

Near real-time analysis of long reads for detection of organisms using MinION sequencer

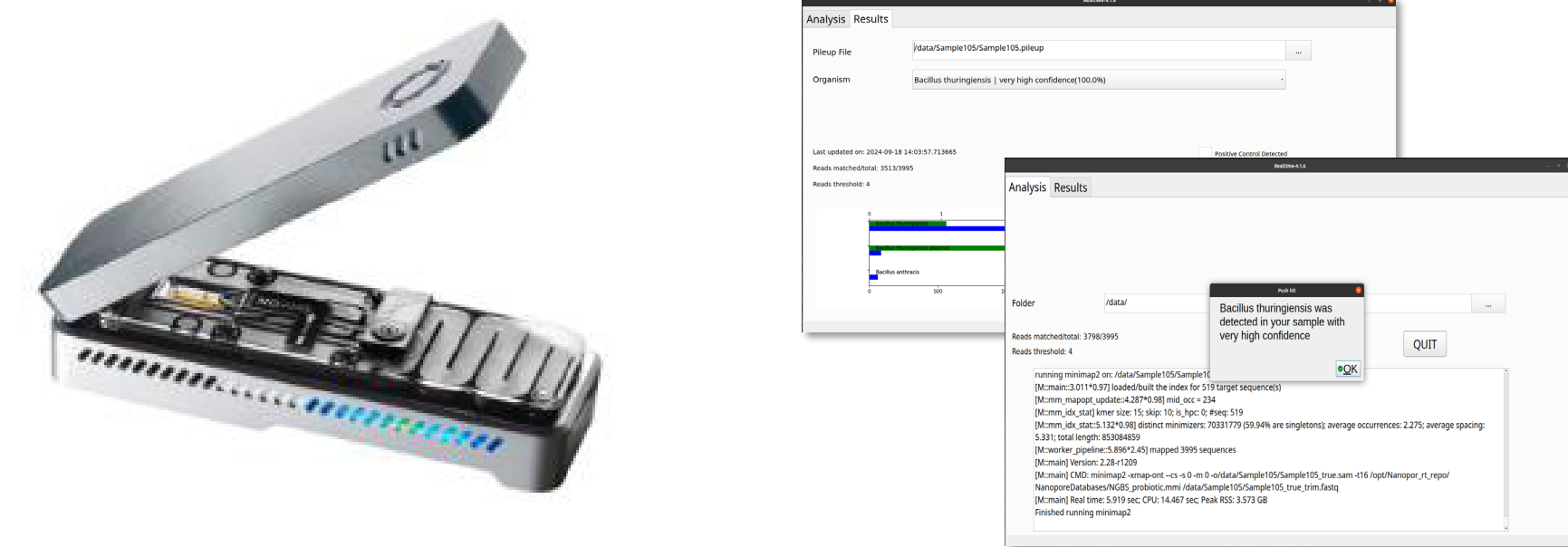
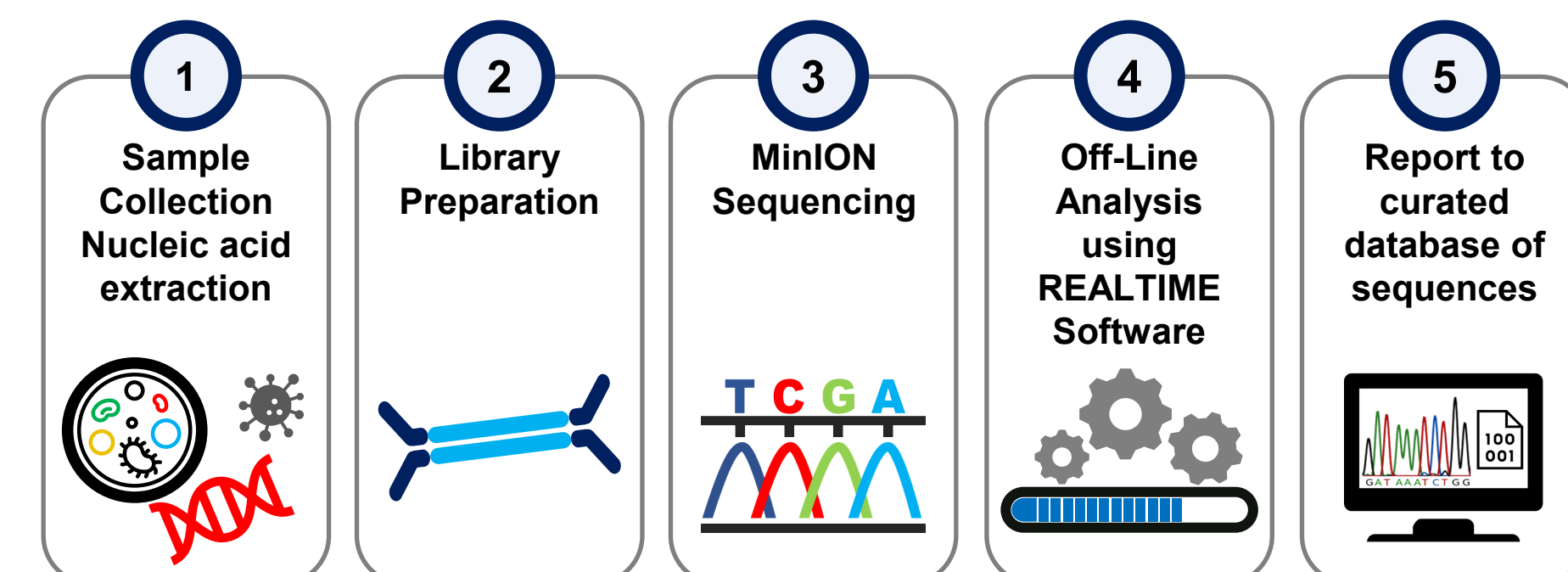
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INTRODUCTION

