

Field deployment of sequencing technology - practical considerations and solutions



Sierra Leone March 2015



DRC December 2019

Professor Ian Goodfellow, Dept of Pathology, University of Cambridge



What do we do "normally"?



RESEARCH ARTICLE
Host-Microbe Biology

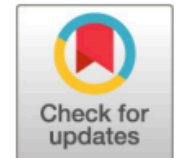


Epigenetic Suppression of Interferon Lambda Receptor Expression Leads to Enhanced Human Norovirus Replication *In Vitro*

Sabastine E. Arthur,^a Frédéric Sorgeloos,^a Myra Hosmillo,^a Ian G. Goodfellow^a



RESEARCH ARTICLE
Host-Microbe Biology



Norovirus Replication in Human Intestinal Epithelial Cells Is Restricted by the Interferon-Induced JAK/STAT Signaling Pathway and RNA Polymerase II-Mediated Transcriptional Responses

Myra Hosmillo,^a Yasmin Chaudhry,^a Komal Nayak,^b Frederic Sorgeloos,^a Bon-Kyoung Koo,^{c,d} Alessandra Merenda,^{c*} Reidun Lillestol,^e Lydia Drumright,^e Matthias Zilbauer,^b Ian Goodfellow^a



RESEARCH ARTICLE



Noroviruses subvert the core stress granule component G3BP1 to promote viral VPg-dependent translation

Myra Hosmillo^{1†}, Jia Lu^{1†}, Michael R McAllaster^{2†}, James B Eaglesham^{1,3}, Xinjie Wang^{1,4}, Edward Emmott^{1,5,6}, Patricia Domingues¹, Yasmin Chaudhry¹, Tim J Fitzmaurice¹, Matthew KH Tung¹, Marc Dominik Panas⁷, Gerald McInerney⁷, Nicolas Locker⁸, Craig B Wilen^{9*}, Ian G Goodfellow^{1*}

LETTER

<https://doi.org/10.1038/s41586-018-0852-1>

Calicivirus VP2 forms a portal-like assembly following receptor engagement

Michaela J. Conley¹, Marion McElwee¹, Liyana Azmi², Mads Gabrielsen³, Olwyn Byron⁴, Ian G. Goodfellow⁵ & David Bhella^{1*}

HOW DID WE REACH
THIS POINT?



Sierra Leone November 2014

DECEMBER 2014 – THE DECISION TO SEQUENCE

December 2014 - Identified a need for real-time in-country genetic sequencing capabilities to aid with epidemiological tracking as contact tracing was ineffective in many instances.

PROBLEM:

- Limited sequence availability (Gire et al., Sept 14, 99 EBOV genomes)
- Limited access to data for those in the field (internet etc)
- Data being produced in a timeframe that was of little use to those on the ground– 3-6 months from samples taken to sequence availability

DECEMBER 2014 – UTILITY OF REAL-TIME GENOMICS FOR EBOV

Identify and/or confirm epidemiological links – particularly essential during the final stages of the epidemic when “atypical” transmission occurs.

Understand virus evolution – had long-term transmission in humans resulted in virus adaptation to a new host?

Determine if repeated human-human transmission compromised the efficiency of detection by diagnostic primer sets.

“Lets move a sequencer to an Ebola treatment centre –

How hard can it be?”

WHAT SEQUENCING PLATFORM TO USE?

- Robust and tolerant of extreme/unconventional environments
- Easy to install – capable of working out of the box
- Low maintenance
- Simple and robust workflow - idiot proof
- High quality data
- High throughput – *need to develop a reference database of sequences*

- A company willing to engage fully with the project and provide innovative solutions to practical problems

