

Kromek Biosequencer

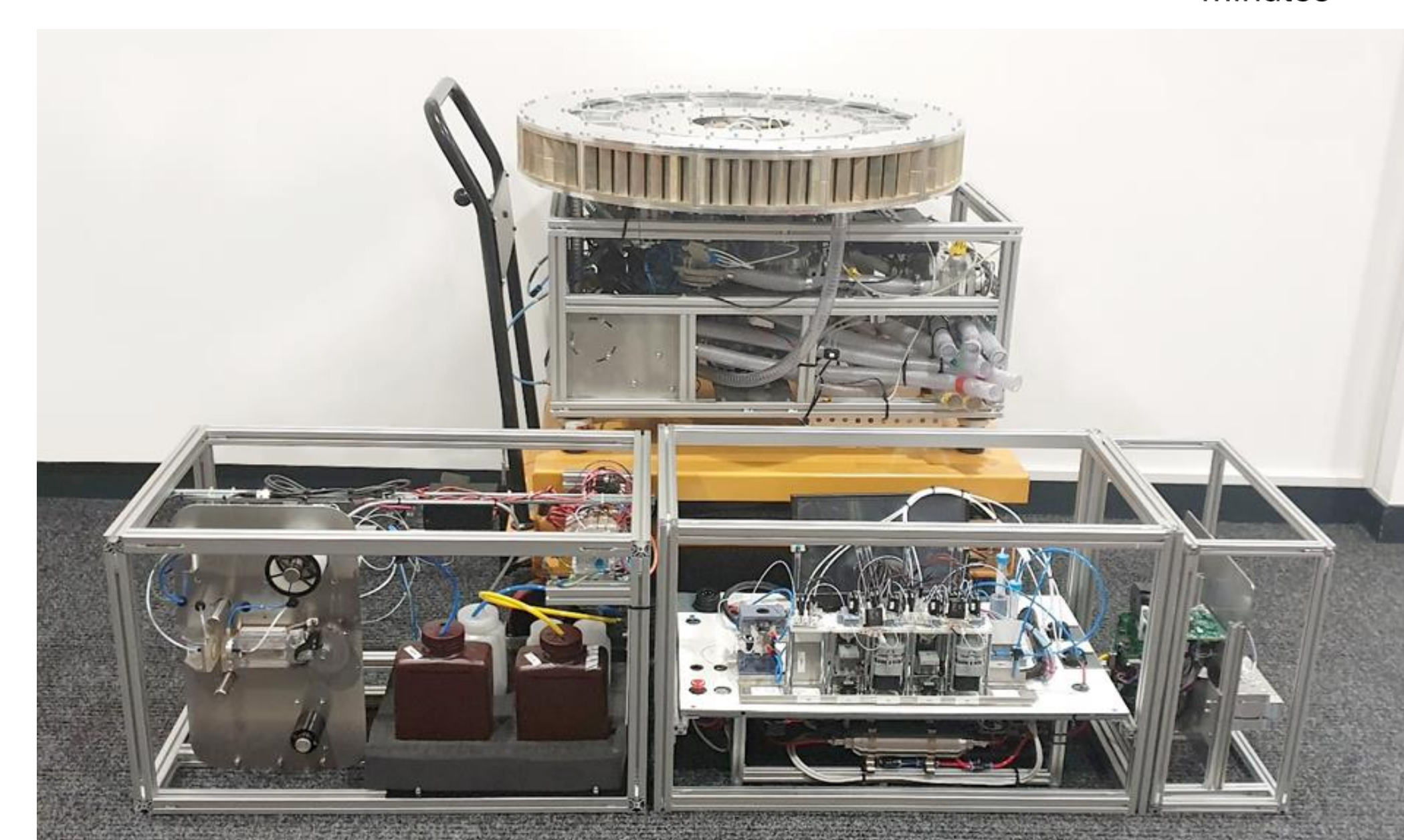
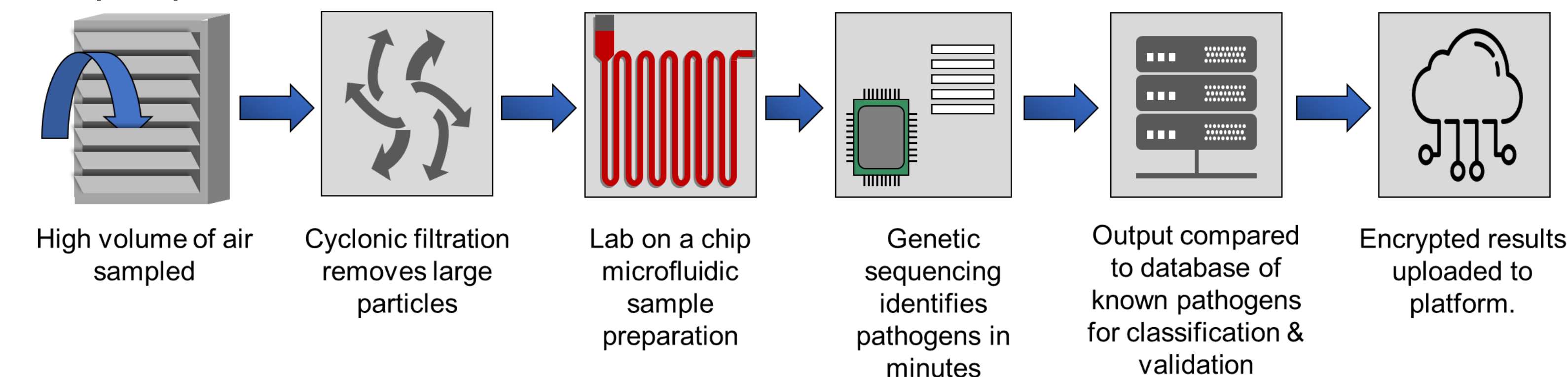
