D-JRP12-WP3.1 Review on current scientific literature and overview of commercially available methods for on-site DNA isolation.

JRP12-AMRSH5-FARMED

Contributing Partners:

APHA

Manal Abu Oun

Indre Navickaite

Sciensano

Sigrid De Keersmaecker

Mathieu Gand

SSI

Søren Persson

ISS

Valeria Michelacci

IZSAM

Francesca Marotta

Marco Di Domenico

Giuliano Garofolo

WBVR

Michael Brouwer

1. Introduction

The development of tools for real-time detection of antimicrobial resistant (AMR) pathogens is a priority topic of the One Health EJP. For real-time analysis to be achievable on-site (away from the laboratory setting), robust culture independent detection methods, employing minimal equipment are required. Metagenomic sequencing using short-read data has provided insight and detailed compositions of a variety of microbial communities, as well as for the detection of potential pathogens and AMR or virulence genes. In addition to the currently bulky nature of short-read technologies and the difficulty to perform this analysis on site, an important limitation is their inability to reliably associate the genetic context of individual genes to bacteria (including pathogens) within a community.

The FARMED project aims to address these issues by using the Oxford Nanopore Technologies (ONT) MinION, comparing to the current gold standard short-read technology, to evaluate its capability for diagnostic use on a range of sample matrices, particularly on-site at/near point of sample collection. This is enabled by the portability of the ONT technology, allowing on-site analysis, in contrast to short read sequencing. An additional advantage of using ONT sequencing or long-read metagenomic sequencing, is that the local genetic context of AMR genes can be derived, and as such, the presence of the AMR genes can be attributed to specific species or plasmids, within the bacterial community. This technology will enable the identification of a plethora of bacterial species and linkage of AMR genes to particular species. However, the successful application of on-site microbial detection/monitoring is influenced by various factors such as resource-limited working environment, sample collection and importantly the quality of the input DNA for sequencing. In addition, the availability of on-site sequencing and subsequent data analysis needs to be taken into account.

This deliverable will review the scientific literature on existing DNA extraction methods and determine which have the potential/are suitable for rapid on-site metagenomic analysis. For on-site DNA extraction, it is essential that the implemented methods use minimal transportable equipment. Furthermore, the method(s) need to be free of hazardous chemicals for field personnel and the working environment, as well as components that require sub-zero transport conditions. We will also discuss the requirements of DNA for long-read sequencing and suitability of the different methods. We consider methods suitable for on-site DNA extraction from different ‘simple’ and ‘complex’ sample matrices, as each has different considerations. Finally, we will deliver recommendations for the FARMED consortium to be tested using ONT sequencing.

2. Review of general DNA extraction techniques

Efficient extraction of biomolecules such as DNA has been the foundation for molecular biology, which are utilised in many downstream processes, and has resulted in a plethora of protocols to be considered, with different principles behind them. Downstream processing of microbial DNA requires different sample criteria (e.g. quality, purity, and quantity of extracted DNA), depending on the scientific question/scope of the project. Currently, many molecular laboratories rely on commercial kits for DNA extraction, especially when the DNA is extracted for Next-Generation Sequencing (NGS). While these commercial kits come at a higher price per sample, they provide a high measure of standardisation and quality control of the components. However, different DNA sequencing techniques and biological questions still require consideration of different parameters and approaches for DNA extraction.

While there are several approaches for the extraction of DNA, all commonly follow three key steps (Tan and Yiap 2009):

1. Lysis of the (bacterial) cells
2. Precipitation of the DNA
3. Purification of the DNA from other cellular components.

Bacterial lysis can be achieved by chemical/enzymatic, thermal, mechanical/physical, or a combination of these approaches (Song, Lee et al. 2018). More recently, a combination of chemical/enzymatic and mechanical/physical shearing of the bacterial membrane by bead beating with glass/silica beads has become a popular choice as exemplified by the number of commercial kits employing this approach (Table 1). However, it is important to bear in mind that not all bacteria lyse equally well under certain circumstances. For example, Gram positive bacteria require harsher lysis to disrupt the cell wall compared to Gram negative bacteria. Therefore, when working with complex microbial communities, careful consideration must be taken to ensure the extraction approach is not biased/detrimental towards specific species.

Following bacterial cell lysis, DNA needs to be separated from the other cellular components. Traditionally, DNA extraction methods based on phenol-chloroform have long been the gold standard for molecular biology, relying on a phase separation of lysed bacteria, during centrifugation, where nucleic acids dissolve in the top aqueous phase and other cellular components such as proteins and membranes remain in the lower organic phase. DNA is subsequently precipitated with alcohol. When the DNA is bound to an insoluble carrier it can be washed before it is eluted into a solvent and ready for the downstream processes it's required for (Farnsworth, Wallace et al. 2020).

