A portable system for rapid bacterial composition analysis using a nanopore-based sequencer and laptop computer

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A portable system for rapid bacterial composition analysis using a nanopore-based sequencer and laptop computer

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Introduction

- Time is of critical importance when identifying pathogens in acute infectious disease.
- In some critical conditions, such as bacteremia, starting antibiotic administration within 1 hour is highly recommended.
- Initial choice of antibiotic is usually empirical.
- Establishing systems for rapid microorganism identification via metagenomics sequencing seems pertinent and practical, especially for clinical use, to facilitate appropriate initial antibiotic treatment.

Cont…

- **Most currently available, next-generation sequencing (NGS) technologies are not designed for the rapid acquisition of sequence data.**
- **NGS obtain huge amounts of nucleotide sequence data, but require days to weeks to complete.**
- **However, a portable USB sequencer MinION (Oxford, UK) produces nucleotide sequence data sequentially, enabling real-time metagenomic analysis.**
- **Moreover, MinION has further advantages including simple sample preparation, portability, rapid, and being relatively inexpensive.**

Cont…

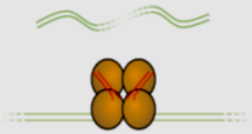
- MinION's rapid library preparation protocol was also released in May 2016, which enables 10-min library preparation from DNA to bacterial identification.
- This protocol only reads the template strand of double-stranded DNA (1D sequencing), and was initially considered to be of relatively low quality.
- In contrast, the original library preparation protocol produced data from both strands (2D sequencing), but required 90 min for library preparation.
- This novel NGS sequencing technology is increasingly being used for detecting pathogens in bacterial infections.