Oxford Nanopore Technologies (ONT) developed a nanopore-based genomic sequencer¹ that has allowed for true mobility of field sequencing with a very small footprint. While the sequencing system has been miniaturized from the laboratory bench to fit inside a backpack, the ability to perform analysis offline as the reads are being acquired by the MinION has been a significant bottleneck.

In this effort, we have developed an easy-to-use analysis software called “**REALTIME**” that maps the reads as soon as they are base called and generates a graphical report that keeps track of taxonomic classification of reads to a curated database of sequences of interest.



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BACKGROUND

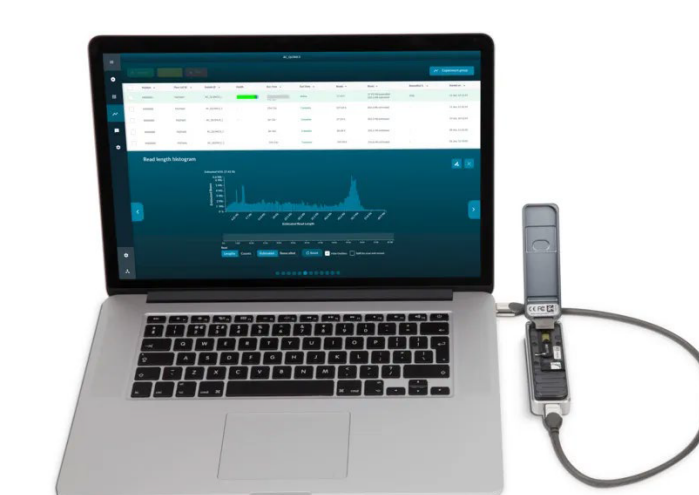
The limitations of next generation sequencing in field can include space constraints, expensive perishable reagents, the logistics of moving & setting up sequencers, trained personnel, and access to internet.



The ONT sequencers provide immediate access to sequencing (long reads) data. New computing hardware enables local base calling without the need for laboratory infrastructure and has been proven in the field.

By implementing the REALTIME software, we have the ability to perform analysis offline as the reads are being acquired by the MinION device, allowing for field analysis without need of internet access.

Using this software, we can identify an organism to the species/strain level within 1–30 minutes of sequencing, thereby eliminating logistical hurdles involved in accessing the internet, as well as data and sample transfer.



The REALTIME software allows for field analysis without need of internet access.

METHODS

Sample Preparation:

Step	Time
Cells/spores are lysed using the OmniLyse	2 mins
DNA purification: Akonni TruTip Kit	~ 3.5 mins
Library prep: SQK-RAD004 (ONT)	~ 10 mins



- Sample preparation is quick and simple for high biomass, low complexity samples
- Complex sample types use DNA amplification

MinION Mk1B

Weight: 87 g
Size: 105 mm (l) x 23 mm (w) x 33 mm (h)