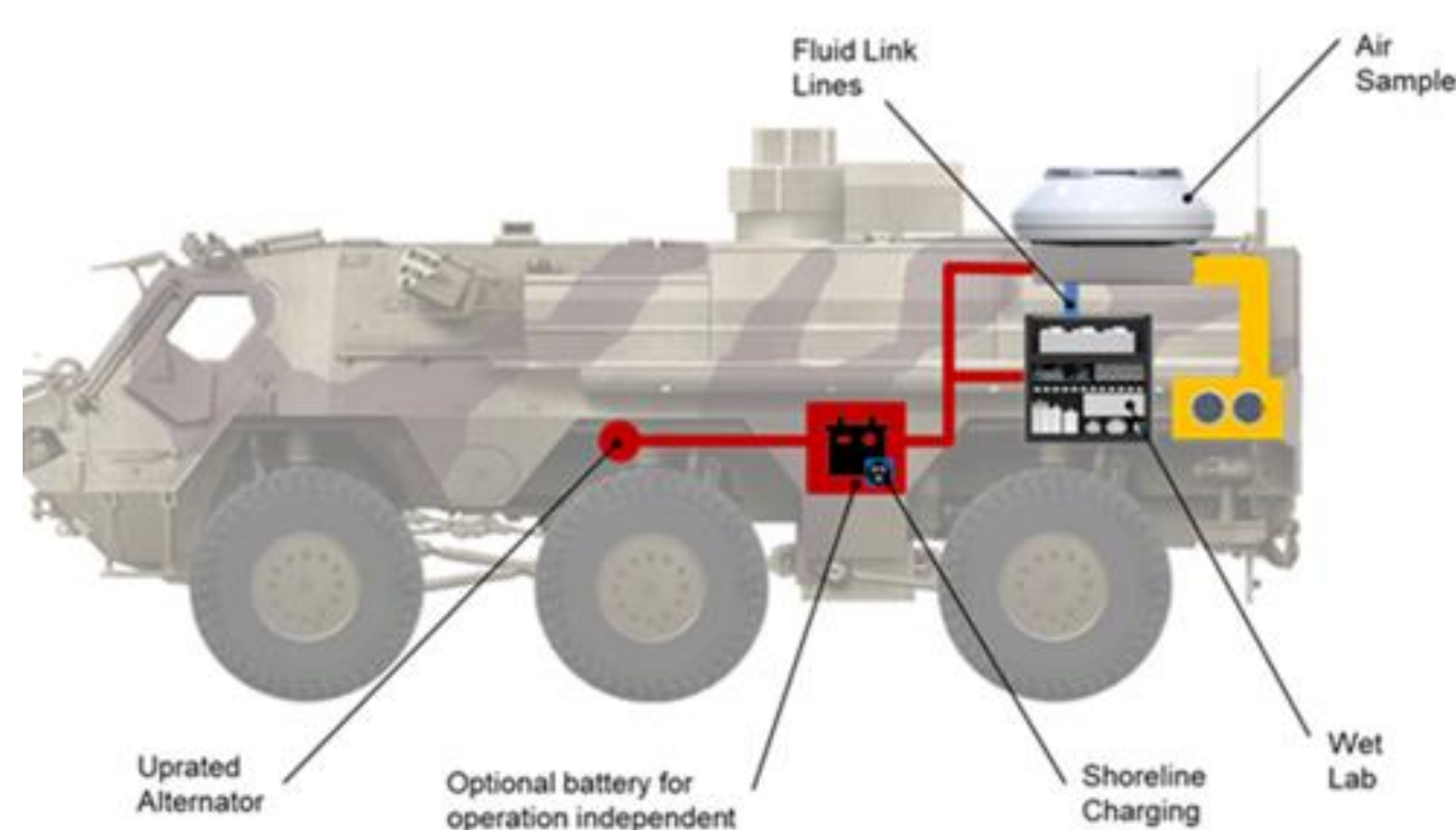
Agnostic airborne pathogen detection and identification via fully automated Long Read Sequencing

