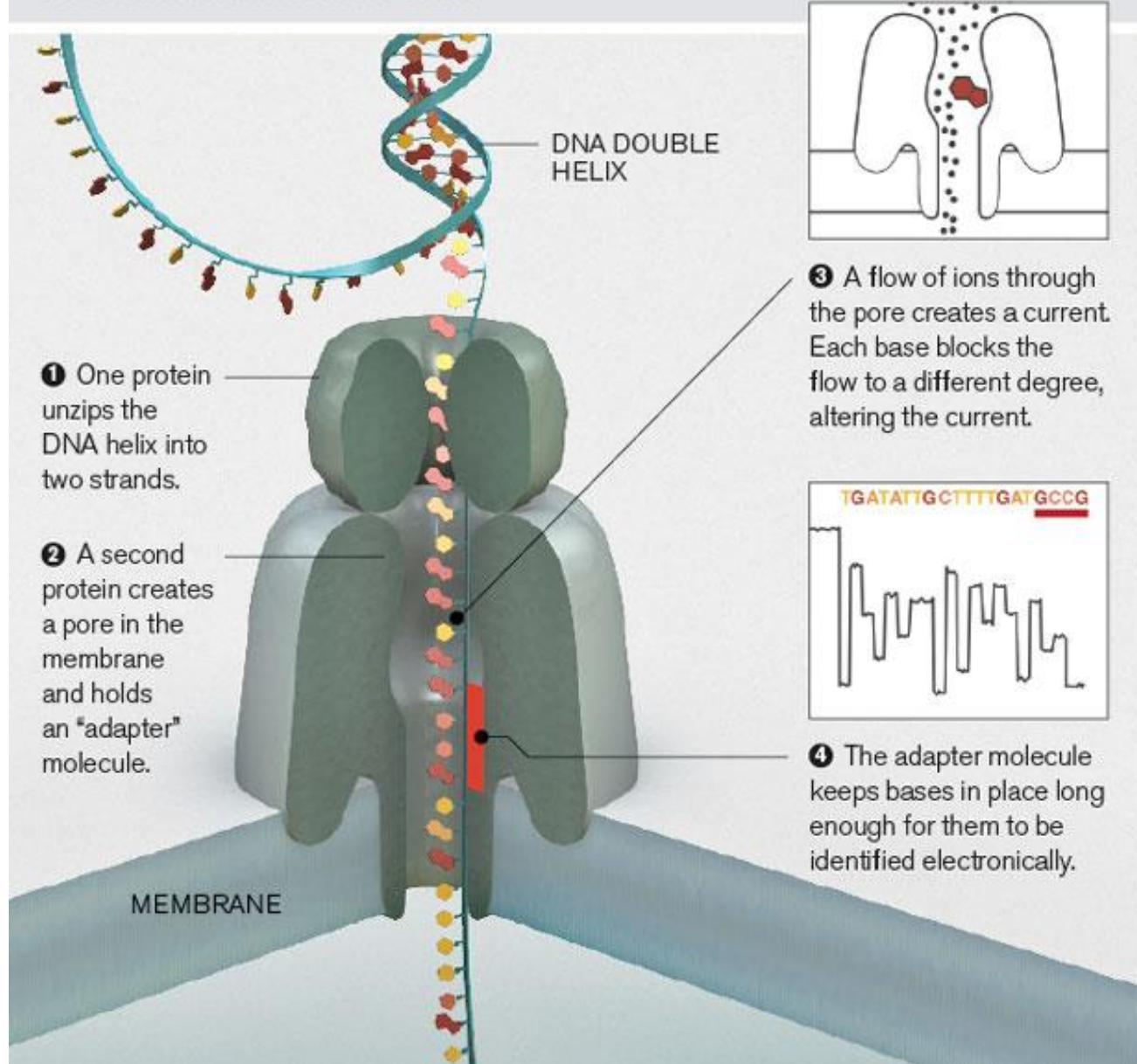
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FAA59275

The MinION device



DNA can be sequenced by threading it through a microscopic pore in a membrane. Bases are identified by the way they affect ions flowing through the pore from one side of the membrane to the other.



1 One protein unzips the DNA helix into two strands.

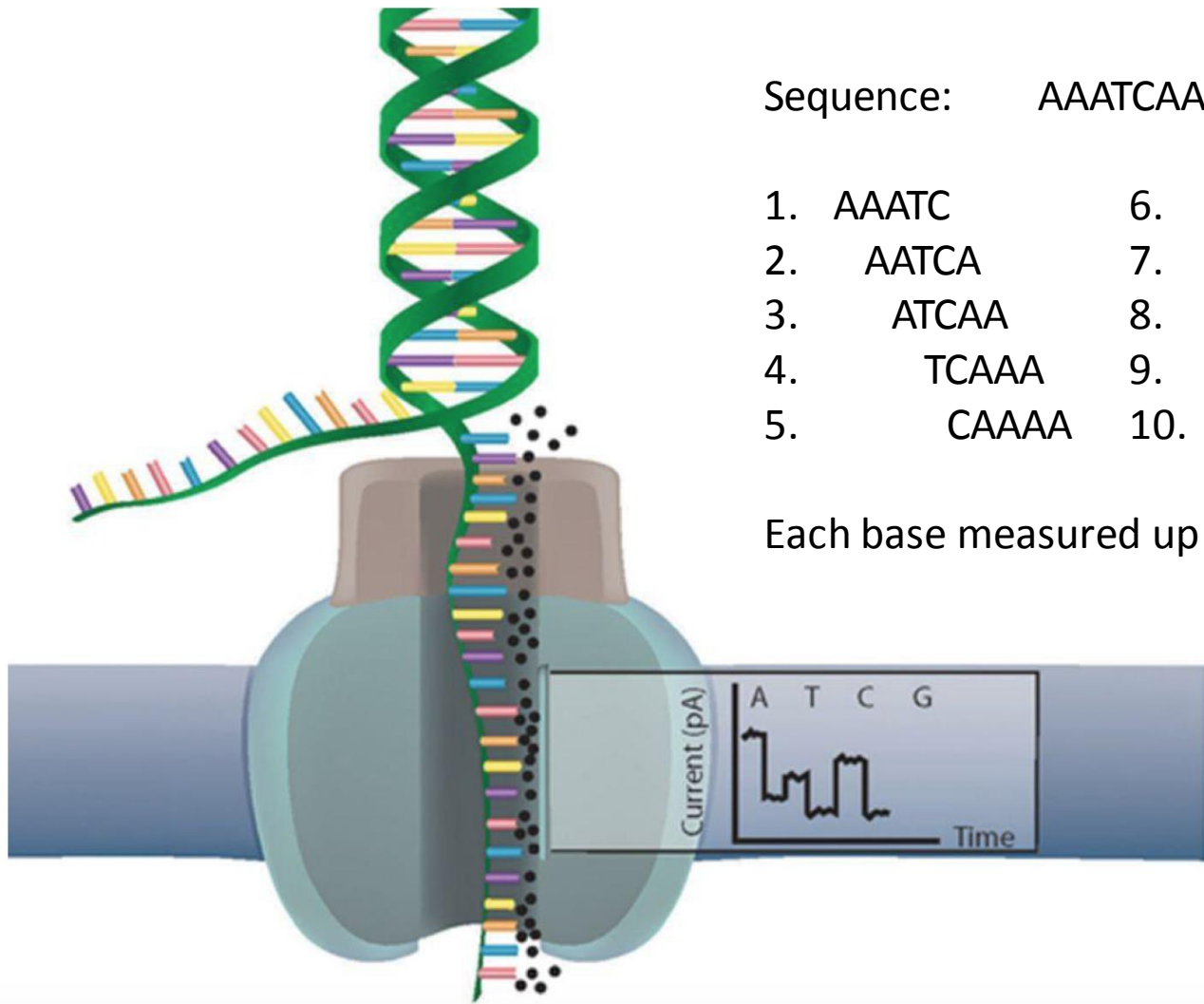
2 A second protein creates a pore in the membrane and holds an "adapter" molecule.

3 A flow of ions through the pore creates a current. Each base blocks the flow to a different degree, altering the current.

4 The adapter molecule keeps bases in place long enough for them to be identified electronically.

TGATATTGCITTTGATGCCG

MEMBRANE



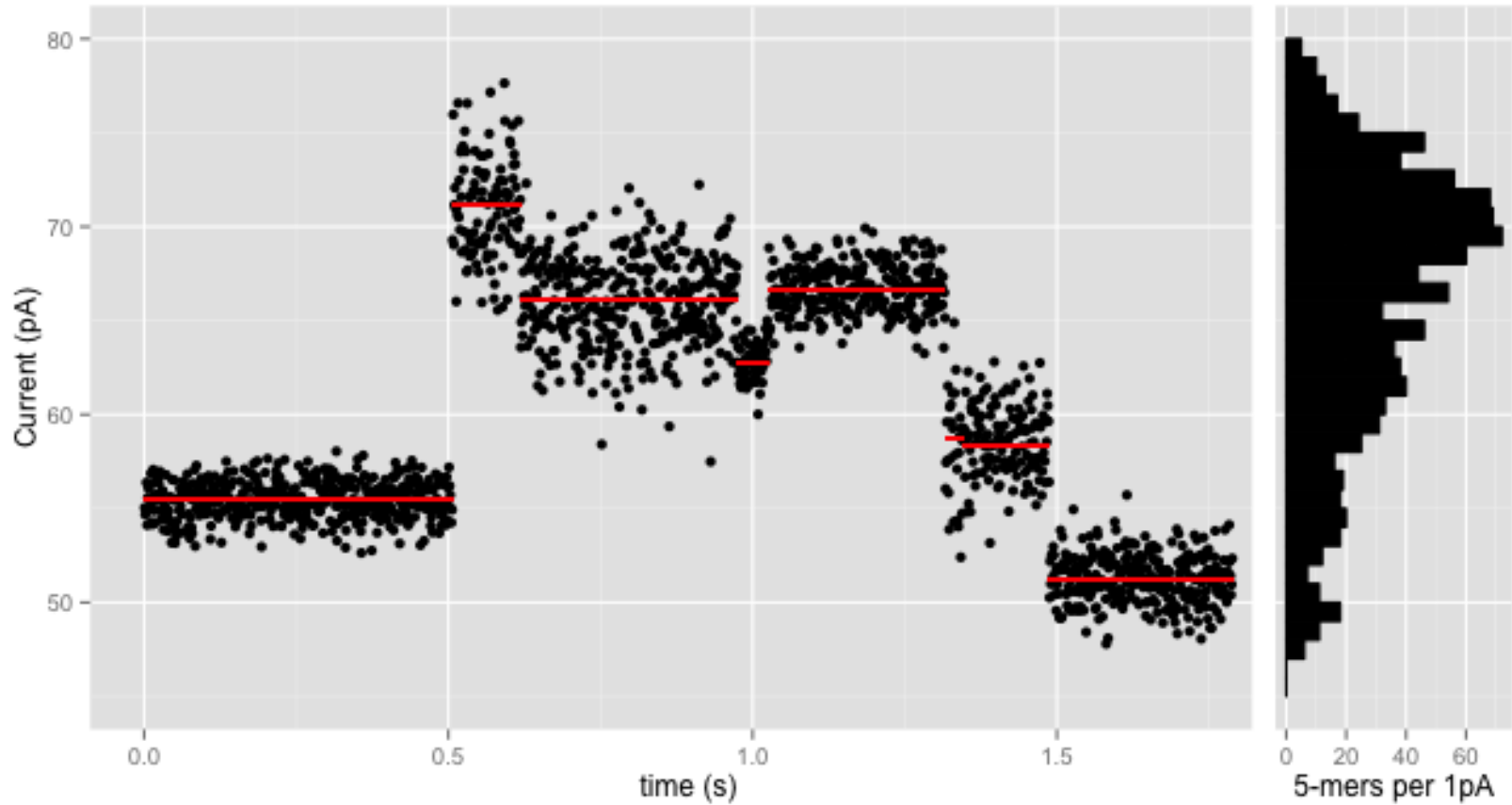
Sequence: AAATCAAAA

- | | |
|----------|-----------|
| 1. AAATC | 6. AAAAC |
| 2. AATCA | 7. AAAPT |
| 3. ATCAA | 8. AACTA |
| 4. TCAAA | 9. ACTAA |
| 5. CAAAA | 10. CTAAA |

Each base measured up to 10 times!

Image: Torsten Seemann

$4^5 = 1024$ signal levels
Dynamic range = 35pA
0.2pA mean separation



Sequencing workflow

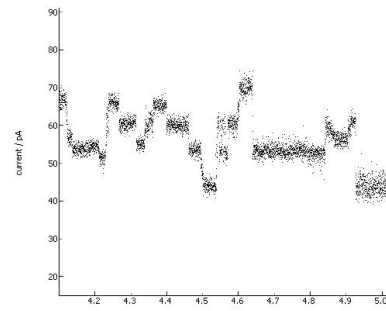
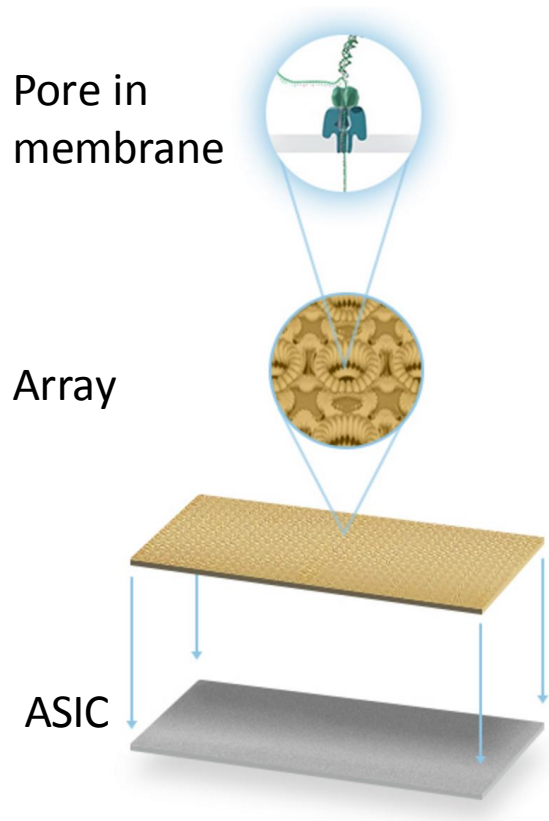
MinION



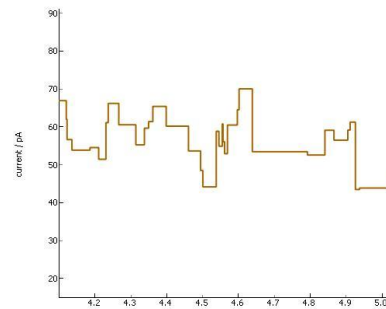
MinKNOW (local)



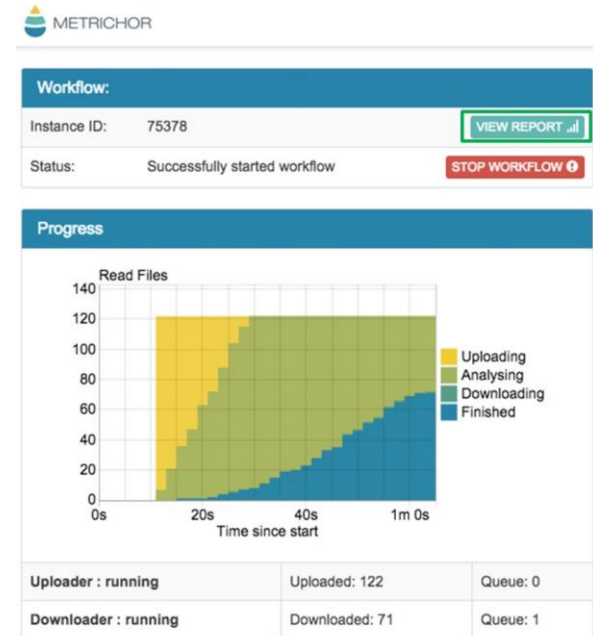
Metrichor (cloud)



Samples



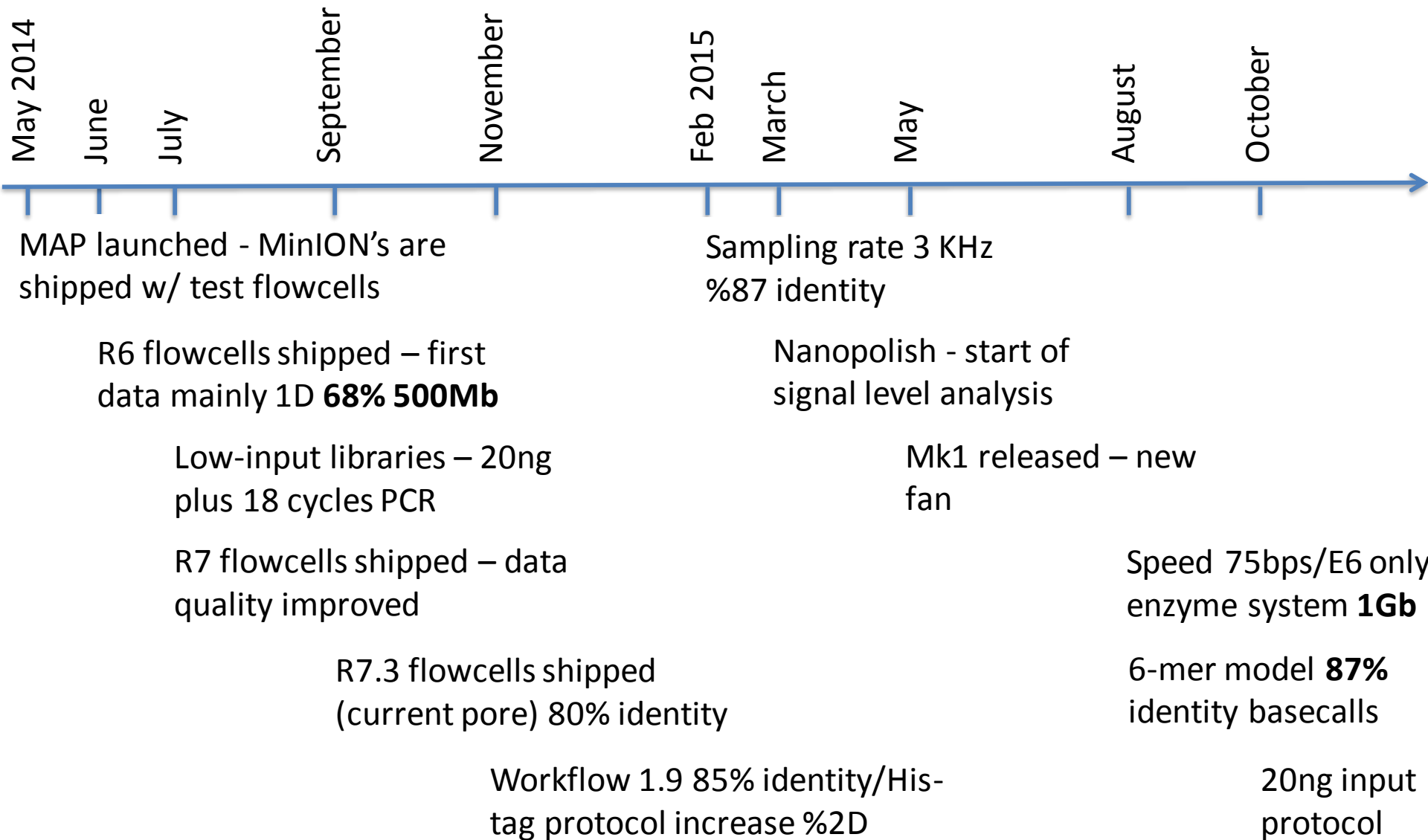
Events



```
>read  
AGCTAGCTACGATCGACT...
```

Basecalls

Development timeline



Development timeline (cont.)

