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PERSONAL VIEW
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Before you go: a packing list for portable DNA sequencing of microbiomes and metagenomes

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INTRODUCTION

This article is being drafted as we pack our genomics kit for our first polar fieldwork since the COVID-19 pandemic began. Nanopore sequencing has certainly proven itself as a disruptive technology during the pandemic, tracking the spread of SARS-CoV-2 (e.g. [1]). However, for microbiologists using nanopore sequencing for its portability, the pandemic has often disrupted their field plans. In this article, we share some reflections from exploits during the pre-pandemic era of portable sequencing. Hopefully these considerations are helpful for others seeking to take DNA sequencing where genomics has never ventured before.

ESTABLISH IF PORTABLE DNA SEQUENCING IS THE OPTIMAL STRATEGY FOR YOUR WORK

Gilbert *et al.* [2] argued Darwin would have been a metagenomic scientist. Had portable DNA sequencers been available, it is more than likely *HMS Beagle* would have one, as it was well equipped with the contemporary state of the art. There is little novelty in bringing your laboratory to the field, however the comparative accessibility of nanopore DNA sequencing does lend itself to field applications. Before embarking on portable DNA sequencing, it is essential to justify carefully if it is optimal for your goals. Relevant factors might include difficulties in transporting preserved samples to the laboratory [3], a need to gain insights to the study system while deployed to direct other field activities, the detection of contamination in high-stakes sampling (e.g. subglacial lake access), emergency response, or to build capacity at the point of need [4]. There is also a line of argument that sequencing on site may have less environmental impact compared with the cold-chain transfer of samples to a laboratory followed by long-term deep-frozen storage of large volumes of samples and reagents. Equally, sample archiving in microbial ecology is a neglected but vital topic [5]. Will portable sequencing discourage the collection of material which then denies the opportunity to archive unique samples for future analyses? Finally, it is important to consider the ethical implications of the work: how will benefits arising from the work be shared? If applicable, is it within the remit of the Nagoya Protocol (https://www.cbd.int/abs/)? Importantly, will the work be transient in benefit, or will it result in lasting enhancement to genomics capacity in the study area [6]?

UNDERSTAND THE ENVIRONMENTAL TOLERANCES OF NANOPORE SEQUENCING

Portable sequencing currently uses ~1000 recombinant protein-based nanopores individually entrapped within synthetic lipid layers to decipher nucleotide sequences [7]. This means nanopores are vulnerable to freezing, air bubbles, the ingress of surfactants, clumsiness, and rapid changes in temperature. Sequencing is also presently conducted within a narrow range of temperature, centred at +34 °C. Helpfully, nanopore flow cells can be stored at room temperature for a month, and many reagents are shipped in sustainable packaging, negating most cold-chain considerations. However, field applications can include sub-zero sequencing, where flow cells would freeze. In 2018, we embarked on an expedition on the Greenland Ice Sheet, sequencing ice cave microbiomes in ambient temperatures of −25 °C. While such conditions are perfect for storing frozen consumables, flow cells would fail. We explored different solutions to this challenge: heat packs, hot water bottles, extreme levels of insulation. However, the only sustainable source of warmth in a winter ice camp is the ration-fuelled bodies of the researchers. We therefore stored our flow cells in insulated containers in our clothing and sleeping bags. Using Bluetooth-enabled thermometers we ensured the flow cells stayed above freezing by exercising if they cooled. This approach