The need

We are developing a novel fully automated and autonomous biosensing system (Kromek Biosequencer) that detects the presence of airborne pathogens (viral and bacterial) based on long read sequencing. Minimising the impact of pathogen-initiated outbreaks, whether naturally occurring or intentional biological attacks, depends on our ability to detect and identify the causative agent, in order to diagnose, properly triage and treat those infected, and gauge the extent and dynamics of an outbreak. DNA sequencing provides a sample's constitution and its pathogenic burden. Advances in genome engineering (e.g., CRISPR) and synthetic biology mean that variations of pathogens can be created as bioweapons which evade detection by standard targeted techniques like PCR. Our integrated environmental monitoring system allows early detection of any biological threat across large areas and can inform appropriate and timely response to a potential threat. The device autonomously and continuously collects air samples which are immediately processed and analysed.

The concept

Our 2-hour end-to-end sample processing pipeline consists of air sampling, wet lab, and data analysis. The Kromek Biosequencer collects air via a wide area air sampler, concentrates the air and collects viral and bacterial pathogens in a liquid phase.



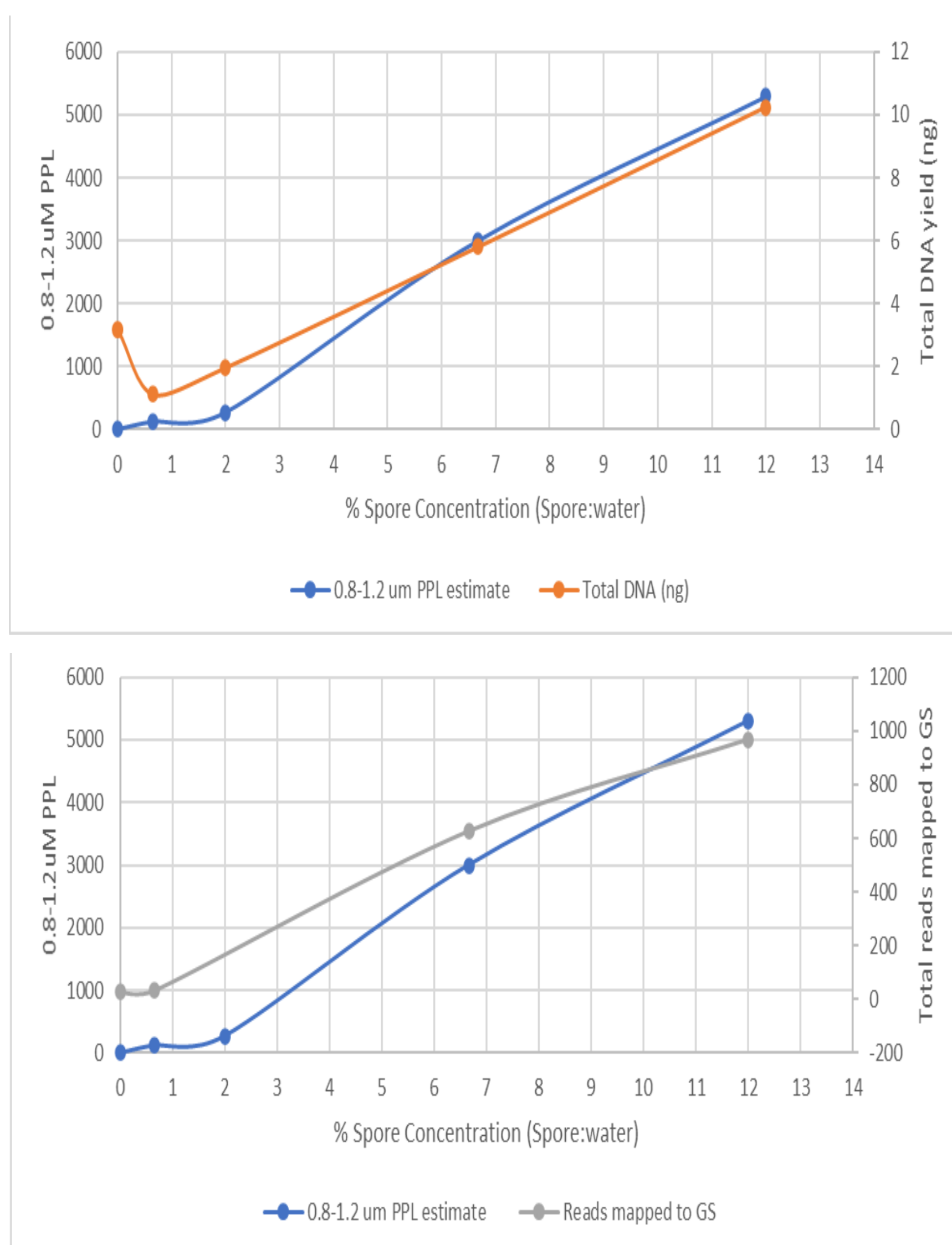
The pathogens are separated by filtration and processed in two parallel pipelines through a modular system of several alternating steps. Proprietary software maps the generated data against all organisms and delivers fast results on a species level in under 20 min.

