CASE STUDY

One size fits all: a simple workflow to characterise any pathogen

Successful surveillance of pathogen outbreaks requires a rapid, scalable, and cost-effective response. To overcome the challenges of monitoring pathogen outbreaks, such as generating real-time epidemiological information, researchers are utilising the benefits of portable, real-time nanopore sequencing technology to perform successful surveillance at sample source.

Genomic epidemiology, the study of how variants in the genomes of pathogens, or their hosts, influence health and disease, aims to track pathogen transmission, spread, and evolution. Sequence information also enables the identification of drug resistance factors. Nanopore technology has played a pivotal role in global pathogen surveillance, from the Ebola outbreak in western Africa in 2013–16 to the present-day COVID-19 pandemic, with greater than one million SARS-CoV-2 genomes sequenced using nanopore technology across over 85 countries[1]. Many of the known RNA viruses, such as SARS, influenza, and HIV, are fast evolving, which means they continually accumulate changes in their genome. Sequence data can guide important control measures, but only if the results are generated quickly enough to inform interventions. Conventional sequencing technologies are typically not accessible in low-resource settings, where requirements, such as continuous power, lab infrastructure, and trained personnel are often not available within outbreak areas, leading to practical difficulties transporting samples to remote sequencing facilities. With this in mind, Josh Quick and his team based at the University of Birmingham, took advantage of the accessibility, affordability, and portability of nanopore sequencing, to successfully perform scalable, field-based genomic surveillance of the Ebola virus in western Africa. Using portable equipment, including the MinION™, which they termed a 'lab-in-a-suitcase'[2], the team highlighted how easily nanopore technology can be deployed to '*remote and resource-limited locations*' to monitor pathogen outbreaks with rapid turnaround times, '*with the sequencing process taking as little as 15–60 min*'[2].

The ARTIC network has since been set up to expand international viral surveillance, enabling worldwide collaboration in sequencing and analysing pathogen outbreaks[3]. Leveraging the portability and streamlined

> ***"real-time genomic surveillance is possible in resource-limited settings and can be established rapidly to monitor outbreaks[2]"***

sample preparation workflows of the MinION, they aimed to target a wide range of DNA and RNA viruses with a simple workflow that could be used worldwide. The approach needed to be applicable, scalable, and rapidly deployable. Sequencing viral genomes directly from clinical research samples can be challenging for viruses with a low viral load, such as the Zika virus. The workflow developed by the ARTIC network used a PCR enrichment approach, suitable for samples containing as few as 50 genome copies per reaction[4]. PCR can provide both target enrichment and amplification in a single step. For complete coverage, a tiled amplicon scheme was devised, and to reduce time, complexity, and cost, a multiplexed sequencing approach was used. The end-to-end workflows allow any researcher to amplify and sequence high- and low-abundance viruses directly from clinical research samples. Users can design their own primer schemes for their virus of interest using the web-based primer design tool, primalscheme[5].

Due to easy adaptability for the amplification and sequencing of different viruses, tiled PCR approaches using nanopore sequencing have been widely adopted (**Table 1**).

With nanopore sequencing, read length is equal to fragment length, enabling tiled PCR and sequencing of viral genomes with long amplicons. Mori and coworkers[6] generated near full-length HIV genomes and obtained distinctive genetic information for the highly genetically diverse recombinant forms (RFs) of HIV-1 using nanopore sequencing of

Virus	Genome size (bp)	Amplicon length (bp)	Number of amplicons
Ebola[2]	~20,000	2,000	11
Zika[4]	~11,000	400	35
Monkeypox[8]	~200,000	2,500	88
SARS-CoV-2 (ARTIC)[9]	~30,000	400	98
SARS-CoV-2 (Midnight)[10]	~30,000	1,200	29
Chikungunya[11]	~12,000	300	44
Yellow fever[12]	~10,000	500	27
Dengue[13]	~11,000	1,000	13
Africa Swine Fever[7]	~190,000	7,000	32
Avian influenza[14]	~13,500	900–2,300	8
Rabies[15]	~12,000	400	39
Tick-borne encephalitis[16]	~10,000	450	37
Hepatitis C[17]	~9,600	~9,000	1
HIV-1[6]	~9,600	5,000–9,000	1–2
Adenovirus-F41[18]	~34,000	1,200	92