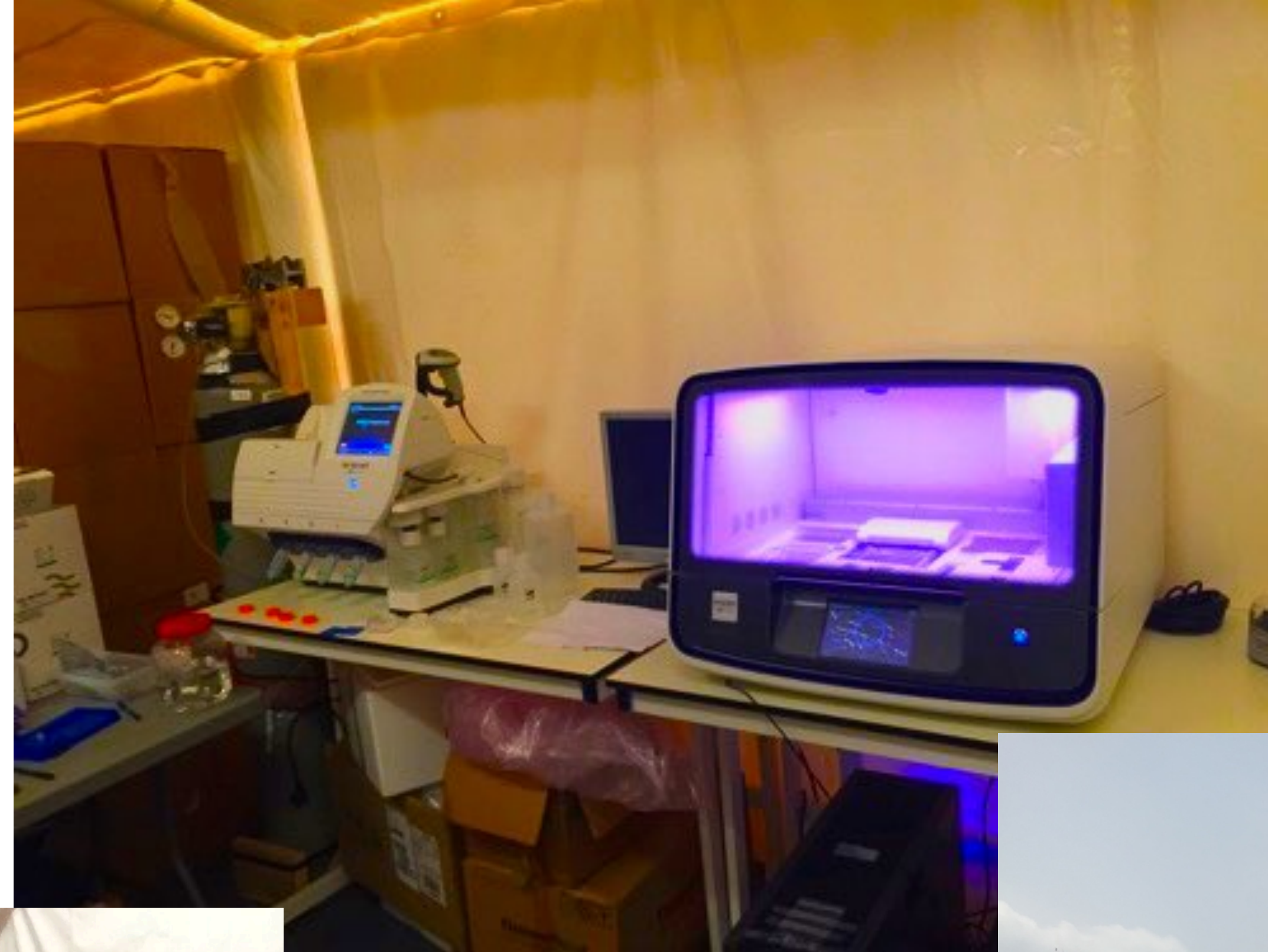


**ThermoFisher Ion Torrent
PGM and Ion Chef**

DEPLOYMENT #3: MARCH 2015



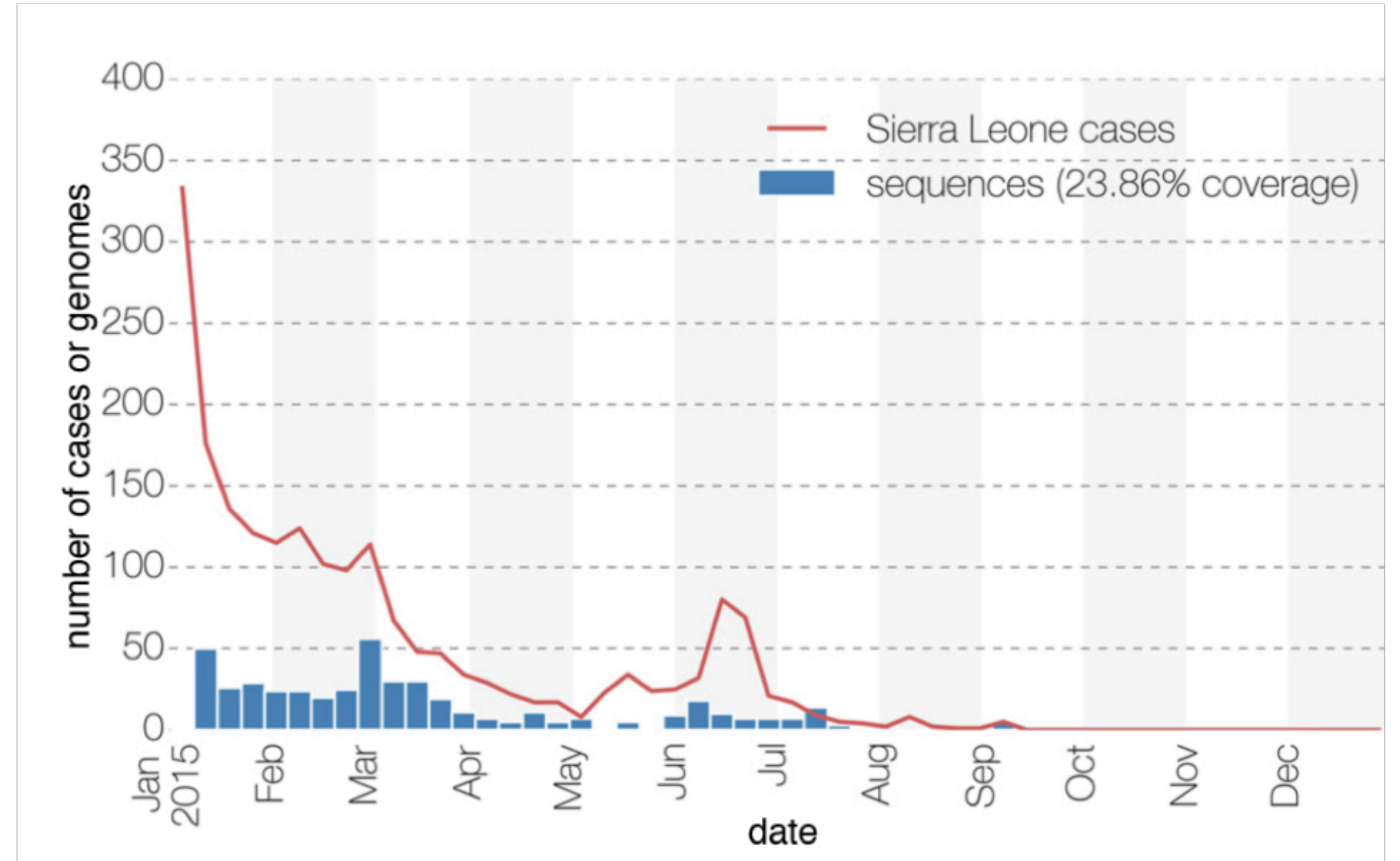
Matteneh Ebola Treatment Centre



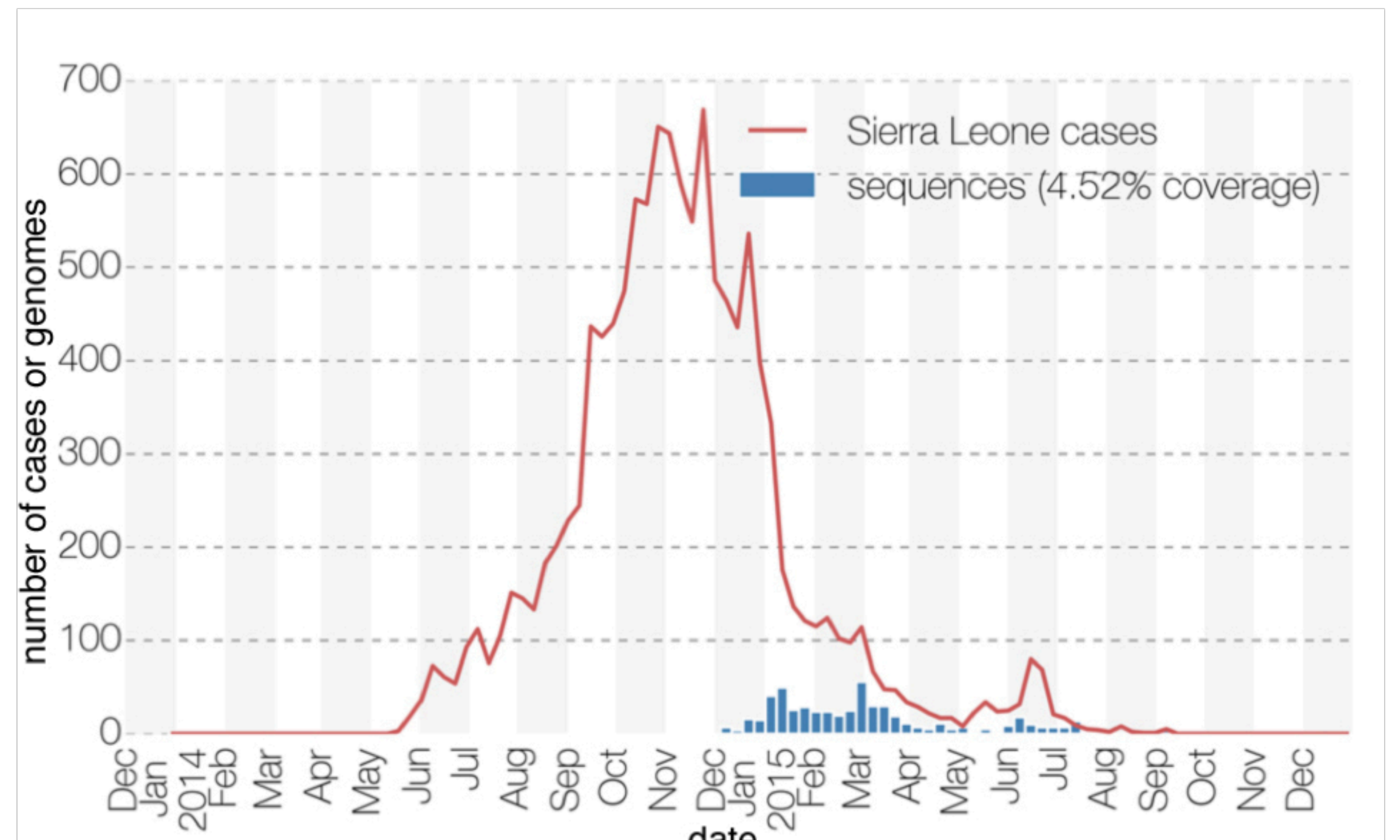
Over ~8 months
>600 genomes
~1/3 of all
genomes from
entire outbreak
Cost ~£450,000



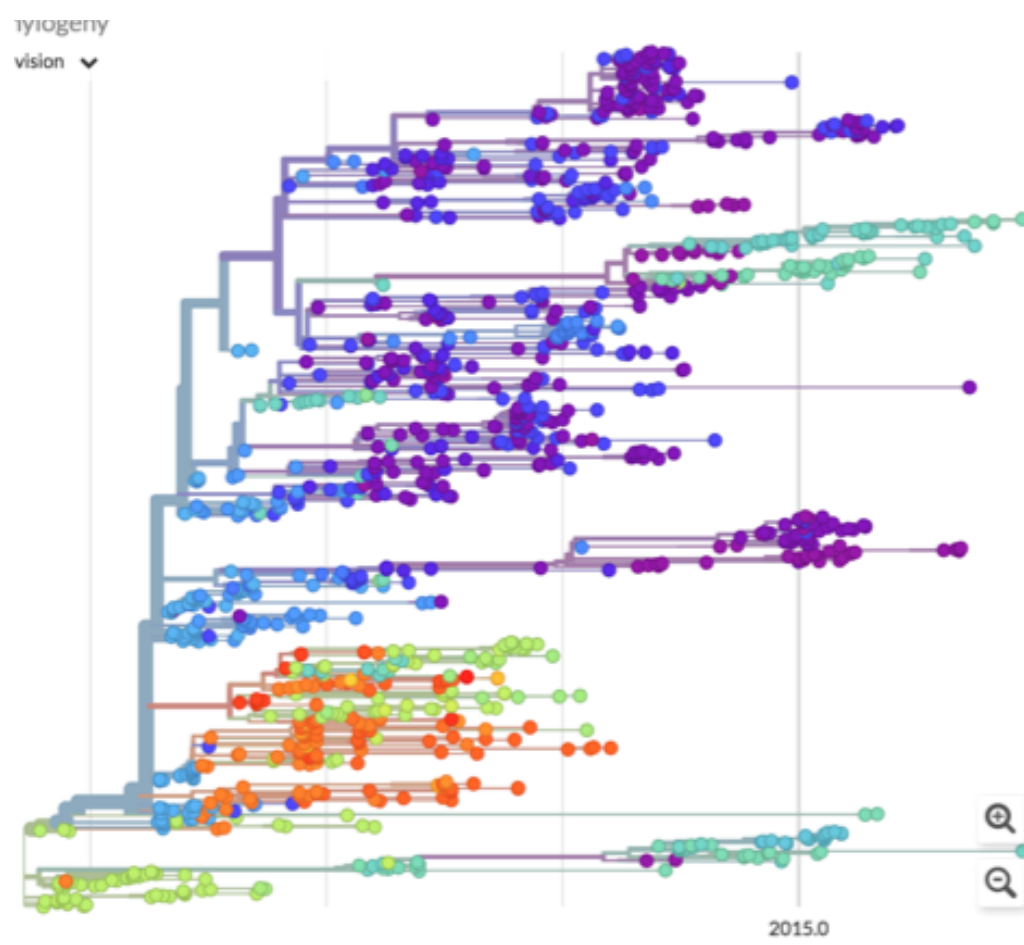
SIERRA LEONE 2015:
 FULL GENOMES FROM 24%
 OF ALL CASES



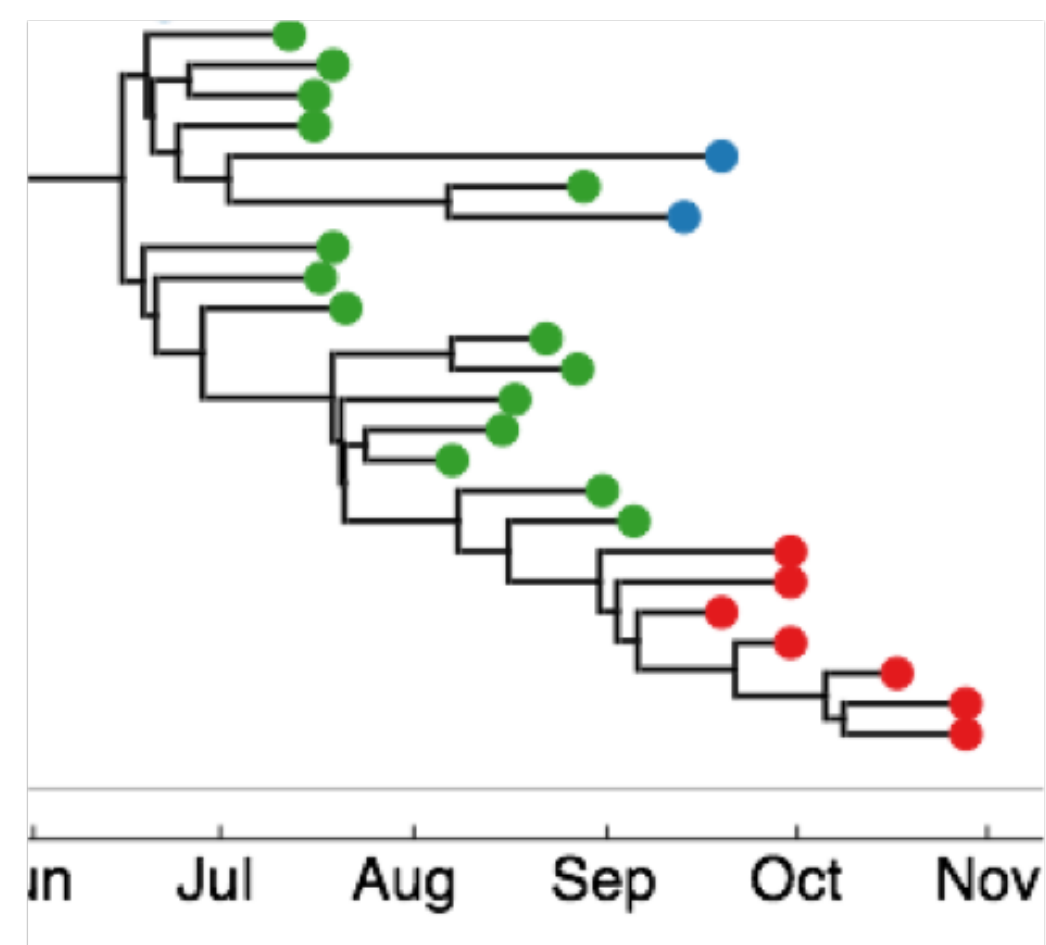
SIERRA LEONE ENTIRE
 OUTBREAK



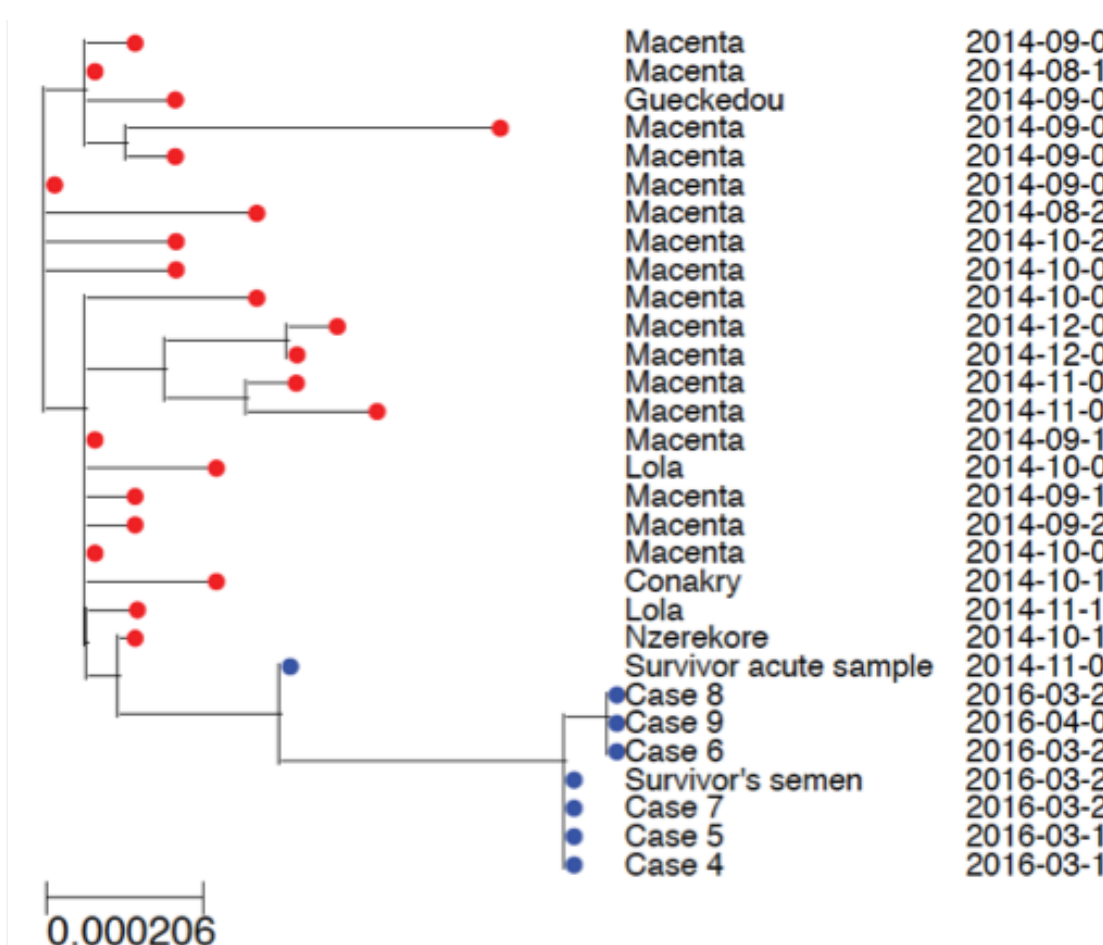
Example application of real-time viral genomics in the Ebola response