The results do not require analysis by trained individuals or specialist laboratories.

The Air Collector



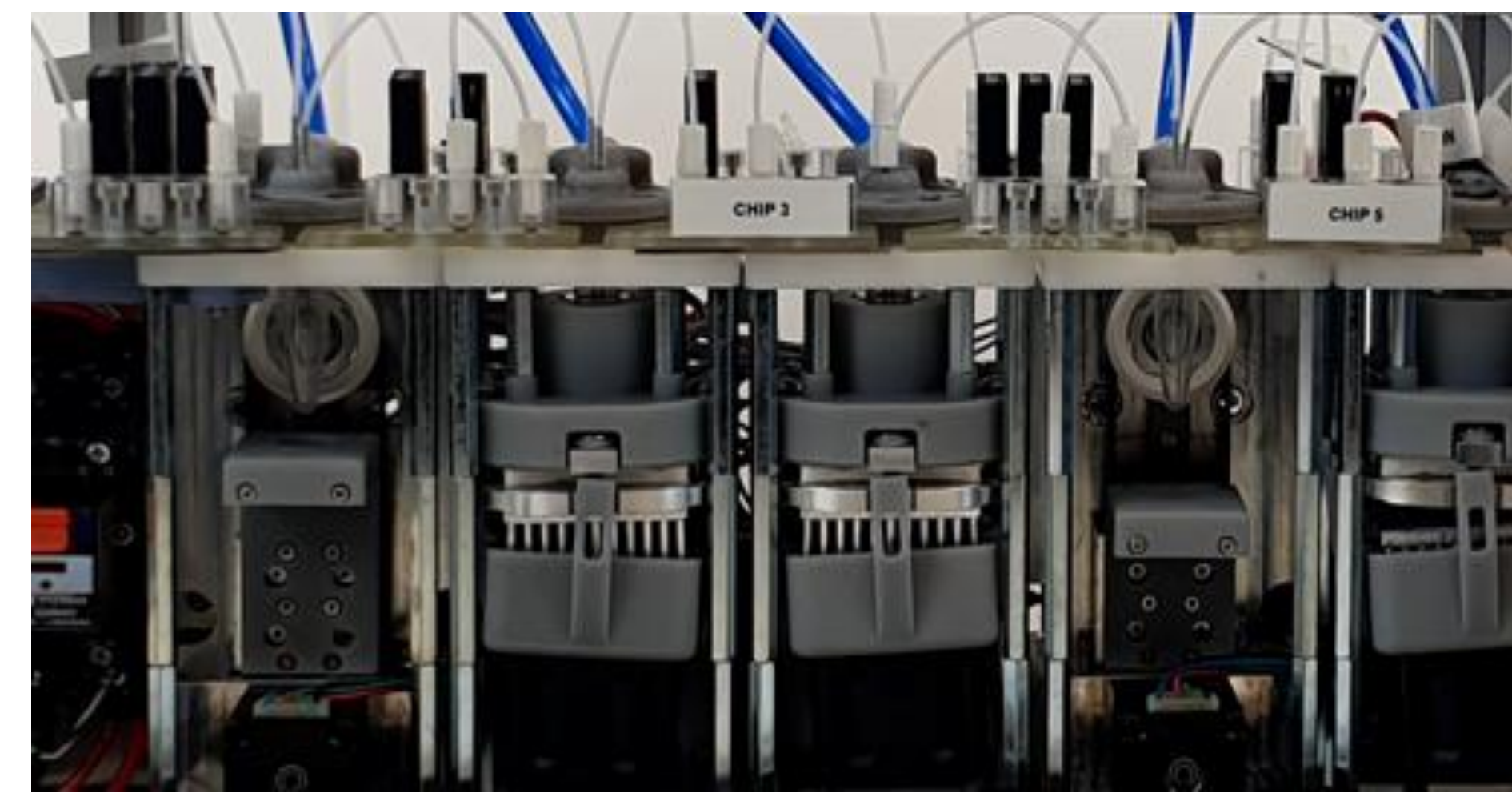
The air collector module is a high airflow air sampler that collects 4800 ml / min of particles in the respirable size range (1 – 10 µm). Testing of the air collector in a chamber revealed a strong correlation between the concentration of spores (*Geobacillus stearothermophilus*) in nebulized water and the amount of DNA extracted (top) and the number of reads assigned to the nebulized species (bottom).