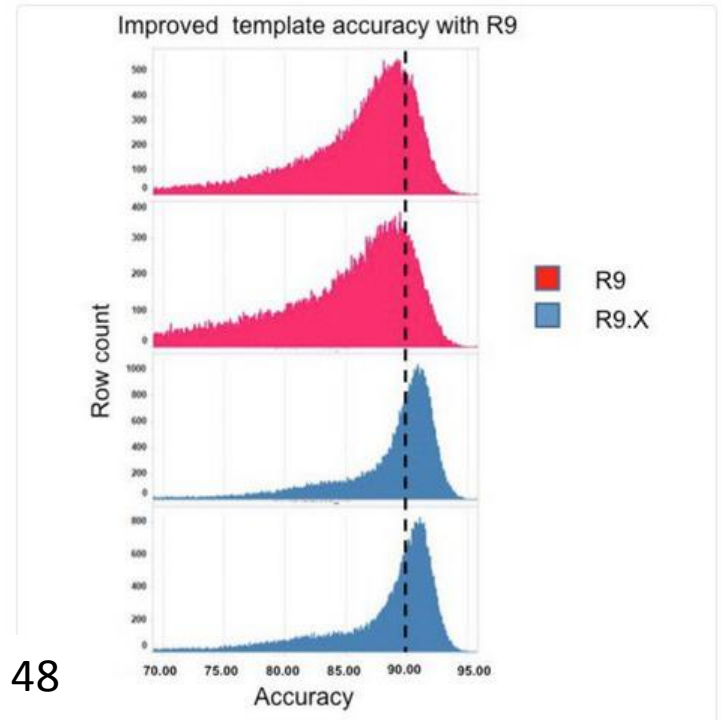


Clive G. Brown
@Clive_G_Brown



Following

@Clive_G_Brown minor R9 upgrade validates c.p. chips available to developer group this week. Only 1D shown, 2D>95%



Fast Mode early access – 550 bps **6Gb**, new MinKNOW

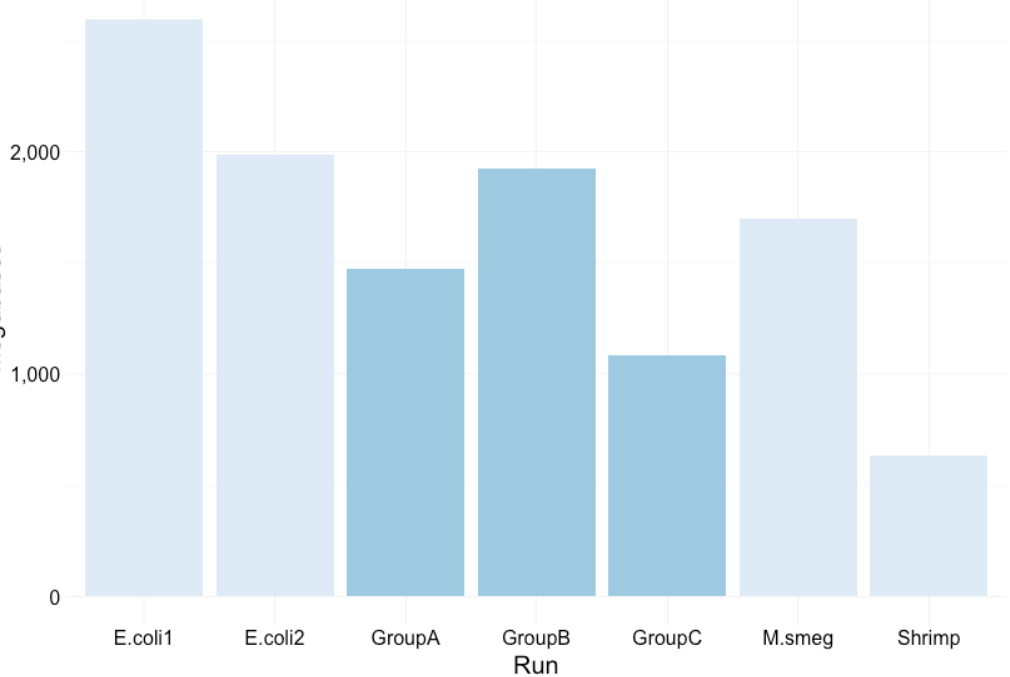
Rapid 1D library preparation – 15 minutes

R9 pore, 270 bps, RNN
85% identity 1D, **95%** 2D

First PromethION shipped, 48 flowcells 144,000 channels

R9.4 pore, 450 bps, 1D by ligation **>90%** identity 1D

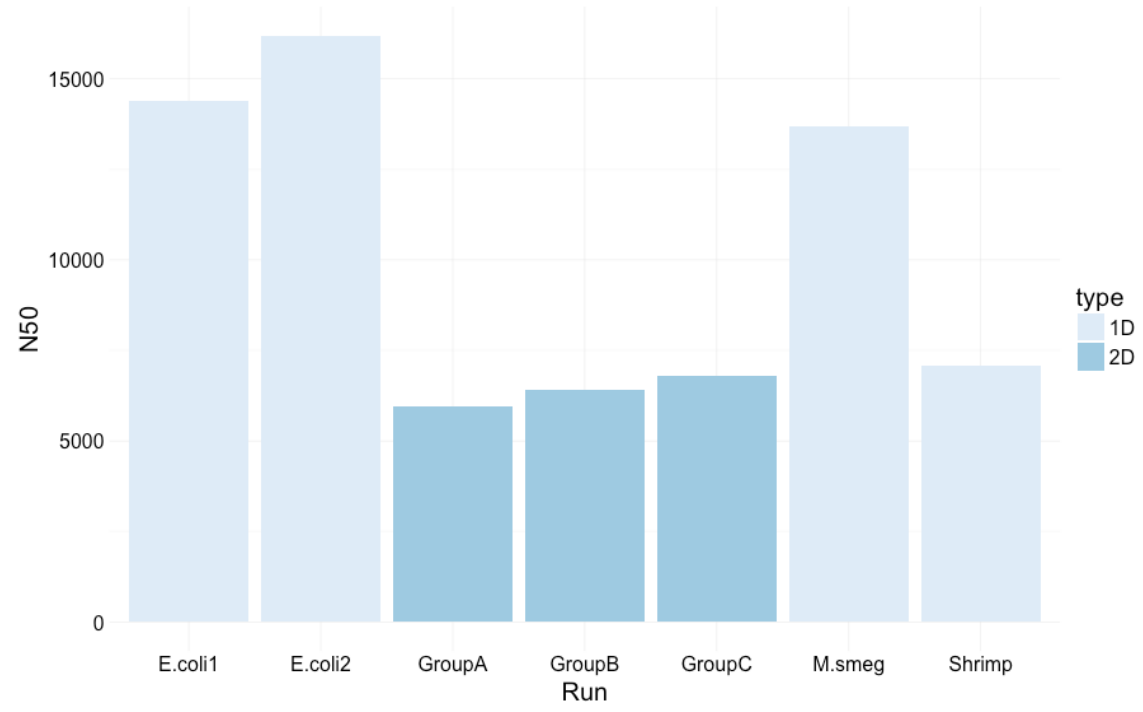
Megabases



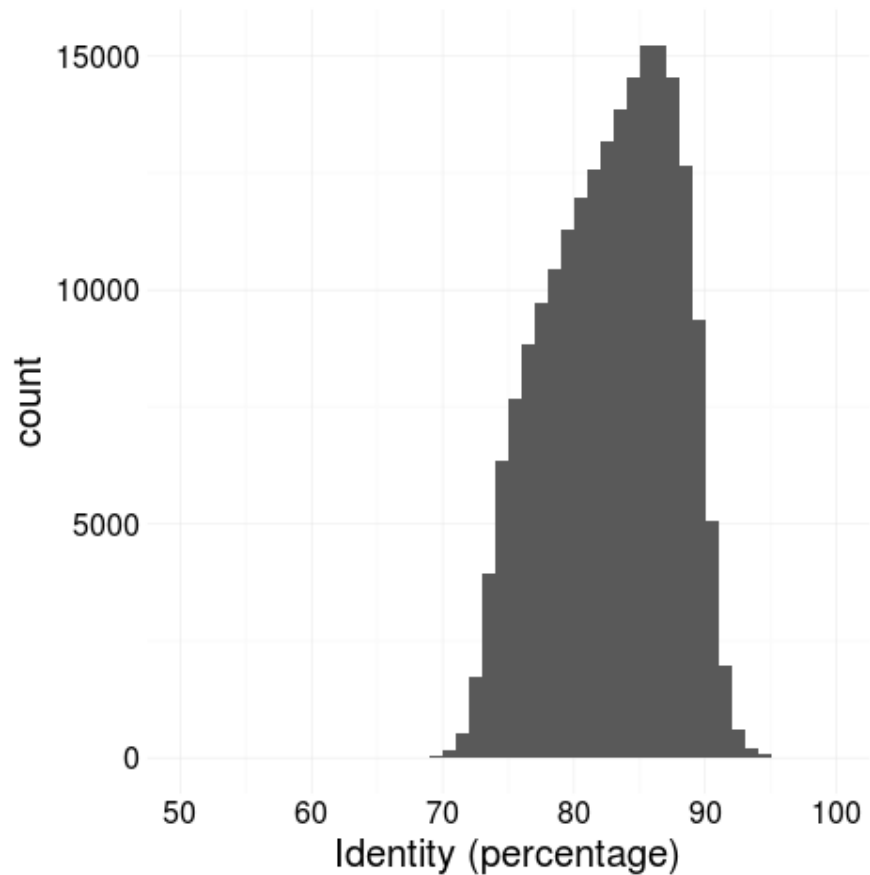
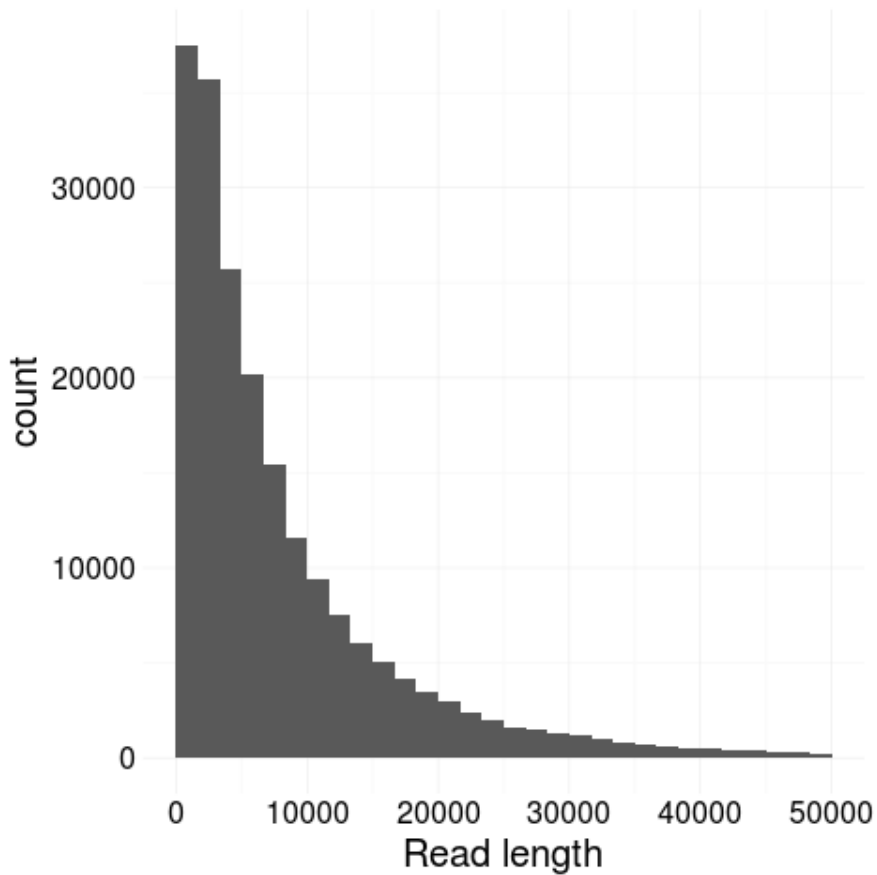
Yields
Max: 2.6Gb
Mean: 1.6Gb

porecamp.github.io/2016

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



R9 Rapid Kit Results (1D)





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Kit for Sierra Leone

- Lab notebook and pen
- ✓ Gloves
- Lab coat
- ✓ Sharps bin
- ✓ Waste bottle
- ✓ Protocol
- ✓ Casio calculator
- Microfuge
- ✓ Heatblock - got
- ✓ Magnetic rack - got
- Tube rack
- ✓ Lab timer
- Marker pen
- ✓ Ice bucket and ice - got
- ✓ P20, P100, P200, P1000 pipettes - got
- ✓ P20, P100, P200, P1000 pipette tips
- ✓ DNA LoBind 2ml tubes
- ✓ Protein LoBind 2ml tubes
- ✓ PCR tubes and caps
- ✓ MinION
- ✓ Laptop
- Tape
- Sample
- Genomic DNA Sequencing Kit (MAPQ005) - got
- ✓ SPRI beads
- NEB End-repair module
- NEB dA-tailing module
- NEB Blunt/TA ligase
- Nuclease-free water (Promega)
- Ethanol 100%
- ✓ MinION flowcells
- ✓ His-tag pull-down beads
- DNAase
- RT
- 2nd strand
- LongAmp
- Qubit DNA - Dye
- Standards
- Qubit RNA - Dye
- Standards

Stones

10 ul tips
200ul tubes

- need eppendorf rotor

Qubit tubes

Holdall

Thermometer

Power bar

✓ Bleach

✓ RNase

Cold

Flowcells

SPRI bead

His-tag

Qubit RNA

Qubit DNA

Frozen

MinION kits

Enzymes - dA tail (2)

- ER (2)

- RT (?)

- 2nd strand (4)

- ligase (1)

- LongAmp (1)

- DNAase. (4)

(250ul x 2)
Standards (2)
- Dye (100ul)
- Buffer (100ul)
- Dye (125ul) (100ul)
- Buffer (50ul)
Standards (2)
(250ul x 2)







<https://twitter.com/igoodfel/>

Rumsfeld Matrix

Known

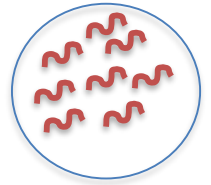
Unknown

 Human background
 Pathogen

Culture enrichment



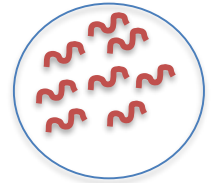
Slow



PCR enrichment



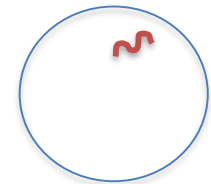
Fast



Selective enrichment of nucleic acids



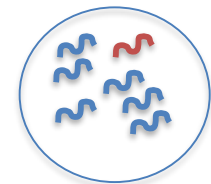
Moderate

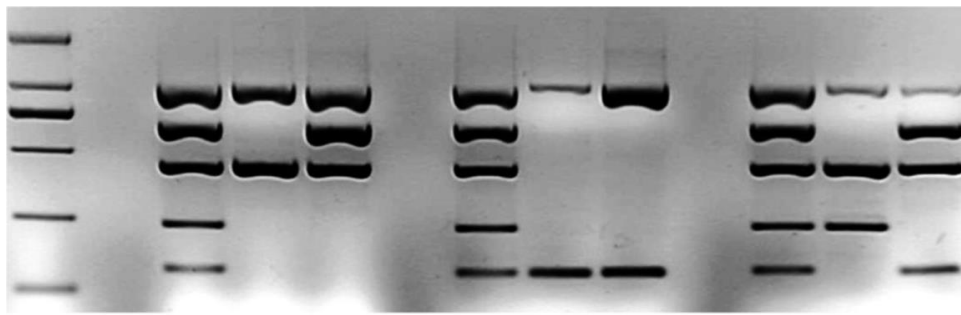


Metagenomics

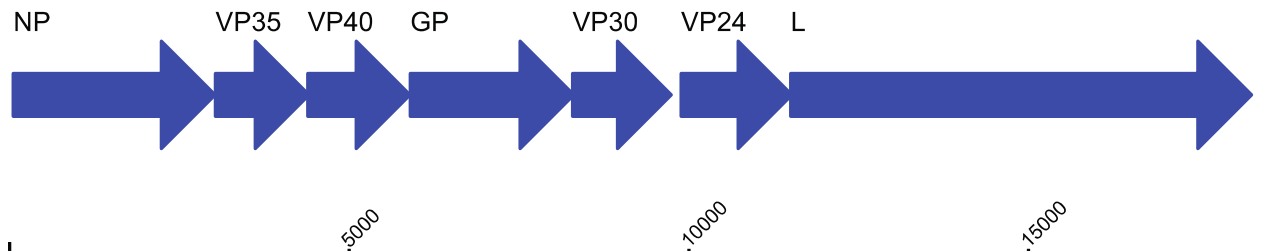


Moderate

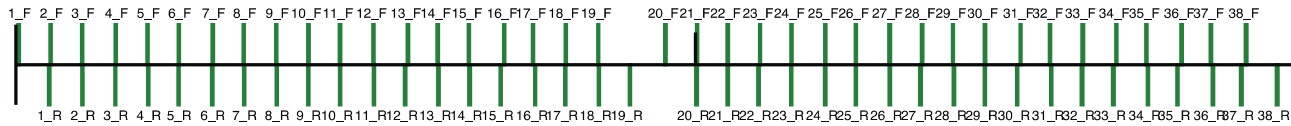




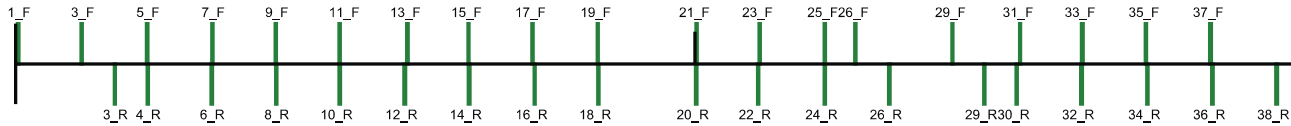
A.