Numerous DNA extraction kits on the market rely on the conditional binding of DNA to silica membranes for the precipitation of DNA. Methods often rely on the use of micro-centrifuges for the purpose of moving a lysate through the membrane. Several washing steps are then followed by elution of the DNA using a buffer with a low basic elution buffer (Esser, Marx et al. 2006). Recently, some extraction kits have moved towards the use of magnetic beads to bind DNA, using the solid-phase reversible immobilization (SPRI) method (DeAngelis, Wang et al. 1995). The method makes use of the DNA binding capabilities in PEG and salt containing buffers, followed by alcohol wash and elution in the absence of PEG and salt. While the method is highly sensitive, other advantages are the possibility to employ a size selection of

the extracted DNA by varying the DNA to bead ratio, reduced shearing of the DNA compared to silica-column based methods and the lack of need for the use of micro-centrifuges.

Automated systems for nucleic acid extraction have become popular in high throughput sequencing facilities and diagnostic laboratories due to the high level of quality standardisation and reduced costs by increased efficiency (Lee, Park et al. 2010, Mallott, Malhi et al. 2019). While the standardised results of these systems would be highly desirable for the on-site analysis of DNA, many automated systems include large machinery that are not suitable for transport outside the laboratory environment.

Table 1. Comparison of popular/on-site commercial microbial DNA extraction and purification kits used for metagenomics.

Extraction kit	Manufacturer	Recommended sample input amount (mg)	Cell lysis	DNA precipitation / purification approach	Approximate hands-on time (min)*
DNeasy PowerSoil	Qiagen	≤250	Mechanical	Spin column	~30
QIAamp Fast DNA stool mini	Qiagen	180-220	Enzymatic and heat	Spin column	<60
QIAamp Power Faecal Pro DNA	Qiagen	≤250	Mechanical	Spin column	~30
GenFind V3	Beckman	Not defined	Enzymatic and heat	Magnetic beads	~240
Quick DNA Faecal/Soil Microbe Miniprep	Zymo Research	≤150	Mechanical	Spin column	~30
NucleoSpin DNA Stool	Macherey-Nagel	≤220	Mechanical and enzymatic	Spin column	<60

* times are based on processing a single sample based on experience or manufacturers claims

3. Requirements for feasible on-site DNA extraction for on-site sequencing

3.1 Definition of on-site sequencing

The term 'on-site' generally describes any environment outside of a specific laboratory setting. This may include: (I) literal work on-site where access to laboratory resources/equipment is scarce such as in nature reserves or at a farm; (II) on-site with basic amenities including a primary care unit such as the office of a general practitioner or the lab of a veterinarian; (III) work at a site that contains basic laboratory equipment such as a secondary care unit or a non-academic hospital. These conditions are compared to (IV) laboratory facilities that are designed for NGS work, see Table 2.

Table 2. On-site DNA sequencing can be performed at various levels.

Level	Facilities	Examples
I	No facilities, all equipment transportable and battery operated, all reagents stored at local temperatures, no internet connection. Personal protection, and proper waste disposal need to be considered.	Nature reserve Farm Difficult to access locations (e.g. Antarctica, Amazon rainforest)
II	Bare facilities, all equipment transportable, (limited) access to local power grid, reagents can be transported chilled. Basic internet connection available.	General practitioner office Veterinarians practice
III	Basic facilities, access to local power grid and -20 °C or 4 °C storage. Access to basic lab equipment such as centrifuges. Access to high-speed internet connection.	Non-specialised laboratory Non-academic hospital
IV	Full facilities, including equipment for quality assessment of DNA. Access to high-speed internet connection.	Specialised molecular lab or sequencing facility.

Currently, most on-site work using ONT sequencing is conducted at levels II or III while some have been described at level I (Figure 1). However, the studies described at level I and II are mostly proof of principle with limited data availability and scientific insights other than the feasibility of the implemented methods (Castro-Wallace, Chiu et al. 2017, Menegon, Cantaloni et al. 2017, Boykin, Sseruwagi et al. 2019). Furthermore, some studies have chosen to perform DNA sequencing on-site (level I) while basecalling (a computationally intense data conversion) and data analysis is performed in a laboratory at a later time.

Figure 1. Left panel: Preparation for ONT sequencing in a rain forest in Tanzania (nanoporetech.com). Right panel: ONT sequencing run conducted aboard the International Space Station (space.com).

For FARMED we aim to develop and test methods for level I where minimal facilities are present. As we aim to perform the full protocol on-site, including basecalling and analysis, the sequencing run that may take up to several hours, which will require assessment of commercially available power supplies.