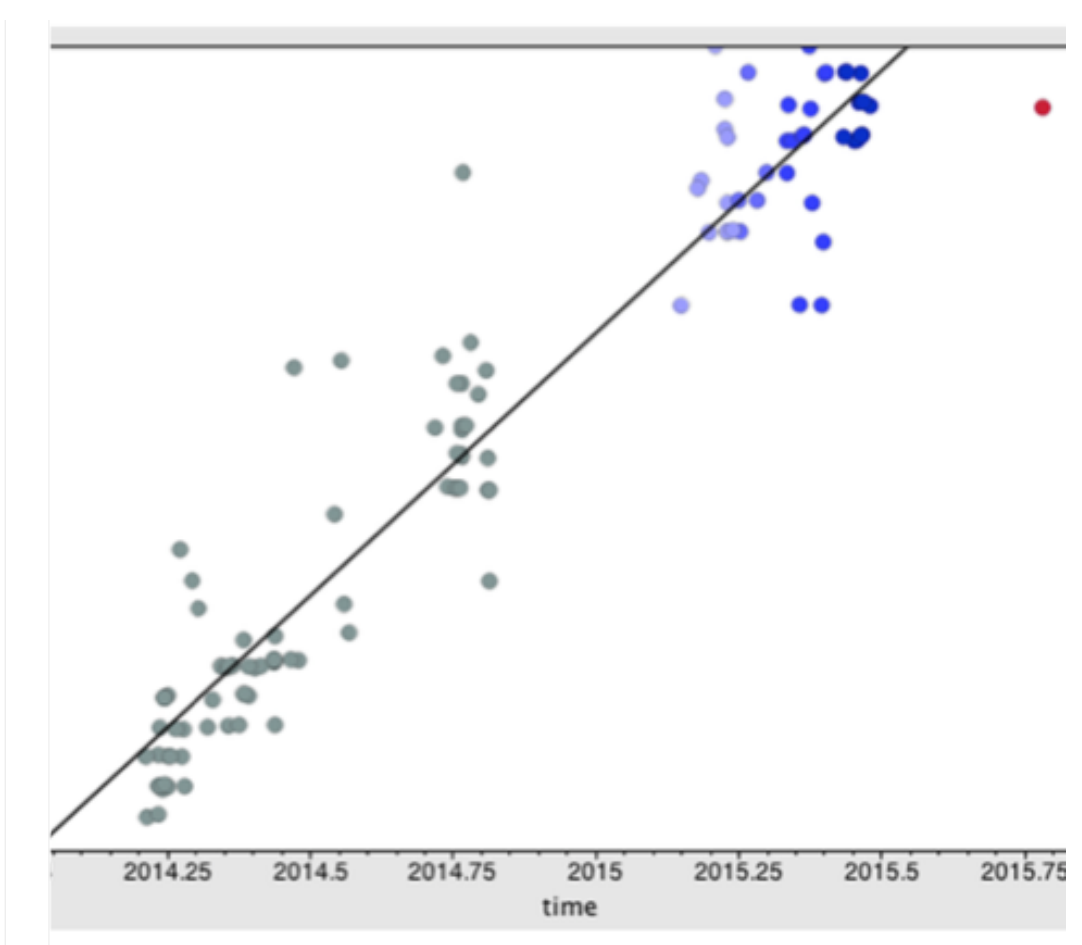
Identify cross-border transmissions through open data sharing



Identify links between cases, particularly in hard to investigate cases



Rapidly identify sources of flare-ups: distinguish between hidden chains, new animal introductions, and survivors



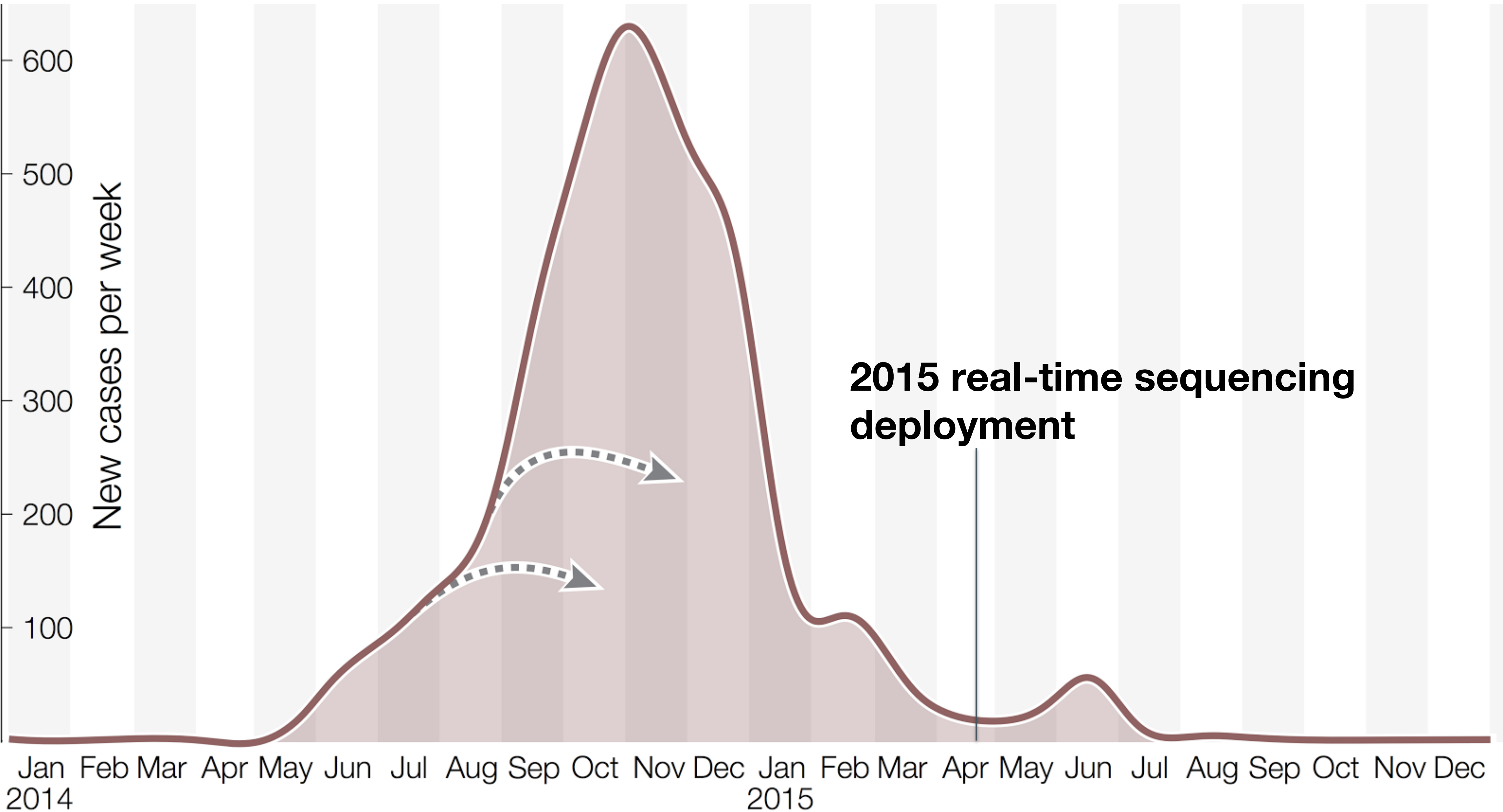
Demonstration of delayed transmission from survivors, in one case infected >500 days previously

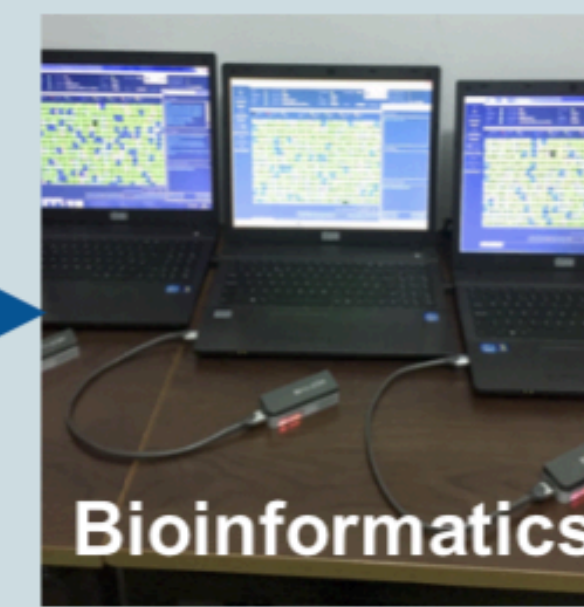
Lessons learnt

- Preparation is key
- When data generation is critical - better to over-prepare and ensure redundancy in all processes
- Process controls are essential to convince the end user that the data you are not producing is NOT "junk"



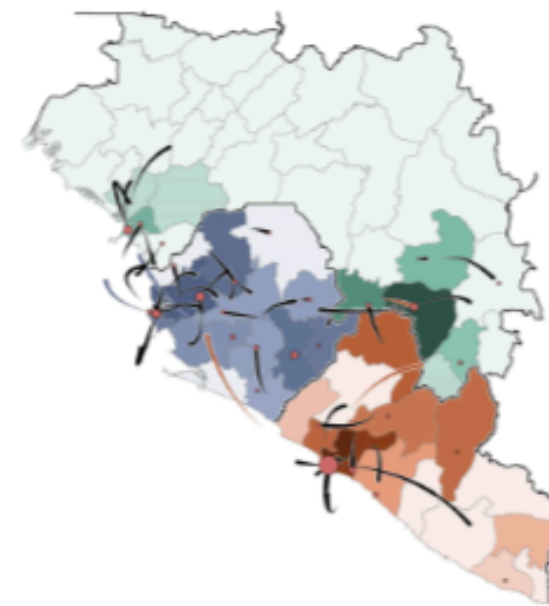
How do we learn from the experience?



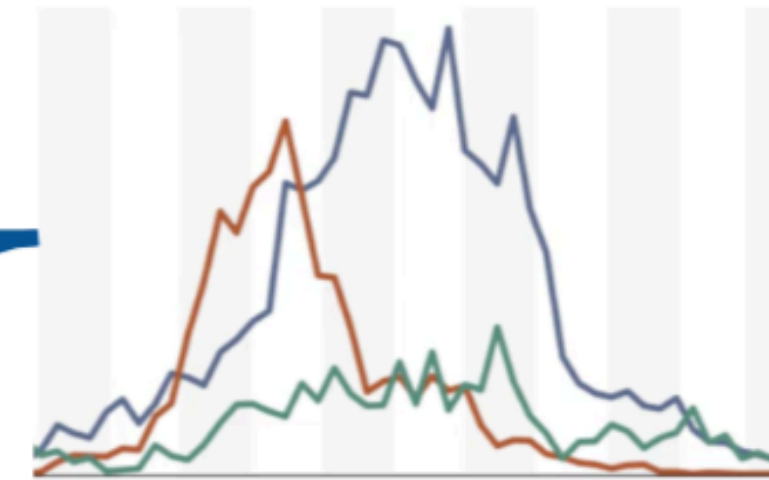


Deployed in-country

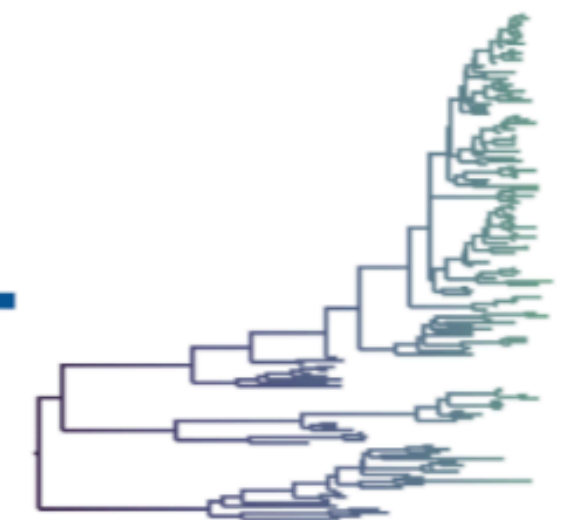
Visualization / Interpretation



Molecular epidemiology



Real-time analysis



Outbreak response

Collaborators

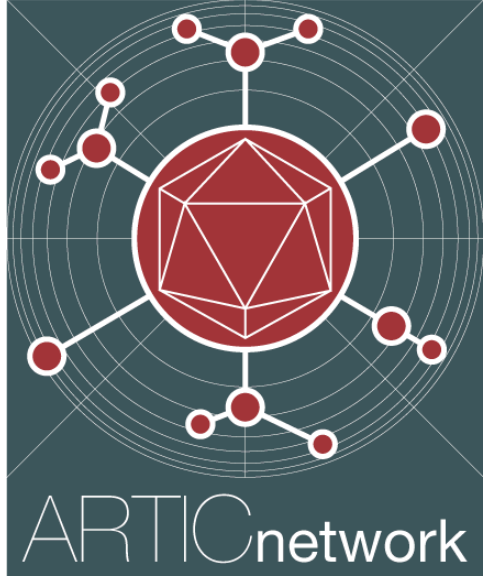
Dhamari Naidoo & Pierre Formenty, WHO
Stephan Günther, European Mobile Lab
Miles Carroll, Public Health England
Pardis Sabeti, Broad Institute
Kristian Andersen, Scripps Institute

Key requirements: Robust, Accurate, Rapid, Fieldable, Easy to use, Interpretable results.

SOPs released: artic.network/ebov



ARTIC Network: Accessible, open, real-time field sequencing



- Wellcome collaborative award with Edinburgh, Birmingham + others
- All protocols, bioinformatic pipelines, reagent lists, phylogenetics schemes, freely available online.
- Protocols are field tested and deployable within hours of arrival at a destination.