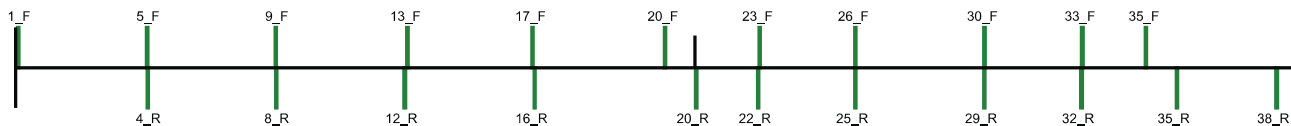
All



19 rxn



11 rxn



B. 19 reactions

| Forward | Reverse | Length |
|---------|---------|--------|
| 1_F | 3_R | 1426 |
| 3_F | 4_R | 973 |
| 5_F | 6_R | 952 |
| 7_F | 8_R | 941 |
| 9_F | 10_R | 940 |
| 11_F | 12_R | 958 |
| 13_F | 14_R | 906 |
| 15_F | 16_R | 974 |
| 17_F | 18_R | 969 |
| 19_F | 20_R | 1445 |
| 21_F | 22_R | 906 |
| 23_F | 24_R | 958 |
| 25_F | 26_R | 947 |
| 26_F | 29_R | 1898 |
| 29_F | 30_R | 946 |
| 31_F | 32_R | 901 |
| 33_F | 34_R | 963 |
| 35_F | 36_R | 977 |
| 37_F | 38_R | 975 |

C. 11 reactions

| Forward | Reverse | Length |
|---------|---------|--------|
| 1_F | 4_R | 1911 |
| 5_F | 8_R | 1901 |
| 9_F | 12_R | 1895 |
| 13_F | 16_R | 1874 |
| 17_F | 20_R | 2406 |
| 20_F | 22_R | 1371 |
| 23_F | 25_R | 1410 |
| 26_F | 29_R | 1898 |
| 30_F | 32_R | 1427 |
| 33_F | 35_R | 1396 |
| 35_F | 38_R | 1921 |

Porton Down validation set, 89.1% coverage



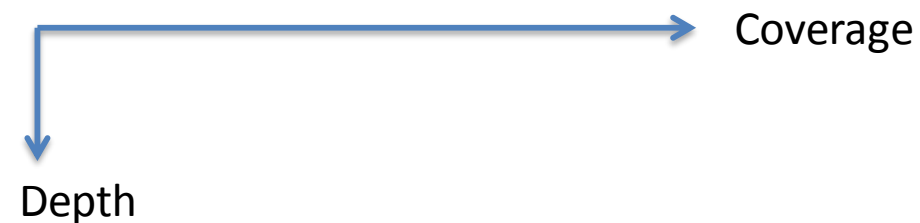
Guinea 19 reactions v1, 98.1% coverage

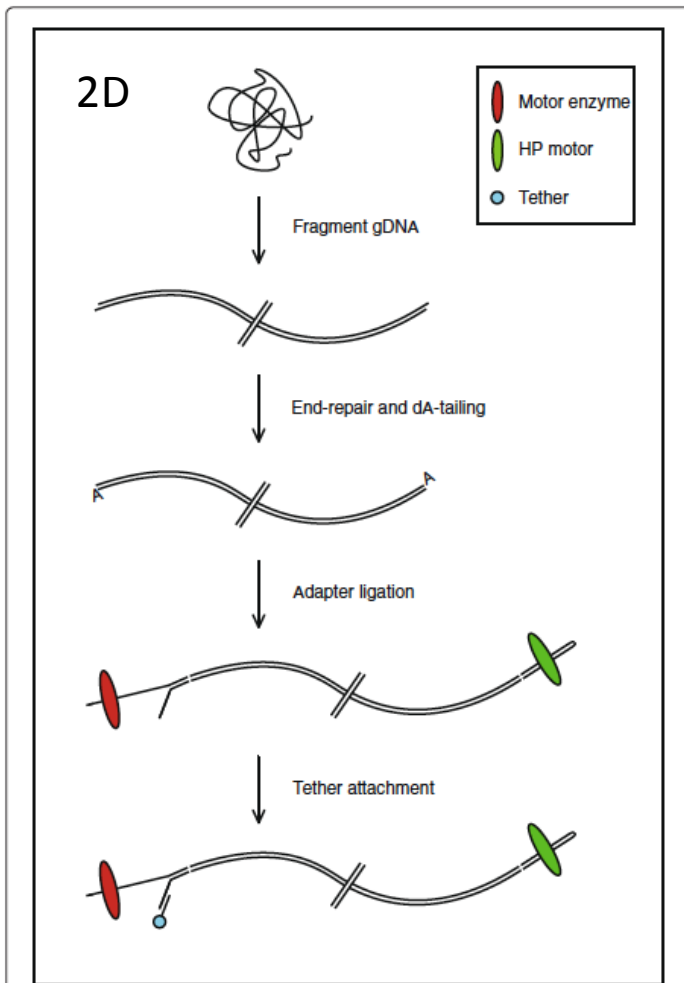


Guinea 11 reactions v1, 95.9% coverage



Guinea 11 reactions v2, 98.4% coverage

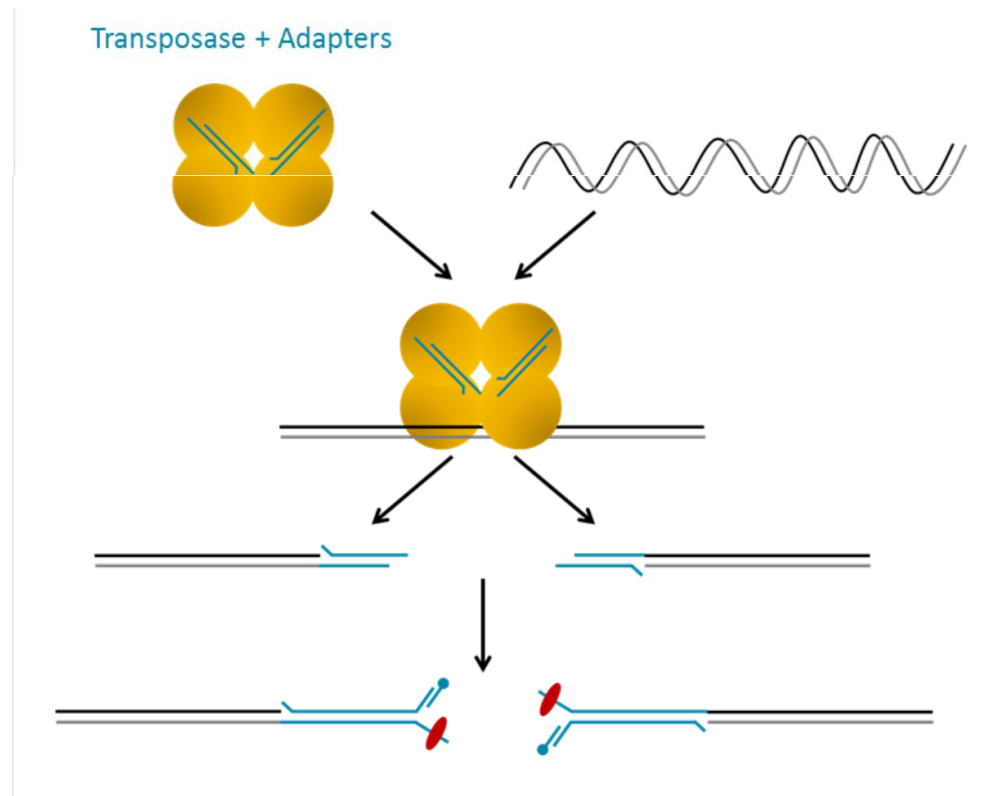




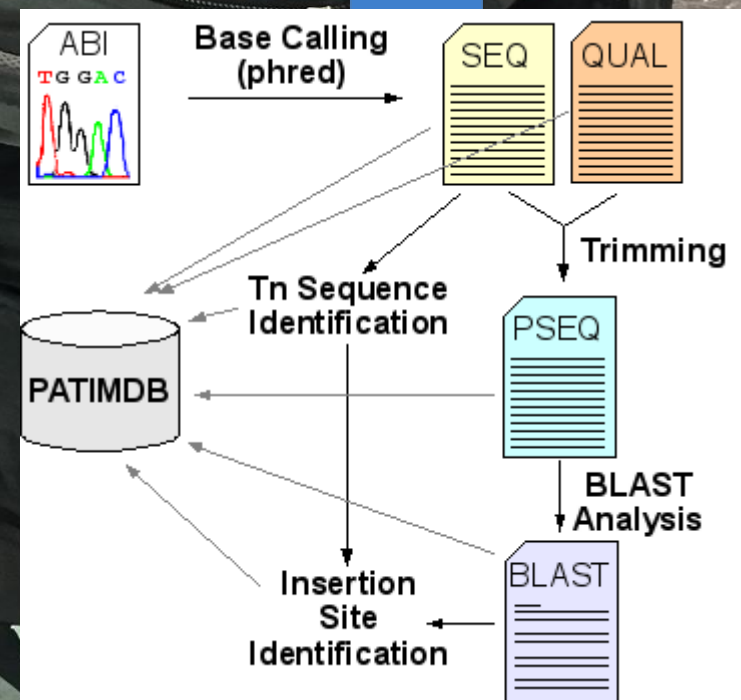
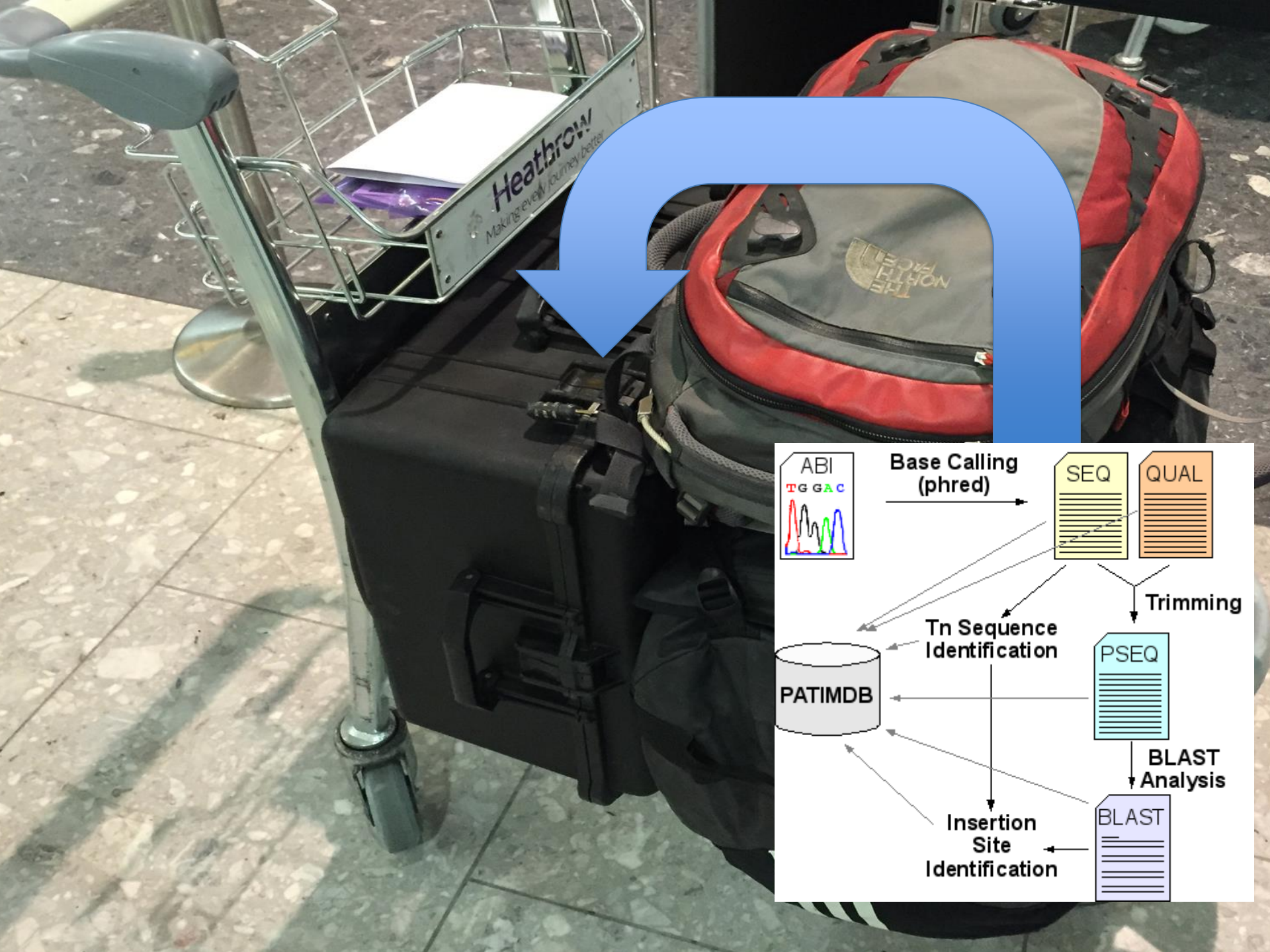
Quick et al. Gigascience 2014

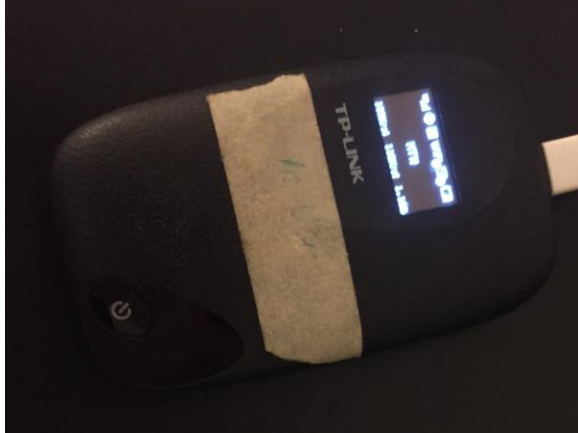
Library prep time: 2 hours

1D

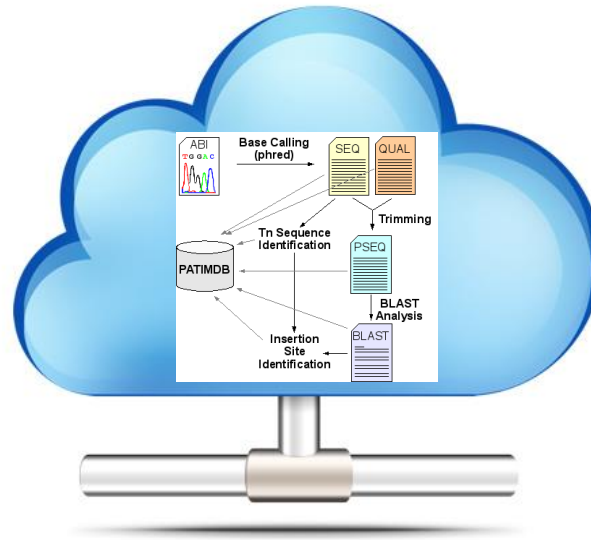
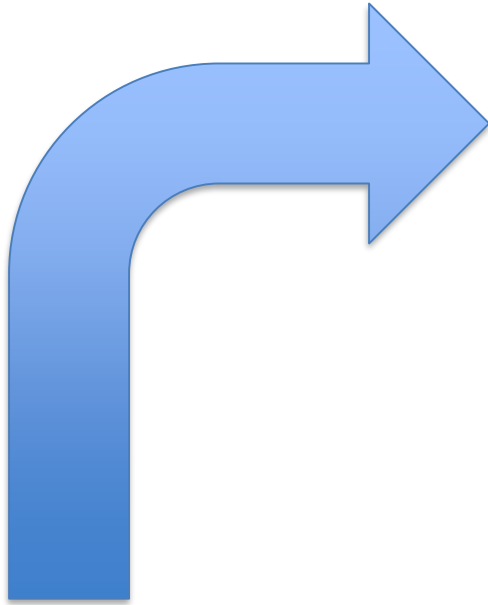


Library prep time: 5 minutes

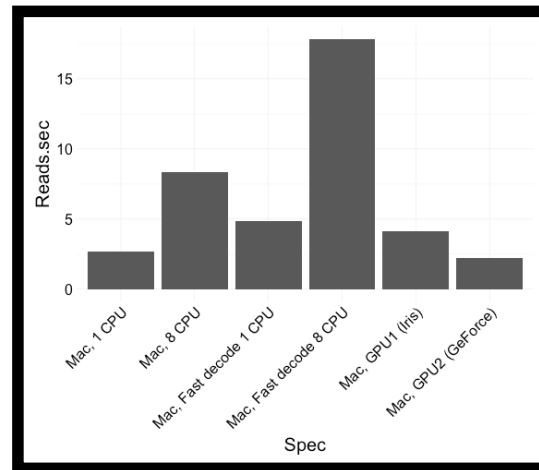




Cost / Mb



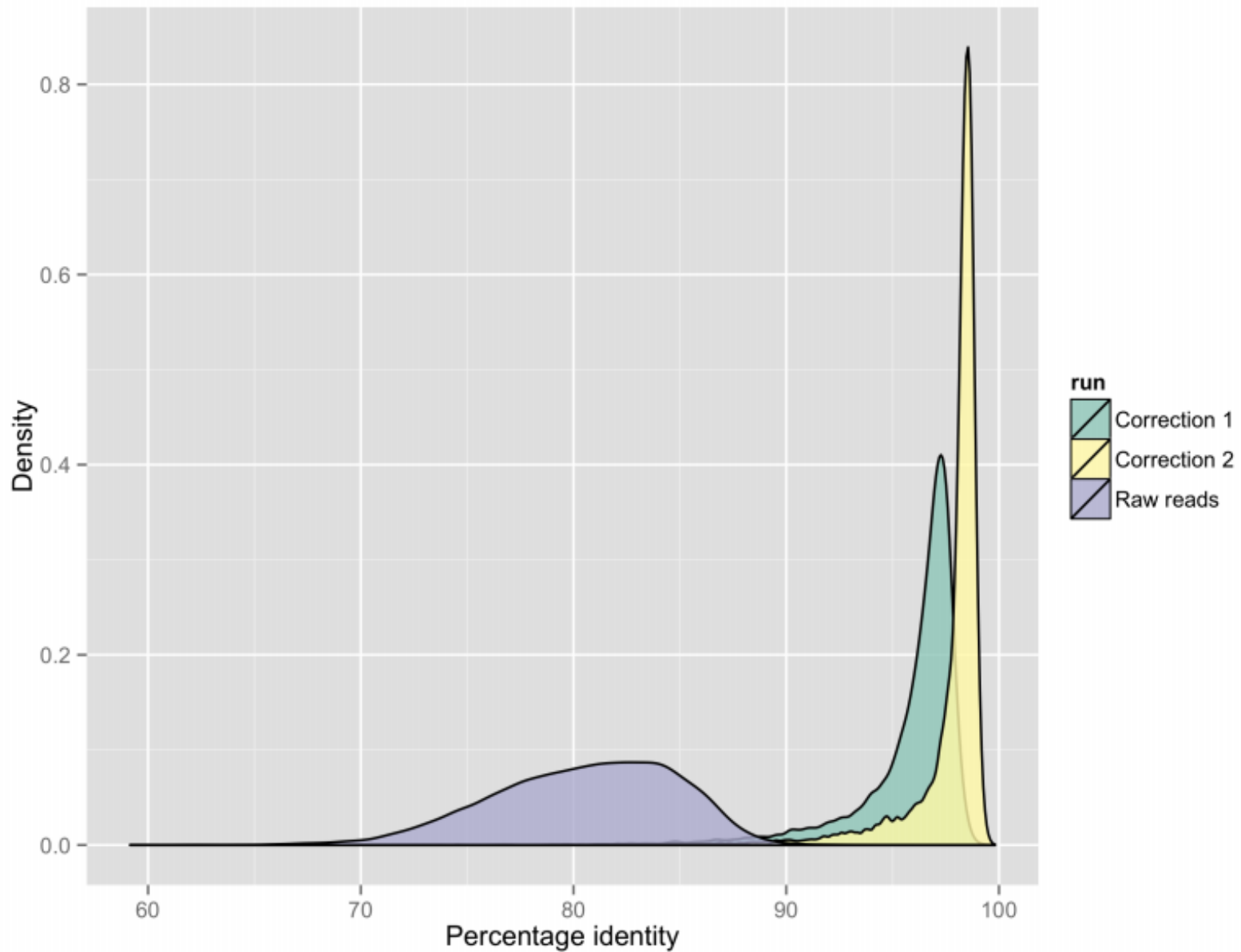
Deep learning:
Nanonet (2D)



Deep learning:
MinKNOW local
Albacore
Deepnano

HMM:
Nanocall

Error Correction



Contig Assembly

Celera Assembler produces one contig at 98.5% identity

```
Query: 61      gacaaccagatttatctgtcgatttcgctccccttcggtaacggtgggtccgcttggctat 120
              |||
Sbjct: 750537  gacaaccagatttatctgtcgatttcgctccccttcggtaacggtgggtccggttggctat 750478

Query: 121     gacatgcaaa-cagtagccacagcaccatacaccgatgtcgtggaacgatacgctggat 179
              |||
Sbjct: 750477  gacatgcaaaacagtagccacagcaccatacaccgatgtcgtggaacgatacgctggat 750418

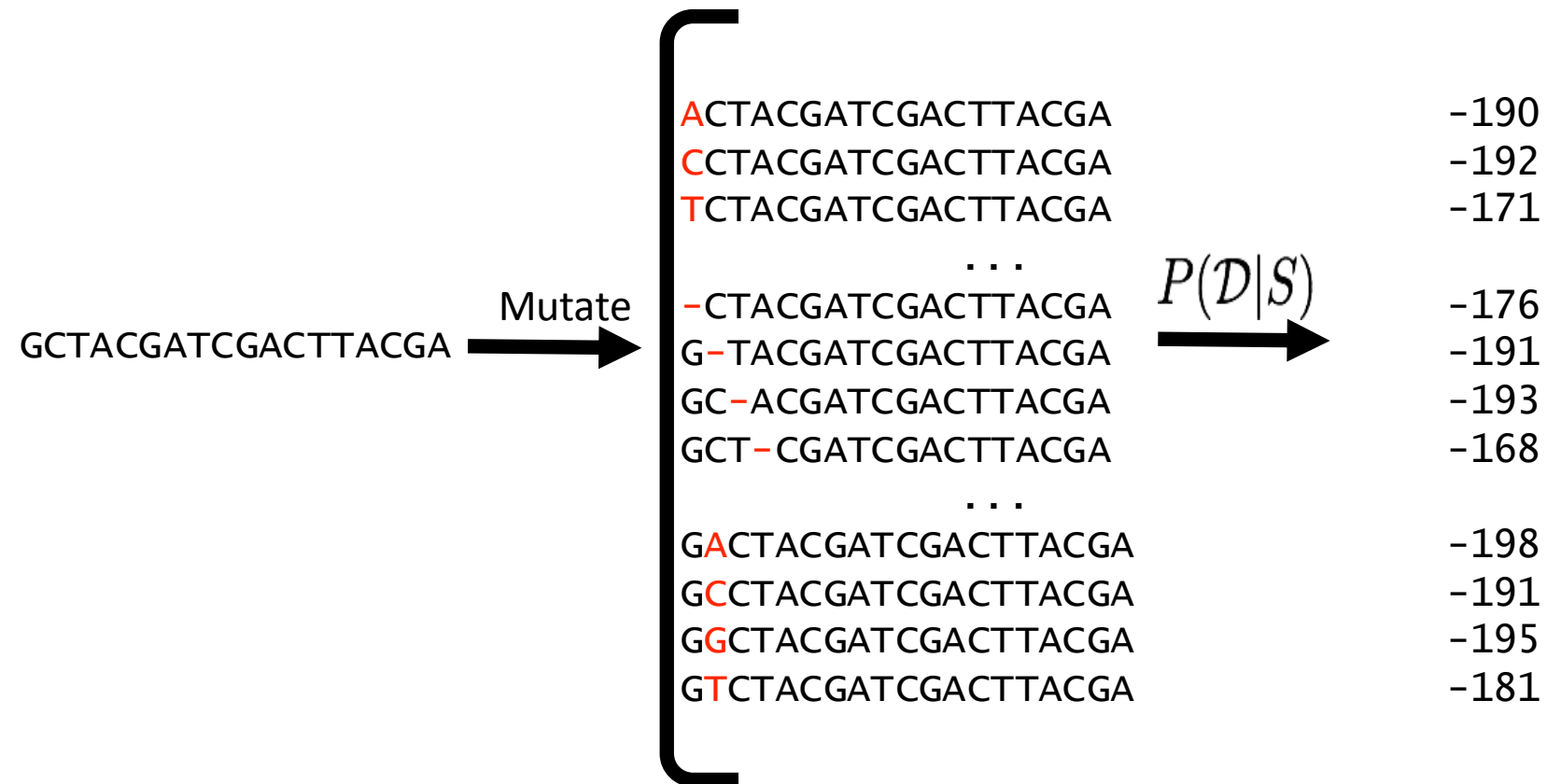
Query: 180     gaacgtaatagctggggcatgtctgccggactgcaatccgatcggttagaacggacaatg 239
              |||
Sbjct: 750417  gaacgtaatagctggggcatgtctgccggactgcaatccgatcgt-----ccggacaatg 750363

Query: 240     gagccca--tgagcggtaactatcagcacctgagttcagcgggtgagtgggatatttctg 297
              |||
Sbjct: 750362  gagcccaggtgagcggtaactatcagcacctgagttcagcgggtgagtgggatatttctg 750303
```