Procedure

Library preparation

Library preparation
~ 10 minutes

As per Rapid Sequencing of genomic DNA for the MinION device using SQK-RAD001



FRAGMENTATION

ADAPTER LIGATION

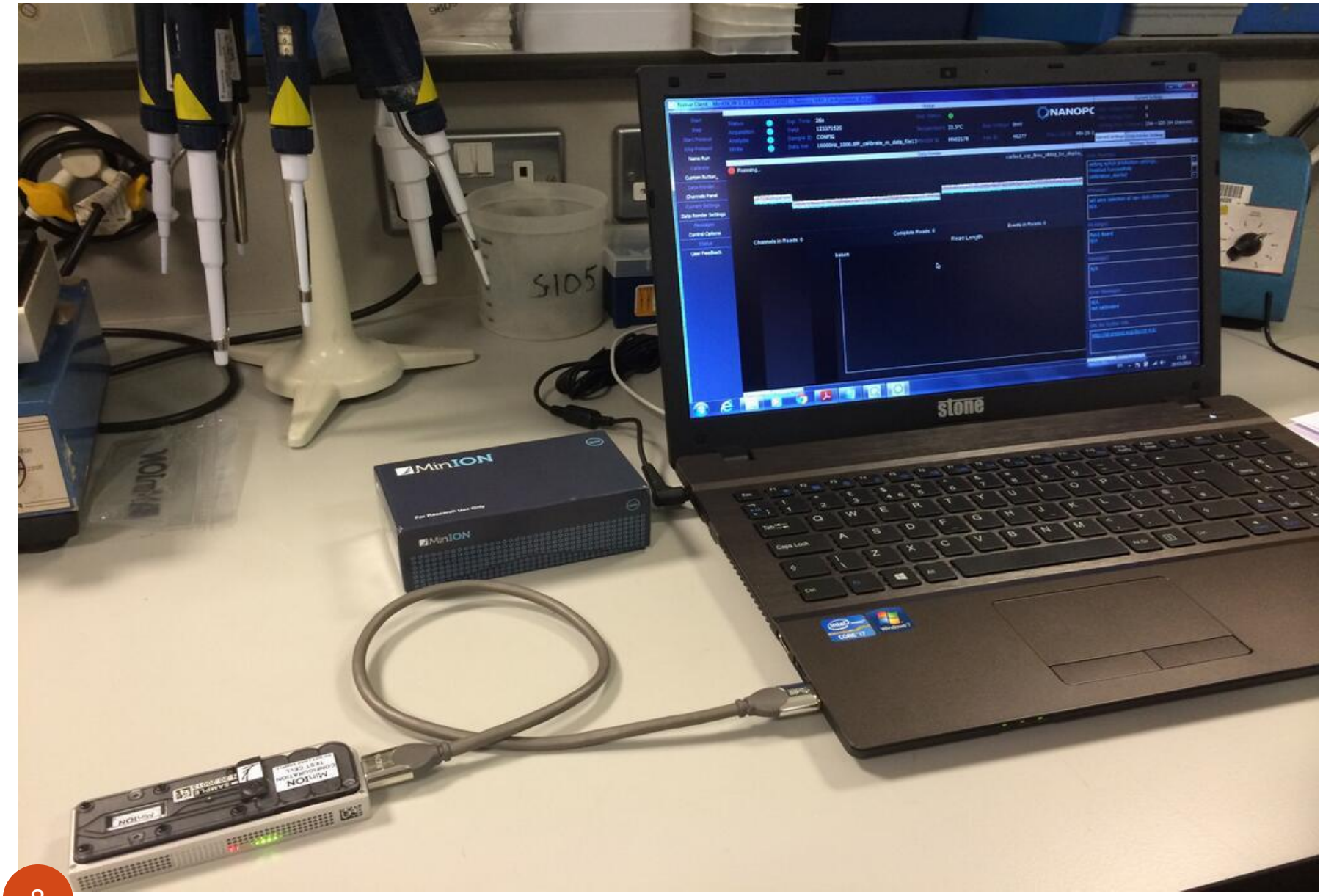
SEQUENCING

200 ng high molecular weight DNA in 7.5 µl transposase fragmentation.
PowerSoil routinely produces DNA fragments of 20-25 kb.

ANALYSIS

Edwards *et. al*, 2016, Extreme metagenomics using nanopore sequencing: a field report from Svalbard, 78°N, BioRxiv, https://doi.org/10.1101/073965