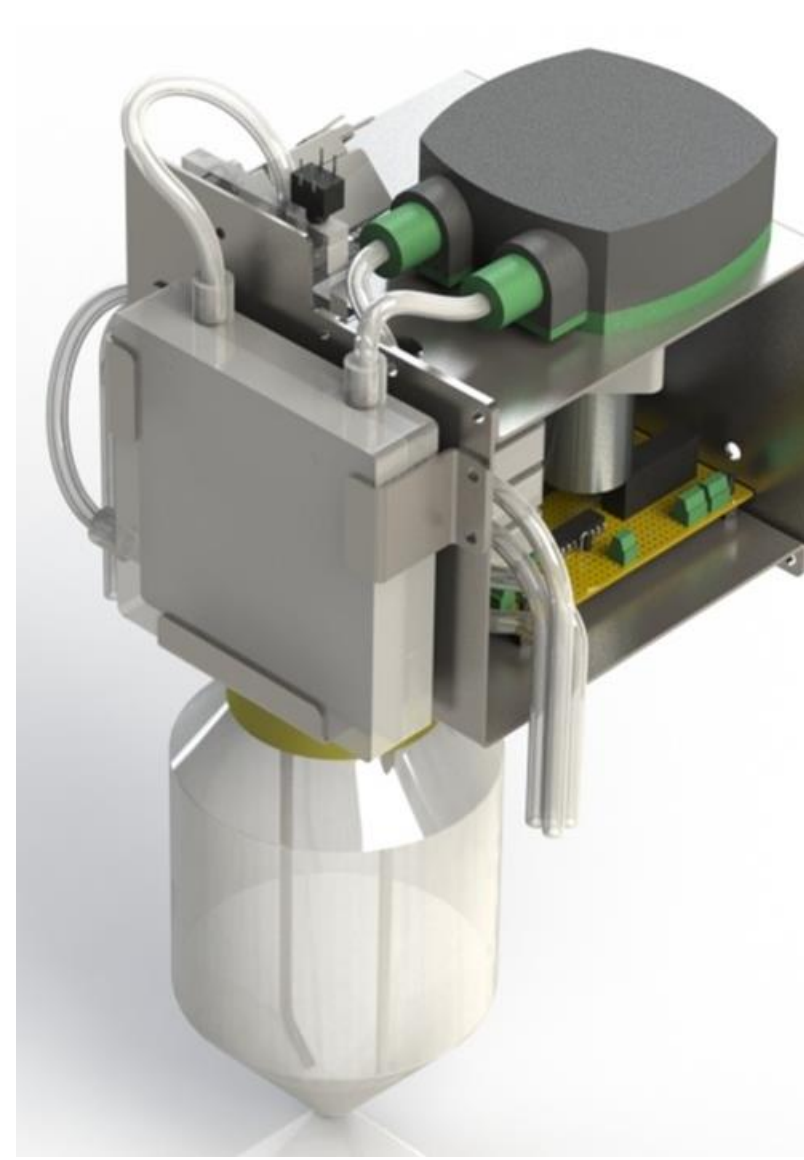
The Automated Wet Lab

The automated wet lab consists of:

- The concentration module, that concentrates the biological matter and separates bacteria and viruses.
- The lysis module that lyses the bacterial matter.
- The nucleic acid extraction and preparation unit, that extracts, amplifies and prepares the DNA library for sequencing. It is able to process both DNA and RNA.
- The sequencing module, consisting of sequencer loader and sequencer which loads the DNA library and performs the long-read sequencing.