Jared Simpson

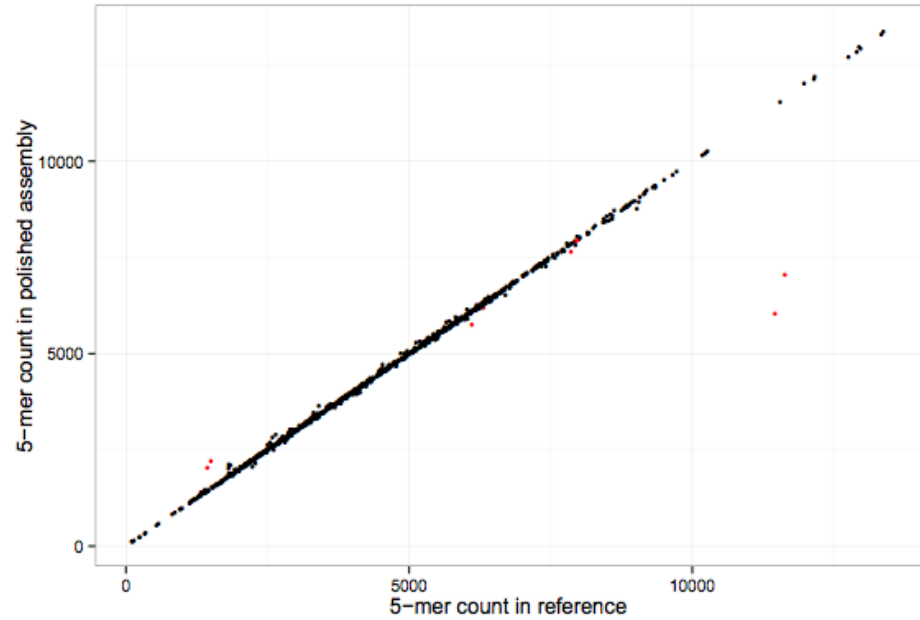
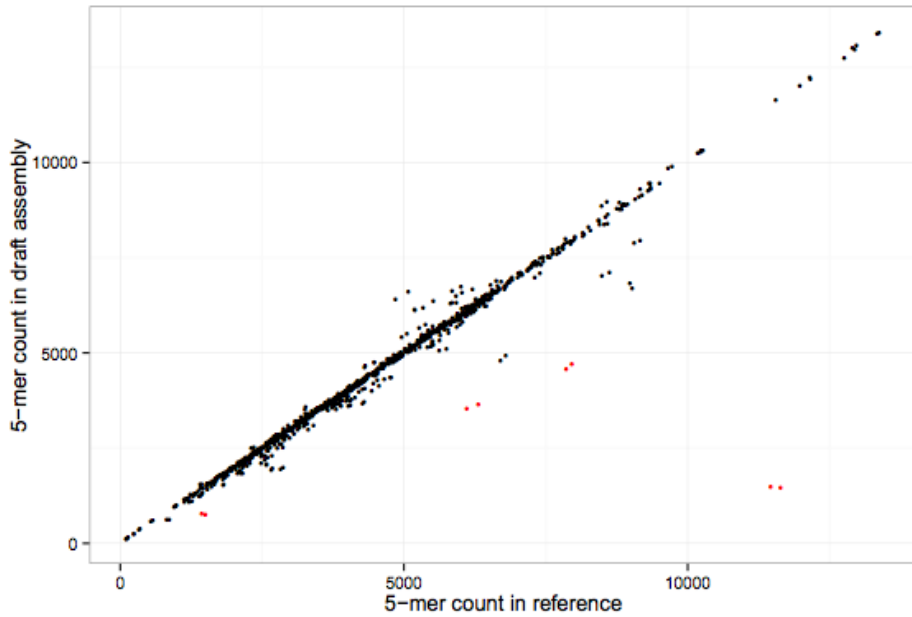
Selecting a Consensus



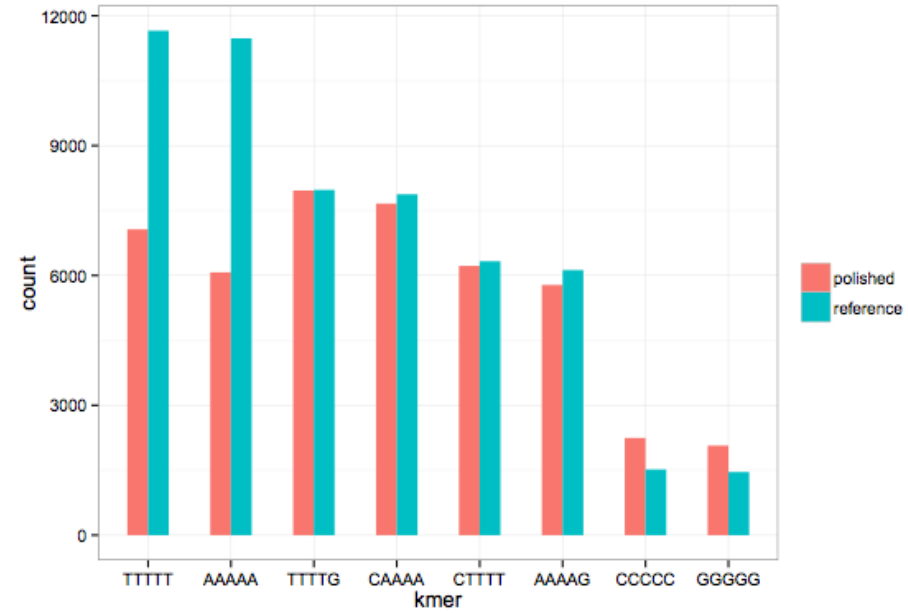
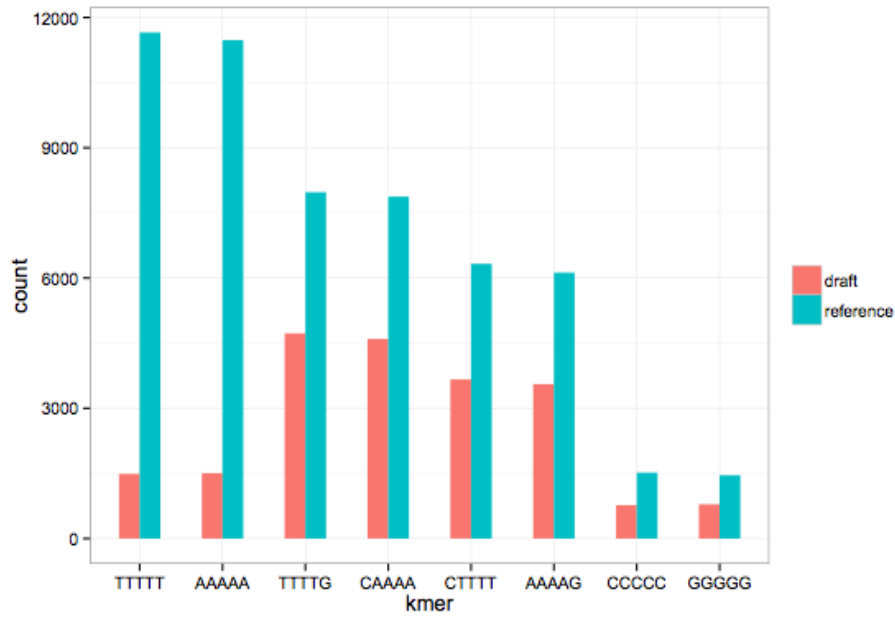
Assembly Accuracy

Draft: 98.5% accuracy

Polished: 99.5% accuracy



Assembly Accuracy



Assembly update

| Kit, Coverage | Percent Identity | # SNPs | # Indels |
|----------------------|-------------------------|---------------|-----------------|
| SQK005, 29X | 99.48% | 1,343 | 22,601 |
| SQK006, 48X | 99.78% | 644 | 9,697 |
| SQK006-PCR, 30X | 99.82% | 222 | 8,200 |

- Current best: R7 + R9 99.98% (>Q30)
- Some success modeling hp dwell times

Read alignment:



SNP

Sequencing error

Consensus:

A G A A G A A A C G A A C C C T C C G G C T

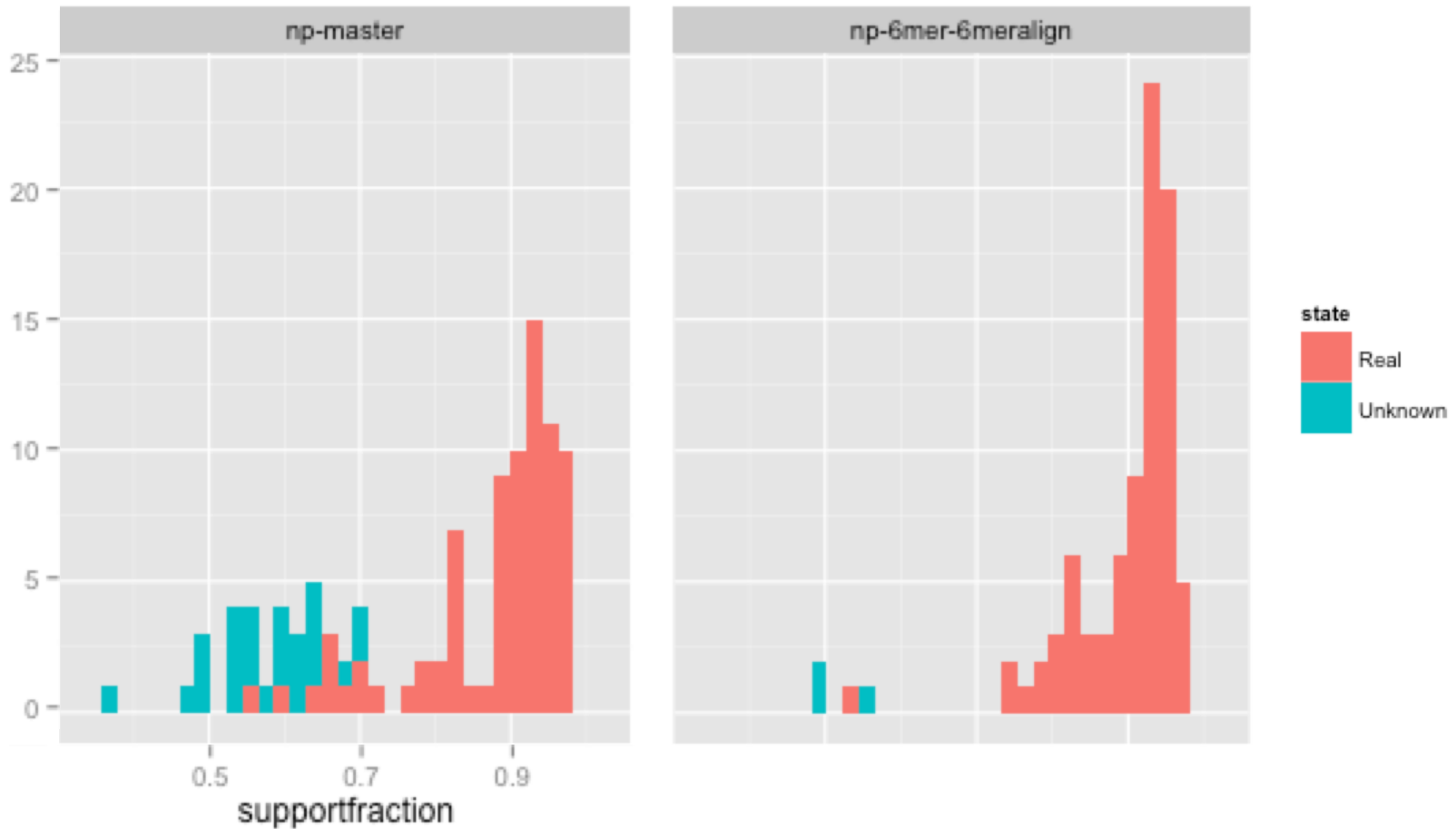
```

351          361          371          381          391          401          411          421          431
ATTAA*CTTTGATTTTGAACCTGAACACCCAGAGGACTGGAGAC*TCAACAACCCTAAAGCCTGGGGTAAAACATTAGAAATAGTTTAAA
.....C...G.....T.....TT..G...T..A...T.....T.....G.....A...
.....*C...G.....T.....TT..G...T..A...T*.....T.....G.....A...
.....*C...G.....T.....TT..G...T..A...T*.....T.....G.....A...
.....*C...G.....T.....TT..G...T..A...T*.....T.....G.....A...
.....*C...G.....T.....TT..G...T..A...T*.....T.....G.....A...
.....*C...G.....T.....TT..G...T..A...T*.....T.....G.....A...
.....*C...G.....T.....TT..G...T..A...T*.....T.....G.....A...
,,,*,c,,,g,,,,,,t,,,,,,tt,g,,,t,,a,,,t*,,,,,,t,,,,,,g,,,,,,a,,,
,,,*,c,,,g,,,,,,t,,,,,,tt,g,,,t,,a,,,t*,,,,,,t,,,,,,g,,,,,,a,,,

```

- Nanopolish can call complex haplotypes

6-mer model



- 6-mer model improves genotyping accuracy

| | | |
|------------------------|---|---|
| Base calling | Convert nanopore squiggles to nucleotide sequences | <code>metrichor-cli -w 380</code> |
| Convert to FASTA/FASTQ | Extract basecalled information from nanopore FAST5 files | <code>poretools fasta --type 2D pass/ poretools fasta --type 2D --high-quality fail/</code> |
| Align to reference | Align sequences to reference | <code>bwa -x ont2d mem EM_079517.fasta reads.fasta</code> |
| Local HMM realignment | Iteratively improve alignment based on nanopore insertion/deletion/substitution rates | <code>marginAlign --inputModel input.hmm EM_079517.fasta reads.fastq out.sam</code> |
| Alignment trimming | Remove alignments outside of primer regions in case of adaptor contamination | <code>align_trim.py</code> |
| Event alignment | Map individual event k-mers to reference genome guided by base alignment | <code>nanopolish eventalign</code> |
| Variant calling | Extract candidate mutations from aligned reads, cluster them and evaluate them using a 6-mer HMM | <code>nanopolish variants</code> |
| Consensus building | Mask positions in the genome with either i) <50x 1-D coverage ii) low-quality variants detected iii) in primer binding site | Refer to IPython Notebook for details |



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Case incidence has remained at 5 confirmed cases or fewer per week for 12 consecutive weeks. Over the same period, transmission of the virus has been geographically confined to several small areas in western Guinea and Sierra Leone, marking a transition to a distinct, third phase of the epidemic. The [phase 3 response](#) coordinated by the [Interagency Collaboration on Ebola](#) builds on existing measures to drive case incidence to zero, and ensure a sustained end to EVD transmission. Enhanced capacity to rapidly identify a reintroduction (either from an area of active transmission or from an animal reservoir), or re-emergence of virus from a survivor, and capacity for testing and counselling as part of a comprehensive package to safeguard the welfare of survivors are central to the phase 3 response framework.

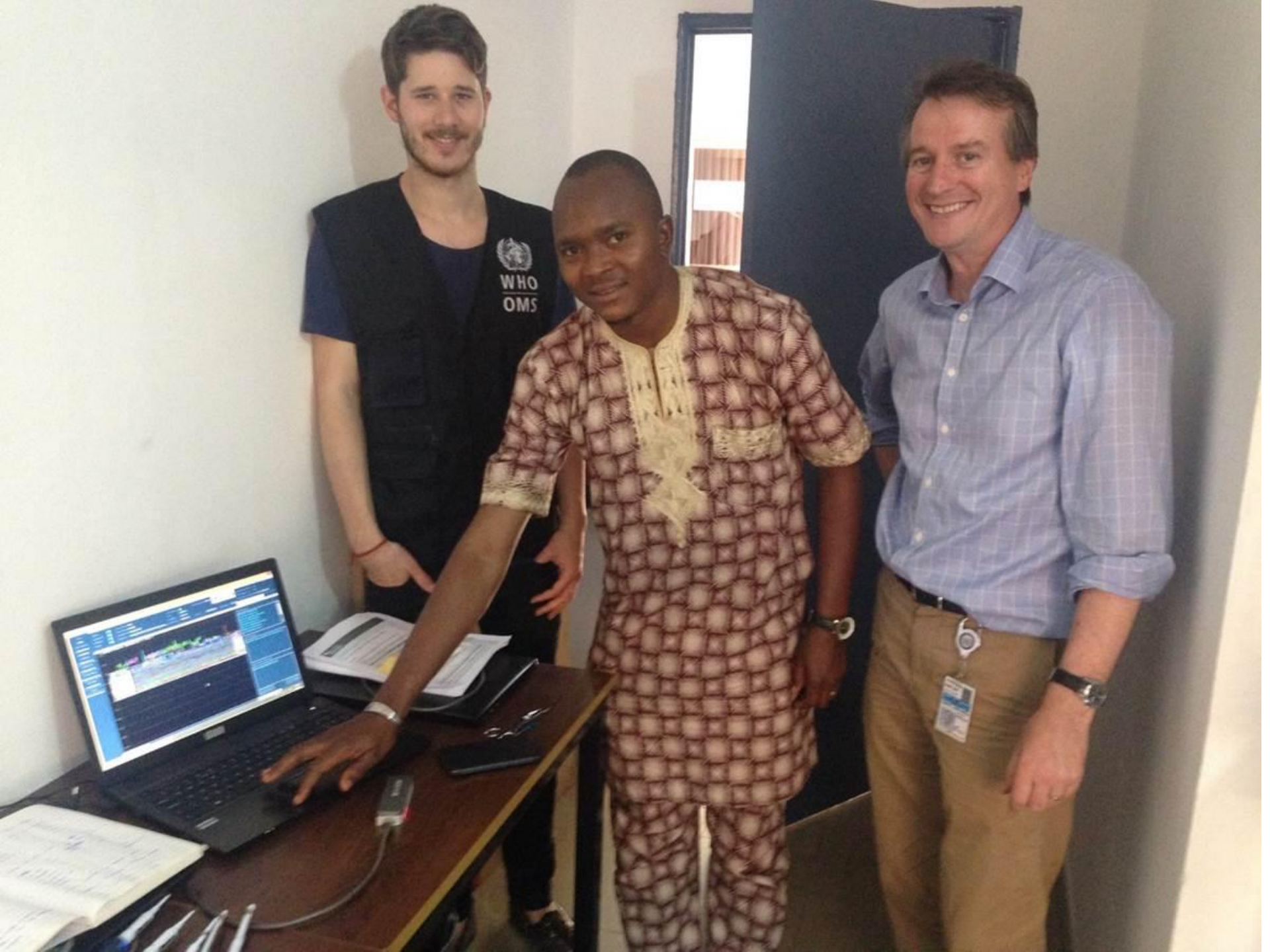
• The case from Conakry, a 21-year-old male, was reported from the Ratoma area of the city. However, he is not a known contact of a previous case, and genomic analyses suggest he was not infected with the strain of Ebola virus responsible for the most recent cases in Conakry and Forecariah. Investigations to identify the origin of infection are ongoing. The first case identified from Forecariah, a 35-year-old woman, was not a registered contact, and was identified after post-mortem testing of a community death. However, genomic analyses suggest she is part of the same chain of transmission—the Ratoma chain—as the 4 cases that were reported from the same subprefecture in Forecariah during the week ending 27 September 2015. The second case identified from Forecariah is her 3-month-old child, and was a registered contact. Of 246 contacts under follow-up in Guinea on 18 October, 42 were located in Conakry with the remainder located in Forecariah.

