# Optimizing sequencing costs in metagenomics: How to reduce costs of pathogen detection in with PaRTI-Seq™

Pathogen detection techniques based on culture PCR, or serology are still regarded as the gold standards for pathogen detection, even though these techniques present significant limitations due to their turnover time, low positivity rates and low sensitivity. Next-generation sequencing (NGS) techniques are capable of addressing many of these shortcomings with the high-throughput approach of nucleic acid sequencing, in which millions to billions of short fragments of DNA (reads) or RNA (transcripts) are massively sequenced in parallel (Behjati and Tarpey, 2013). With the help of bioinformatics, these fragments can then be assembled and mapped against a reference database for fast and accurate pathogen identification. NGS-based approaches have the potential to significantly improve throughput capabilities of clinical laboratories, while also enhancing detection sensitivity and resolution.

While the cost of sequencing and turn-around times (TAT) associated with NGS have been consistently decreasing (Wetterstrand, 2021), host DNA interference still presents a major limitation that withholds NGS from mass adoption in clinical settings today. A major driver for the reduction of cost and TAT of the sequencing run is an effective host depletion method. The products offered by Micronbrane, Devin™ filter and PaRTI-Seq™, offer healthcare providers a new complete solution that makes NGS pathogen detection for clinical diagnostics of infectious diseases faster, and at the same time, more efficient and affordable. This white paper gives a detailed explanation on how Micronbrane's products (PaRTI-Seq™) can save up to 75% of sequencing costs and reduce more than a half of overall costs for such tests in clinical settings while providing accurate results within 24 hours.

## *Identifying key costs across the NGS value-chain*

NGS workflows typically involve three steps: sample preparation, DNA/RNA extraction and library preparation, and finally, sequencing and data analysis (Fig. 1). Despite the increasing affordability of sequencing per megabase of DNA (Wetterstrand, 2021), sequencing costs still represent a major portion (up to 80%) of the total costs associated with the employment of NGS-based techniques in clinical settings (Fig. 1).

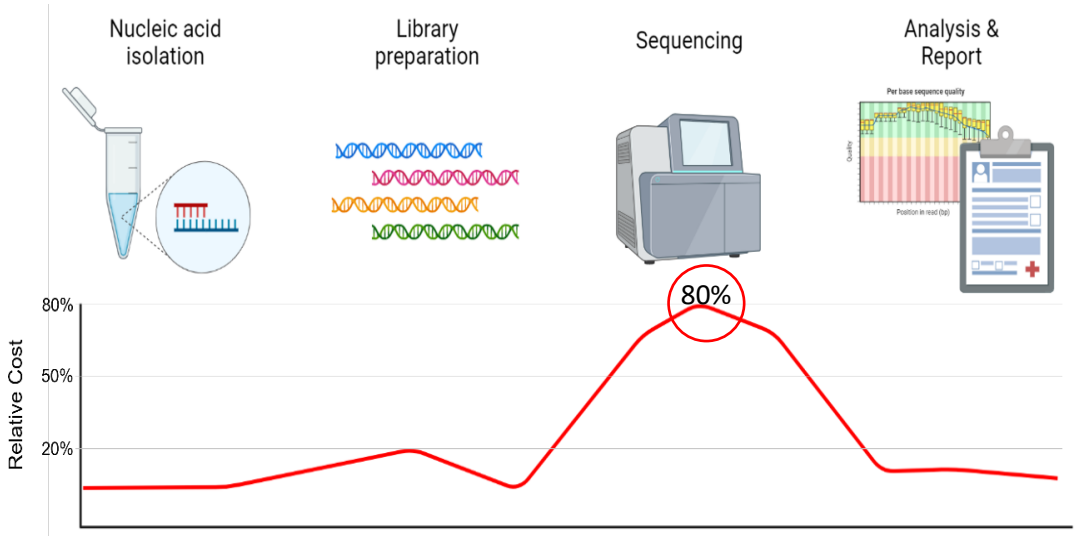


**Figure 1**. Typical NGS workflow and an estimation of the relative cost of each step.

Total reagent cost per sample, including cost of sequencing chemistries, represents a variable expense that is highly dependent on the required sequencing depth and analytical output, i.e., number of samples processed. A major driver for the reduction of cost and TAT of the sequencing run is the need for an effective host depletion method. A recent review of clinical studies using NGS applications for pathogen detection has shown that a large majority of the sequencing outputs corresponded to human DNA, which frequently accounted for a median of 79% (range, 7%-98%) of the DNA sequenced (Govender et al., 2020). To circumvent these impacts, the studies in the analysis typically required longer (up to 10 hours) and therefore more expensive sequencing runs per sample analysed (up to $685 on average), to achieve a satisfactory sequencing output (up to 20M reads/sample) (Govender et al., 2020). In other cases, the influence of host DNA was so overwhelming that sequencing repetitions were required, increasing the associated costs 2 or 3-fold (Govender et al., 2020).