MinION


MinION
For Research Use Only
MinION
NANOPC
stone

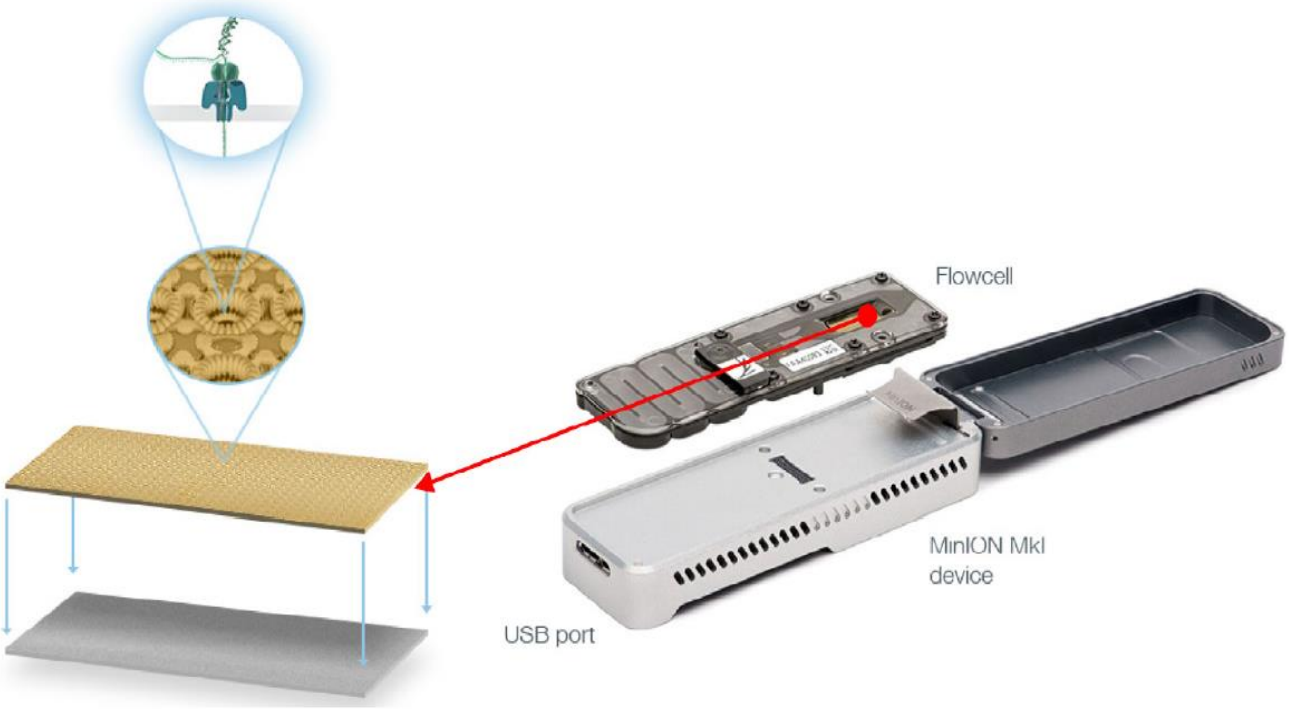
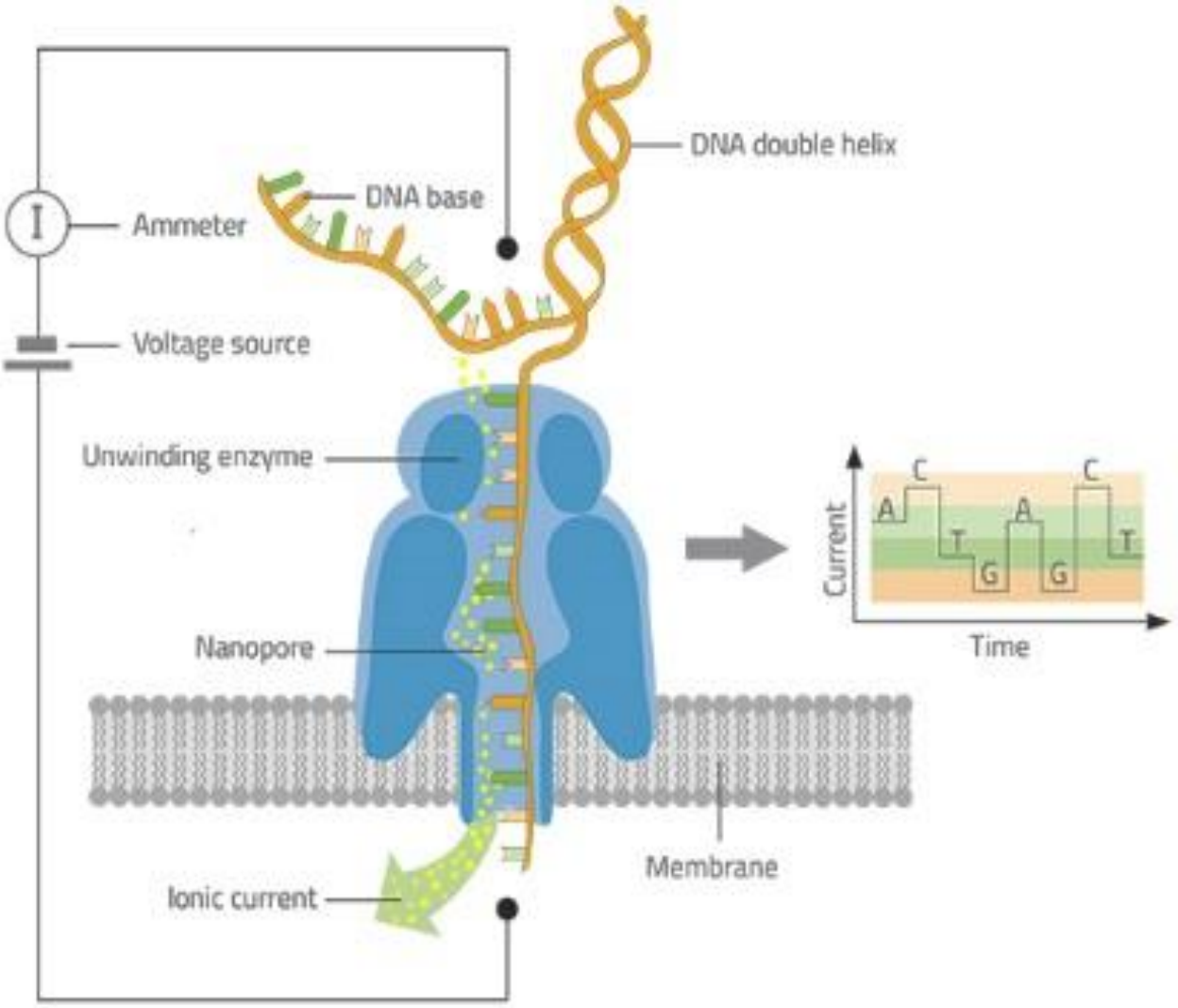