• In Sierra Leone, all contacts linked to the country's 2 most recently active chains of transmission, Bombali and Kambia, have completed 21-day follow-up. In addition, the last case to receive treatment was confirmed free of EVD after a second consecutive negative test on 25 September. The country will be declared free of EVD transmission on 7 November if no further cases are reported. However, 2 high-risk contacts—one from Bombali and one from Kambia—remain untraced. Efforts to trace these contacts will continue until 42 days have elapsed since the last reported case in each district.

CHU DE CONAKRY
HOPITAL NATIONAL DONKA
URGENCES MEDICO CHIRURGICALES
ET CONSULTATIONS EXTERNES

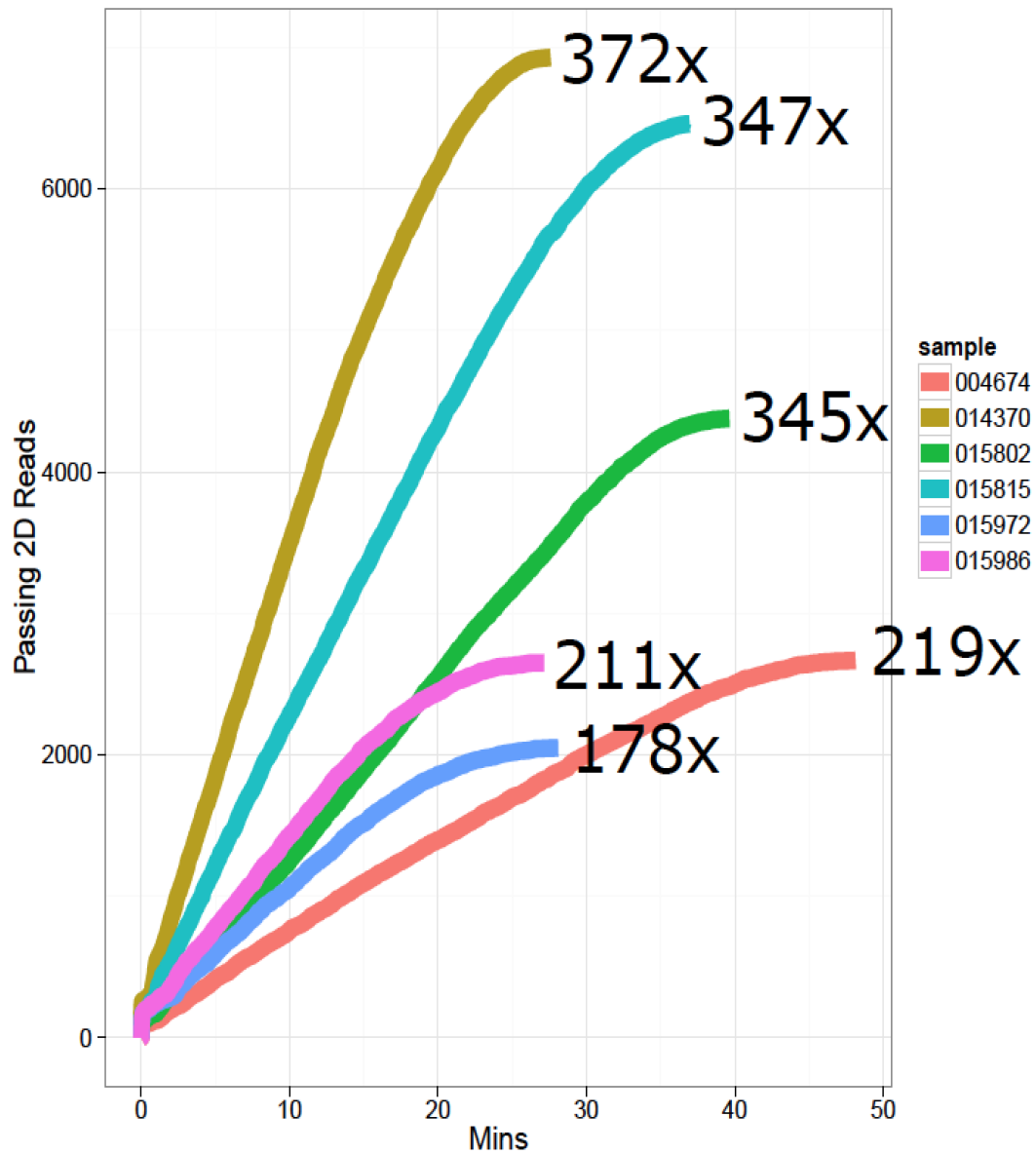
STATIONNEMENT INTER-
URGENCES → PERSONNEL

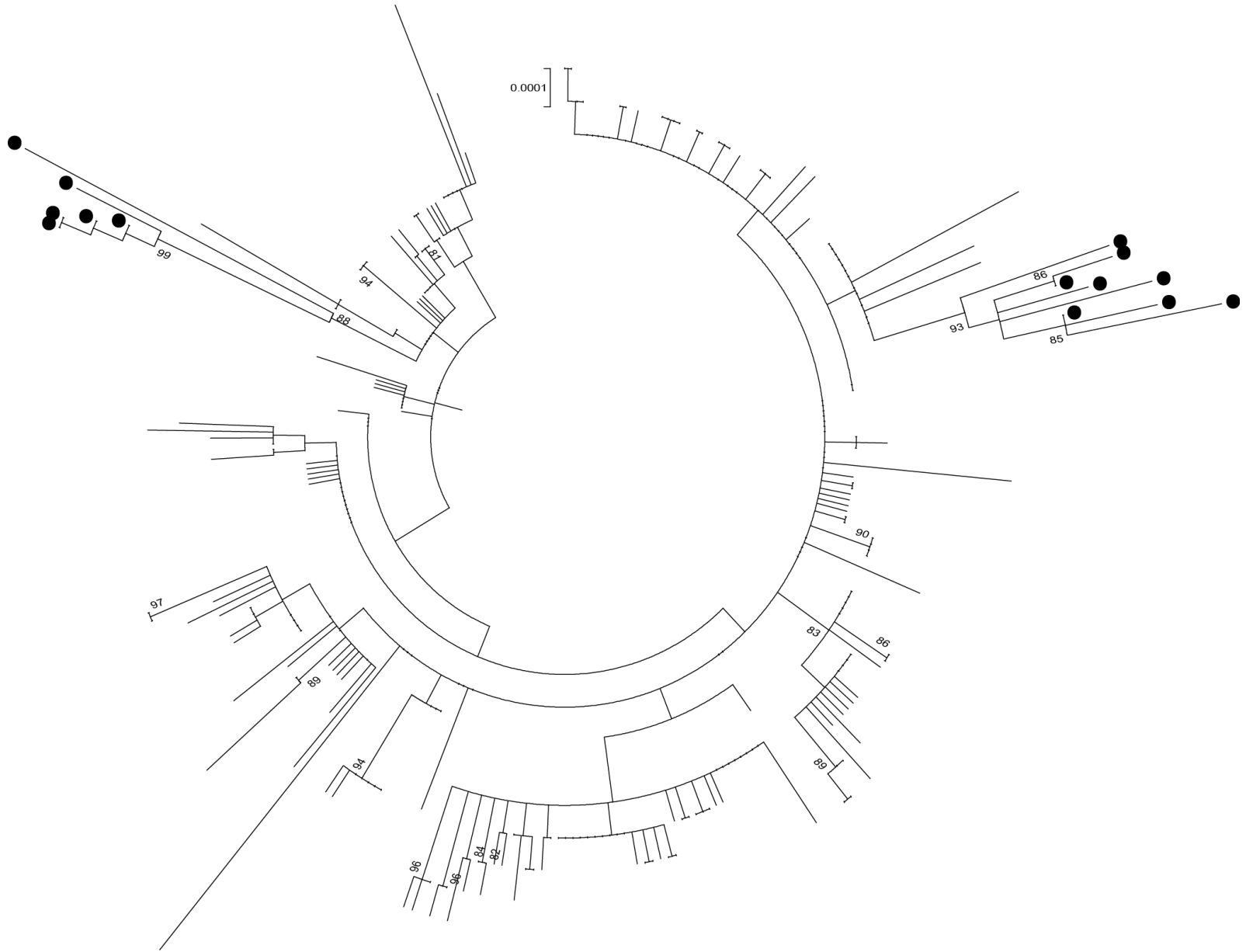




WHO
OMS







Sophie Duraffour

Lauren Cowley

Raymond Bore

Joseph Koundouno

Antonio Mazarelli

Liana Kafetzopoulou





Handwritten notes on a grid paper, including a table with columns for 'Date', 'Time', 'Temp', 'pH', 'Conc', 'Vol', 'Wt', 'No. of plates', and 'No. of wells'. The table contains several rows of data, including dates like '2023/08/01' and '2023/08/02', and various numerical values. There are also some diagrams and additional notes written in the margins.

| Date | Time | Temp | pH | Conc | Vol | Wt | No. of plates | No. of wells |
|------------|-------|------|-----|------|-----|-----|---------------|--------------|
| 2023/08/01 | 10:30 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 11:00 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 11:30 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 12:00 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 12:30 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 13:00 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 13:30 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 14:00 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 14:30 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 15:00 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 15:30 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
| 2023/08/01 | 16:00 | 25.0 | 7.2 | 1.0 | 100 | 100 | 1 | 96 |
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Lauren Cowley @LaurenCowley4 · Jun 5

#Ebola #MinION sequencing right in the field, all in a days work



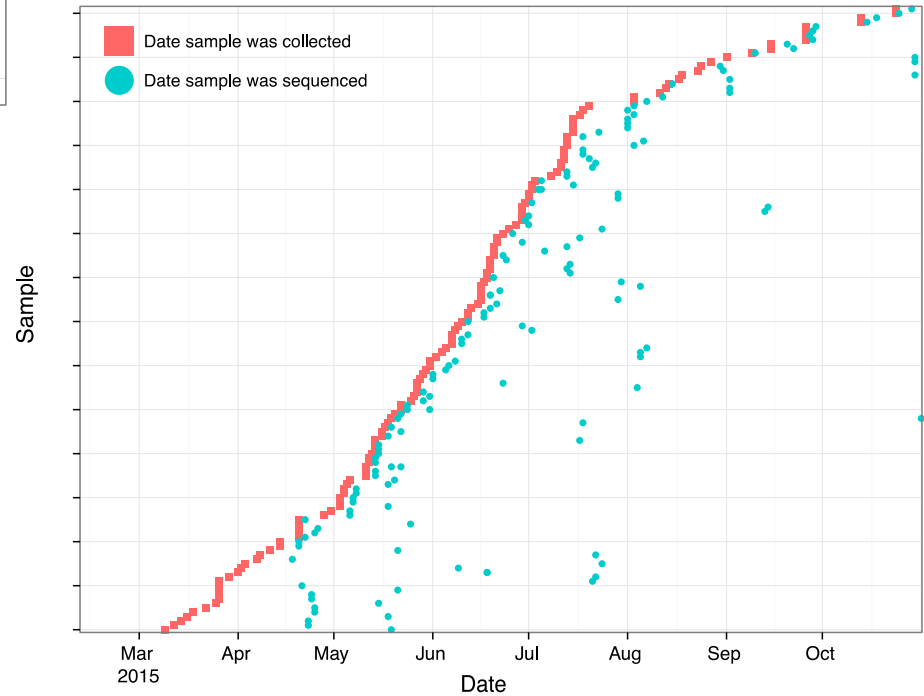
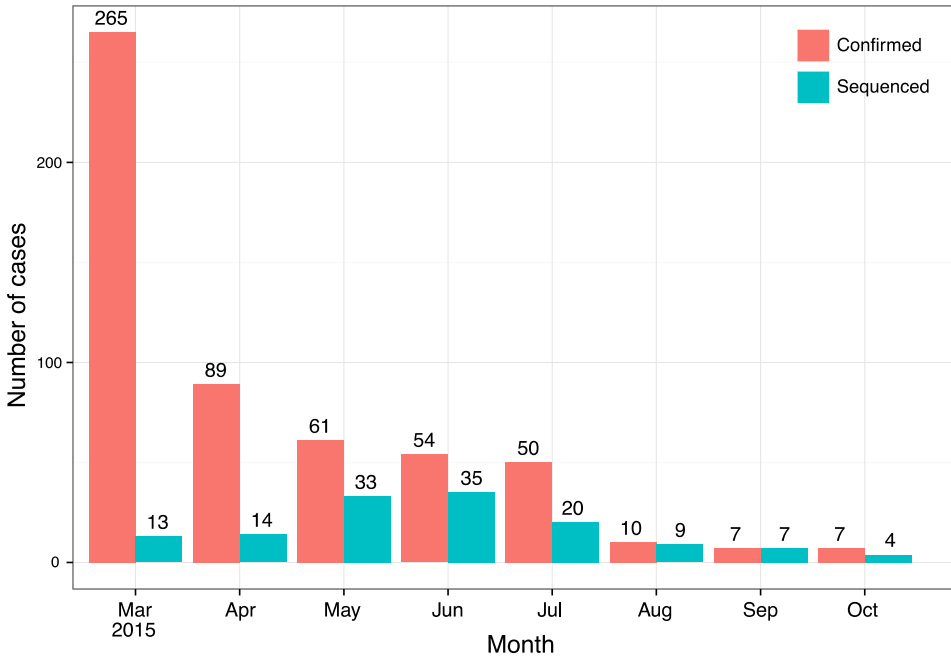
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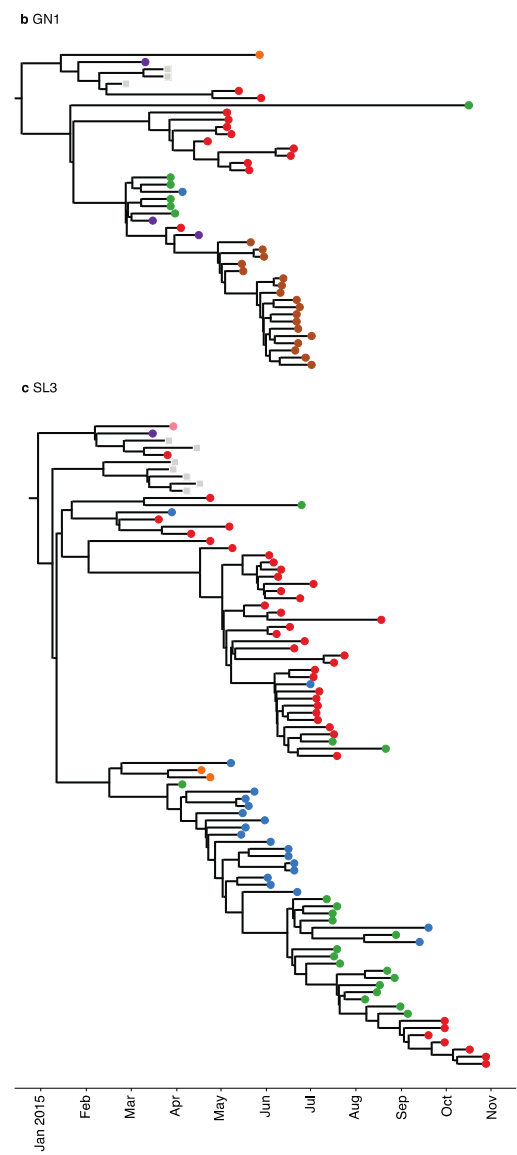
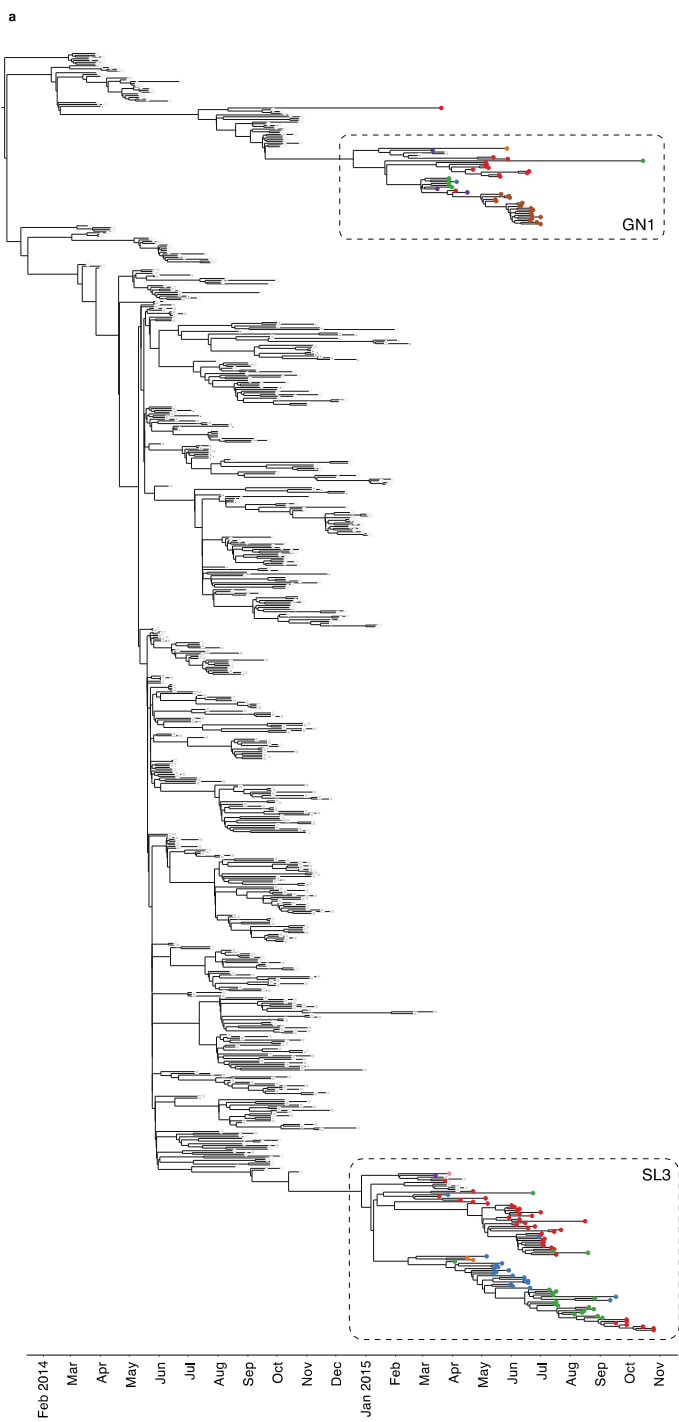