There are a number of factors which should be considered for on-site DNA extraction and sequencing which will be discussed below:

- Collection of the sample matrix
- Storage of sample prior to DNA extraction
- Consistency of sample matrix and cell lysis
- Robustness of equipment and reagents
- Quality and purity of the extracted DNA

3.2 Collection of the sample matrix and impact on downstream processes

In laboratory settings, samples must be safely sampled to avoid both laboratory personnel infections and contamination of individual samples from the environment or from other samples. However, when implementing on-site methods, sample collection and contamination factors must be carefully considered to ensure effective sampling and analysis (Yarbrough, Kwon et al. 2018, Farnsworth, Wallace et al. 2020). Existing laboratory procedures have been optimized to deal with cross-contamination (e.g. working in sterile conditions, PPE, access to disinfectants).

In studies describing on-site sampling conditions, the required level of care to prevent cross-contamination and infection of field-personnel is not currently considered much in literature. The risk involved varies greatly depending on the type of sample collected (such as environment, animals, or persons). Proper hand hygiene and use of personal protective equipment, as well as training of personnel, will be essential for on-site DNA extraction and DNA sequencing to ensure the risk for contamination and infection of users is minimised. Other considerations include the contamination of the on-site equipment and contamination of the environment itself (Farnsworth, Wallace et al. 2020).

The choice of DNA extraction method is largely dictated by the sample matrix of interest. Summaries of challenges and recommendations for best DNA extraction practices from a

variety of simple (e.g. water and urine) and complex samples (e.g. soil, faeces, animal and plant tissue) has been covered in the literature (Hermans, Buckley et al. 2018). Within FARMED, environmental water and faeces are considered the reference sample matrices for simple and complex matrices, respectively, although both pose challenges. It is important to understand possible limitations/difficulties in DNA extraction from such sample matrices.

- ***Faeces***: can be humic-rich (humic substances may affect DNA detection and quantification); sample quantity used for extractions is generally low (<0.25g) thus robust homogenisation is necessary to ensure equal sampling (see below); there is also the aspect of sample consistency (i.e. if faecal samples are dry it is recommended to add water to rehydrate, so as to ensure that the extraction buffers/solutions are not absorbed by the dried material, thus compromising the extraction efficiency).

- ***Water***: generally the DNA yield is low, thus requiring huge amounts of water to be collected and filtered (litres). It is also worth keeping in mind the special variability of samples (detected species might be originating from the sampling site and/or from different sites of the water source). Water samples can be processed by precipitation and centrifugation or filtration, recommended filter size for bacterial samples is 0.22µm. Although filtration is influenced by different pore sizes affecting species detection and the amount of water that can be filtered before filters become clogged, it does provide with higher DNA yields.

In the case of all sample matrices, a limiting factor is the quantity of sample needed to achieve optimal extraction of DNA. Comparison of samples will require standardisation of sample quantities to ensure optimal DNA extraction.

3.3 Storage of sample prior to DNA extraction and sequencing

Once sampling has been completed, samples may need to be stored until analysis, which in the case of on-site means relative short term, as analytical equipment will be present close to the site of sample collection. Several studies have investigated the degradation of DNA in clinical samples under different storage conditions, like temperature (-20, +4 °C and room temperature) and DNA preserving chemicals (RNA Later, Omnigene-Gut, FTA, DNA Shield and 95% ethanol) compared to native clinical samples (Song, Lee et al. 2018, Wu, Chen et al. 2019). These investigations have showed 1) the reagents mentioned above can preserve DNA for up to 4 weeks when the samples are stored at room temperature, 2) storage at -20 °C is needed when storing native samples for more than 4 weeks and 3) native samples before DNA extraction are stable at +4 °C for 4 hours at room temperature.
While DNA preservation is not a key aspect for on-site analysis as samples are processed rapidly thus avoiding DNA degradation, the sampling and storage conditions were nevertheless contemplated for the FARMED protocols. This was to ensure that a re-analysis of on-site processed samples in the laboratory for benchmarking purposes was possible. However, water samples should be processed immediately as it has been observed that DNA yield and quality rapidly decrease in unfiltered samples even if stored at -20 °C.