Figure 1 The MinION sequencing device

DNA sequencing is performed by adding the sample to the flowcell. When DNA molecules pass through or near the nanopore, there will be a change in the magnitude of the current in the nanopore, which is measured by a sensor. The data streams are passed to the ASIC and MinKNOW, the software that generates the signal-level data. ASIC, application-specific integrated circuit.

DNA: nanopore sequencing

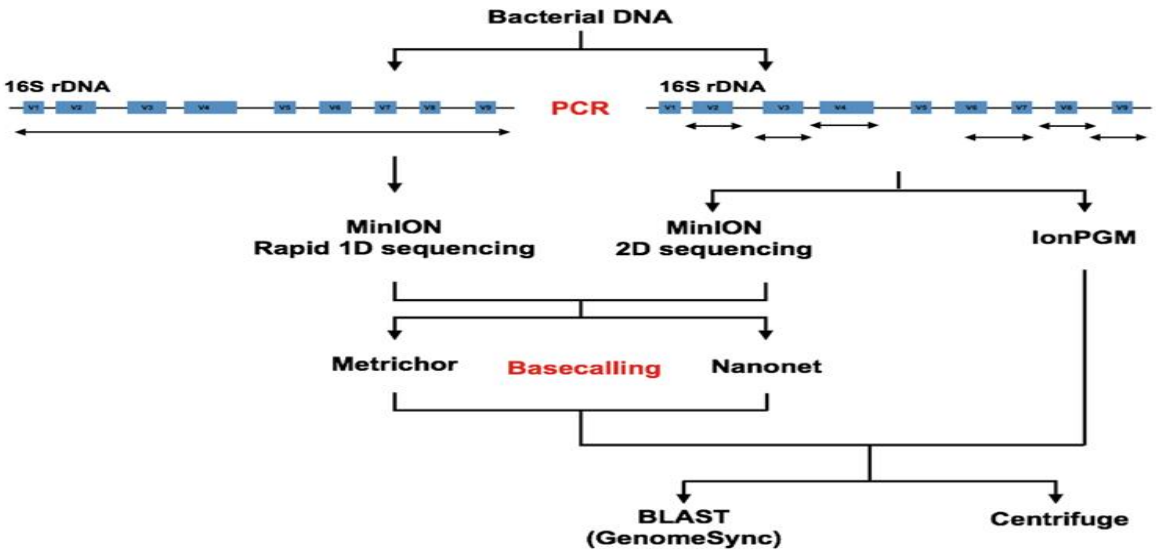


PGM

Materials and Methods

a

b



c

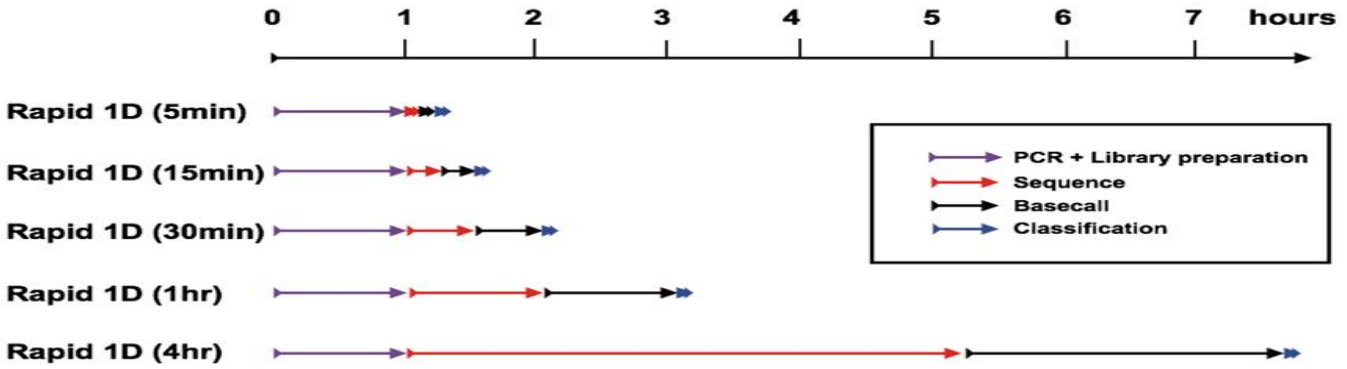


Figure 1. Portable system for bacterial determination and overview of our study. (**a**) Our system for bacterial composition determination. (**b**) Schematic outline of our experiment protocols. We used primer sets that amplified the same region of V2, V3, V4, V6–7, V8, and V9 for MinION 2D and IonPGM sequencing. For Rapid 1D sequencing, we amplified nearly the full-length of the16S rRNA genes. Metrichor and Nanonet software were used for base-calling. Results were analysed both with *Centrifuge* and BLAST-based searching methods. (**c**) Rapid 1D sequencing data at different time points, 5 min, 15 min, 30 min, 1 h, and 4 h, were collected and analysed to assess time-effectiveness. Figure shows the time course after sample DNA preparation.. Detailed time count for "PCR + Library preparation" is as follows: PCR reaction, 41 minutes; DNA purification, 10 minutes; and library preparation using Rapid Sequencing Kit, 10 minutes.

Sequencer	Method	Reads*	Time							
				Sequencer		Data analysis				
			Amplicon preparation	Sequencer preparation	Sequencing	Ion Reporter	Base call by Metrichor and Poretools	Basecall by Nanonet	Centrifuge	BLAST
IonPGM	Ion Metagenomic kit	330,124	6 hr	~3 hr	3 hr	5–6hr	NA	NA	<1 min	NA