Table 1
Examples of tiled amplicon protocols applied to viral genomes with nanopore sequencing, detailing genome size, amplicon length, and number of amplicons to complete whole-genome sequencing.

amplicons (**Figure 1**). The team highlighted that drug resistance-associated mutations are located outside the target sequence regions of the traditional HIV-1 genotyping assay and suggested that long sequencing reads are now required for full analysis.

Outbreaks of mosquito-borne viruses, such as Zika, chikungunya, yellow fever, and dengue, have all been characterised using tiled amplicon workflows with nanopore sequencing, and together with broader epidemiology data could potentially be used to inform vaccination strategies. When vaccine levels are low, critical decisions on geographic areas to be targeted require a detailed understanding of the spatial spread of the virus and predictions of where it is most likely to spread.

> ***"Our new nanopore sequencing platform is applicable to identify the full-length HIV-1 genome structure of intersubtype RFs as well as dual infection heterologous HIV-1[6]"***

Nanopore workflows have also been adapted to study viruses known to affect animals, such as rabies, avian influenza, and African swine fever virus (ASFV). ASFV is highly contagious and has a mortality of up to 100% in domestic pigs, which severely impacts pork production and local economies. There have been major outbreaks throughout the world, making pathogen surveillance essential. However, its large genome size (170–190 kb), ability to acquire large deletions and insertions, and the presence of highly mutagenic hypervariable regions make sequencing the ASFV genome very challenging. Using the MinION, Amanda Warr and colleagues[7] developed a