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Abbreviations: DIY-biology, do-it-yourself biology; self-guided exploration of biological topics and techniques by laypersons; GPU, Graphical Processing Unit; SPRI, solid phase reversible immobilization.
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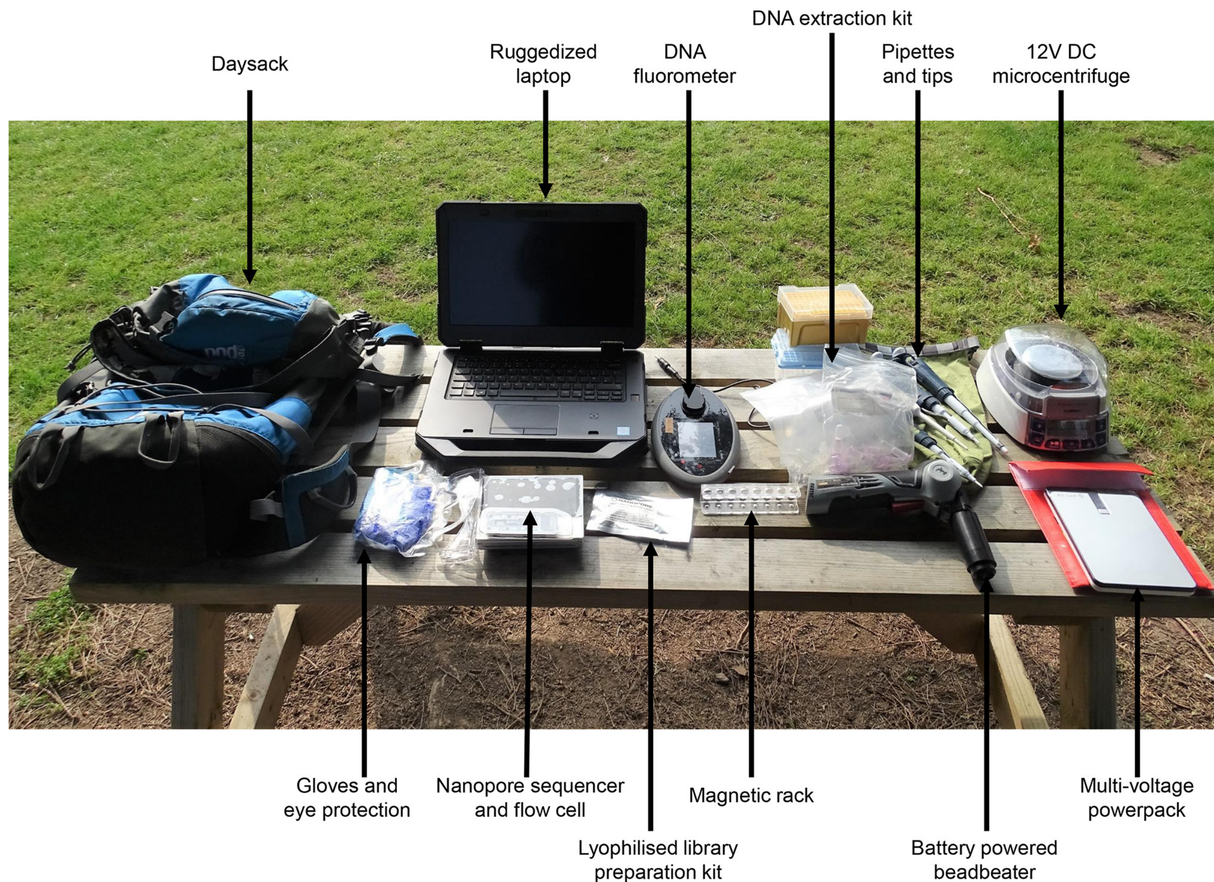

Fig. 1. A packing list for a typical daysack-scale portable DNA sequencing laboratory. While weighing <10 kg and fitting in a typical cabin-baggage daysack, this laboratory is capable of conducting shotgun metagenomics experiments in the field, without access to mains electricity.

was successfully replicated by Gowers *et al.* [8] during a ski traverse of an Icelandic ice cap. For sequencing experiments, conducted at 40–50 °C above ambient temperatures of −10 to −20 °C, a recycled polystyrene freezer box containing a single chemical heat pack was sufficient to sustain sequencing temperatures when coupled with the heat released from the sequencer. Researchers working in hotter climates may have the opposite challenge, of keeping flow cells and experiments cool. Here, ensuring good air flow around the device and potentially seating the device on a cooled surface can help. A key lesson is that pre-deployment trials help eliminate error.

If sub-zero sequencing relies on the normal body temperature of the researchers, then this reinforces a related point. The health, safety and well-being of the researchers must be considered a priority too. A field lab needs to be secure and functional for hours to days. Most of our field sequencing experiments are conducted in less than photogenic environments: camps, field stations, hotel rooms, vehicles, trailers, huts. These are chosen for their ability to protect the sequencing experiment by being dry, secure, warm, wildlife-resistant and stable. Purists might reject these as *ex situ* but the key criterion is whether the site can be sustained for the experiment's duration.

PACK YOUR GENOMICS KITBAG CAREFULLY

The composition of a portable genomics lab is varied (e.g. Fig. 1) precluding one fixed packing list. In basic terms, beyond the resources required to sustain a field team, there is the need to prepare nucleic acids, to handle liquid reagents, to control the sequencer, analyse the results and mitigate contamination/health and safety issues. Our smallest set-up for this weighed just 2 kg, and our largest ca. 50 kg, comprising mostly off-the-bench equipment. Housing the kit in a well-padded rucksack is preferable to hardened suitcases, as it enables the researcher to travel over rough ground.

Below, we discuss challenges and their solutions for each phase of an experiment.

CONTROLLING CONTAMINATION

A field lab will rarely match the standards of a clean lab. However, some basic precautions can reduce the impact of contamination. As standard, aerosol-resistant filter tips and gloves should be used, and with good aseptic technique. Working into a fresh breeze outdoors can reduce the likelihood of airborne contaminants for sensitive steps. Sample bags can provide a sterile working surface if cut open, and household bleach can be diluted to provide a decontamination solution. Chlorine-based water disinfection tablets provide a travel-friendly way to prepare a decontamination solution once at the destination. Site-specific consideration needs to be given for the safe storage, transport, or disposal of waste reagents and plasticware. Given the ad hoc nature of these measures, the normal emphasis on sequencing negative controls to detect contaminants is reinforced [9].