3.4 Consistency of sample matrix and cell lysis

Faeces is considered one of the most relevant and also challenging sample matrices for the goals set out for FARMED. A method that successfully extracts DNA from faeces for use in on-site ONT sequencing will likely be able to handle many other challenging clinical sample matrices. The consistency of the sample matrix is key for successful DNA extraction from faeces. Clinical samples with solid consistency may not have a uniform representation of target bacteria and this uneven distribution may relate to internal physical compartments with different growth conditions and surfaces being exposed to oxygen, which can fluctuate the bacterial populations. Therefore, some sort of homogenization is necessary to ensure uniform sampling. Several methods are available, listed here with increasing intensity: (1) shaking by hand, (2) pipetting, (3) vortexing, (4) bead beating. Vortexing and bead beating require equipment that are not easily transported on-site. However, since bead beating is currently a common part of many commercial DNA extraction kits and on-site solutions may be feasible, such as the RotaPrep Monolyser, workflows containing bead beating will be considered. Bead beating increases the efficiency of cell lysis for Gram-positive bacteria, resulting in more representative communities that can be detected. However, care must be taken to not adversely affect the length of DNA, which would hinder the association between bacterial species and target genes (AMR or virulence) aimed for using ONT sequencing (discussed below).

3.5 Suitability of reagents and equipment for on-site DNA extraction and sequencing

The reagents used to isolate DNA and prepare on-site sequencing, must be carefully considered, regarding safety and stability as the procedures are being optimised for use outside of a laboratory setting. Reagent stability is a factor that is mostly considered for on-site environments where there is no/limited access to fridge and freezer. As the current kits for sequencing library preparation of ONT require certain reagents to be stored chilled, it is considered that the DNA extraction methods tested for FARMED may also contain some reagents that require storage at -20 or +4 °C.

Phenol/chloroform extraction has for many years been considered the gold standard in molecular biology. However, this method is not considered here, due to the toxic nature of these chemicals. Safe usage and disposal of these chemicals is considered impractical for on-site usage and risks for personal and environmental safety are too great outside of the laboratory setting.

Care and consideration must also be given to the necessary equipment as space and a power source are likely to be limited, as well as ease of transportation. Such equipment includes pipettes, magnetic racks, centrifuges, heat blocks, PCR machines and bead beaters, for which the last three specific on-site models exist that run on battery packs are now available (heat block https://www.medicalexpo.com/prod/amplexdiagnostics/product-128395-944468.html, Palm PCR http://www.ahrambio.com, bead beater https://www.zymoresearch.com/products/rotaprep-monolyser). However, reliability of these machines compared to laboratory models and reproducibility of the treated samples remains to be determined.

In terms of sequencing equipment, ONT has improved the feasibility of on-site sequencing and

data analysis with the most recent release of the MinION, version Mk1C, and the VolTRAX, further discussed below. While the previous version of the MinION, Mk1B, relied on a laptop to operate, the Mk1C can operate autonomously (figure 2). With local basecalling being done on the Mk1C, a standard laptop will be required to analyse the produced data in real-time.

Figure 2. Bottom: MinION Mk1B which requires a laptop for on-site DNA sequencing. Top: MinION Mk1C which can perform DNA sequencing fully autonomous (nanoporetech.com).

3.6 Quality and purity of the extracted DNA

The quality and the level of purity of the resulting DNA after extraction must also be considered. Many protocols rely on microcentrifuges during the purification which can be substituted using hand-powered methods or power-drill adapters. However, using centrifugal forces requires high precision in order to reduce shearing of DNA and to efficiently elute DNA from the column. Spin column protocols are not considered for FARMED as a suitable alternative exists, such as using magnetic beads, which require only a magnetic stand to separate the DNA bound to beads, from a solution. Quality and quantity assessment of on-site isolated DNA is limited with current technology. One option is the Qubit, which is a portable fluorometer, with few reagents/consumables required, although it needs a power source (no built in battery). FARMED will consider the best approach to achieve robust sequencing results.

4. Specific Requirements for ONT sequencing

Apart from the feasibility to perform on-site sequencing, the main advantage of ONT sequencing is the fact that the process will analyse long DNA molecules. Short-read technology generally analyses only 2 x 250 basepairs of the input molecule, ONT sequencing will typically analyse molecules of 3000 to 8000 basepairs although using specific protocols, reads above 2,000,000 basepairs have been reported (Payne, Holmes et al. 2019). These long reads allow determination of the genetic context of target genes harboured by specific bacterium or plasmid, which is currently not feasible with short-read metagenomic sequencing.

Fragmentation is of particular concern to ONT sequencing, as long sequence reads are absolutely dependent on intact DNA. Several steps from sample collection to DNA purification are known to introduce DNA fragmentation, including heating, physical/mechanical and harsh chemical conditions. Therefore, the methods necessary for lysing the bacterial cell wall, must be moderated not to introduce excessive DNA breakage (e.g. avoiding extreme force during handling of DNA with pipettes) and reduce DNase activity. Furthermore, the amount of the purified DNA is a critical parameter for ONT sequencing library preparation. Nanopore sequencing kits require an input of approximately 100x higher DNA amounts than Illumina (short read) sequencing. This is feasible with many commercially available laboratory based DNA extraction kits but is an important parameter to consider for on-site sequencing.