Sequencing workflow key considerations

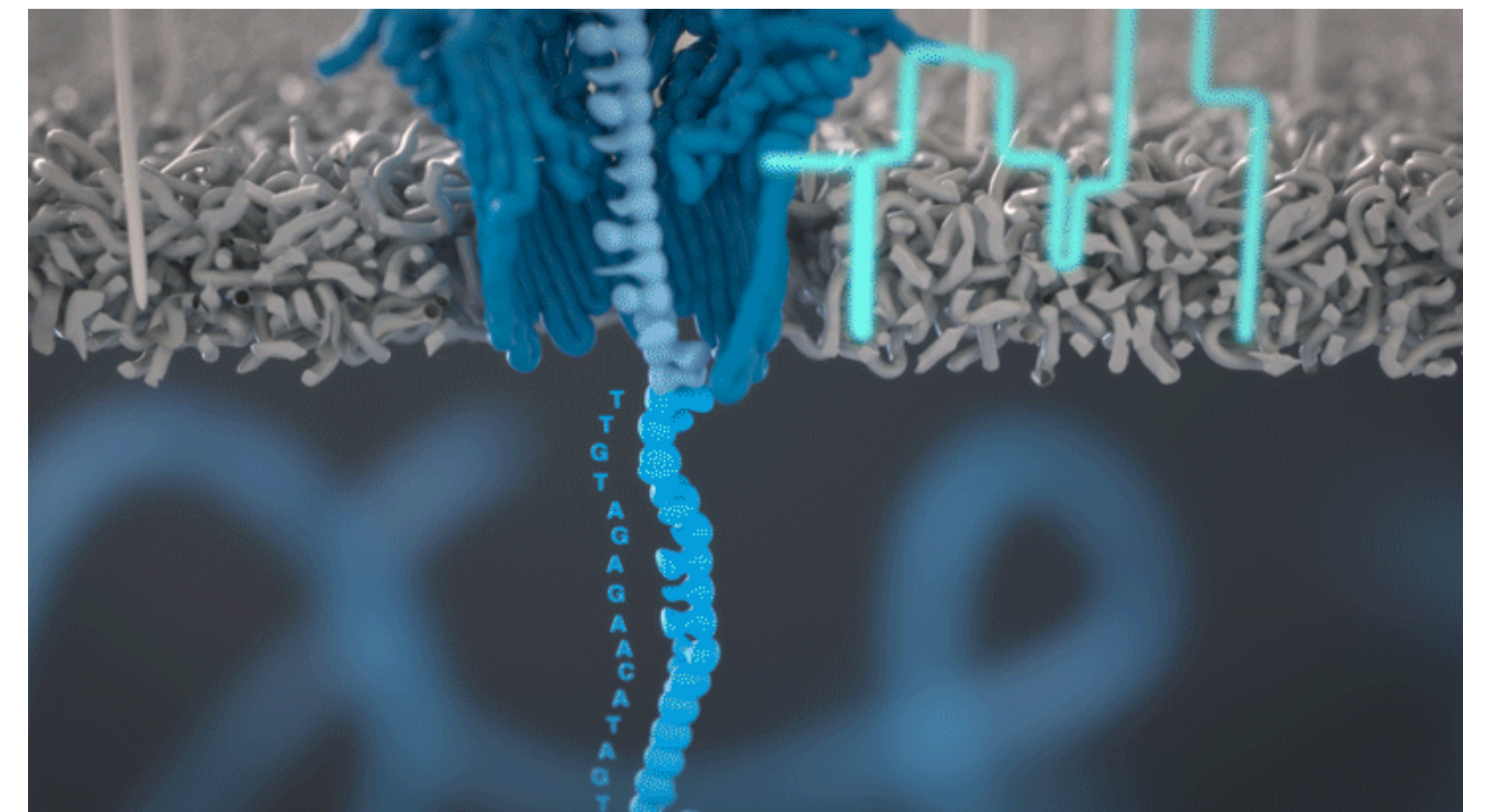


- Portability
- Usability
- Robustness – must avoid any single point of failure
- Maintaining sample integrity and provenance
- Contamination control is critical



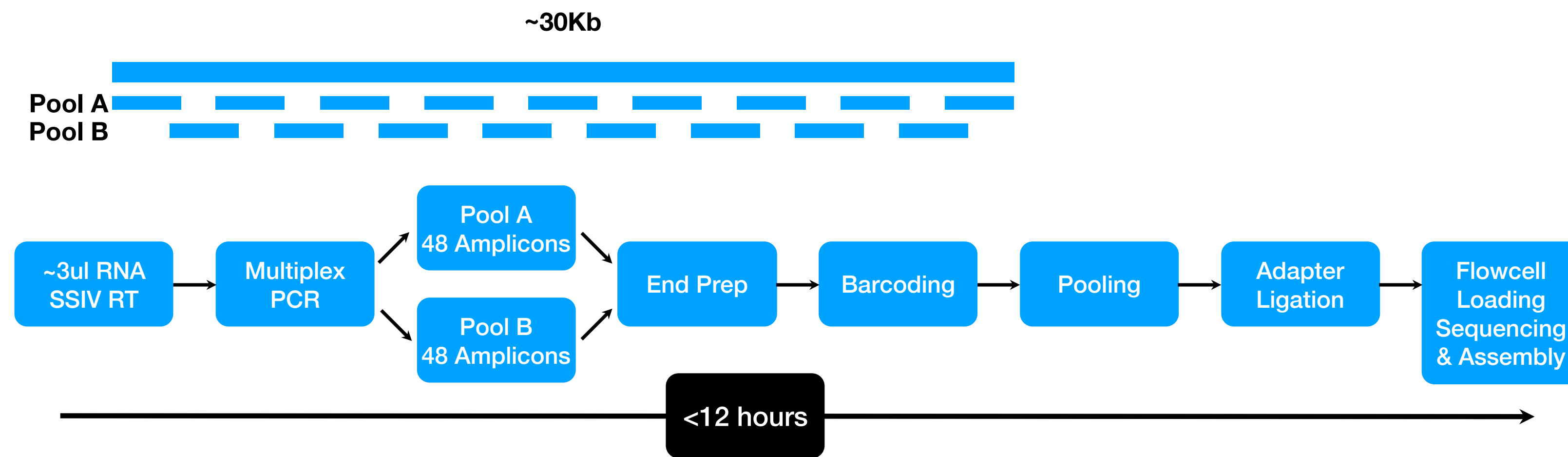
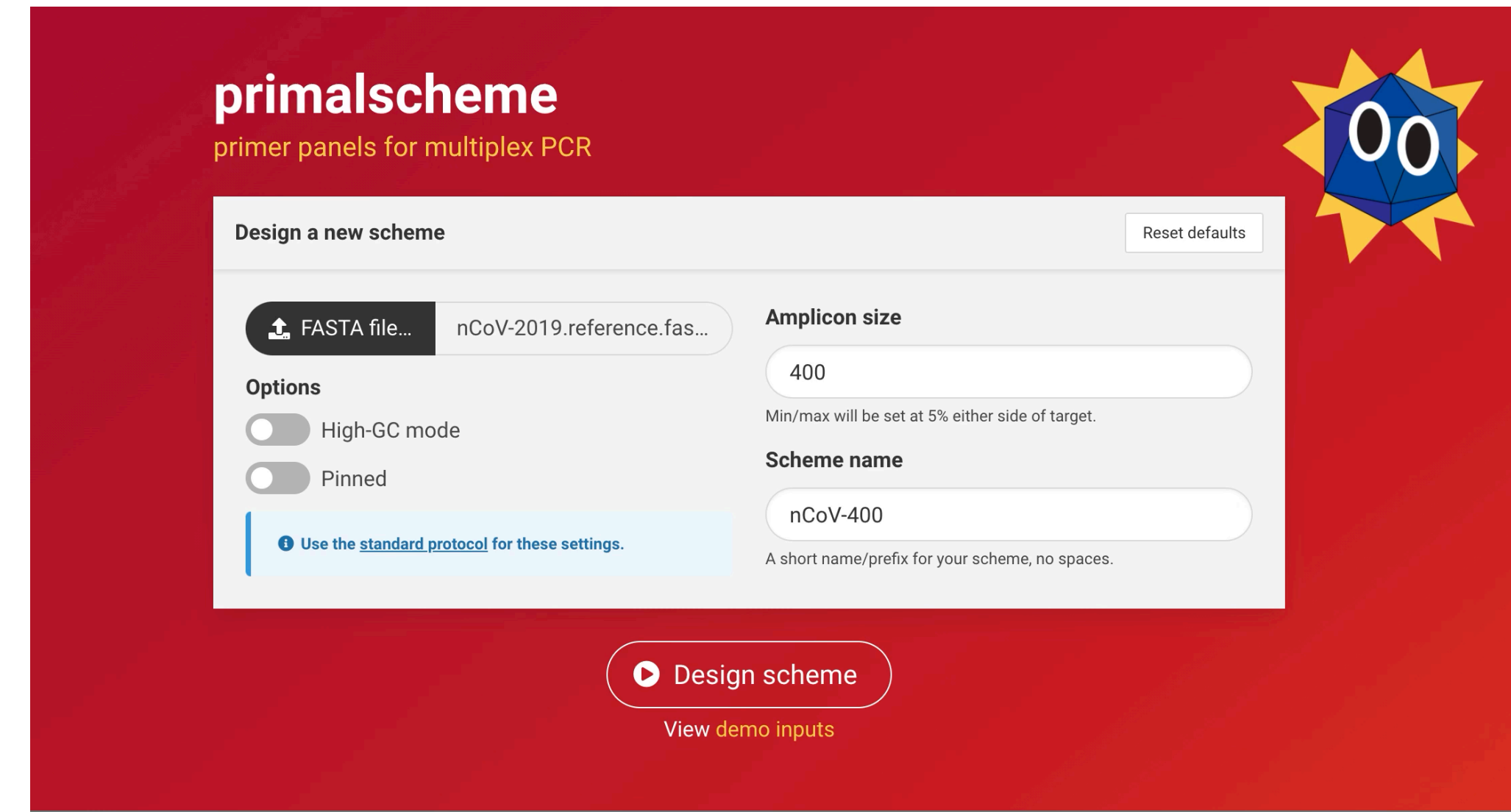
Nanopore sequencing

- Portability
 - Usability
 - Robustness
-
- Error rate – 98.3% accuracy at single reads – however >99.9% consensus accuracy
-
- *Advantages:*
 - *No maintenance*
 - *Low cost*
 - *Fast (6-12 hour runs for 96 samples)*
 - *96-384 samples per flowcell*
 - *Ability to run for as long as needed*
 - *Flowcells can be re-used*



ARTIC Sequencing protocol development

- Josh Quick and Nick Loman (Birmingham)
 - Multiplex PCR protocol developed
 - Provides a robust and sensitive approach
 - Primalscheme – online design tool
-
- >75% of all COVID sequences generated to date have used ARTIC protocols



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Article | [Open Access](#) | Published: 09 December 2020

Analytical validity of nanopore sequencing for rapid SARS-CoV-2 genome analysis

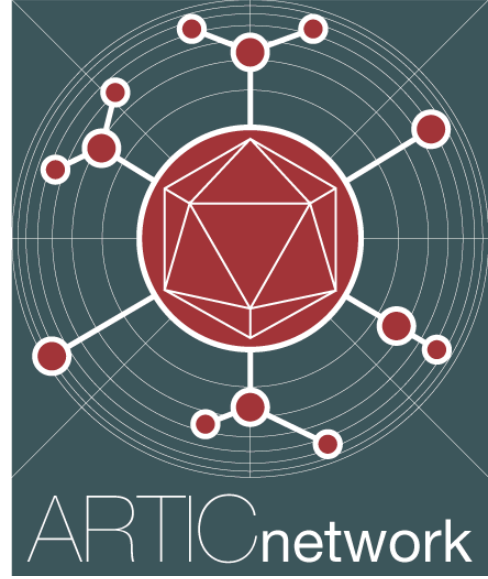
Rowena A. Bull, Thiruni N. Adikari, James M. Ferguson, Jillian M. Hammond, Igor Stevanovski, Alicia G. Beukers, Zin Naing, Malinna Yeang, Andrey Verich, Hasindu Gamaarachchi, Ki Wook Kim, Fabio Luciani, Sacha Stelzer-Braid, John-Sebastian Eden, William D. Rawlinson, Sebastiaan J. van Hal & Ira W. Deveson [✉](#)

Nature Communications 11, Article number: 6272 (2020) | [Cite this article](#)

14k Accesses | 18 Citations | 145 Altmetric | [Metrics](#)