MinION	Rapid 1D (5 min)	1,379	10 min	20 min	5 min	NA	38 min	7 min	<20 sec	NA
MinION	Rapid 1D (15 min)	3,703	10 min	20 min	15 min	NA	101 min	19 min	<20 sec	NA
MinION	Rapid 1D (30 min)	6,906	10 min	20 min	30 min	NA	174 min	34 min	<20 sec	NA
MinION	Rapid 1D (1hr)	11,174	10 min	20 min	1 hr	NA	273 min	58 min	<20 sec	NA
MinION	Rapid 1D (4hr)	24,202	10 min	20 min	4 hr	NA	659 min	141 min	<20 sec	NA
MinION	2D (6 primer set)	348,973	90 min	20 min	48 hr	NA	4373 min	NA	<30 sec	NA

Table 1. Time scale of each protocol. *Number of fast5 files for MinION. Note that this does not necessarily mean reads count.

Centrifuge analysis

- **The BLAST-based search portion of classification analysis is time-consuming, even when using computer clusters.**
- **To reduce the computational time required for species detection, we tested a newly developed species classification suite, *Centrifuge*.**
- ***Centrifuge* is a novel microbial classification software that enables rapid and accurate identification of species, and can even be run on laptop computers.**
- **Rapid analysis using *Centrifuge* on a laptop computer enables us to determine major bacterial lineages within 2 hours, even in a small laboratory environment.**

Rapid 1D sequencing and analysis for pleural effusion derived DNA

- **Finally, to examine whether this rapid sequencing protocol is applicable to clinical samples, we sequenced the total DNA samples extracted from the pleural effusion of a patient with empyema, in which microbiological examination identified the *Streptococcus anginosus* group and unculturable Gram-negative rods.**

- **We compared sequencing results between IonPGM sequencing and MinION rapid 1D sequencing.**

- **All sequencing methods showed Prevotella as the major taxon in the pleural effusion from this patient.**