In Table 3, four of the ONT sequencing kits used to prepare the extracted DNA for sequencing are considered. Other kits are not considered here because of the necessity to use a thermal cycler during the preparation. While the Field Sequencing Kit and the Rapid Sequencing Kit are superior in terms of preparation time and the quantity of DNA required, these kits usually result in lower output of sequence results per hour. The Ligation Sequencing Kit is advertised to generate the highest data throughput, however, due to its long protocol and required high DNA input amount, the kit is not easily applicable for on-site work.

Table 3. Characteristics of different ONT library preparation kits.

ONT Sequencing Kit	Prep time	Input DNA	Typical output (6 hours)	(Dis)advantages
Field Sequencing Kit	10 minutes	400 ng	1-2 Gb	+ Minimal handling of components
Rapid Sequencing Kit	10 minutes	400 ng	1-2 Gb	- Pipetting of multiple components
Ligation Sequencing Kit	60 minutes	1000 ng	2-3 Gb	- Long protocol - Sensitive kit components + High output of sequences
VolTRAX	10 minutes + 50 incubation	475 ng	2-3 Gb	- No control when preparation fails + Automatic preparation of sequencing libraries + High output of sequences

The VolTRAX is a small machine (similar in size to the MinION sequencer) that automates the sequencing library preparation, using a combining of the rapid and ligation sequencing kits, thus simplifying production of high quality libraries. As the product has only recently been introduced it requires further optimisation in consultation with ONT. Furthermore, the costs of using VolTRAX are currently high (€ 157 per sequence reaction) compared to other ONT seuencing kits which cost € 89 per sequence reaction. To increase sequencing throughput, samples can be multiplexed and labelled using specific barcodes, and sequenced in a single run. Thus, the VolTRX would be ideal for library preparation on-site, where the added cost for using this system may be modest due to the reduced hands-on time of the technician.

The development of the VolTRAX is currently still continuing at ONT and, although the

deadlines are often postponed, in the past the company has always delivered on forecasts for future products. The company has been developing additional workflows and/or cartridges that enable new functions for the equipment. An example is the new workflow that allows a multiplexed analysis including reverse transcription and PCR amplification on the VolTRAX of the SARS-CoV-2 virus. Future workflows that are under development include the lysis and isolation of DNA on the VolTRAX (figure 3).

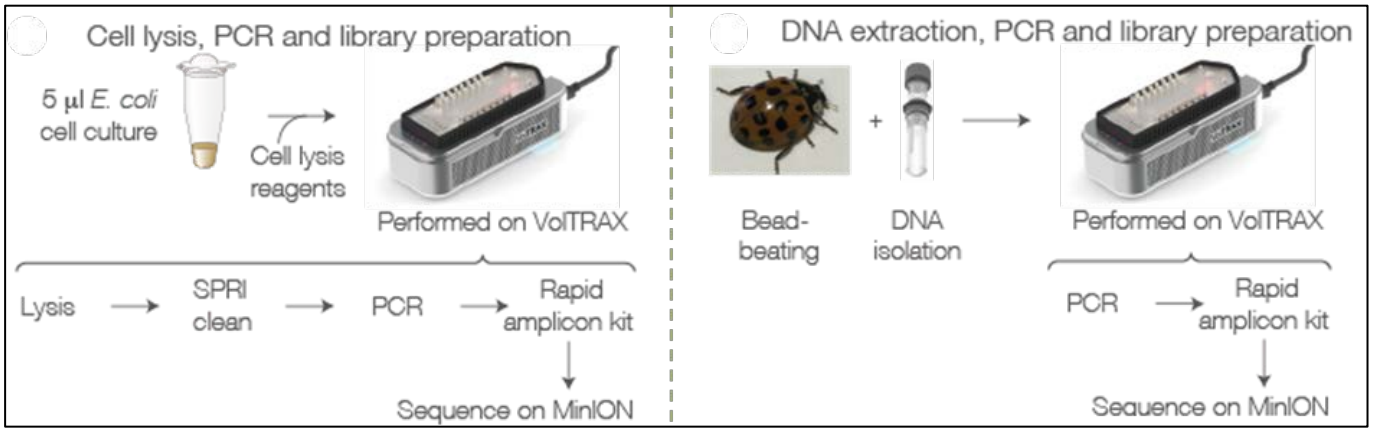

Figure 3. Left panel: future upgrade of the VolTRAX system that includes lysis and DNA extraction steps within the workflow. Right panel: upgraded workflows include PCR on the VolTRAX cartridges, this is currently in place in the SARS-CoV-2 workflow. (Figures adopted from nanoporetech.com).