Through the streamlining of the required sequencing depth, NGS becomes more affordable. A higher number of samples can be processed using the same volume of reagents. This can further maximize cost optimization, as it will allow multiplexing of each sequencing run, which can reduce sequencing costs up to 5-fold (Govender et al., 2020). This will also enable faster sequencing (TAT) for samples and allow the use of smaller NGS machines. Achieving rapid TAT (e.g., < 24 hours) is urgently needed in certain clinical settings, especially in hospitalized patients in a critical care environment like the ED or ICU.

## *Optimizing sequencing costs with PaRTI-Seq™*

Effective host depletion techniques are pivotal for robust pathogen detection, especially given that even a small fraction of human genetic interference in a clinical sample may lead to high interferences due to the large size of human genomes relative to the genomes of prokaryotic pathogens. However, the current for human DNA depletion techniques for NGS applications in clinical settings have limitations, which ultimately translate into additional costs and are time-consuming, ineffective and cumbersome depletion. Depending on the techniques, they tend to require large amounts of template DNA that is often unavailable (e.g., methyl-CpG-based eukaryotic DNA depletion), are time-consuming (e.g., chemical and enzymatic depleting approaches) and lead to inconsistent recoveries of microbial DNA for downstream analyses. Poor and slow depletion efficiencies can ultimately affect the sensitivity of pathogen detection, particularly if the pathogen DNA/RNA are present in low amounts. This reduces their practicality for clinical applications due to their large and cumbersome operational demands.

Devin™ fractionation filter presents a novel technology of fast and effective host DNA depletion. At Devin's core is the patented antifouling Zwitterionic coating (ZISC) that specifically captures leukocytes (>99%), while allowing other blood components to flow through the membrane. Devin™ fractionation filter can deplete host DNA within 5 minutes and provides a fast and cost-effective solution to reduce the presence of human DNA while increasing the proportion of microbial pathogen DNA in human blood samples (10 – 1,000-fold). This reduces the required sequencing depth for pathogen identification. The PaRTI-Seq™ assay combined with the Devin™ filter optimizes sample preparation, utilizing dedicated proprietary analytical software PaRTI-Cular™ allowing savings of 75% in sequencing costs (Fig. 2).