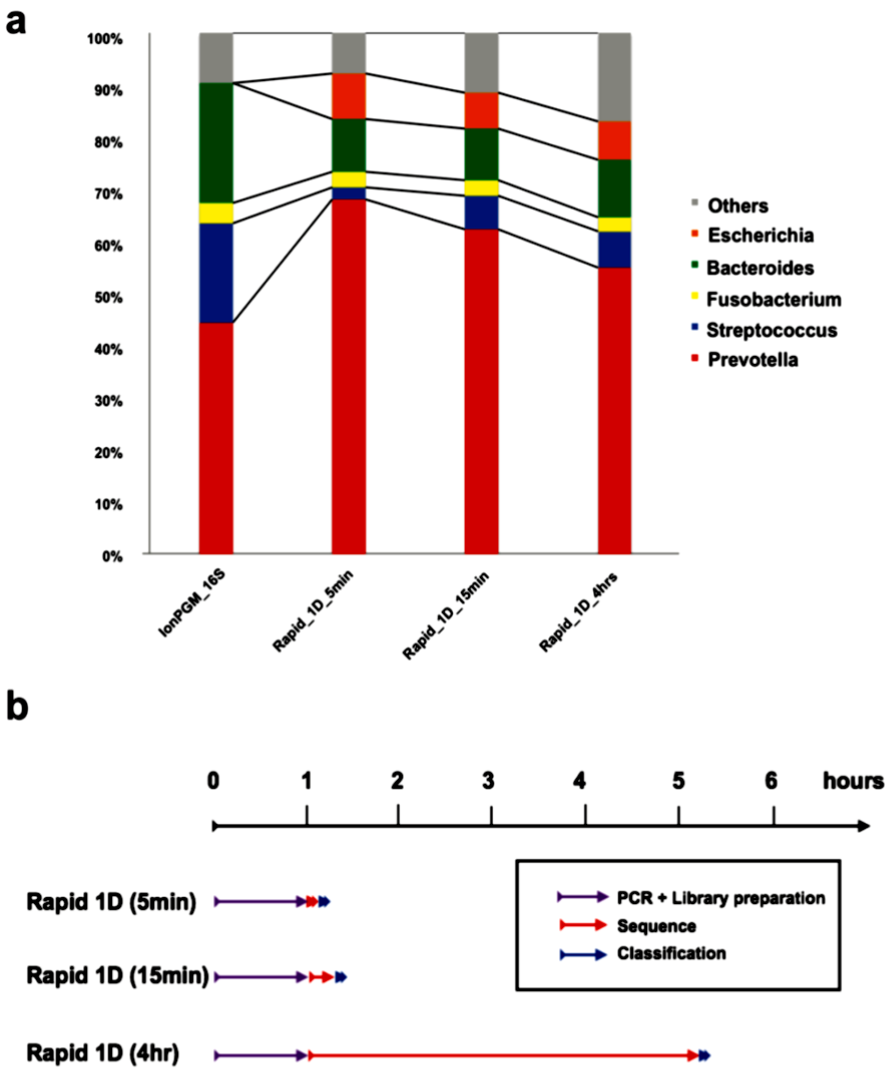


Figure 4. Clinical application of our system for determining bacterial composition in a pleural effusion from a patient with empyema. (**a**) Pleural effusion sample sequenced using three different methods indicating that *Prevotella* was the predominant bacteria. (**b**) Timescale for the experiment. Flow cell preparations took ~20 min for quality check and priming, which can be done during the PCR.

Sequencer	Method	Reads	Time						
				Sequencer		Data analysis			
			Amplicon preparation	Sequencer preparation	Sequencing	Ion Reporter	MinKNOW Base call and poretools*	Centrifuge	BLAST
IonPGM	Ion Metagenomic kit	429,974	~6 hr	~6 hr	3 hr	5–6 hr	NA	<1 min	NA
MinION	Rapid 1D (5 min)	1,012	10 min	20 min	5 min	NA	<20 sec	<20 sec	NA
MinION	Rapid 1D (15 min)	3,523	10 min	20 min	15 min	NA	<20 sec	<20 sec	NA
MinION	Rapid 1D (4hr)	54,544	10 min	20 min	4 hr	NA	<1 min	<20 sec	NA

Table 2. Time scale for the pleural effusion sample sequencing. *Base-calling was performed simultaneously while sequencing using MinKNOW software.