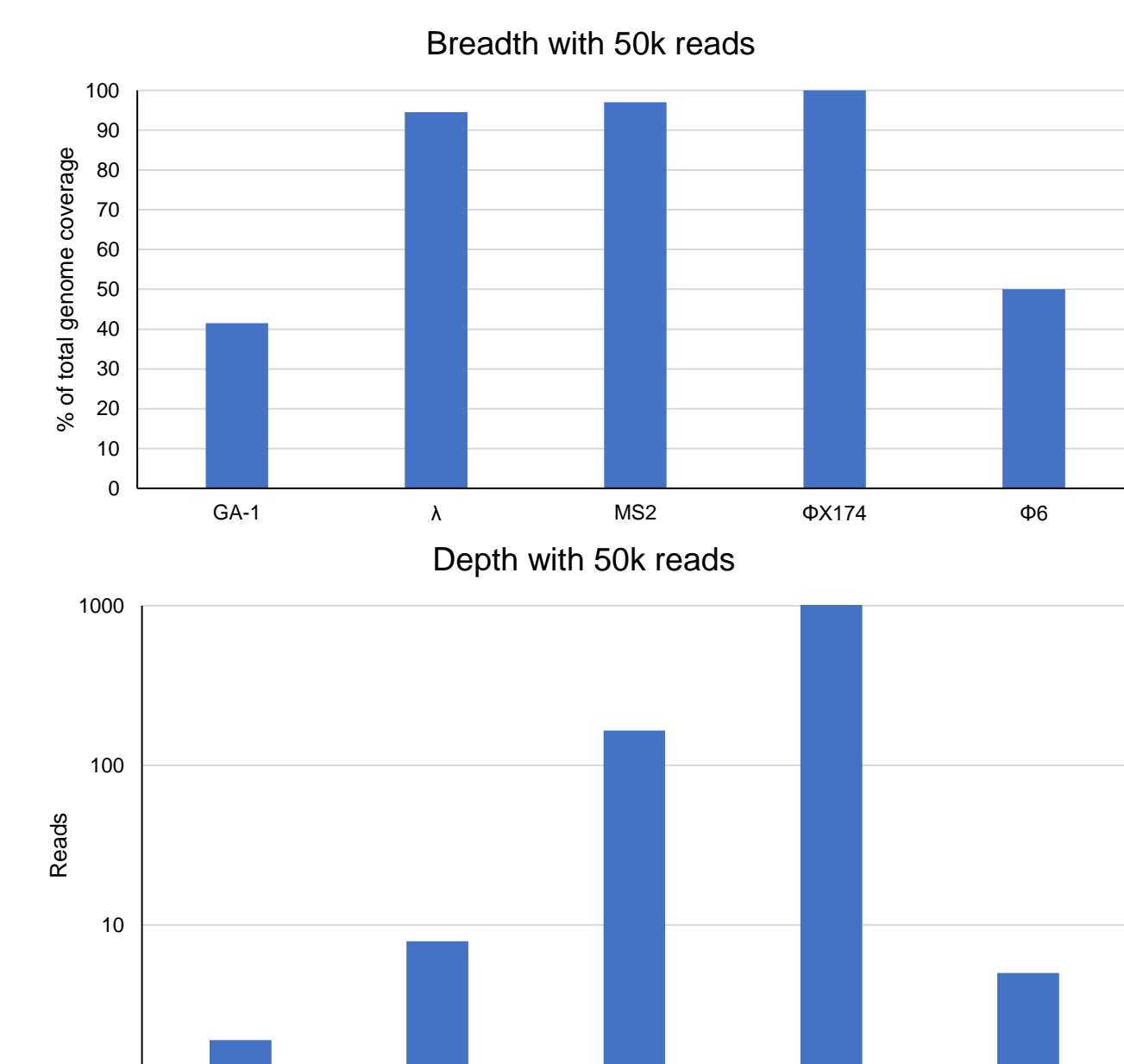