35



Outbreak coverage



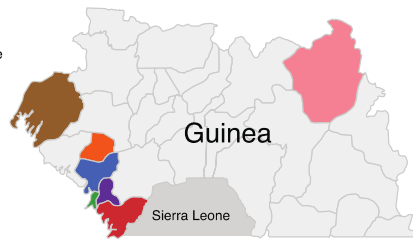


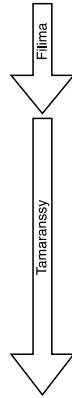
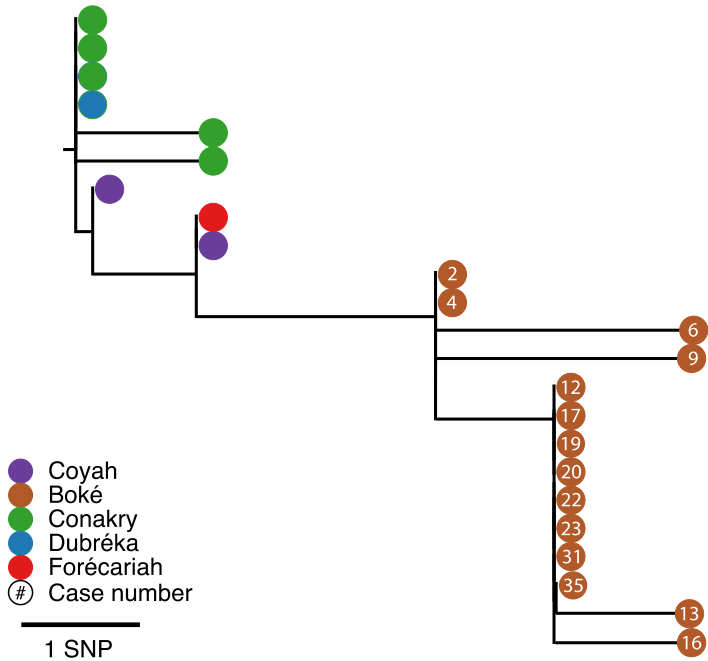
Country

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- Sierra Leone
- ▲ Liberia

Prefecture

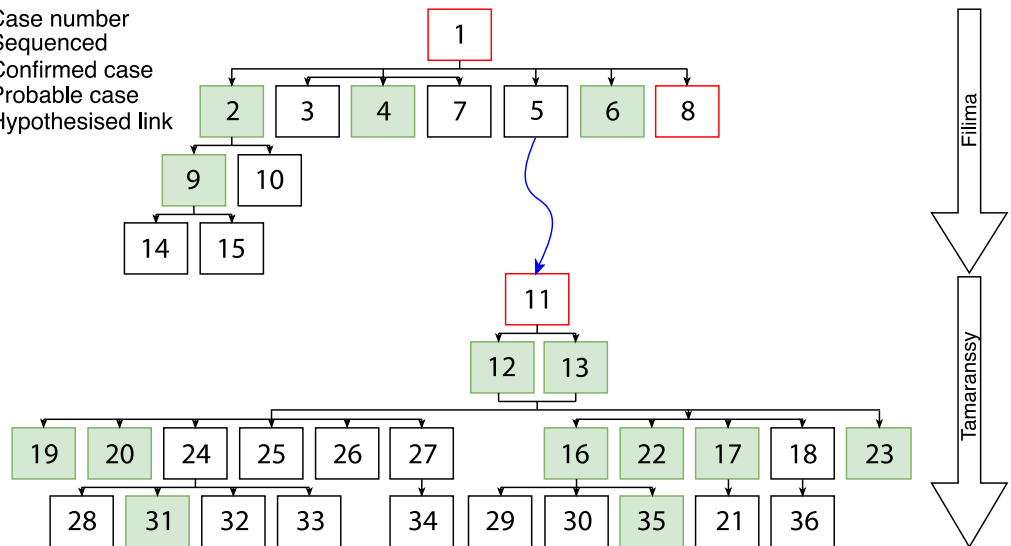
- Siguiri
- Boké
- Fria
- Conakry
- Dubréka
- Coyah
- Forécariah



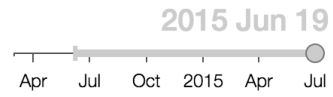


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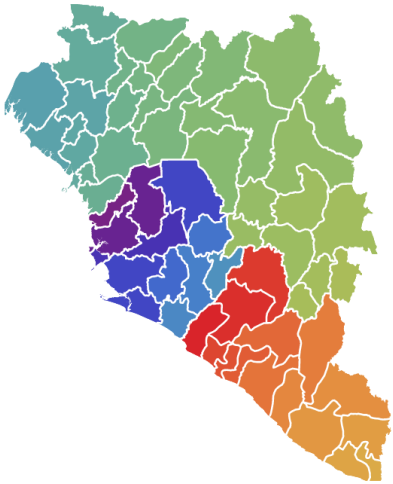
- # Case number
- Sequenced
- Confirmed case
- Probable case
- ~ Hypothesised link



Real-time analysis of Ebola virus evolution



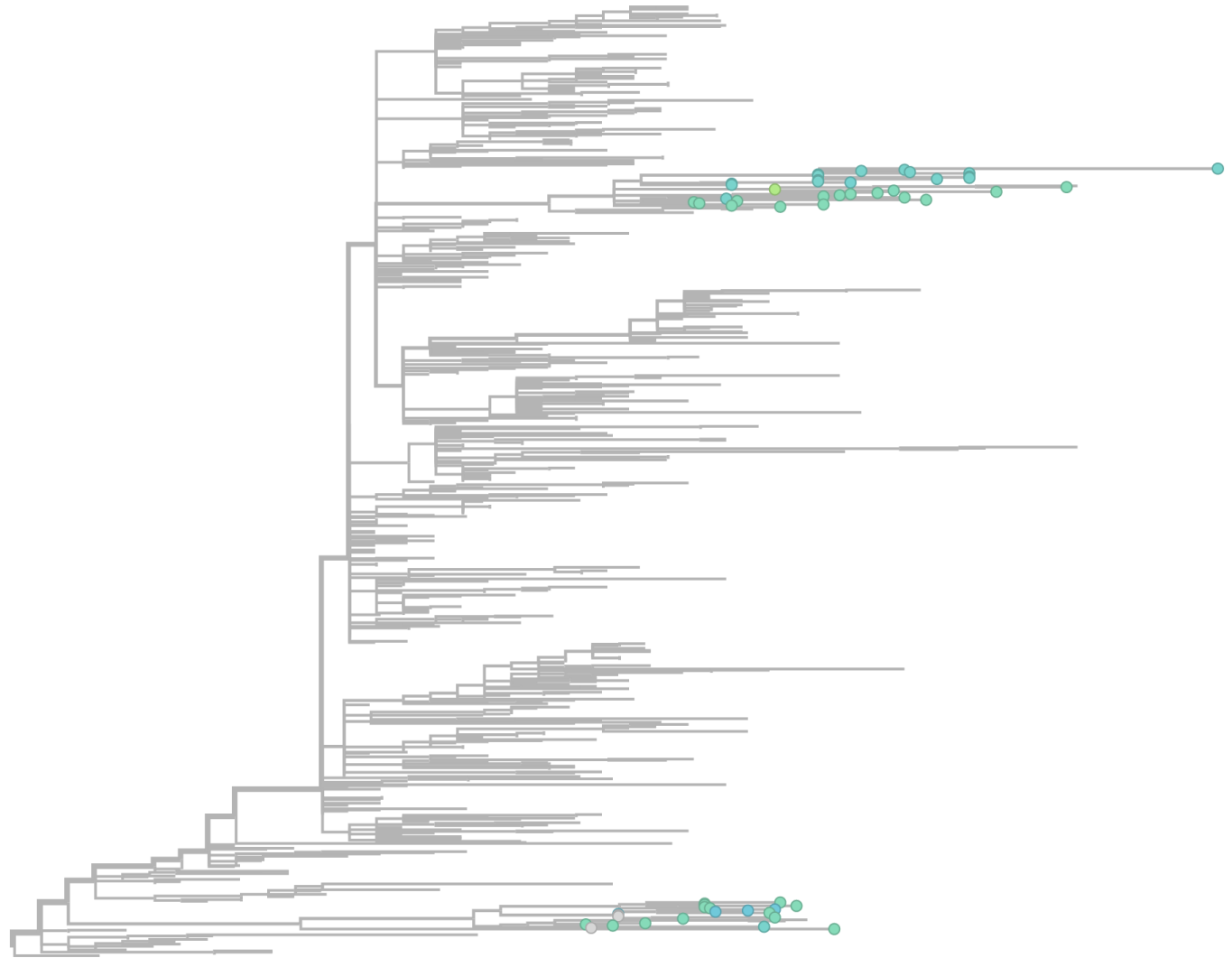
Region



Color by

Or

Region



Real-time, portable genome sequencing for Ebola surveillance

Joshua Quick^{1*}, Nicholas J. Loman^{1*}, Sophie Durauffour^{2,3*}, Jared T. Simpson^{4,5*}, Ettore Severi^{6*}, Lauren Cowley^{7*}, Joseph Akoi Bore², Raymond Koundouno², Gytis Dudas⁸, Amy Mikhail⁷, Nobila Ouédraogo⁹, Babak Afrough^{2,10}, Amadou Bah^{2,11}, Jonathan H. J. Baum^{2,3}, Beate Becker-Ziaja^{2,3}, Jan Peter Boettcher^{2,12}, Mar Cabeza-Cabrerizo^{2,3}, Álvaro Camino-Sánchez², Lisa L. Carter^{2,13}, Juliane Doerrbecker^{2,3}, Theresa Enkirch^{2,14}, Isabel García-Dorival^{2,15}, Nicole Hetzelt^{2,12}, Julia Hinzmann^{2,12}, Tobias Holm^{2,3}, Liana Eleni Kafetzopoulou^{2,16}, Michel Koropogui^{2,17}, Abigael Kosgey^{2,18}, Eeva Kuisma^{2,10}, Christopher H. Logue^{2,10}, Antonio Mazzarelli^{2,19}, Sarah Meisel^{2,3}, Marc Mertens^{2,20}, Janine Michel^{2,12}, Didier Ngabo^{2,10}, Katja Nitzsche^{2,3}, Elisa Pallasch^{2,3}, Livia Victoria Patrono^{2,3}, Jasmine Portmann^{2,21}, Johanna Gabriella Repits^{2,22}, Natasha Y. Rickett^{2,15,23}, Andreas Sachse^{2,12}, Katrin Singethan^{2,24}, Inês Vitoriano^{2,10}, Rahel L. Yemanaberhan^{2,3}, Elsa G. Zekeng^{2,15,23}, Trina Racine²⁵, Alexander Bello²⁵, Amadou Alpha Sall²⁶, Ousmane Faye²⁶, Oumar Faye²⁶, N'Faly Magassouba²⁷, Cecelia V. Williams^{28,29}, Victoria Amburgey^{28,29}, Linda Winona^{28,29}, Emily Davis^{29,30}, Jon Gerlach^{29,30}, Frank Washington^{29,30}, Vanessa Monteil³¹, Marine Jourdain³¹, Marion Bererd³¹, Alimou Camara³¹, Hermann Somlare³¹, Abdoulaye Camara³¹, Marianne Gerard³¹, Guillaume Bado³¹, Bernard Baillet³¹, Déborah Delaune^{32,33}, Koumpingnin Yacouba Nebie³⁴, Abdoulaye Diarra³⁴, Yacouba Savane³⁴, Raymond Bernard Pallawo³⁴, Giovanna Jaramillo Gutierrez³⁵, Natacha Milhano^{5,36}, Isabelle Roger³⁴, Christopher J. Williams^{6,37}, Facinet Yattara¹⁷, Kuiama Lewandowski¹⁰, James Taylor³⁸, Phillip Rachwal³⁸, Daniel J. Turner³⁹, Georgios Pollakis^{15,23}, Julian A. Hiscox^{15,23}, David A. Matthews⁴⁰, Matthew K. O'Shea⁴¹, Andrew McD. Johnston⁴¹, Duncan Wilson⁴¹, Emma Hutley⁴², Erasmus Smit⁴³, Antonino Di Caro^{2,19}, Roman Wölfel^{2,44}, Kilian Stoecker^{2,44}, Erna Fleischmann^{2,44}, Martin Gabriel^{2,3}, Simon A. Weller³⁸, Lamine Koivogui⁴⁵, Boubacar Diallo³⁴, Sakoba Keita¹⁷, Andrew Rambaut^{8,46,47}, Pierre Formenty³⁴, Stephan Günther^{2,3} & Miles W. Carroll^{2,10,48,49}

The Ebola virus disease epidemic in West Africa is the largest on record, responsible for over 28,599 cases and more than 11,299 deaths¹. Genome sequencing in viral outbreaks is desirable to characterize the infectious agent and determine its evolutionary rate. Genome sequencing also allows the identification of signatures of host adaptation, identification and monitoring of diagnostic targets, and characterization of responses to vaccines and treatments. The Ebola virus (EBOV) genome substitution rate in the Makona strain has been estimated at between 0.87×10^{-3} and 1.42×10^{-3} mutations per site per year. This is equivalent to 16–27 mutations in each genome, meaning that sequences diverge rapidly enough to identify distinct sub-lineages during a prolonged epidemic^{2–7}. Genome sequencing provides a high-resolution view of pathogen evolution and is increasingly sought after for outbreak surveillance. Sequence data may be used to guide control measures, but only if the results are generated quickly enough to inform interventions⁸. Genomic surveillance during the epidemic has been sporadic

owing to a lack of local sequencing capacity coupled with practical difficulties transporting samples to remote sequencing facilities⁹. To address this problem, here we devise a genomic surveillance system that utilizes a novel nanopore DNA sequencing instrument. In April 2015 this system was transported in standard airline luggage to Guinea and used for real-time genomic surveillance of the ongoing epidemic. We present sequence data and analysis of 142 EBOV samples collected during the period March to October 2015. We were able to generate results less than 24 h after receiving an Ebola-positive sample, with the sequencing process taking as little as 15–60 min. We show that real-time genomic surveillance is possible in resource-limited settings and can be established rapidly to monitor outbreaks.

Conventional sequencing technologies are difficult to deploy in developing countries, where availability of continuous power and cold chains, laboratory space, and trained personnel is restricted. In addition, some genome sequencer instruments, such as those using optical

science

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From Ebola to Zika, tiny mobile lab gives real-time DNA data on outbreaks

A genomic surveillance system which fits in a suitcase can help health workers to quickly understand the spread of viruses and break the chain of infection

Alert! There's a dangerous new viral outbreak: Zika conspiracy theories



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Cancer treatment development to be speeded up by new London research hub



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10,366



German scientists to conduct nuclear fusion experiment



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100

All today's stories

The laboratory in a suitcase

How genome sequencing can be quickly deployed to monitor outbreaks



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SCIENCE HEALTH REPORT

0
COMMENTS

This 'lab in a suitcase' helped track Ebola cases last year

By **Russell Brandom** on February 3, 2016 01:00 pm [Email](#) [@russellbrandom](#)



PART OF THIS STORYSTREAM

49 UPDATES TO
Tracking the deadly ebola outbreak in West Africa



Bill Gates [@BillGates](#)