Discussion

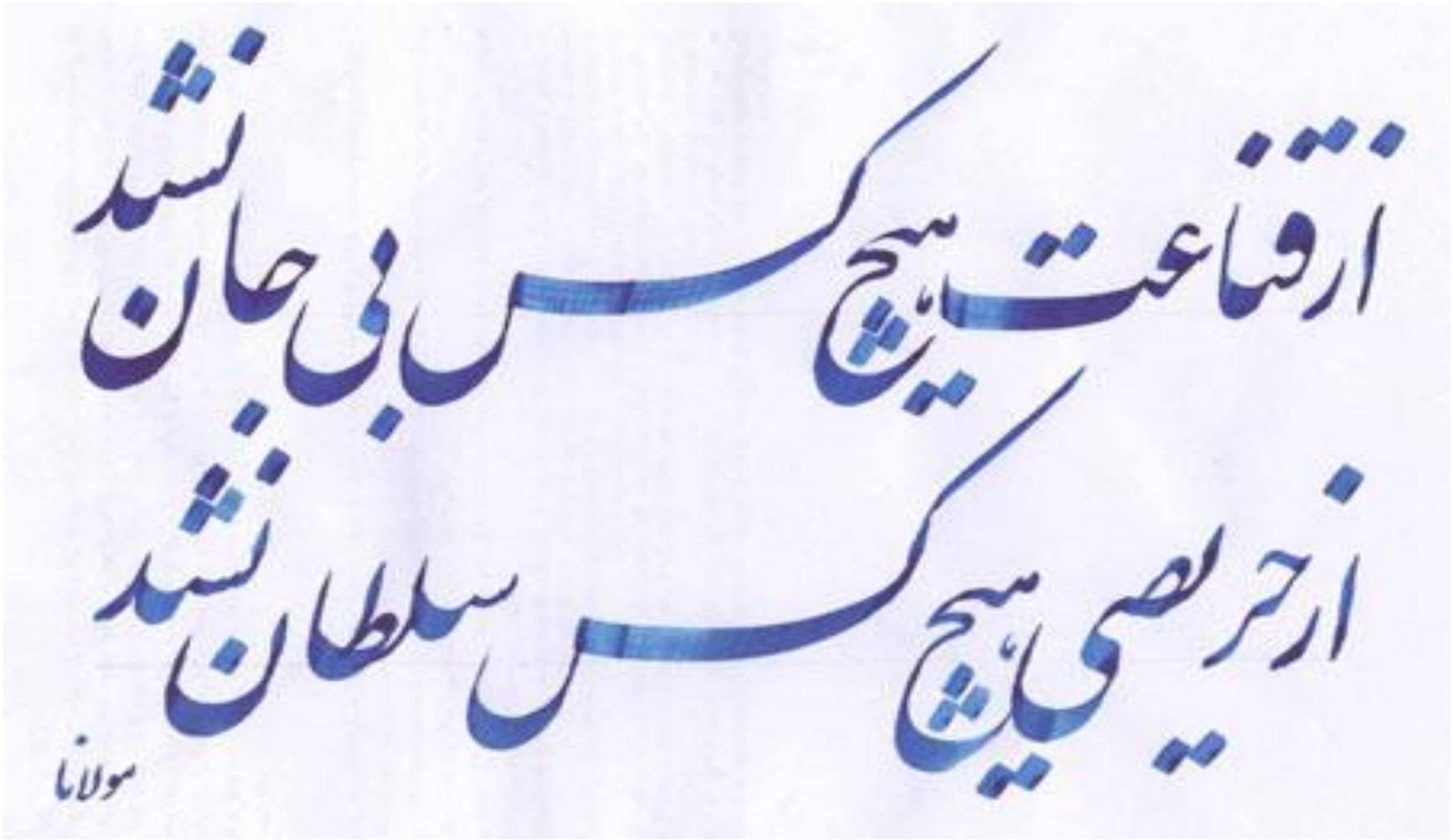
- **Performing rapid, on-site sequencing of clinical samples containing pathogenic bacteria for determining a first choice antibiotic regime would prove incredibly informative.**

- **Although our study is still in trial stages, it provides preliminary evidence that the species composition of a mock bacterial community can be successfully detected within 2 hours using MinION sequencing and data analysis on laptop computers.**

- ***Centrifuge* is very rapid, and could detect all the bacteria in our samples at the genus level; however, as our study revealed, it may misclassify some bacteria at the species level.**

Conclusions

- **Our results suggest that the 2-hour rapid determination of bacterial composition using a MinION sequencer and laptop computer is feasible, and that the system and the protocol presented in this study may be applicable to clinical use as a diagnostic support tool in hospitals or small laboratories in the near future.**



Thank you