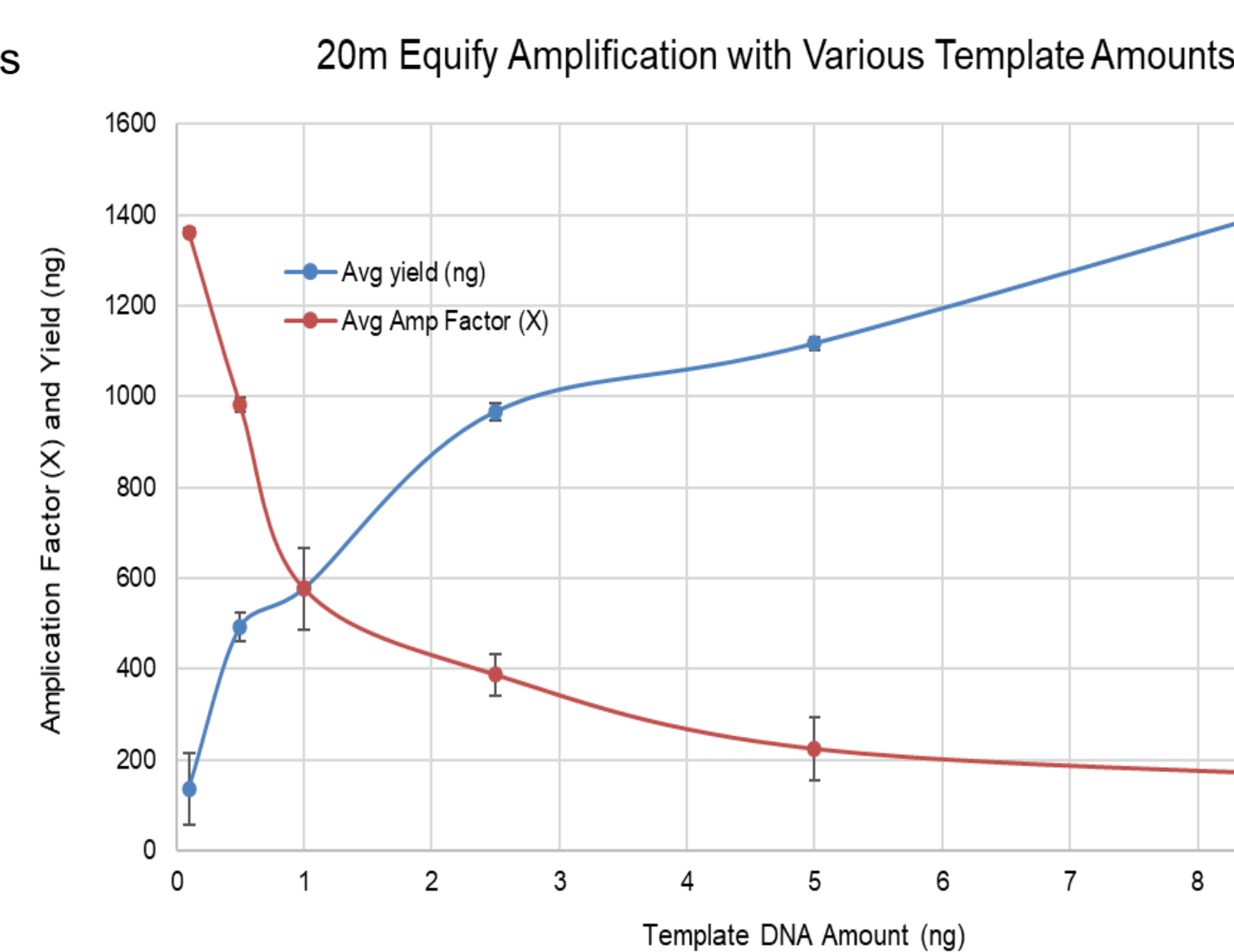