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zibraproject.org



ZIKA IN BRAZIL REAL TIME ANALYSIS





Heathrow
Making every journey better

THE NORTH FACE

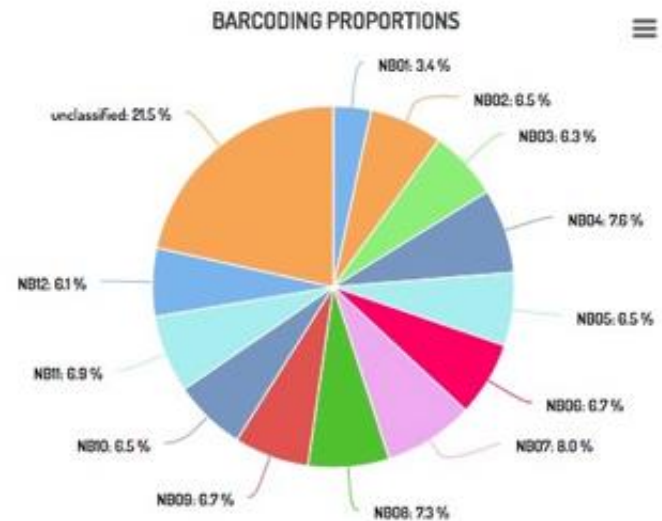


Project Goals

- ▶ 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







ZiBRA management team

team1

Database
RNA extractions

team2

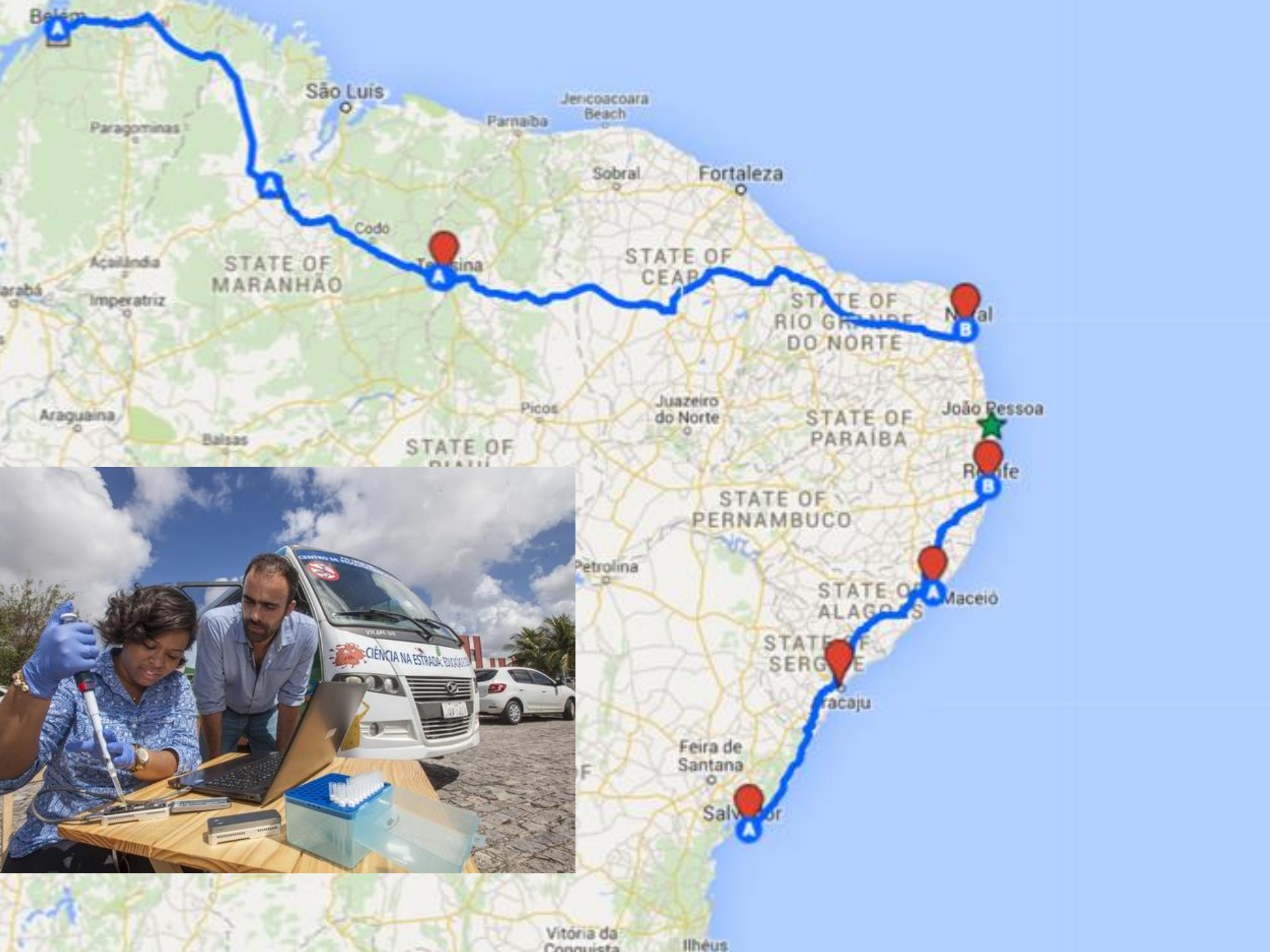
RT-PCR detections
Virus sequencing

team3

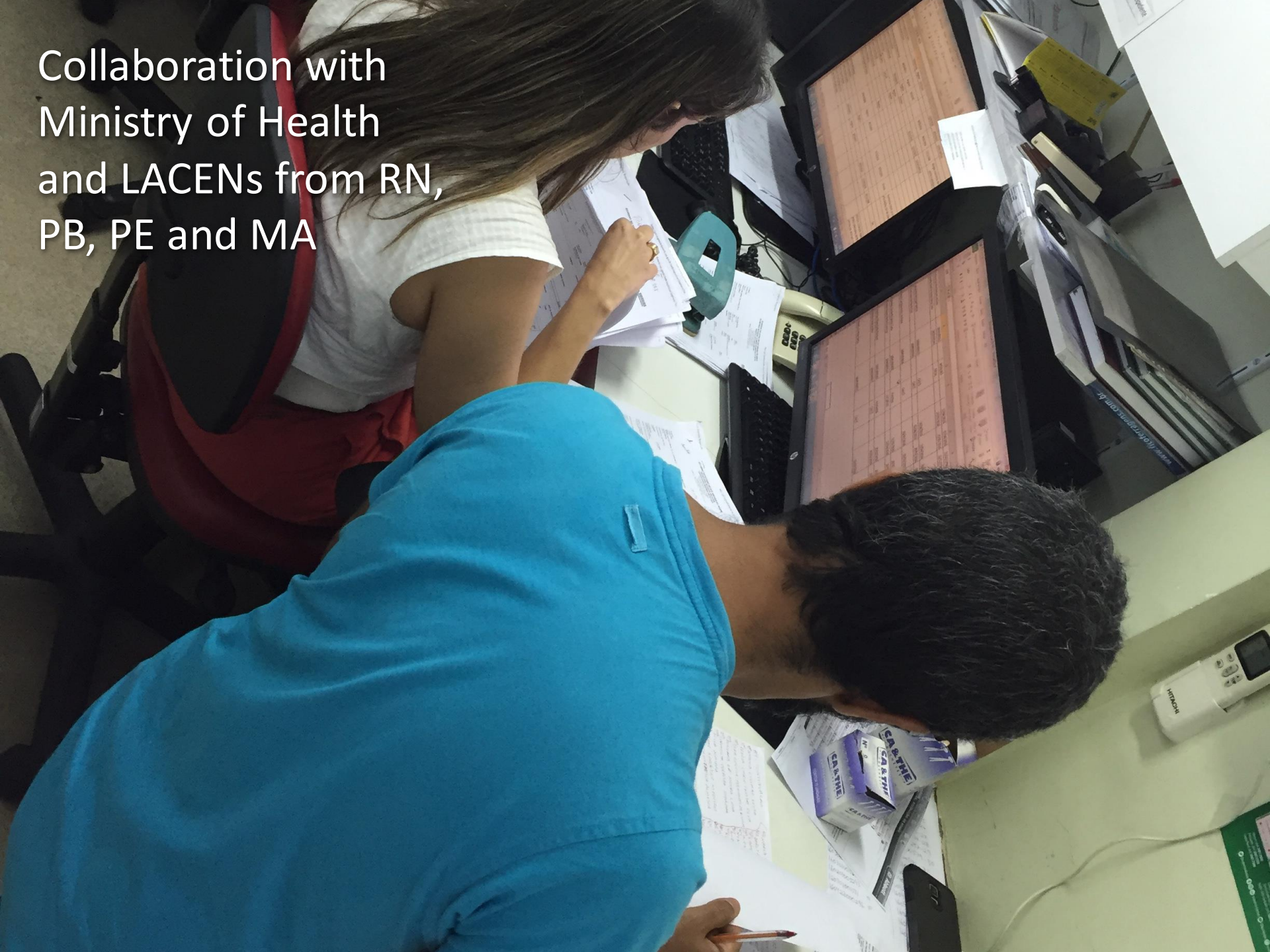
Mosquito capture
and identification

team4

IT and analyses
Media coverage

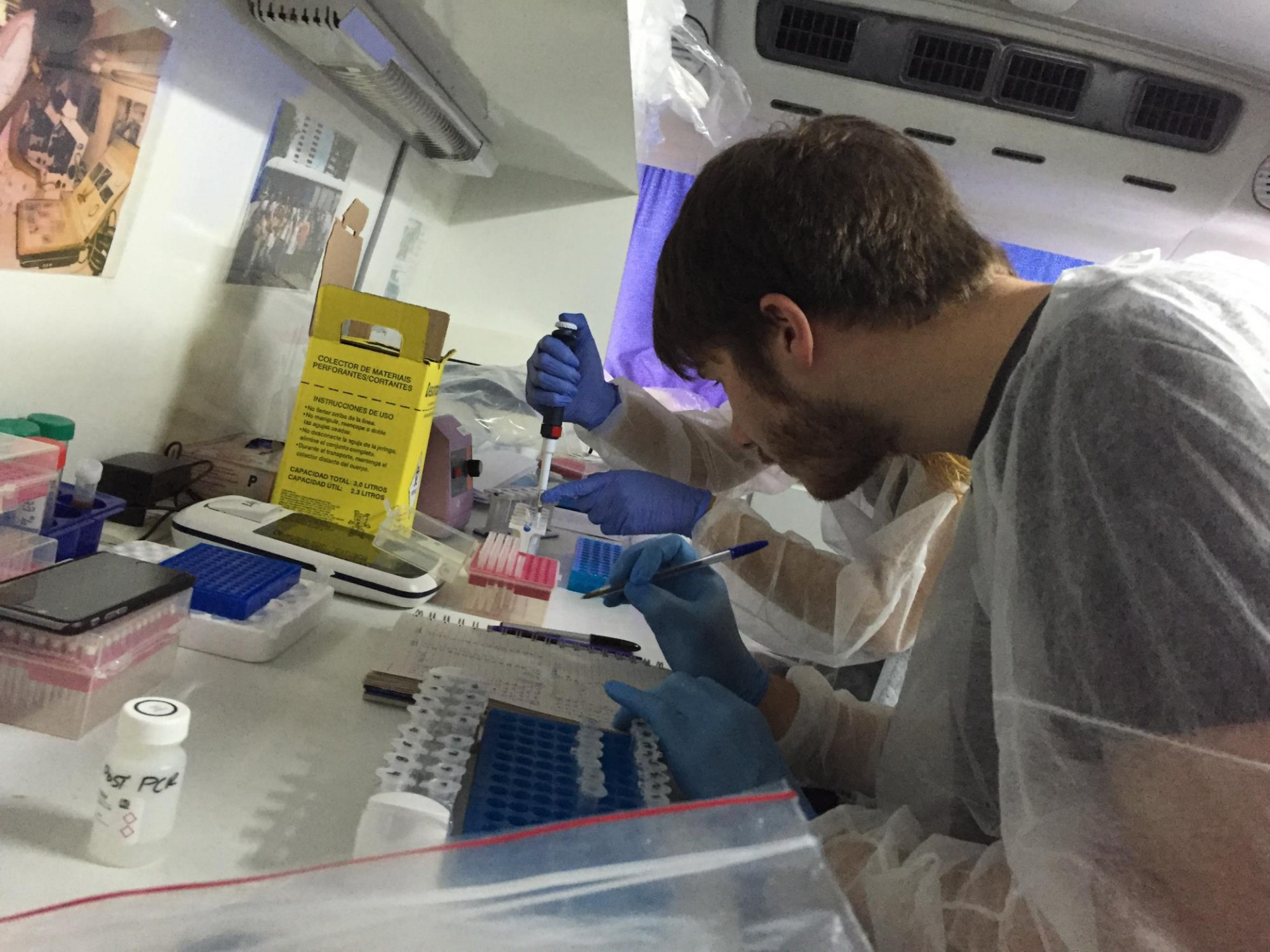


Collaboration with
Ministry of Health
and LACENs from RN,
PB, PE and MA









COLECTOR DE MATERIAIS
PERFORANTES/CORTANTES

INSTRUCCIONES DE USO

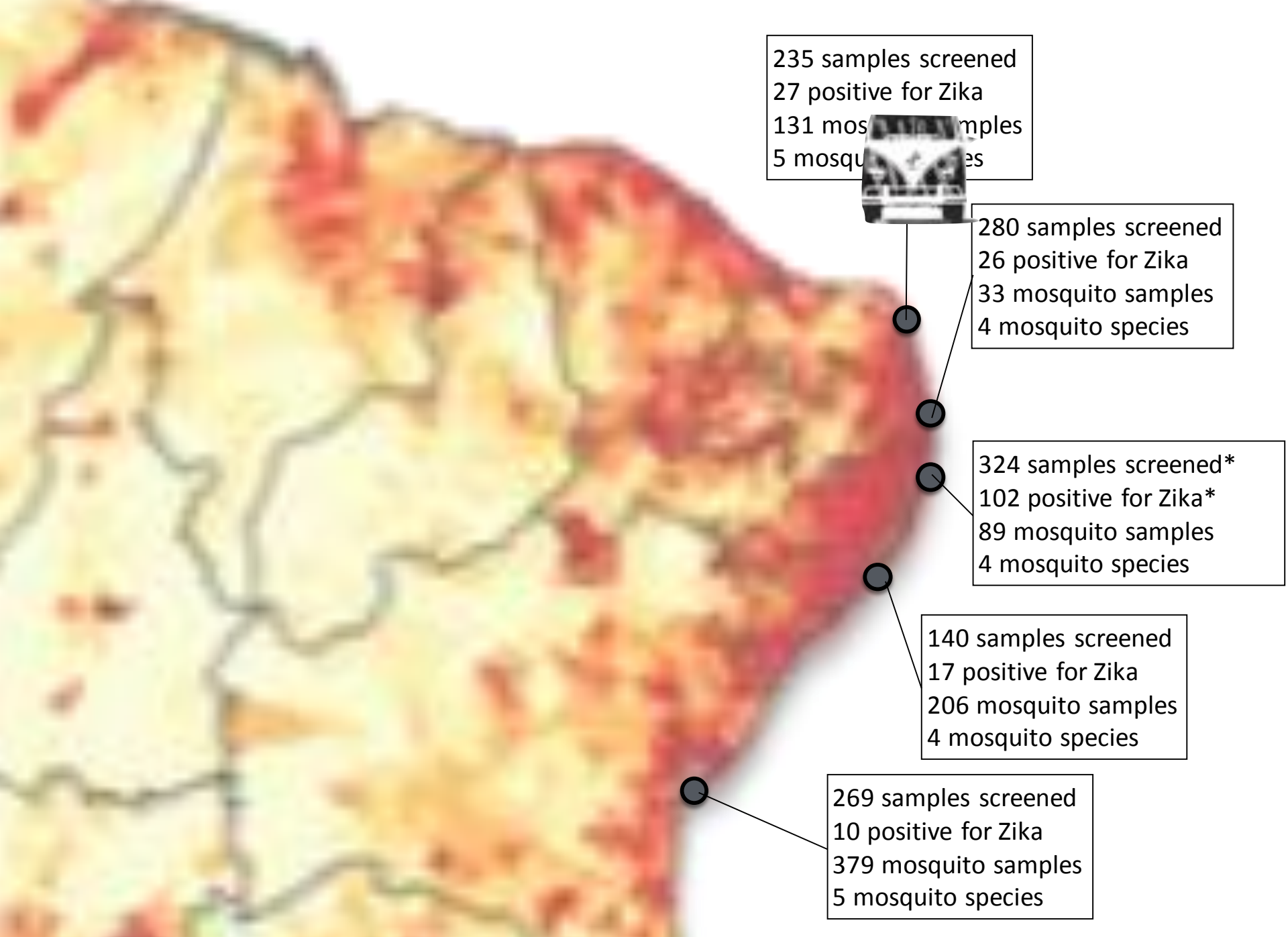
- No tener aprietas de la línea.
- No manipular presiones o golpes en las agujas cortantes.
- No desconectar la aguja de la jeringa, alimite el conjunto completo.
- Durante el transporte, mantenga el colector distante del cuerpo.

CAPACIDAD TOTAL: 3.0 LITROS
CAPACIDAD UTIL: 2.3 LITROS

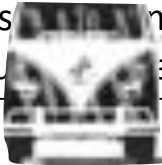
1st PCR







235 samples screened
27 positive for Zika
131 mosquito samples
5 mosquito species



280 samples screened
26 positive for Zika
33 mosquito samples
4 mosquito species

324 samples screened*
102 positive for Zika*
89 mosquito samples
4 mosquito species

140 samples screened
17 positive for Zika
206 mosquito samples
4 mosquito species

269 samples screened
10 positive for Zika
379 mosquito samples
5 mosquito species

| Federal state | <i>Aedes aegypti</i> | <i>Aedes albopictus</i> | <i>Haemagogus leuc</i> | <i>Culex quinquefasciatus</i> | <i>Wyeomyia spp</i> | <i>Aedes escapularis</i> | <i>Aedes taeniorhynchus</i> | <i>Aedes fluviatilis</i> |
|---------------------------------------|----------------------|-------------------------|------------------------|-------------------------------|---------------------|--------------------------|-----------------------------|--------------------------|
| Rio Grande Norte (Natal) | 16 | 29 | 2 | 46 | 38 | | | |
| Paraíba (Joao Pessoa) | 5 | 22 | | 3 | | 3 | | |
| Pernambuco (Recife) | 42 | 31 | | 9 | | 7 | | |
| Alagoas (Maceio) | 86 | 1 | | 118 | | | 1 | |
| Bahia (Feira de Santana and Salvador) | 75 | 75 | | 178 | | 50 | | 1 |

Absent 1 10 20 30 40 50 % of total population



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

Laboratories involved

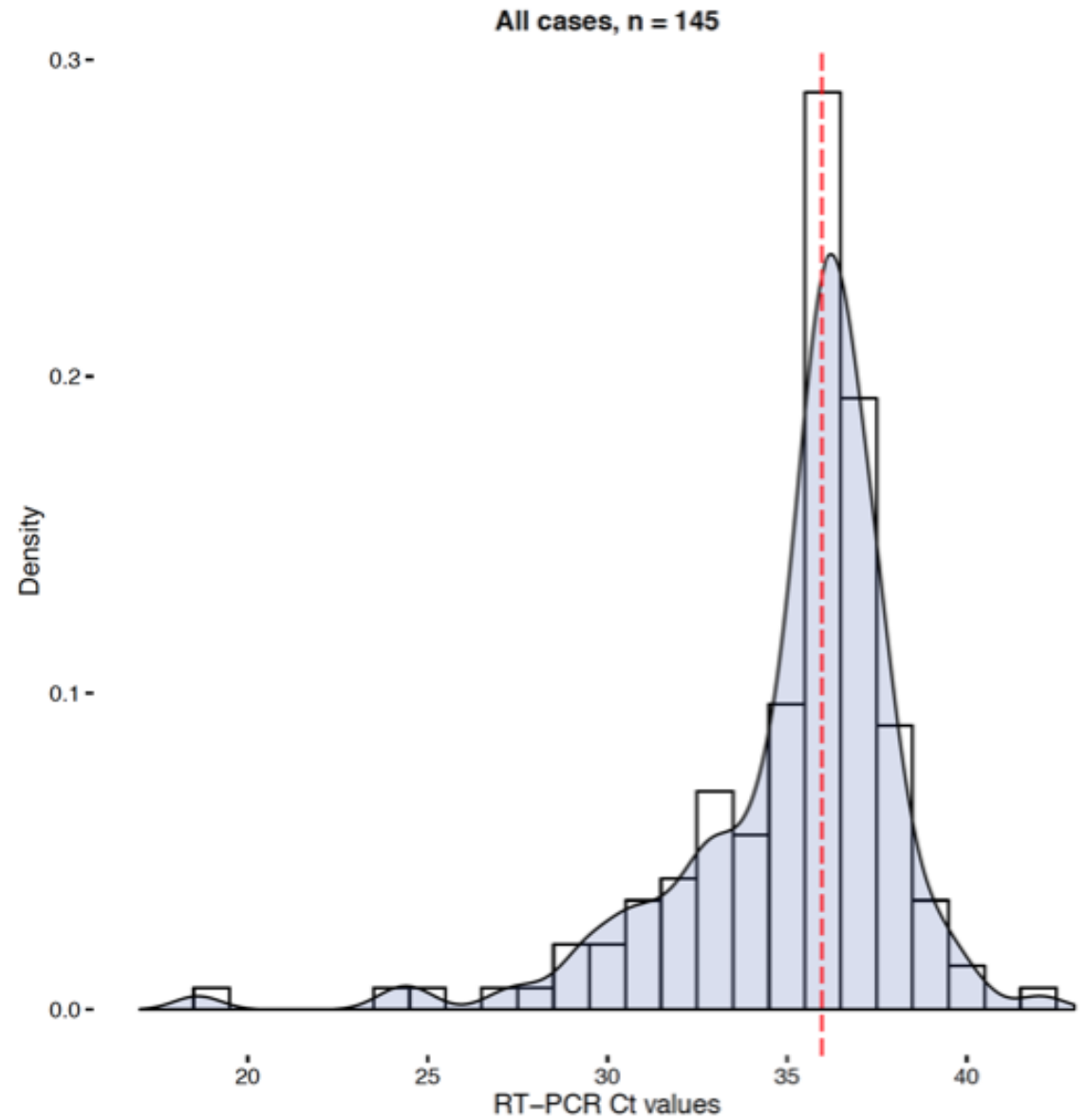
| | | Positive | % | |
|---------------------------------------|-------------|------------|----------|---|
| Universidade de São Paulo (Tocantins) | 10,302 | 633* | 70* | GO, TO, RR, AP, RS, PR, SP, MS, MT, ES, MG, AL, PI, DF |
| LACEN Rio Grande do Norte (Natal) | 431 | 27 | 6 | RN |
| LACEN Paraíba (João Pessoa) | 280 | 26 | 9 | PB |
| FioCruz/LACEN Pernambuco (Recife) | 324 | 102* | 32* | PE |
| LACEN Alagoas (Maceio) | 140 | 17 | 12 | AL |
| FioCruz Bahia (Salvador) | 232 | 11 | 5 | BA, MS |
| Total | 1407 | 212 | 8 | 17 states |