5. Feasibility of current DNA extraction methods for FARMED

Several published studies have already described on-site sequencing of diverse sample types, but many have used DNA or RNA extraction techniques that would not be considered feasible (1) for large scale on-site analysis due to pieces of equipment that were used, (2) reliance on transport of the samples to specialised laboratories, (3) implementation of phenol-chloroform extractions, (4) time consuming processing and extraction steps (Quick, Loman et al. 2016, Castro-Wallace, Chiu et al. 2017, Singh, Bezdan et al. 2018, Hamner, Brown et al. 2019, Hu, Green et al. 2019, Maestri, Cosentino et al. 2019, Edwards, Cameron et al. 2020, Stubbs, Blacklaws et al. 2020). While these studies have acted as pioneers to deliver proof of principle for on-site DNA extraction and sequencing, the methods will not be considered for the reasons described.

Recent studies have described methods that could fit FARMED needs in terms of extracting DNA in sufficient quantity and high quality DNA suitable for downstream on-site ONT sequencing from faecal material (Wei, Hung et al. 2020) or from water (Acharya, Blackburn et al. 2020), although both methods still rely on kits that require microcentrifuges. At Wageningen University, an in-house kit for on-site extraction (USEB) of DNA has been developed which relies on Chelex and buffering components to lyse cells, degrade proteins and extract DNA (personal communication, Anne van Diepeningen). Although the recipe of the components cannot be disclosed at this moment, this kit will be made available to WBVR and other FARMED partners for testing with ONT sequencing. Additionally, Sciensano is testing the Claremont Bio Express DNA extraction kits for suitability for metagenomics ONT

sequencing. This kit uses a battery-operated bead beating method in the presence of ClaremontBio's proprietary binding and elution buffers to quickly shear open cells and simultaneously bind nucleic acid to the surface of beads inside of the chamber.

During the literature search, other promising methods for DNA extraction were identified such as the use of electrophoretic DNA extraction, however, this technology currently has only been proven to work for single isolates of bacteria and needs further maturation before it can be considered for metagenomic sequencing (Kang, Kim et al. 2020). A more feasible option might be the PDQeX DNA extractor by Microgem (figure 4). As far as we are aware, this is the first commercially available automated DNA extractor that has acceptable dimensions for on-site use and for which successful downstream metagenomic sequencing using the MinION has been reported (Boykin, Sseruwagi et al. 2019). The PDQeX has a rapid protocol (under 30 minutes) and uses disposable tubes to prevent cross-contamination between samples.

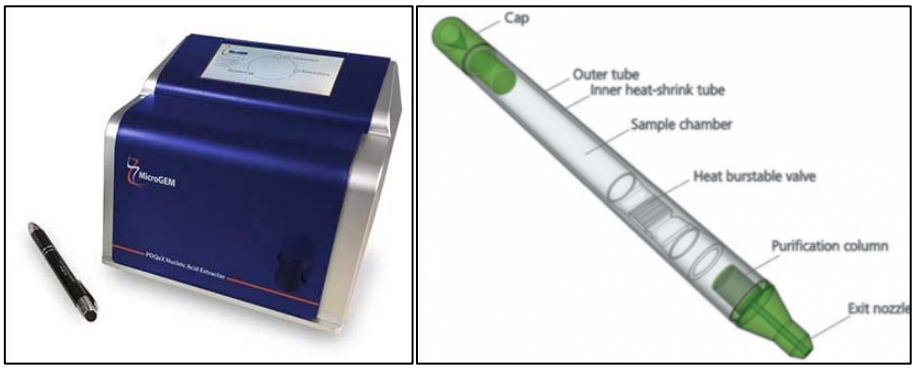

Figure 4. Left panel: Microgem PDQeX portable and automated DNA extractor. Right Panel: PDQeX uses a closed tube system which handles all steps of DNA extraction.

6. *Concluding recommendations for on-site DNA extraction in FARMED*

In order to perform this literature study, the FARMED consortium has reviewed the current literature on DNA extraction methods and considered their suitability for on-site usage. Reducing the amount of equipment, using portable equipment, and using reagents that do not contain hazardous materials have prioritised particular methods that will be tested during the project.

Currently, a proficiency test is being performed by FARMED partners, in which a defined set of six bacterial species was used to spike faecal sample that were then distributed to all partners. Using various kits that are routinely utilised in their laboratories to perform metagenomic DNA extractions, the DNA will be extracted and sequenced using the same methods. This analysis aims to highlight 2-3 methods which can be explored in further detail to determine if they can be deployed on-site. In addition, FARMED will also investigate other newly released on-site DNA extraction methods. On-site protocols currently being tested:

1. SSI will test magnetic particle DNA purification with lysis through bead beating versus

enzymatic lysis.