Independent validation of our protocols

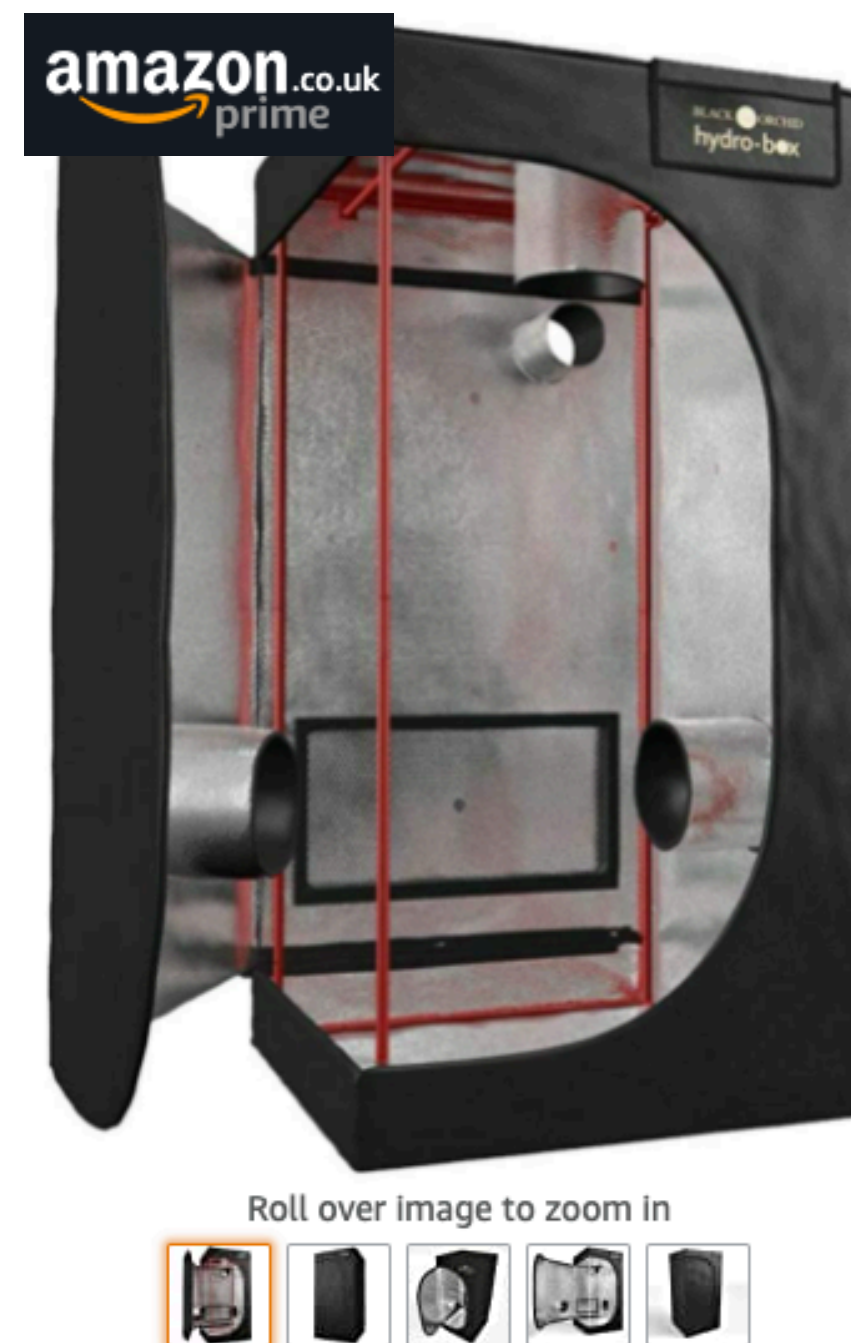
Maintaining a clean workspace – the “clean “cabinet”



- Contamination control is critical.
- Monitor via inclusion of extraction & RT-PCR controls in each batch that are then put through the entire process & sequenced
- PCR cabinets are heavy and expensive



£1500-£5000
32Kg



BLACK ORCHID 50x50x100 Extra Strong Lightproof Indoor Grow Tent Hydroponics Hydroponic Plant Growing Dark Room

Brand: BLACK ORCHID
★★★★☆ 121 ratings | 37 answered questions

RRP: £68.62
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You Save: **£21.63 (32%)**

Pay ~~£46.99~~ **£16.99**: get a **£30 Amazon Gift Card** on approval for the Amazon Platinum Mastercard. Terms apply.

Note: This item is eligible for **FREE Click and Collect** without a minimum order subject to availability. [Details](#)

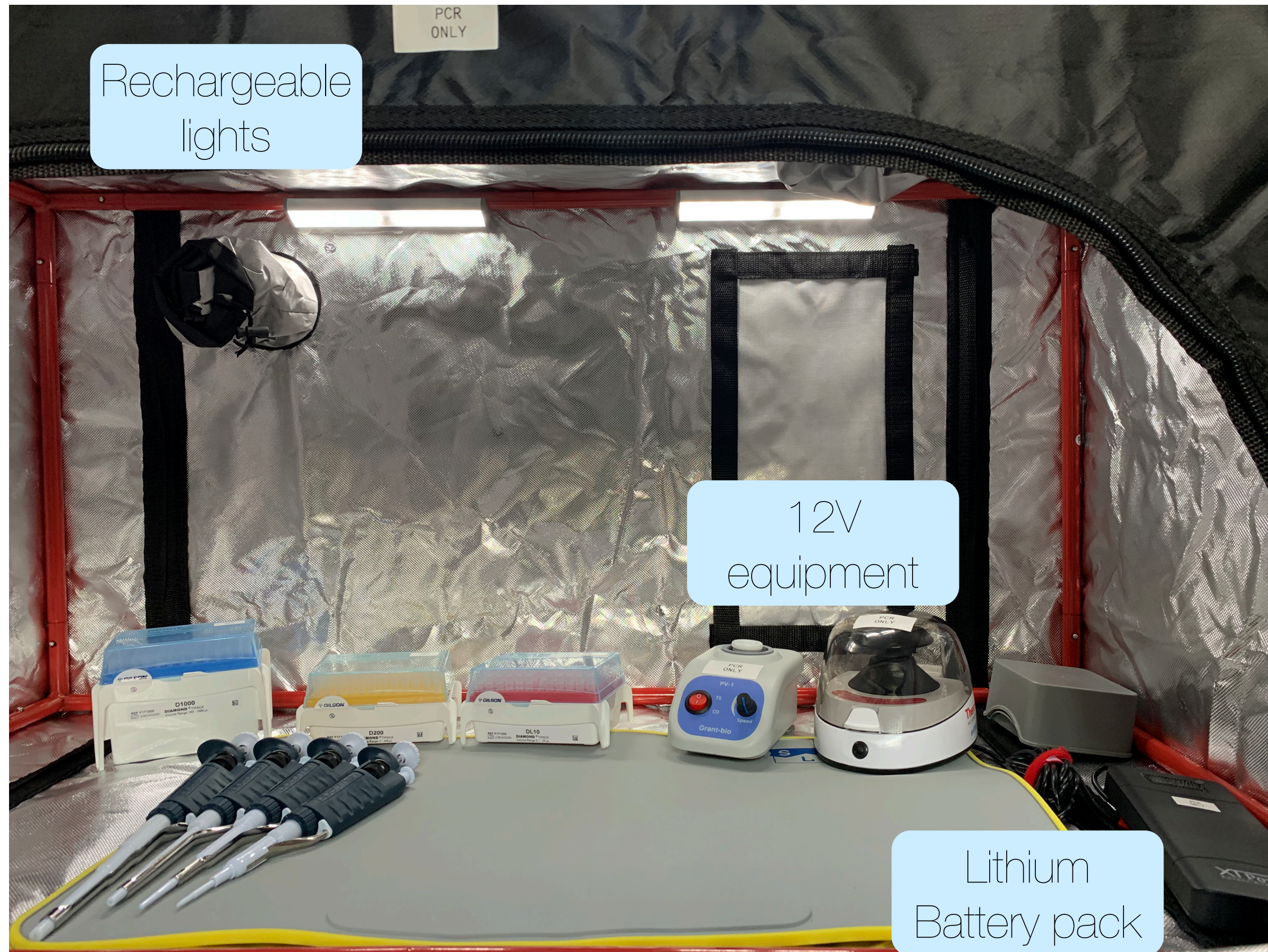
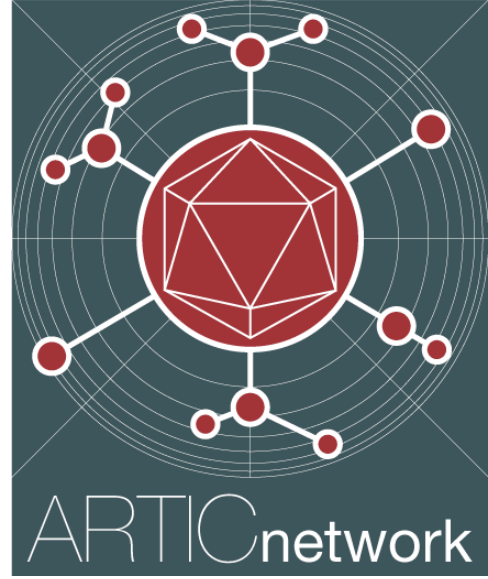
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Size Name: **50 cm wide, 50 cm deep, 100 cm high**

40x40x100 cm	50 cm wide, 50 cm deep, 100 cm high		
60x60x140 cm	60x60x160 cm	60x60x200 cm	70x70x160 cm
70x70x200 cm	80x80x200 cm	140x140x200 cm	
240x240x200 cm			

£47
<5Kg

Minimising power requirements



Berocia UV Disinfection Lamp Ozone Germicidal Lamp UV Light Sterilizer Sterilization Lamp Portable USB Rechargeable for Travel Home Hotel Wardrobe Toilet Car Pet Area Phone Kills 99.9% Virus Bacteria

Brand: Berocia

★★★★☆ 36 ratings

Price: **£12.77** ✓prime FREE One-Day Delivery

Pay ~~£12.77~~ **£0.00**: get a **£30 Amazon Gift Card** on approval for the Amazon Platinum Mastercard. Terms apply.

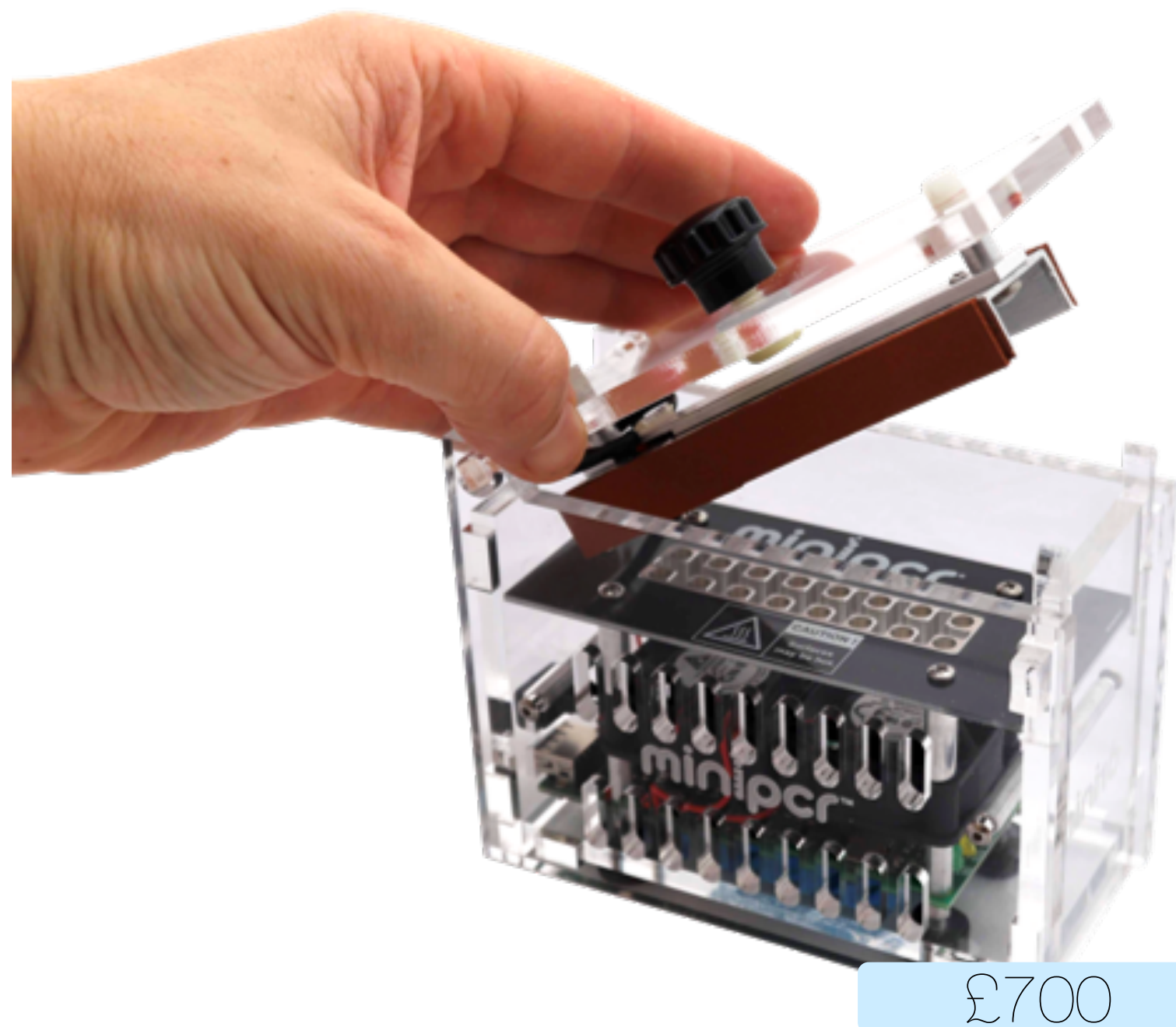
Note: This item is eligible for **FREE Click and Collect** without a minimum order subject to availability. [Details](#)