REALTIME

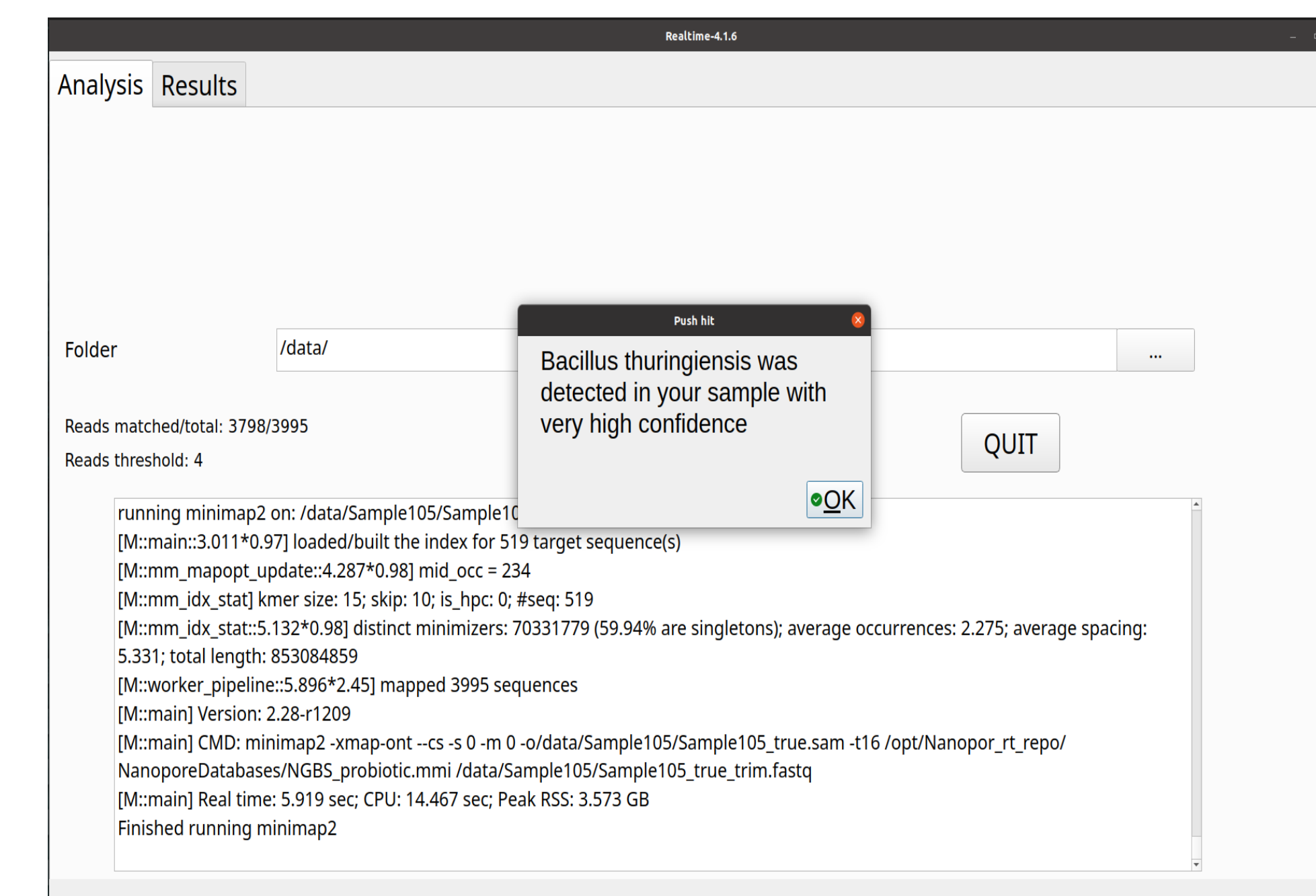
- RefSeq and nt database
- minimap2² index
- Python 3.10, pandas³
- QT5.0 – GUI
- Flow cell (R9), MinKNOW 24.06.14 & Dorado basecaller 7.4.1

The mapped reads assigned to an organism are provided with a computed confidence metric that uses various parameters.