2. APHA have recently acquired the PDQeX system described above and will test this commercially available system.
3. WBVR will test the USEB method described above which will be made available to further FARMED partners upon satisfactory results in combination with nanopore sequencing.
4. Sciensano will test the ClaremontBio Express DNA extraction kit.

7. Scientific literature

Acharya, K., A. Blackburn, J. Mohammed, A. T. Haile, A. M. Hiruy and D. Werner (2020). "Metagenomic water quality monitoring with a portable laboratory." Water Res **184**: 116112.

Boykin, L. M., P. Sseruwagi, T. Alicai, E. Ateka, I. U. Mohammed, J. L. Stanton, C. Kayuki, D. Mark, T. Fute, J. Erasto, H. Bachwenkizi, B. Muga, N. Mumo, J. Mwangi, P. Abidrabo, G. Okao-Okuja, G. Omuut, J. Akol, H. B. Apio, F. Osingada, M. A. Kehoe, D. Eccles, A. Savill, S. Lamb, T. Kinene, C. B. Rawle, A. Muralidhar, K. Mayall, F. Tairo and J. Ndunguru (2019). "Tree Lab: Portable genomics for Early Detection of Plant Viruses and Pests in Sub-Saharan Africa." Genes (Basel) **10**(9).

Castro-Wallace, S. L., C. Y. Chiu, K. K. John, S. E. Stahl, K. H. Rubins, A. B. R. McIntyre, J. P. Dworkin, M. L. Lupisella, D. J. Smith, D. J. Botkin, T. A. Stephenson, S. Juul, D. J. Turner, F. Izquierdo, S. Federman, D. Stryke, S. Somasekar, N. Alexander, G. Yu, C. E. Mason and A. S. Burton (2017). "Nanopore DNA Sequencing and Genome Assembly on the International Space Station." Sci Rep **7**(1): 18022.

DeAngelis, M. M., D. G. Wang and T. L. Hawkins (1995). "Solid-phase reversible immobilization for the isolation of PCR products." Nucleic Acids Res **23**(22): 4742-4743.

Edwards, A., K. A. Cameron, J. M. Cook, A. R. Debbonaire, E. Furness, M. C. Hay and S. M. E. Rassner (2020). "Microbial genomics amidst the Arctic crisis." Microb Genom **6**(5).

Esser, K., W. Marx and T. Lisowsky (2006). "maxXbond: first regeneration system for DNA binding silica matrices." Nat Methods **3**.

Farnsworth, C. W., M. A. Wallace, A. Liu, A. M. Gronowski, C. D. Burnham and M. L. Yarbrough (2020). "Evaluation of the Risk of Laboratory Microbial Contamination during Routine Testing in Automated Clinical Chemistry and Microbiology Laboratories." Clin Chem **66**(9): 1190-1199.

Hamner, S., B. L. Brown, N. A. Hasan, M. J. Franklin, J. Doyle, M. J. Eggers, R. R. Colwell and T. E. Ford (2019). "Metagenomic Profiling of Microbial Pathogens in the Little Bighorn River, Montana." Int J Environ Res Public Health **16**(7).

Hermans, S. M., H. L. Buckley and G. Lear (2018). "Optimal extraction methods for the simultaneous analysis of DNA from diverse organisms and sample types." Mol Ecol Resour **18**(3): 557-569.

Hu, Y., G. S. Green, A. Milgate, E. A. Stone, J. P. Rathjen and B. Schwessinger (2019). "Pathogen Detection and Microbiome Analysis of Infected Wheat Using a Portable DNA Sequencer." PBIOMES **3**(2).

Kang, J. H., Y. T. Kim, K. Lee, H. M. Kim, K. G. Lee, J. Ahn, J. Lee, S. J. Lee and K. B. Kim (2020). "An electrophoretic DNA extraction device using a nanofilter for molecular diagnosis of pathogens." Nanoscale **12**(8): 5048-5054.

Lee, J. H., Y. Park, J. R. Choi, E. K. Lee and H. S. Kim (2010). "Comparisons of three automated systems for genomic DNA extraction in a clinical diagnostic laboratory." Yonsei Med J **51**(1): 104-110.

Maestri, S., E. Cosentino, M. Paterno, H. Freitag, J. M. Garces, L. Marcolungo, M. Alfano, I. Njunjic, M. Schilthuizen, F. Slik, M. Menegon, M. Rossato and M. Delledonne (2019). "A Rapid and Accurate MinION-Based Workflow for Tracking Species Biodiversity in the Field." Genes (Basel) **10**(6).

Mallott, E. K., R. S. Malhi and K. R. Amato (2019). "Assessing the comparability of different DNA extraction and amplification methods in gut microbial community profiling." Access Microbiol **1**(7): e000060.