NUCLEIC ACID EXTRACTION AND PURIFICATION

This phase is the crux for portable sequencing, as the yield and quality of nucleic acid is critical for the experiment's success. However, the equipment and power supply demands for nucleic acid extraction are the most challenging. For extractions from microbial communities bound to environmental matrices or filters, bead-beating remains the most practical option for lysis in the field and can still provide long reads [10]. Battery-powered bead beaters exist (e.g. Zymo Research Terralyzer, MP BIO SuperFastPrep-2) but are limited in throughput and endurance. Once lysed, standard benchtop nucleic acid protocols (e.g. Qiagen PowerSoil, MP BIO FastDNA) can be adapted for field use with minimal impact on performance. Importantly, these kits can easily be adapted to challenging conditions for nucleic acid extraction, e.g. low biomass, high/low pH, high mineral content. The modifications can easily be accommodated in a nucleic acid extraction field kit [11].

Centrifugation is challenging for few battery-powered centrifuges are available. Our current solution is the Thermo Scientific MySpin12 centrifuge, which can provide benchtop microcentrifuge speeds while powered by a 12 V external power bank. Protein precipitation steps often require cold incubation, which in the absence of ice or snow, can be provided with an instant-ice pack placed in a cold-water bath.

If the performance of a DNA extraction protocol in terms of purity and DNA integrity has been validated in the laboratory, its success in-field is best verified using a portable fluorometer. Qubit fluorometers (Life Technologies) are widely used in molecular laboratories thus reducing their significant outlay, and can be operated using a suitable power bank.

LIBRARY PREPARATION

Depending on the objective, different library preparation protocols can be followed. Lyophilized 'rapid' kits are available for the instant-add-DNA-and mix generation of (meta-)genomic libraries, but other non-lyophilized options include ligation sequencing of amplicons or (meta-)genomes. Solid-phase reversible-immobilization (SPRI) beads are frequently used for clean-up steps in sequencing library preparation. These can be conducted in the field using a handheld magnet, or a 3D-printed magnetic rack. Enzymatic manipulation steps can be performed in the field. Ambient or +37 °C steps can be performed by placing the samples on a work surface or holding them tightly in a gloved hand, while heat-based inactivation can be improvised with hot water held in an insulated mug. More sophisticated processes (e.g. PCR) can be conducted using field-portable cyclers (e.g. MiniPCR, Amplyus). Devices powered by external batteries typically have 8–16 wells, limiting throughput, but this may be adequate for a typical field-based workflow.

SEQUENCING, PROCESSING AND ANALYSIS

Once the challenges for extraction and library generation have been solved, sequencing itself is typically straightforward. Maintaining a stable temperature for sequencing can require additional measures for warming or cooling. Contemporary nanopore sequencers rely upon graphical processing unit (GPU)-powered basecalling within the sequencing device, however in-field analysis of the data generated typically requires a laptop. Portable sequencing devices can now include integrated GPU units, but field-deployable, external GPUs can also be used for basecalling metagenomics sequencing datasets in remote conditions. Processing of amplicon sequencing data, some assembly or rapid taxonomic classification of metagenomes can be performed on a high-performance laptop. If working in extreme conditions, a rugged specification high-performance laptop will be required. For more demanding analyses, reliable internet links to server-based tools are still required. This can pose difficulties for researchers in many regions, so it is important to have confirmed the extent of internet access, its costs, and potential contingencies in the case of outages. Currently, sequencing and initial analysis in field conditions while returning to the home laboratory for more intensive (and leisurely) in-depth analyses are valid goals, but as GPU-based tools become more portable, performing more computationally intensive analyses in the field may break the last tethers with the home laboratory.

While operating in more conventional settings such as field stations or hotels with mains AC electricity, the endurance of sequencing or analysis activities is likely to be near normal. However, if reliant on internal or external batteries these will constrain

data generation and analysis. In our experience, portable generators are prone to failure, whereas Gowers *et al.* [8] successfully conducted solar-panel powered sequencing for 24 h, off-grid sequencing remains an area for development.

FUTURE DEVELOPMENTS

The principle and utility of portable sequencing has been proven on every continent, e.g [12]., as well as at sea, e.g [4], inside the Earth [11], and in low-Earth orbit [13]. Lessons learnt from portable sequencing can also be transferred to the laboratory, resulting in more streamlined protocols and equipment with a lighter footprint. Future areas for innovation include the streamlining of nucleic acid extraction to reduce dependencies on power-heavy instruments or pipettors, and the development of bioinformatics tools optimized for internet-free portable sequencing [14]. Furthermore, whether genomics becomes democratized or anarchized through the widespread availability of portable sequencing is unclear, however innovative low-power and low-cost tools and instruments exist within DIY-biology or maker communities (e.g. http://samtomindustrys.best/), which can enable rapid prototyping and fabrication of bespoke equipment for portable sequencing. Ultimately, while many aspects of portable sequencing compare favourably in their sustainability compared to the retrograde transfer of samples for storage and analysis in large-scale facilities, a portable sequencing experiment is limited in endurance. Further development in terms of efficient supply chains, room-temperature stable reagents and supporting portable bioinformatics will continue to normalize portable sequencing.

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Author contributions
All authors contributed to the writing of the manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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