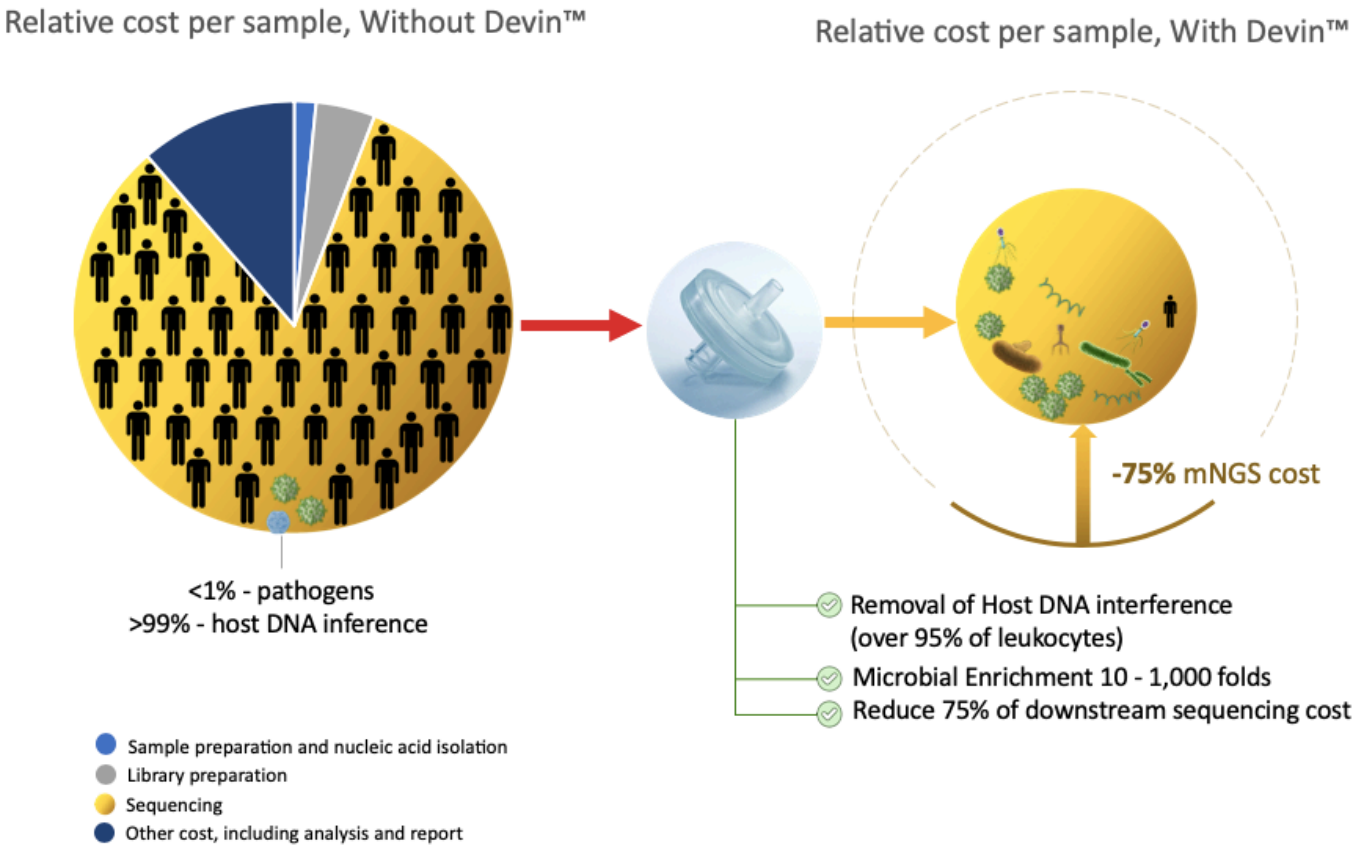


**Figure 2.** How PaRTI-Seq™, with the Devin™ technology, can reduce sequencing costs. Normally each whole blood sample requires 20 million sequencing reads. By removing over 95% of leucocytes, microbial DNA enrichment can be improved 10 to 1,000-fold with less sequencing reads (5 millions of sequencing reads) required to reach the same sensitivity and test results. By reducing 75% of sequencing cost, relative cost per sample can be reduced by more than half in comparison with samples not processed with Devin™ filter.

## *How exactly sequencing cost is reduced?*

Devin™ filter can effectively remove leukocytes from whole blood, plasma and other body fluids within 5 minutes, while ensuring a high microbial passing efficiency due to its large pore size (15-20 μm). This ensures an effective mitigation of host DNA contamination and allows the enrichment of high-molecular-weight microbial DNA, thus enabling improved NGS analysis focused on accurate pathogen detection. By effectively reducing host contamination, the sequencing depth required to achieve a representative microbiome sample decreases up to 75% (as low as 5 million sequencing reads per sample instead of the standard requirement of 20 million sequencing reads per sample for whole blood). This drastically reduces the sequencing costs, as it shortens the sequencing runs which reduces the volume of reagents required. Using one of the most

current and mature NGS platforms currently in the market, Illumina®, the incorporation of Devin™ filter in the sample preparation phase can lead to a reduction of up to 3-fold of the sequencing costs per sample (Fig. 3). The time efficiency enabled by Devin™ filter, especially when used within the PaRTI-Seq™ assay, also allows healthcare providers to maximize throughput, by improving turnover rate, with shorter sample preparation steps, as well as reduced sequencing repetitions and shortened sequencing runs. This is achieved due to the enrichment of high-quality microbial DNA with minimal host contamination. By using Devin™ filter and PaRTI-Seq™ test, healthcare providers not only optimize cost of testing, but also maximize utilization of sequencing machines and potential revenue by streamlining the efficiency and throughput of their pathogen detection pipelines (Fig. 3).