Menegon, M., C. Cantaloni, A. Rodriguez-Prieto, C. Centomo, A. Abdelfattah, M. Rossato,

M. Bernardi, L. Xumerle, S. Loader and M. Delledonne (2017). "On site DNA barcoding by nanopore sequencing." PLoS One **12**(10): e0184741.
Payne, A., N. Holmes, V. Rakyan and M. Loose (2019). "BulkVis: a graphical viewer for Oxford nanopore bulk FAST5 files." Bioinformatics **35**(13): 2193-2198.
Quick, J., N. J. Loman, S. Duraffour, J. T. Simpson, E. Severi, L. Cowley, J. A. Bore, R. Koundouno, G. Dudas, A. Mikhail, N. Ouedraogo, B. Afrough, A. Bah, J. H. Baum, B. Becker-Ziaja, J. P. Boettcher, M. Cabeza-Cabrerizo, A. Camino-Sanchez, L. L. Carter, J. Doerrbecker, T. Enkirch, I. G. G. Dorival, N. Hetzelt, J. Hinzmann, T. Holm, L. E. Kafetzopoulou, M. Koropogui, A. Kosgey, E. Kuisma, C. H. Logue, A. Mazzarelli, S. Meisel, M. Mertens, J. Michel, D. Ngabo, K. Nitzsche, E. Pallash, L. V. Patrono, J. Portmann, J. G. Repits, N. Y. Rickett, A. Sachse, K. Singethan, I. Vitoriano, R. L. Yemanaberhan, E. G. Zekeng, R. Trina, A. Bello, A. A. Sall, O. Faye, O. Faye, N. Magassouba, C. V. Williams, V. Amburgey, L. Winona, E. Davis, J. Gerlach, F. Washington, V. Monteil, M. Jourdain, M. Bererd, A. Camara, H. Somlare, A. Camara, M. Gerard, G. Bado, B. Baillet, D. Delaune, K. Y. Nebie, A. Diarra, Y. Savane, R. B. Pallawo, G. J. Gutierrez, N. Milhano, I. Roger, C. J. Williams, F. Yattara, K. Lewandowski, J. Taylor, P. Rachwal, D. Turner, G. Pollakis, J. A. Hiscox, D. A. Matthews, M. K. O'Shea, A. M. Johnston, D. Wilson, E. Hutley, E. Smit, A. Di Caro, R. Woelfel, K. Stoecker, E. Fleischmann, M. Gabriel, S. A. Weller, L. Koivogui, B. Diallo, S. Keita, A. Rambaut, P. Formenty, S. Gunther and M. W. Carroll (2016). "Real-time, portable genome sequencing for Ebola surveillance." Nature **530**(7589): 228-232.
Singh, N. K., D. Bezdan, A. Checinska Sielaff, K. Wheeler, C. E. Mason and K. Venkateswaran (2018). "Multi-drug resistant Enterobacter bugandensis species isolated from the International Space Station and comparative genomic analyses with human pathogenic strains." BMC Microbiol **18**(1): 175.
Song, E. J., E. S. Lee and Y. D. Nam (2018). "Progress of analytical tools and techniques for human gut microbiome research." J Microbiol **56**(10): 693-705.
Stubbs, S. C. B., B. A. Blacklaws, B. Yohan, F. A. Yudhaputri, R. F. Hayati, B. Schwem, E. M. Salvana, R. V. Destura, J. S. Lester, K. S. Myint, R. T. Sasmono and S. D. W. Frost (2020). "Assessment of a multiplex PCR and Nanopore-based method for dengue virus sequencing in Indonesia." Virol J **17**(1): 24.
Tan, S. C. and B. C. Yiap (2009). "DNA, RNA, and protein extraction: the past and the present." J Biomed Biotechnol **2009**: 574398.
Wei, P. L., C. S. Hung, Y. W. Kao, Y. C. Lin, C. Y. Lee, T. H. Chang, B. C. Shia and J. C. Lin (2020). "Characterization of Fecal Microbiota with Clinical Specimen Using Long-Read and Short-Read Sequencing Platform." Int J Mol Sci **21**(19).
Wu, W. K., C. C. Chen, S. Panyod, R. A. Chen, M. S. Wu, L. Y. Sheen and S. C. Chang (2019). "Optimization of fecal sample processing for microbiome study - The journey from bathroom to bench." J Formos Med Assoc **118**(2): 545-555.
Yarbrough, M. L., J. H. Kwon, M. A. Wallace, T. Hink, A. Shupe, V. J. Fraser, E. R. Dubberke and C. A. Burnham (2018). "Frequency of Instrument, Environment, and Laboratory Technologist Contamination during Routine Diagnostic Testing of Infectious Specimens." J Clin Microbiol **56**(6).