- This product uses 185-254 nano-meter ultraviolet ray and Ozone to kill bacteria and eliminate odor. It can be used under many occasions, such as in the car, kitchen, washroom, cabinet, closet, pets house, etc
- 15 minutes, give you a place full of sunshine smell. Sterilization and disinfection. Eliminate odor and purify air. Portable. Can be used for disinfection of pets and patients. Healthy and no Chemical, unlike regular air fresheners and purifiers which emit chemical fragrance, formaldehyde, ammonia and chloroform gases
- It eliminates colibacillus, mites, staphylococcus, mycobacterium tuberculosis, influenza virus, parasite and much more bacteria / allergens at the Source without adding Harmful Chemicals To your Air. Remove bad

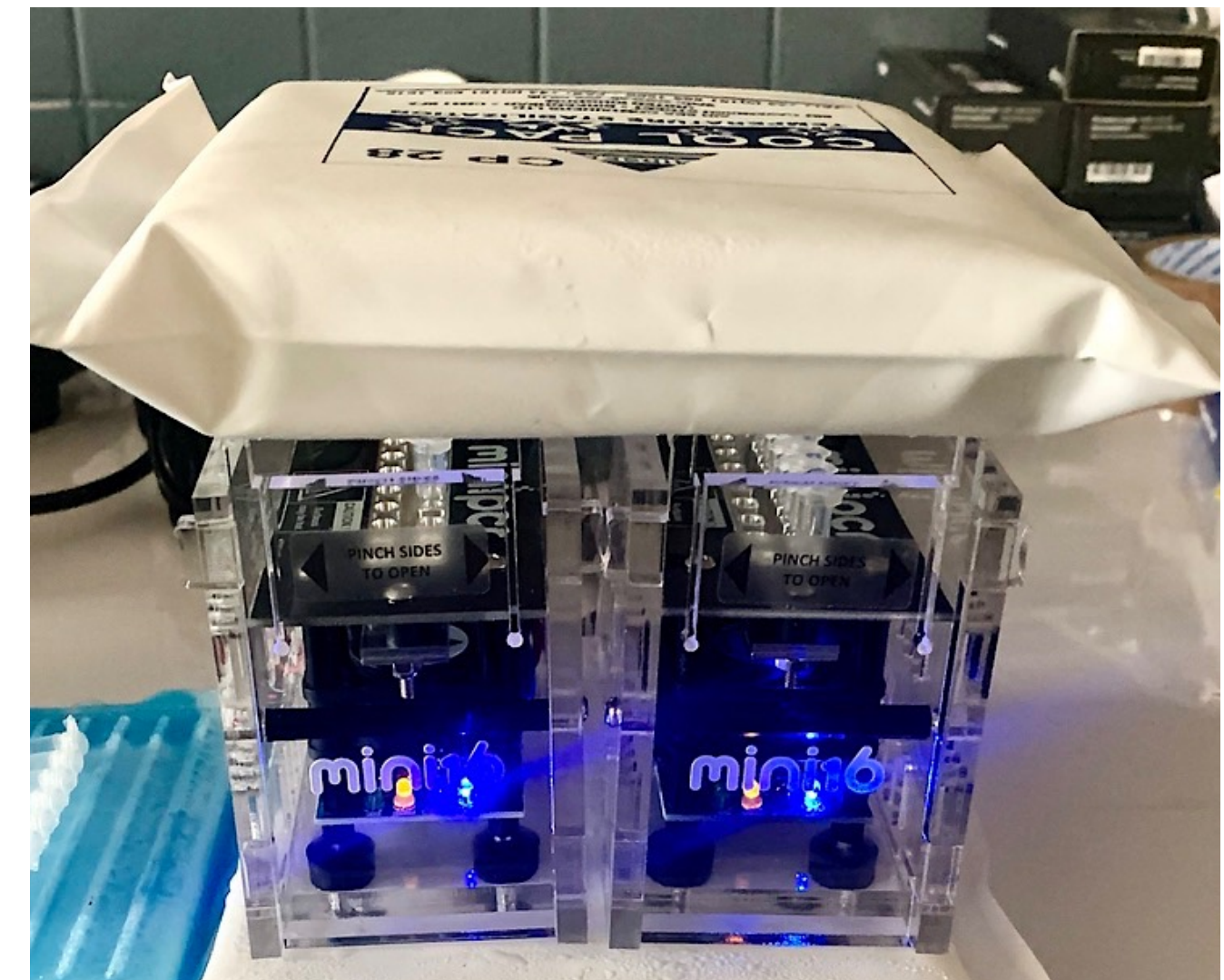
- USB powered UV lights
- 1% sodium hypochlorite solution (bleach) is a cheap and very effective contamination control measure

The Mini-PCR machine

- Light, robust, inexpensive – use multiple machines avoids single point of failure
- 16 samples per batch
- Can run off battery packs when required



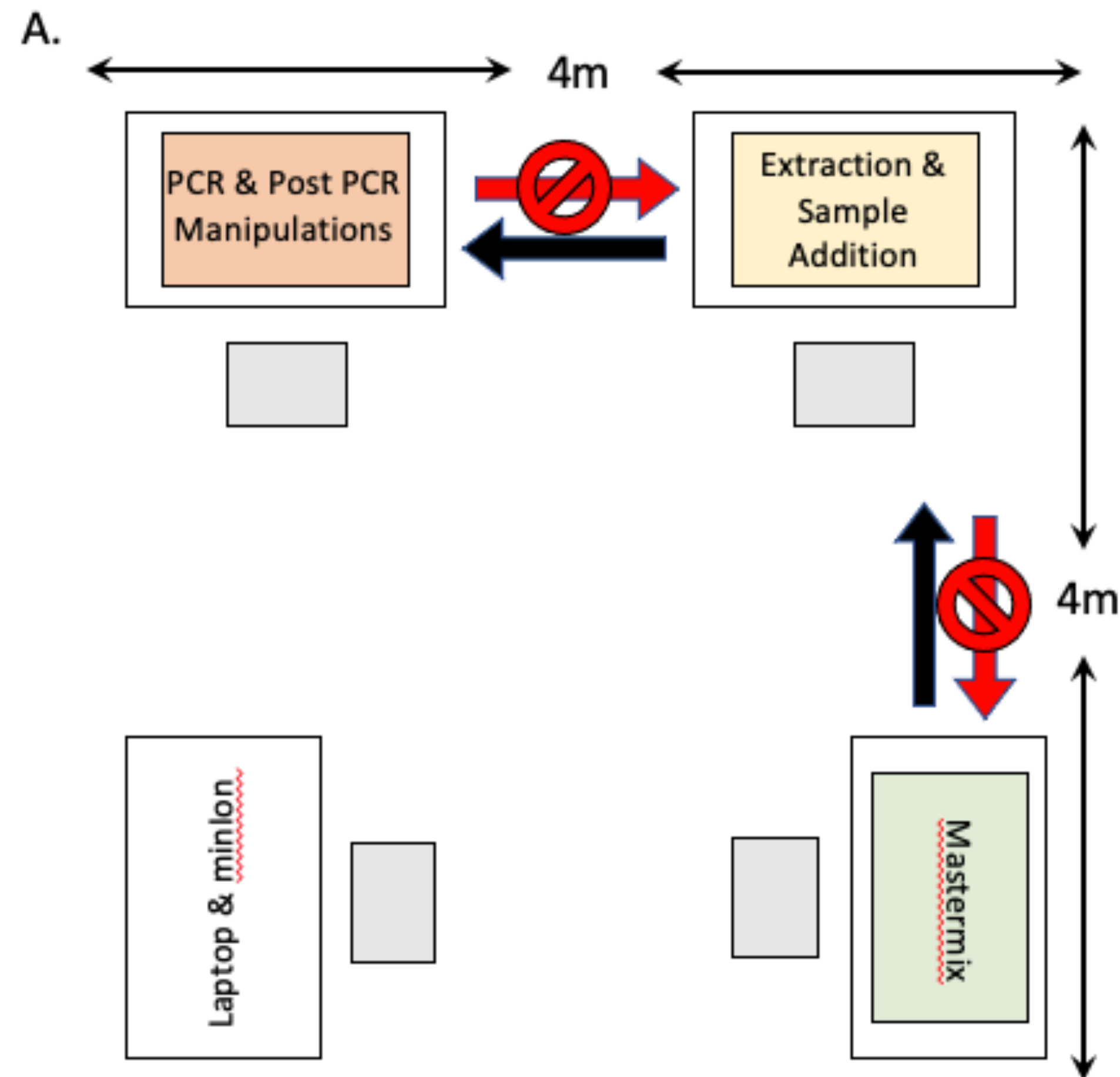
£700



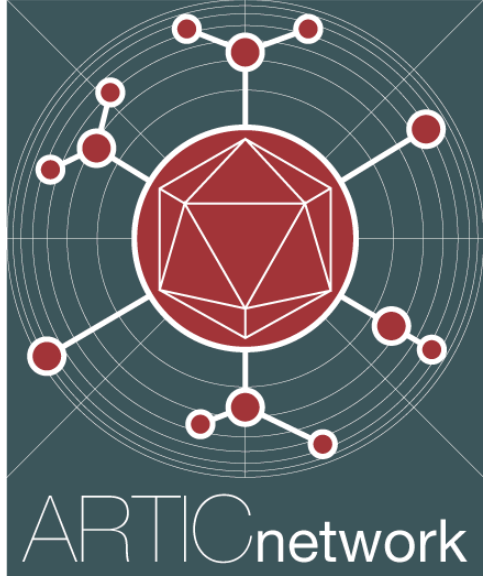
Use of freezer blocks for ligation steps

Three “cabinet” directional workflow

- Maintaining directionality critical to preventing contamination
- Each cabinet has dedicated equipment
- Partner with testing lab for provision of nucleic acid extracts
- Sample inactivation prior to arrival (Swabs into DNA/RNA Shield)



Pre-installed bioinformatics pipelines – lab on SSD



- Pre-configured solid state drive images
- Plug and play basecalling, assembly and bioinformatics