The Concentration Module



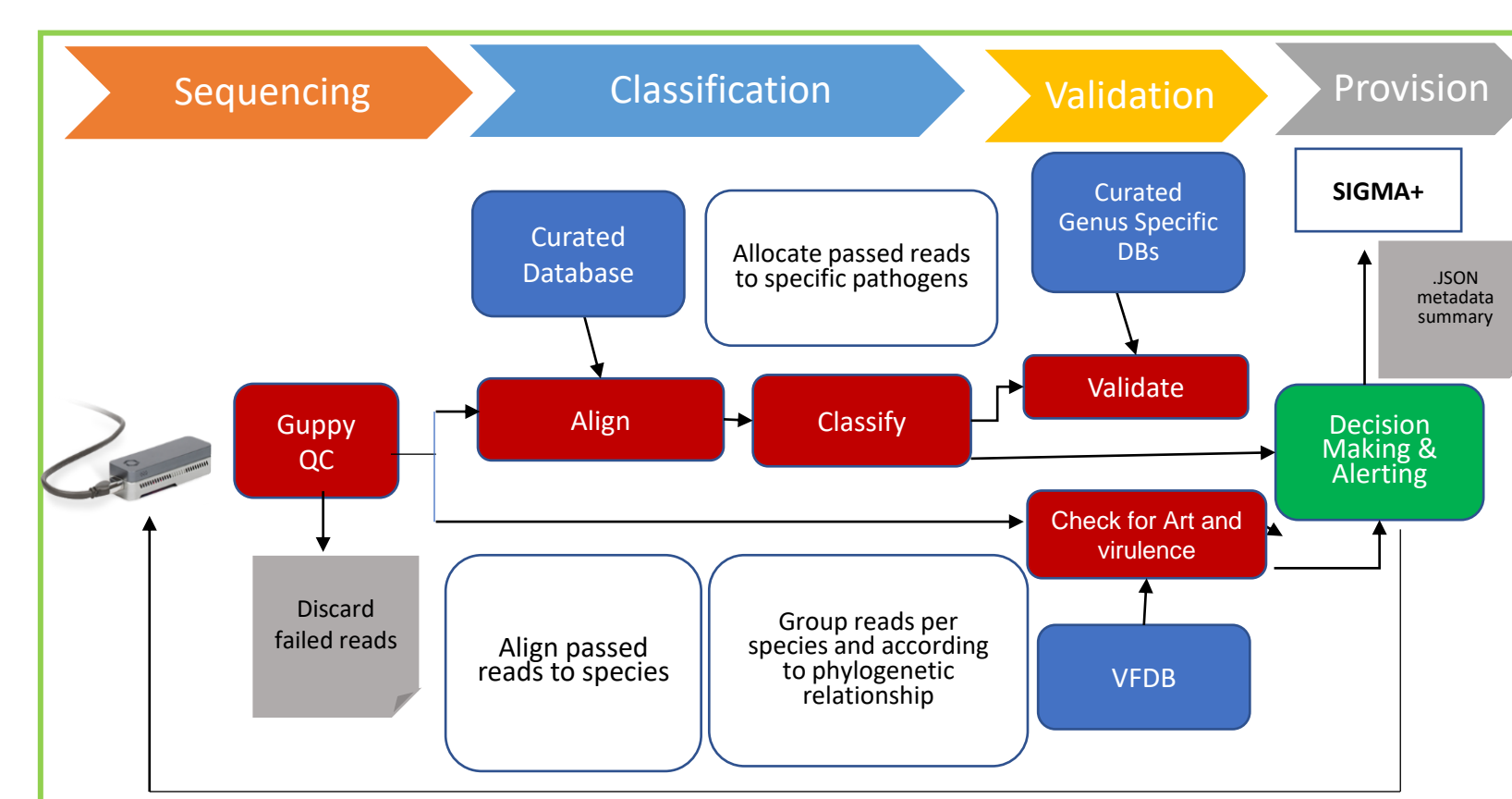
Highly efficient and reusable automated filtration modules are used for bacterial and viral capture. Bacterial concentration is based on size exclusion of bacteria through a filter (left) and consequent elution from the filter. The filtrate is directed to viral capture unit (right) and concentrated.

The nucleic acid preparation module



We have developed a DNA processing pipeline which consists of a rapid amplification protocol that can amplify DNA in 20 minutes (A.). We also demonstrate the development of a rapid viral identification protocol (B & C). It is able to identify a sample of 5 DNA and RNA viruses on a laboratory run of the protocol.

Bioinformatics and data analysis



Metric	Current
Pathogens	29 bacteria + 11 viruses
Sensitivity	0.01 PPL
False Positive Rate	0 in 800k reads
Sequencing time	15-20
Analytical time	8 - 12 minutes
Probability of ID	99.96%

We have developed a bioinformatics analysis pipeline that is able to process 50,000 reads from a complex environmental sample in under 15 minutes post sequencing and can process multiple samples in parallel. It has a low predicted false positive rate (FPR) and a high probability of ID.

Future work

Our ongoing systems integration for non-scientific end-users and cost-down work will result in a commercial system which can achieve widespread adoption within mobile and static CBRN applications to make ubiquitous DNA sequencing a reality and finally transform DNA sequencers into sensors

Acknowledgements

The authors wish acknowledge the support of DARPA, under Contract No: HR0011-19-C-0031

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