~55 libraries sequenced in 1 week over 5 runs

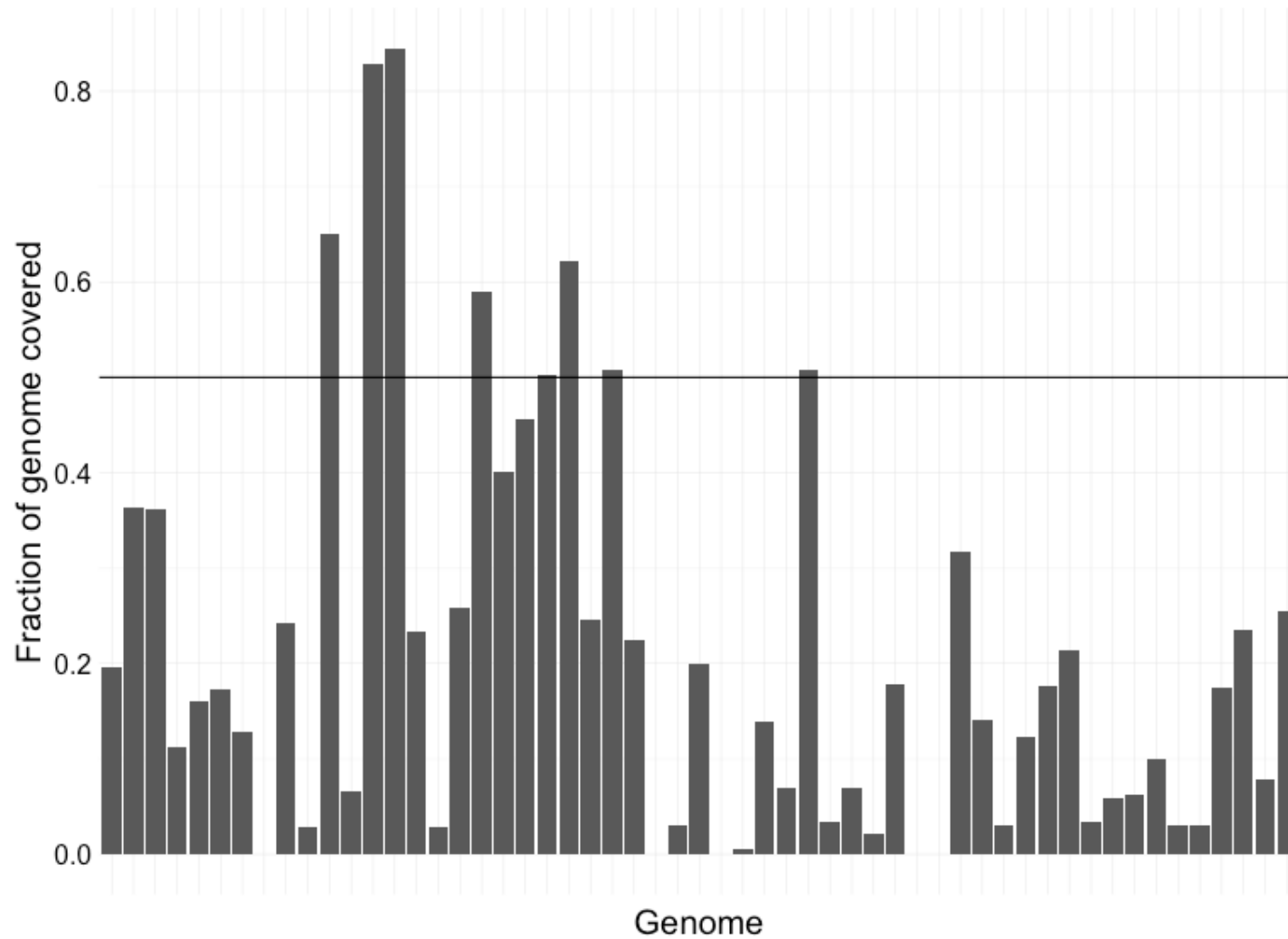




Technical challenges: Zika
Ct values are very low



Just eight genomes have >50% coverage



Zika virus pilot

By Karthik Gangavarapu | April 5, 2016 | Data

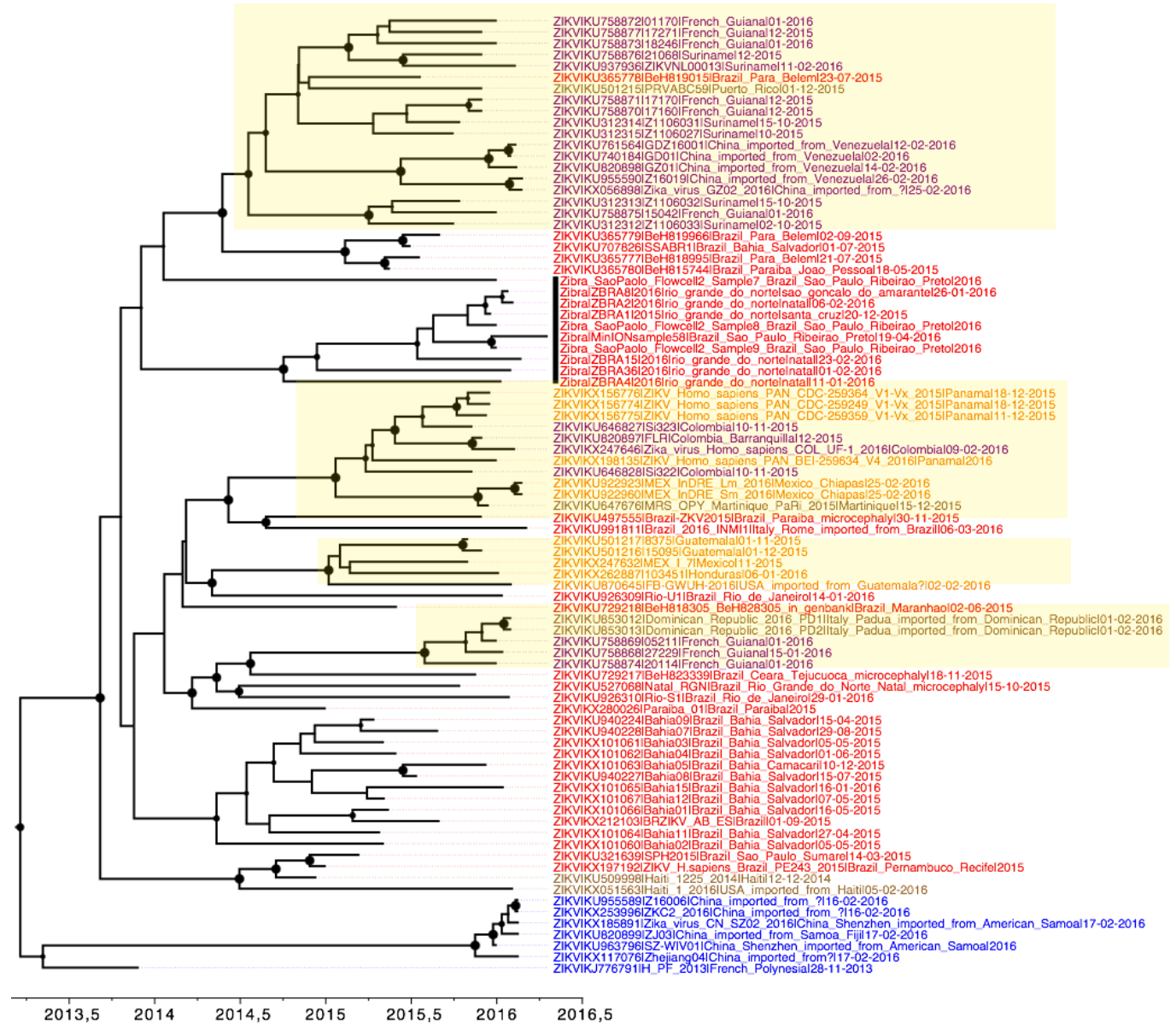
We recently received plasma samples from Zika virus (ZIKV) patients in Colombia. We performed QC on the samples and unfortunately very few had detectable levels of ZIKV by qPCR. We extracted RNA from two of these patient samples (Z184 and Z186), as well as a positive control (seed stock of the Malaysian 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/>

Phylogenetic tree shows multiple exports from Brazil



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

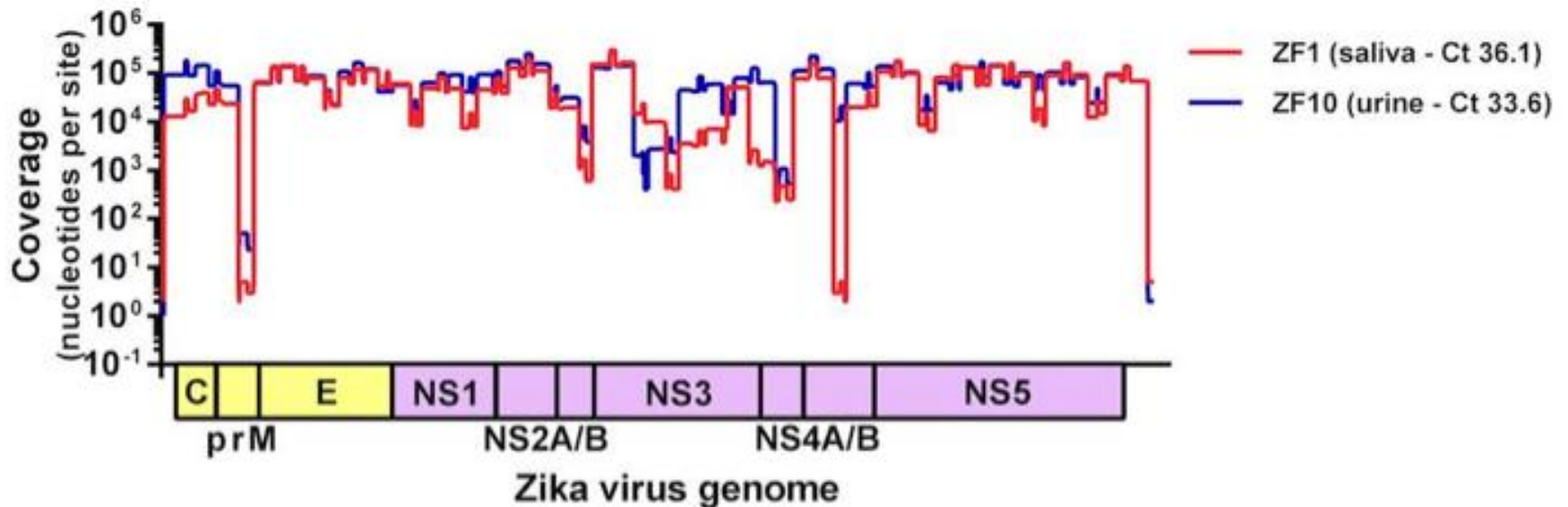
| Region No. | Pool | Left Primer Name | Left Primer Sequence |
|------------|------|------------------|----------------------------|
| 1 | 1 | 400_1_LEFT_3 | AGCAGAAAGTGTCAGTTGTAAAGCA |
| 2 | 2 | 400_2_LEFT_1 | AGCAATGCAGTTCTTTGAGGGG |
| 3 | 1 | 400_3_LEFT_4 | GAGCAAAATATCAGGACAAAACACCG |
| 4 | 2 | 400_4_LEFT_2 | ATGAAGACTGTTCAGGGCTGGT |
| 5 | 1 | 400_5_LEFT_0 | GGGTCAAGTCAGATCCCTAAATGC |
| 6 | 2 | 400_6_LEFT_4 | AACCAGAAGTCCAGAAGCTGTCT |
| 7 | 1 | 400_7_LEFT_4 | GATCTTCGTCATCCGAGTGCT |
| 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 | AAAAACCTCTCCTGTGTCAGC |
| 12 | 2 | 400_12_LEFT_0 | ATTAGCTTTGTCAGGAGCCCCA |

primal.zibraproject.org

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

Table 1: Run statistics

| directory | uncalled | uploaded | pass | fail |
|---------------------|----------|----------|--------|--------|
| 20160915_library20 | 4 | 505748 | 129464 | 296354 |
| 20160910_library13 | 5 | 51996 | 13454 | 37886 |
| 20160920_library21 | 317593 | 136357 | 38329 | 83664 |
| 20160913_library16 | 4 | 271357 | 82175 | 165015 |
| 20160922_Library_22 | 17735 | 462119 | 112428 | 270343 |
| 20160912_library15 | 4 | 682199 | 228341 | 412485 |
| 20160927_Library_23 | 113321 | 169031 | 31837 | 94364 |
| 20160914_library18 | 7 | 266579 | 75997 | 182341 |
| 20160914_library19 | 5 | 219886 | 63111 | 145844 |
| 20160914_library17 | 4 | 237227 | 74614 | 159002 |
| 20160910_library14 | 2040 | 377714 | 99675 | 250667 |

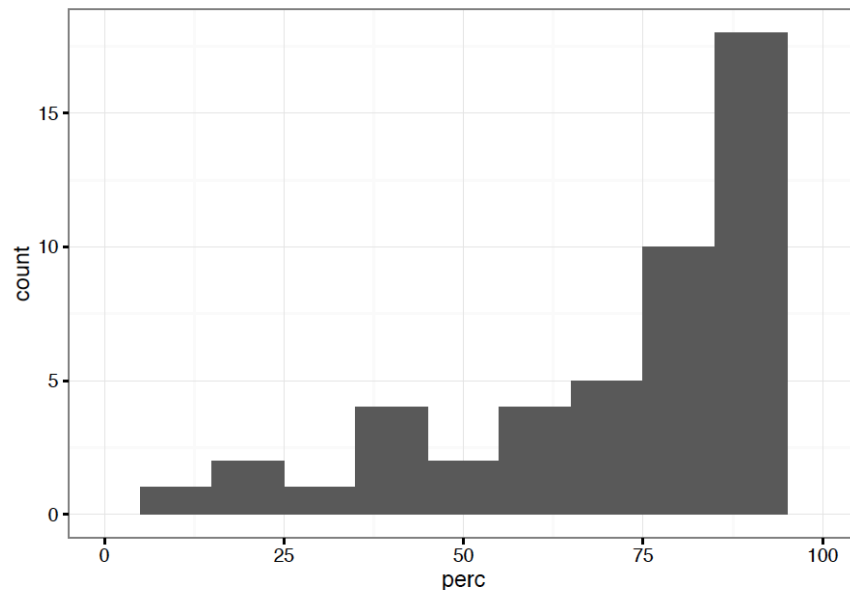
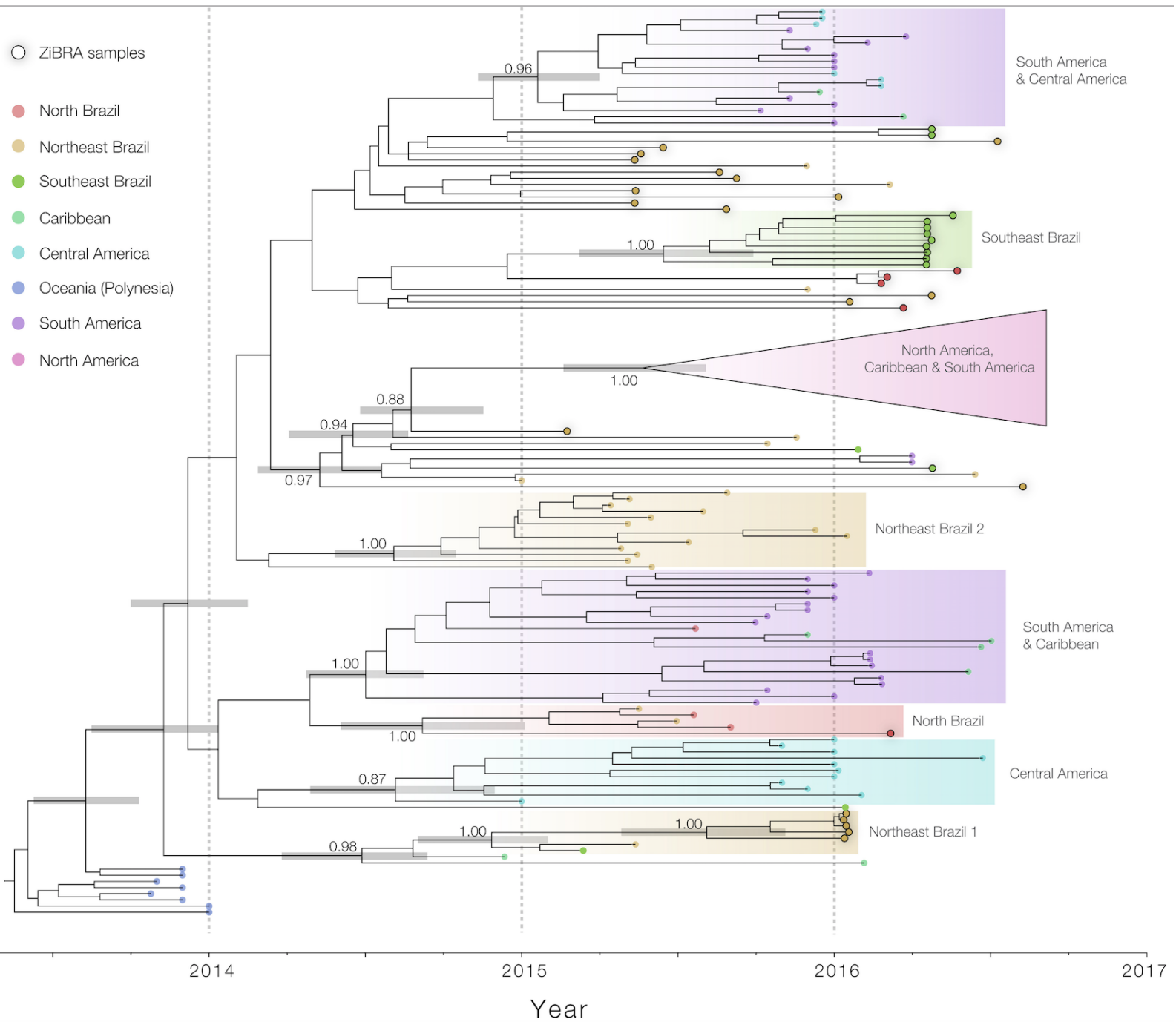


Table 2: Run statistics

| run | sample | reads | mapped | basescovered | basescovered25x | perc |
|---------------------|---------|-------|--------|--------------|-----------------|------|
| 20160922_Library_22 | ZBRX4 | 13708 | 13682 | 10609 | 10608 | 98 |
| 20160914_library18 | ZBRX4 | 14806 | 14722 | 10609 | 10608 | 98 |
| 20160914_library19 | ZBRX13 | 11950 | 11809 | 10609 | 10608 | 98 |
| 20160914_library19 | ZBRX15 | 14529 | 14226 | 10609 | 10605 | 98 |
| 20160910_library14 | ZBRC319 | 17805 | 17316 | 10609 | 10608 | 98 |
| 20160915_library20 | ZBRX130 | 30709 | 30517 | 10473 | 10313 | 95 |
| 20160922_Library_22 | ZBRX12 | 21390 | 19447 | 10609 | 10332 | 95 |
| 20160914_library18 | ZBRX1 | 9674 | 9243 | 10609 | 10303 | 95 |
| 20160920_library21 | ZBRX137 | 9766 | 8160 | 10440 | 9985 | 92 |
| 20160912_library15 | ZBRD116 | 22370 | 21211 | 10345 | 9959 | 92 |
| 20160910_library14 | ZBRC302 | 26847 | 26331 | 10602 | 9993 | 92 |
| 20160915_library20 | ZBRX16 | 21596 | 21513 | 10607 | 9871 | 91 |
| 20160920_library21 | ZBRX130 | 9778 | 8686 | 10588 | 9867 | 91 |
| 20160922_Library_22 | ZBRX2 | 24850 | 24105 | 10608 | 9897 | 91 |
| 20160912_library15 | ZBRA105 | 82728 | 58128 | 10494 | 9934 | 91 |
| 20160910_library14 | ZBRC301 | 21187 | 20826 | 10427 | 9923 | 91 |
| 20160920_library21 | ZBRX103 | 10541 | 9230 | 10280 | 9733 | 90 |
| 20160927_Library_23 | ZBRX100 | 6783 | 5976 | 10609 | 9751 | 90 |
| 20160914_library18 | ZBRX2 | 14850 | 13650 | 10344 | 9735 | 90 |
| 20160914_library19 | ZBRX12 | 14234 | 10999 | 10609 | 9733 | 90 |

○ ZIBRA samples

- North Brazil
- Northeast Brazil
- Southeast Brazil
- Caribbean
- Central America
- Oceania (Polynesia)
- South America
- North America



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

nextstrain / Zika -
Real-time tracking of Zika virus evolution

Phylogeny

2016 Aug 30

Apr Jul Oct 2016 Apr Jul

- Region
- French Polynesia
 - American Samoa
 - China
 - Brazil
 - Colombia
 - French Guiana
 - Suriname
 - Venezuela
 - Panama
 - Honduras
 - Guatemala
 - Mexico
 - Martinique
 - Puerto Rico
 - Dominican Republic
 - Viet

Color by: geographic region

Or: Genomic position
branch labels

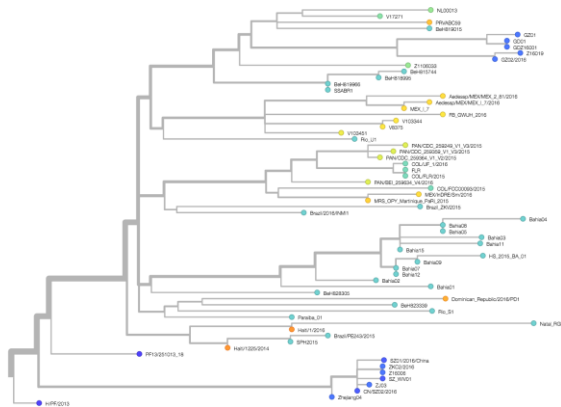
Study: all

Region: all

search strains...

clear

reset layout



'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 mosquitoes have infected 15 people with the Zika virus. Photograph: ddp USA/Rex/Shutterstock

Ebola

Nick Loman, University of Birmingham

Jared Simpson, OICR

Simon Weller, Dstl Porton Down

Jamie Taylor, Dstl Porton Down

Phil Rachwal, Dstl Porton Down

Carl Mayers, Dstl Porton Down

Miles Carroll, Public Health England

Lauren Cowley, Public Health England

Sophie Daffour, EMLab

Martin Gabriel, EMLab

Stefan Gunther, EMLab

Andrew Rambaut, Edinburgh

Gytis Dudas, Edinburgh

Julian Hiscox, Liverpool

Georgios Pollakis, Liverpool

Dave Matthews, Bristol

Oxford Nanopore



Zika

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

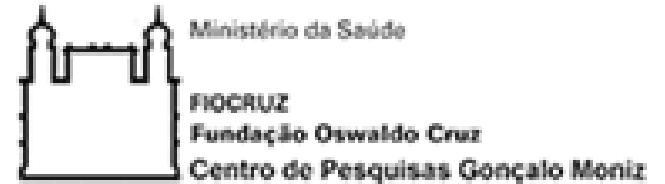
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Oliver Hartwell, Oxford Nanopore Technologies

Richard Ronan, Oxford Nanopore Technologies

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

<https://twitter.com/zibraproject>



Thank you