artic-network/**lab-on-an-SSD**

Scripts for building the ARTIC lab-on-an-SSD

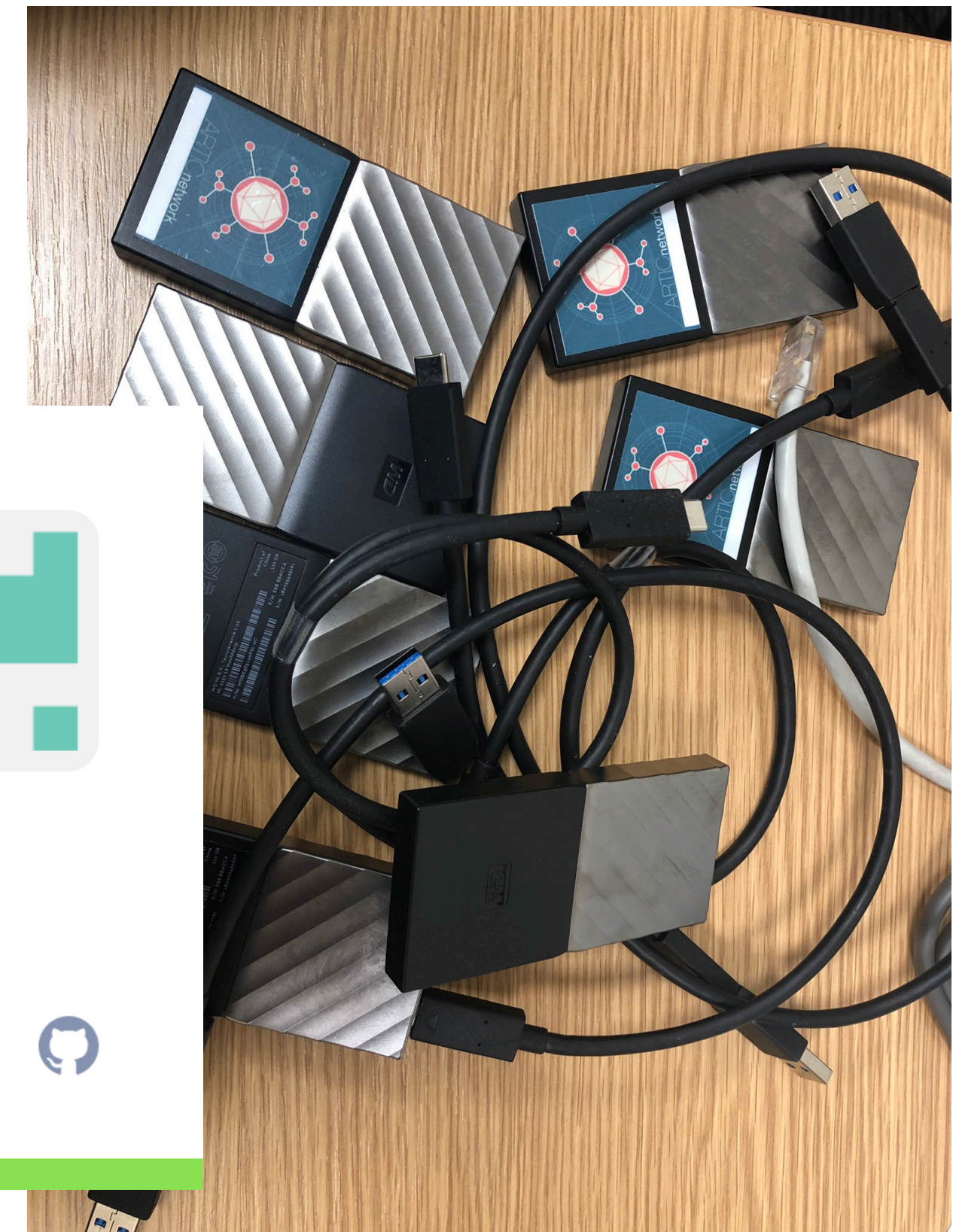


2 Contributors

0 Issues

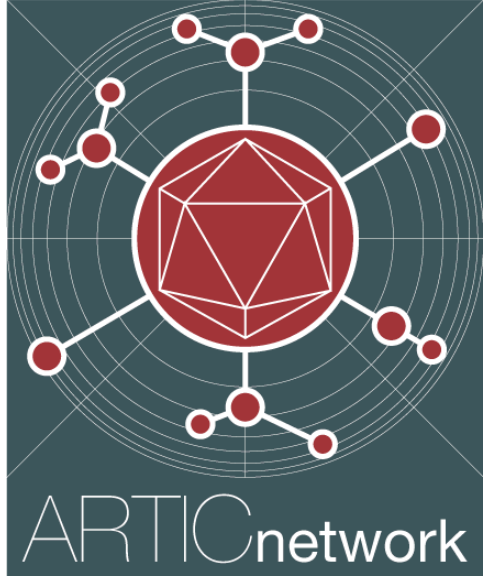
10 Stars

4 Forks

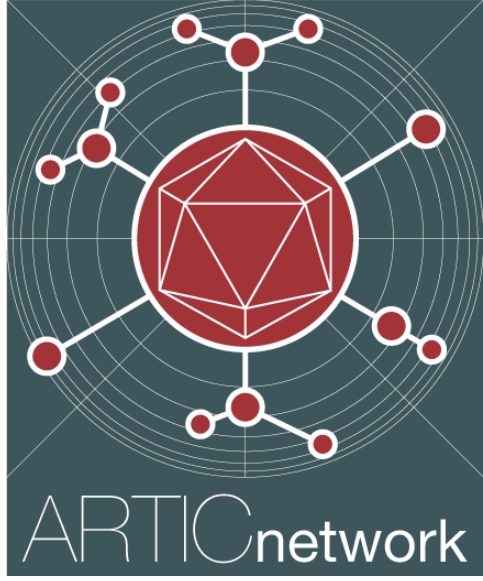


Live monitoring of sequencing runs

- RAMPART allows for real time mapping of data as it is generated
- Immediately identifies issues with library prep
- Provides an estimated required run time
- Useful for monitoring possible contamination

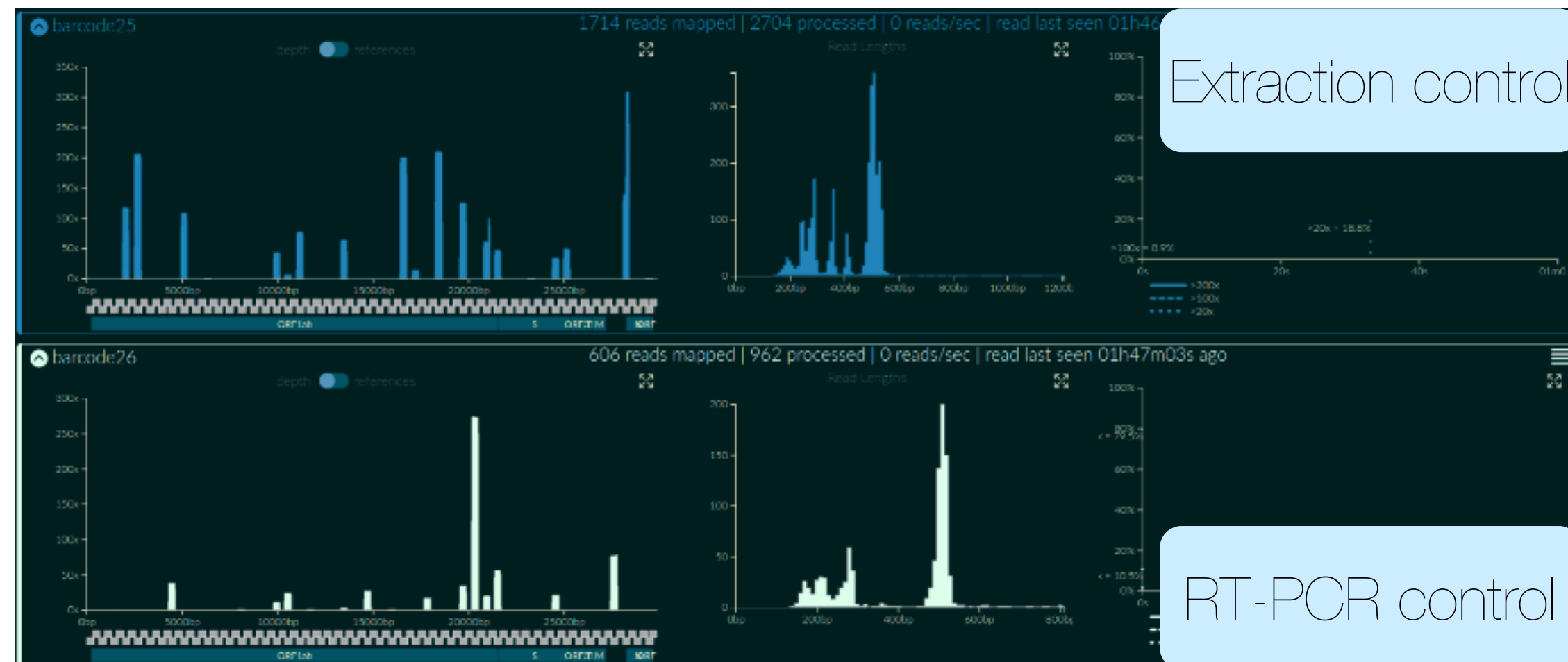


When things go wrong

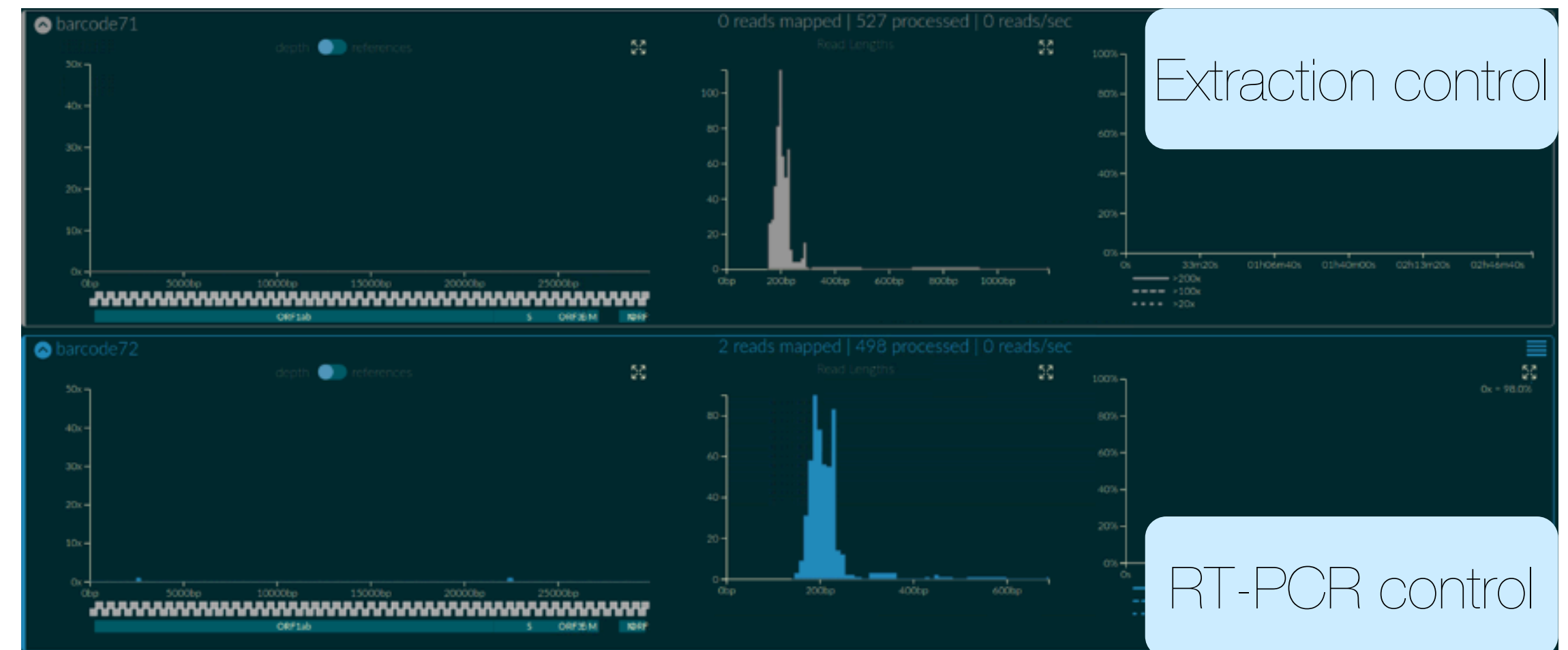


- Contamination control is critical.
- Monitor via inclusion of extraction & RT-PCR controls in each batch that are put through the entire process – this is really essential QC
- Should things go wrong, it is essential to perform a deep clean – UV and bleach, dispose of all PPE and reinforce directional workflow

Evidence of contamination

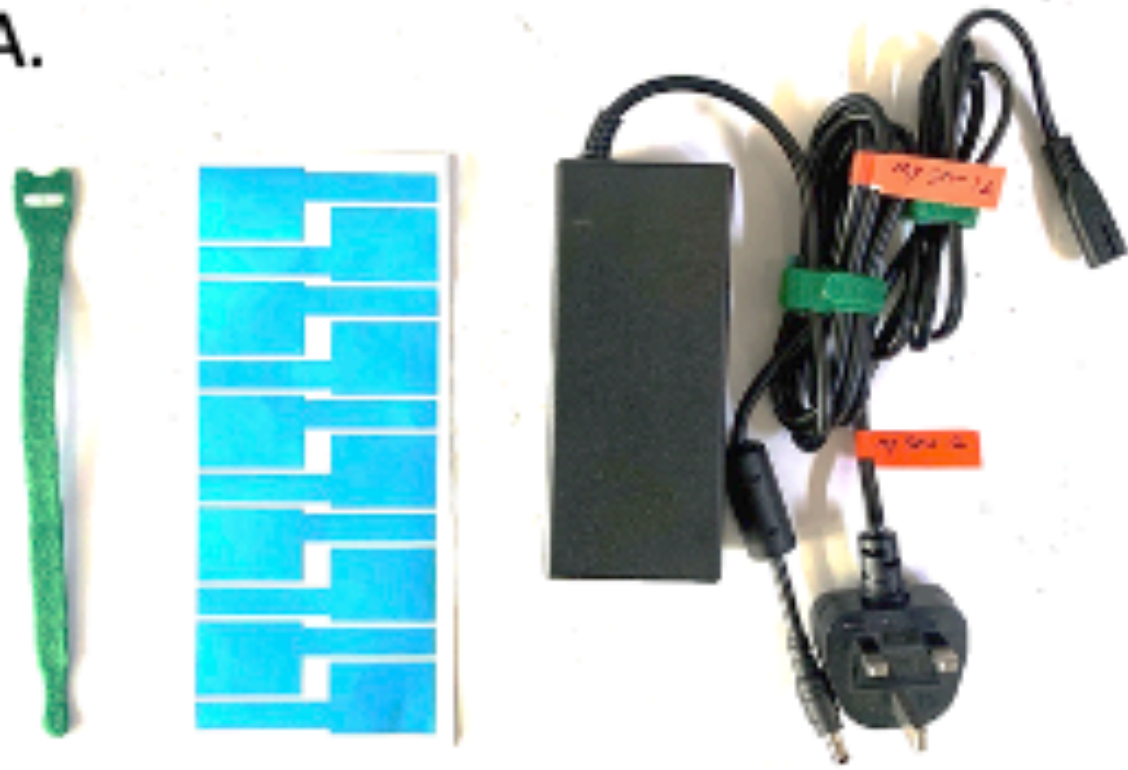


A clean run



The packed equipment

A.