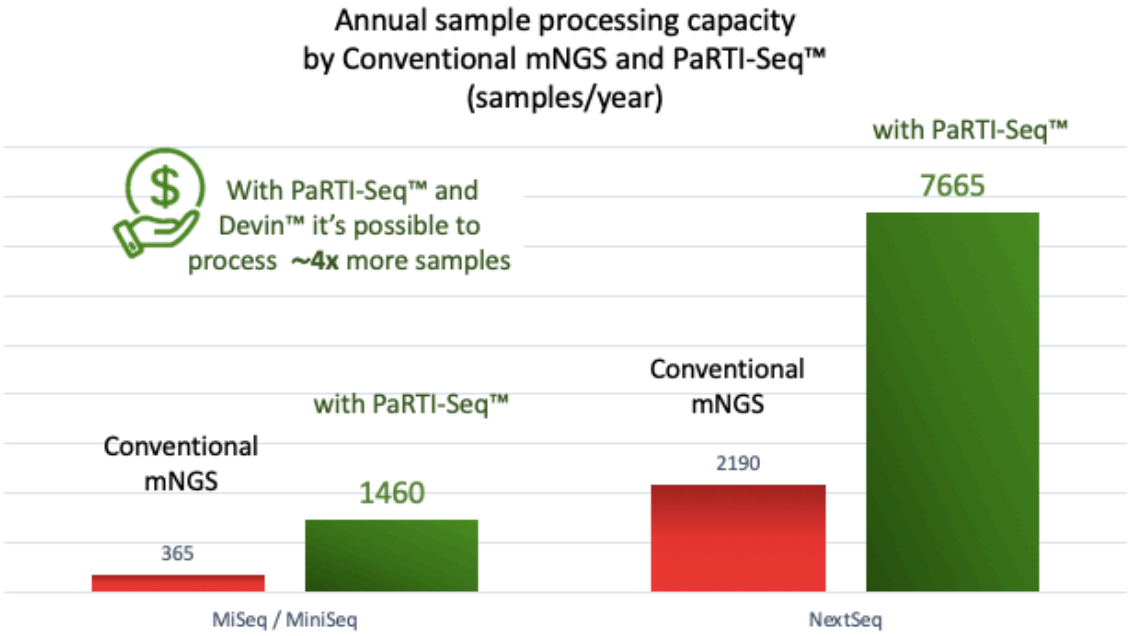


**Figure 3.** Sample processing capacity (annual number of samples) by single sequencing platform (Illumina MiSeq, MiniSeq and NextSeq) using PaRTI-Seq™ vs Conventional mNGS.

## *Impact for the healthcare*

One of the major drawbacks associated with contemporary methods for pathogen detection, such as blood culture, is associated with high rates of negative results and the frequent need for repeat cultures, which not only increases the invasiveness for a patient but can also translate into increased use of resources and a prolongation of the diagnosis timeline (Mushtaq et al., 2019). NGS-based approaches for pathogen detection may also be prone for repetition if there is a high influence of host DNA on the sequencing output, as emphasized earlier, but with an effective host depletion approach, such as the

Devin™ filter, sensitivity can be increased and therefore the accuracy of the diagnosis can be improved significantly.

Improved and precise pathogen diagnostics has clear advantages, such as avoiding the risk of unnecessary or inappropriate antimicrobial therapy, reducing the duration of hospitalization, and consumption of other hospital resources (Fig. 4) (Coburn et al., 2012; Gkika et al., 2018; Schmitt et al., 2019). PaRTI-Seq™ offers precise pathogen identification in less than 24 hours, and can allow the early diagnosis of life-threatening infections. This can offer significant benefits for patients, as well as economic advantages to healthcare systems and their providers (Lin et al., 2021; Zhou et al., 2021).


**Fig 4.** Early diagnostics' impact on hospital length of stay

*due to early diagnostics of infectious diseases including point-of-care testing

## *How it benefits health providers and patients*

The NGS solutions offered by Micronbrane Medical, namely Devin™ filter and PaRTI-Seq™, offer healthcare providers fast, effective and cost-efficient pathogen detection, both in terms of sensitivity, throughput, and cost. This is achieved by mitigating up to 75% the sequencing depth requirements thus reducing the volume of reagents, and allowing a faster sample turnover (as quick as 24 hours). This improved mNGS pipeline will translate into a direct reduction of fixed and variable costs of NGS-based pathogen detection, while also improving the quality of the clinical diagnosis which can lead to reduced hospital length of stay and costs.

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