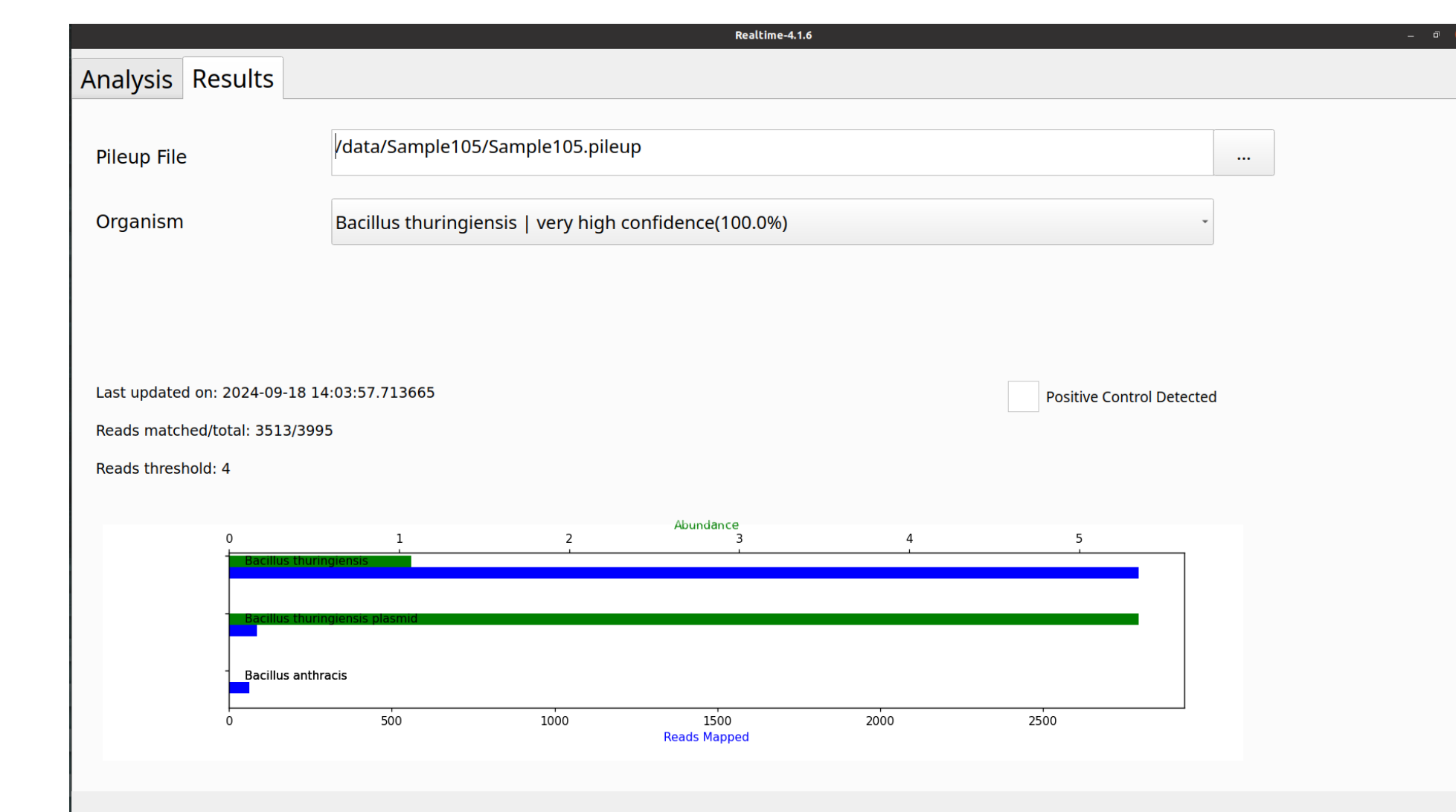
REALTIME software is implemented on an Ubuntu operating system and has a graphical user interface (GUI) for ease of operation by end users in the field.

RESULTS

Sequencing results are displayed in a pop-up message box for the end user. A sample analysis report is also generated at the end of the sequencing analysis.



Screenshot of REALTIME displaying the analysis of the sample along with the name of organism identified.



Screenshot of REALTIME results, showing the number of reads mapped to an organism and its abundance.

CONCLUSION

Automated sample classification is achieved using the REALTIME software while the sequencer is running. Offline sample classification can be achieved using only the MinION sequencer and a laptop. This allows for field analysis of samples *without* need of internet access. REALTIME was also installed on a modified MinION Mk1C⁴ (with an integrated battery) for a standalone device and has been used in numerous field-testing events.



FUTURE DIRECTIONS

Future work will see the REALTIME software tested with V14/R10 flow cells and analyzed for its effectiveness using multiplexed samples.

REFERENCES

1. <https://nanoporetech.com/products/minion>
2. Li, Heng. "Minimap2: pairwise alignment for nucleotide sequences." *Bioinformatics* 34.18 (2018): 3094-3100.
3. McKinney, Wes. "pandas: a foundational Python library for data analysis and statistics." *Python for high performance and scientific computing* 14.9 (2011): 1-9.
4. <https://nanoporetech.com/products/sequence/minion#tabs-0=mk1c>

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