B.



C.



Never underestimate the value of an effective labelling system

D.



E.

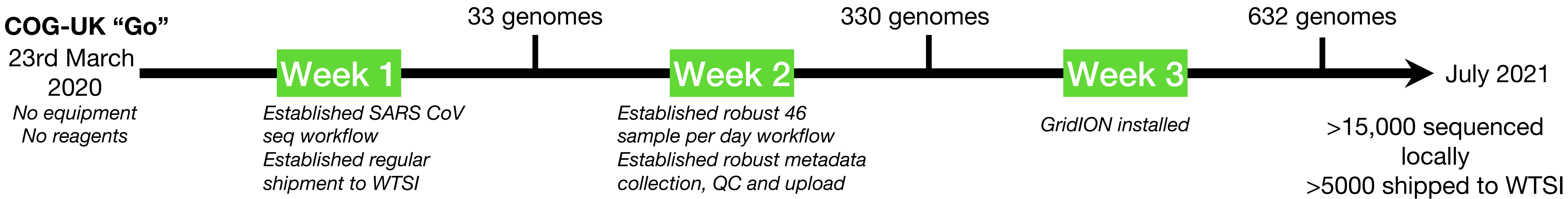


Packing lists are critical

F.



Use of ARTIC workflow for COVID sequencing - Cambridge COG-UK site



Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: a prospective genomic surveillance study

Luke W Meredith†, William L Hamilton*†, Ben Warne, Charlotte J Houldcroft, Myra Hosmillo, Aminu S Jahun, Martin D Curran, Surendra Parmar, Laura G Caller, Sarah L Caddy, Fahad A Khokhar, Anna Yakovleva, Grant Hall, Theresa Feltwell, Sally Forrest, Sushmita Sridhar, Michael P Weekes, Stephen Baker, Nicholas Brown, Elinor Moore, Ashley Popay, Iain Roddick, Mark Reacher, Theodore Gouliouris, Sharon J Peacock, Gordon Dougan, M Estée Török*‡, Ian Goodfellow*‡*

Summary
Background The burden and influence of health-care associated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections is unknown. We aimed to examine the use of rapid SARS-CoV-2 sequencing combined with detailed epidemiological analysis to investigate health-care associated SARS-CoV-2 infections and inform infection control measures.

Lancet Infect Dis 2020
Published Online
July 14, 2020
[https://doi.org/10.1016/S1473-3099\(20\)30562-4](https://doi.org/10.1016/S1473-3099(20)30562-4)

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COVID-19 infection dynamics in care homes in the East of England: a retrospective genomic epidemiology study

William L Hamilton, Gerry Tonkin-Hill, Emily Smith, Charlotte Houldcroft, Ben Warne, Luke Meredith, Myra Hosmillo, Aminu Jahun, Martin Curran, Surendra Parmar, Laura Caller, Sarah Caddy, Fahad Khokhar, Anna Yakovleva, Grant Hall, Theresa Feltwell, Malte Pinckert, Iliana Georgana, Yasmin Chaudhry, Nicholas Brown, Sonia Goncalves, Roberto Amato, Ewan Harrison, Mathew Beale, Michael Spencer Chapman, David Jackson, Ian Johnston, Alex Alderton, John Sillitoe, Cordelia Langford, Gordon Dougan, Sharon Peacock, Dominic Kwiatowski, Ian Goodfellow, M. Estee Torok, COVID-19 Genomics Consortium UK

doi: <https://doi.org/10.1101/2020.08.26.20182279>

This article is a preprint and has not been peer-reviewed [what does this mean?]. It reports new medical research that has yet to be evaluated and so should not be used to guide clinical practice.

Abstract | Info/History | Metrics | [Preview PDF](#)

Time to upload

- Generating sequence data is just the first step
- Essential data is rapidly available for analysis
- Linked metadata critical

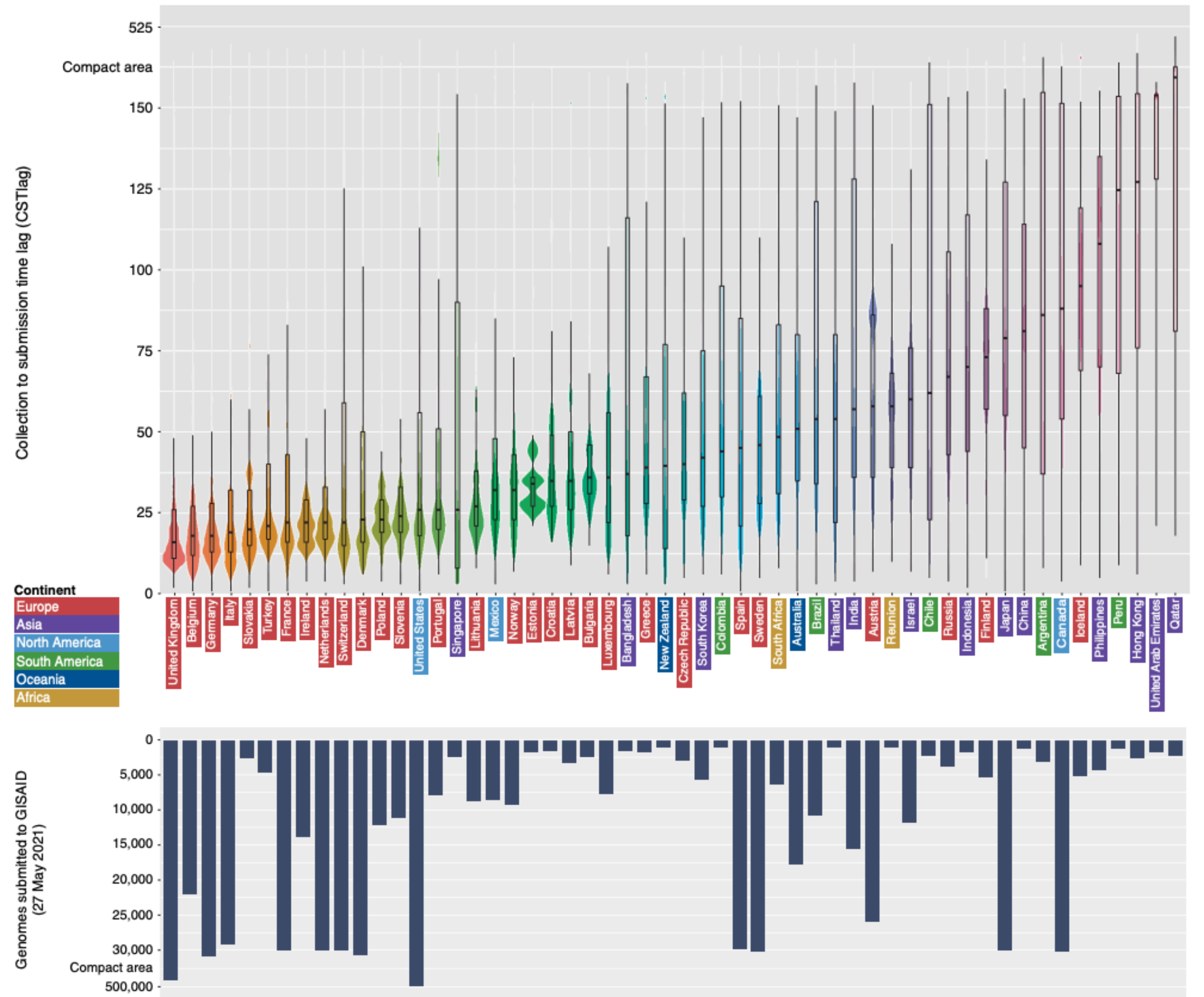


Fig. 1 | Violin plot illustrating the CST lag values for the 54 countries that have sequenced over 1,000 genomes. The box plot inside the violin plot depicts the median CST lag per country. Outlier CST lag entries are not shown. Country name is color-coded according to continent. We have also graphed the relative distribution of the number of genome sequences submitted by each country as a bar plot.

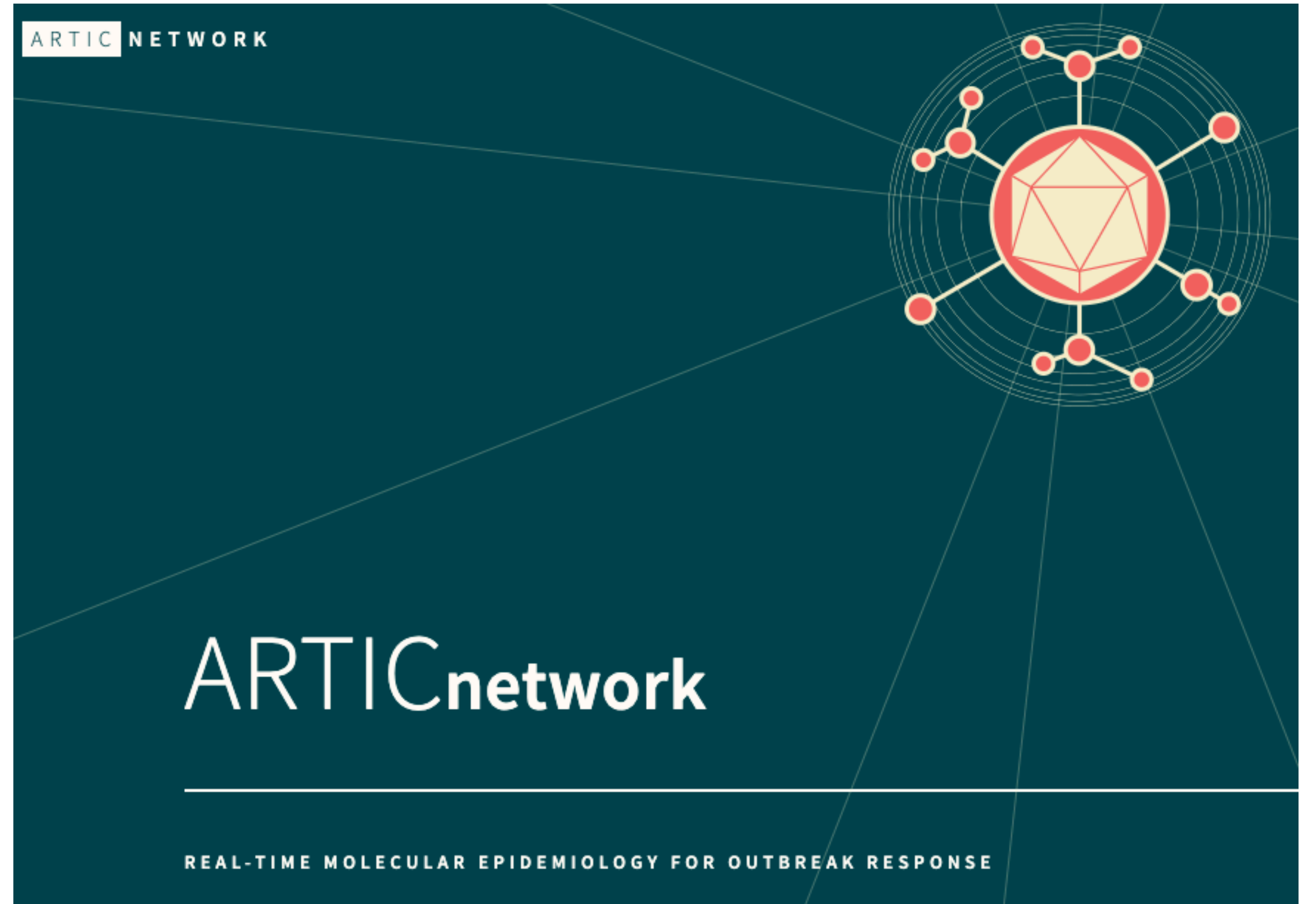
Summary

- Most practical issues associated with rapid viral genome sequencing have been solved
- Issues remain around data analytics and data sharing
- Data sharing is important but scientists frequently abuse data access
- Viral genomics has a role to play in epidemic response *BUT* its utility will differ from epidemic to epidemic
- Effective linkage to patient data and epidemiological information critical for extracting the full utility of viral genomics



<https://artic.network/>

<https://www.cogconsortium.uk/>



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