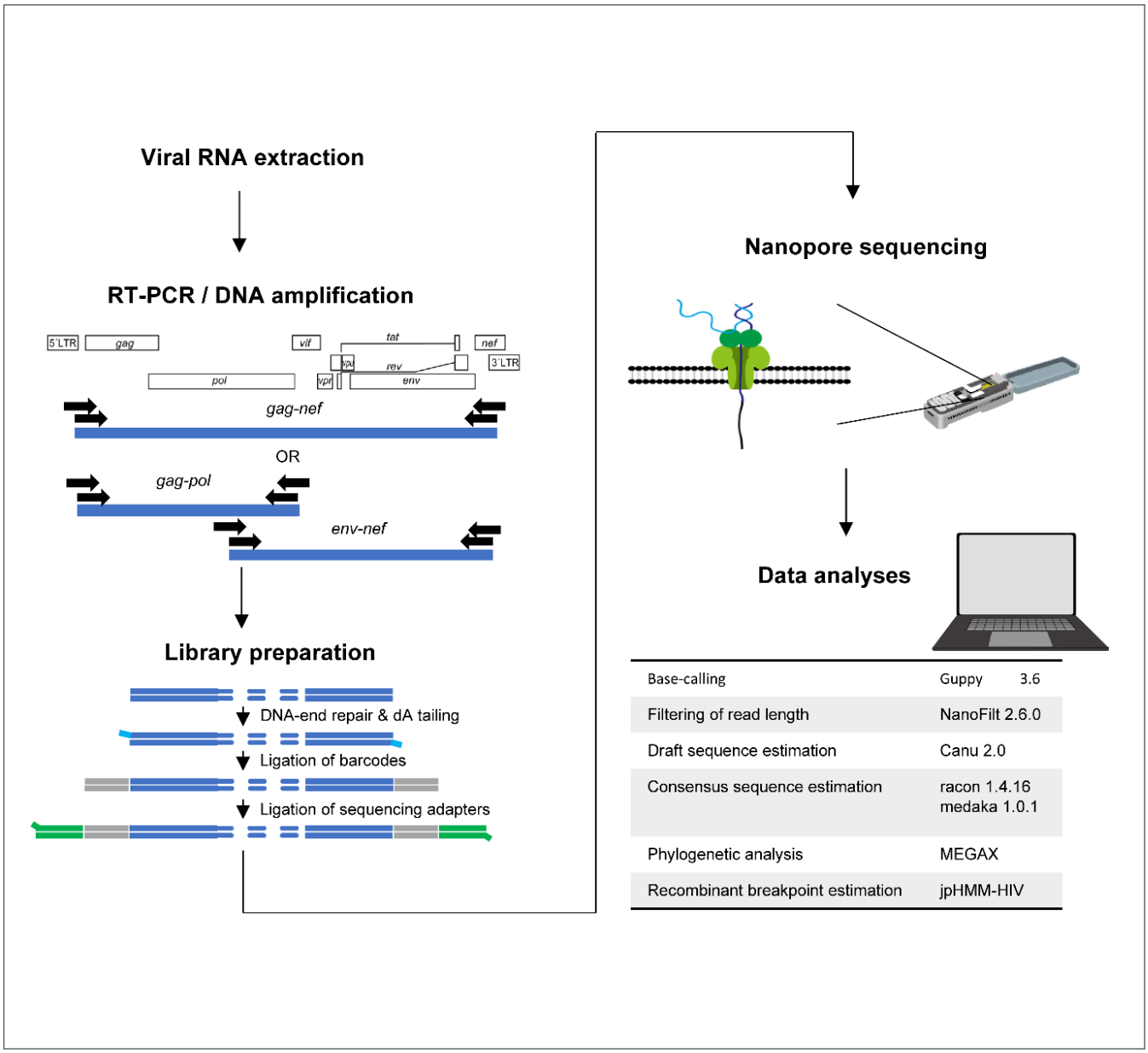

Figure 1
Nanopore sequencing protocol applied to the near full-length HIV-1 genome. Image from Mori *et al.*[6] and available under Creative Commons license (creativecommons.org/licenses/by/4.0).

tiled-amplicon approach to sequence ASFV that could enable near-live monitoring of outbreak situations with rapid turnaround times, and no challenging transportation of samples. Amanda described how '*multiplexing,... washing and reuse of the most expensive component of sequencing, the flow cells*' allowed for lower cost sequencing than other methods and the long reads improved assembly potential, particularly of highly repetitive genomes[7]. Amplification of the genome in 32 fragments of 7 kb, with 1 kb overlaps, generated near-complete genomes.

> ***"Rapid detection and response are of the upmost importance for containing viral outbreaks and supporting control measures[14]"***

Studying virus populations within infected hosts can provide essential information in understanding virus-host interactions and new approaches to outbreak response. During the recent SARS-CoV-2 pandemic, Oxford Nanopore and the ARTIC network were quick to refine the targeted amplicon approach with the Midnight protocol. With the ability to multiplex up to 96 samples, both time and costs have been substantially reduced. The Midnight protocol has become a popular method within the public health community because hands-on time is minimal, workflows are simple, and researchers can go from RNA to real-time sequencing within one working day.

Oxford Nanopore is proud to have worked with the scientific community in pathogen surveillance and, in collaboration with the ARTIC network and other research groups, continuously develops its protocols, kits, and bioinformatic pipelines. The cost-effective workflows and scalability to large numbers of samples during outbreak surges suggests the multiplex, tiled PCR approach with Oxford Nanopore technology will remain an essential method in pathogen surveillance.

Products used

Kits	Ligation Sequencing Kit Rapid Sequencing Kit
Devices	MinION | GridION™
Tools	Primalscheme

Find out more: nanoporetech.com/products

NANOPORE SEQUENCING

- provides a rapid, affordable, and scalable solution for pathogen sequencing and surveillance
- has been used by researchers worldwide to deliver novel genomic insights to support disease control strategies

"The availability of a portable sequencing technology opens new doors to travel to outbreak locations, sequence, and analyze samples without needing to transport them[7]"

Find out more about microbial sequencing using nanopore technology: nanoporetech.com/applications/microbiology

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