

Advancing bacteriophage-based microbial diagnostics with synthetic biology

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Synthetic biology is an emerging engineering field focused on designing artificial biological systems with novel functionalities for use in therapeutics, basic science, biotechnology, and diagnostics [1,2]. Continuous advancements in DNA synthesis and sequencing technologies coupled with new techniques for genomic modification and assembly have opened the door for harnessing the power and diversity of biology for applications. For example, natural bacteriophage products, such as ListShield (Intralix) and Agriphage (Omnilytics), are commercially available for reducing unwanted bacterial contamination. Natural bacteriophages can be genetically modified to deliver engineered payloads into bacteria, thus selectively functionalizing target bacterial populations to produce active biomolecules. This strategy can endow bacteriophages with the ability to efficiently destroy bacterial biofilms or increase the bactericidal efficacy of antibiotics used in combination with phages by many orders of magnitude [3]. In addition, bacteriophages can be engineered as near-real-time microbial diagnostics by using them to transform target bacteria into factories for detectable molecules (Figure 1A) [4].

Near-real-time microbial diagnostics are needed for food, clinical, industrial, and other environmental settings. The current state of the art for microbial diagnostics in food and environmental settings requires an enrichment step during which the pathogen of interest is selectively amplified over incubation times that can range from 10 to 24 h or more. The need for enrichment is to achieve adequate sensitivity and specificity, especially when dealing with complex samples. Although modern diagnostics can be sensitive and specific in laboratory settings, these techniques generally do not perform well in a heterogeneous mixture of competing microbes and environmental inhibitors without enrichment. These methods include traditional plating assays and antibody-based assays. Thus, in the absence of enrichment, most current tests are not sufficiently sensitive to detect a single or a small number of target microbes. By contrast, techniques such as PCR and hybridization-based assays may be sensitive but do not differentiate between living cells and dead cells, and require an enrichment step for specificity. In addition to being time consuming, enrichment necessarily increases the number of pathogens, a source of concern in food, clinical, and industrial settings, leading

to the segregation of testing facilities from production and processing facilities and thus increased cost and time for microbial testing.

These limitations can be potentially overcome by engineering bacteriophages as microbial diagnostics. For a detailed review on bacteriophage-based diagnostics research, the interested reader is referred to [4]. Bacteriophages naturally provide specificity in targeting specific bacterial types for infection, and thus, cocktails of

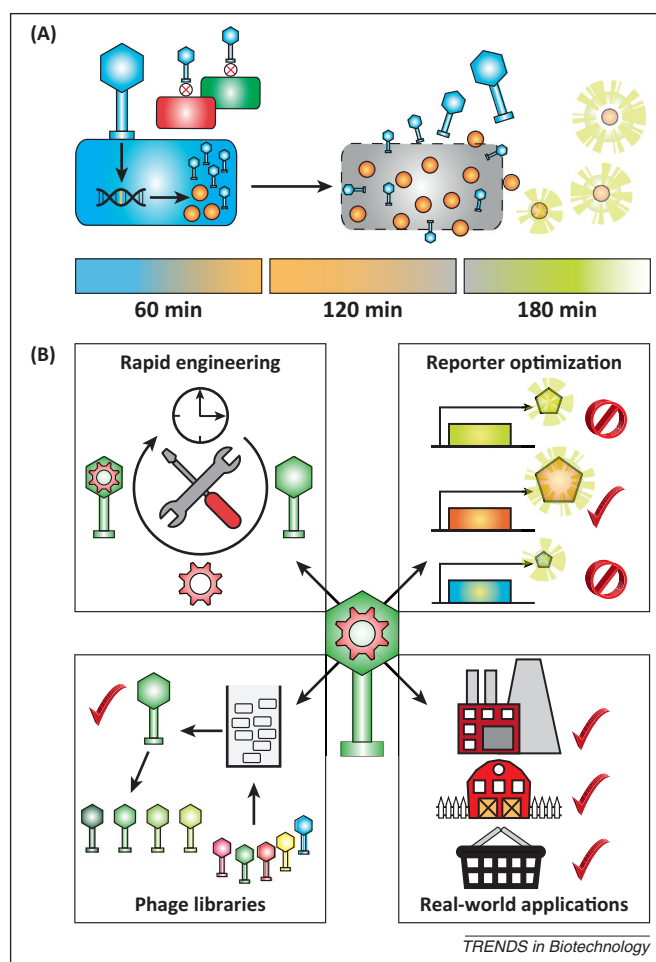


Figure 1. Engineered bacteriophages can be assembled into sensitive, specific, and rapid microbial diagnostics. (A) Bacteriophages can be designed to express reporter payloads during specific microbial infection, leading to the production of detectable outputs. (B) Designing synthetic bacteriophages as microbial diagnostics requires the ability to characterize and rapidly assemble phage libraries, to genetically engineer reporter payloads in these phages and maximize their signal output, and optimize these phage diagnostics in the context of complex real-world samples.

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phages or phage-derived recognition proteins can be assembled together to specifically detect desired bacterial spectra. Rapid detection enabled by bacteriophage-based diagnostics can enhance the tracking and remediation of microbial contamination. For example, the bioMerieux Vidas assay uses phage tails for affinity-based detection of target bacteria. However, natural phages do not produce common or convenient reporters for signal detection. Thus, an FDA-approved phage assay for methicillin-resistant *Staphylococcus aureus* produced by MicroPhage uses phage-specific antibodies to detect the amplification of natural phages upon encountering target microbial populations [5]. Other assays leverage quantitative PCR (qPCR) to identify phage DNA amplification upon infection [6]. Another strategy for bacteriophage-based microbial diagnostics is to genetically engineer phages to produce reporters upon infecting their target bacteria (Figure 1A) [7]. These reporters can generate a variety of different outputs, including luminescence, fluorescence, and colorimetric signals. Proof-of-concept demonstrations that such reporter phages can be used for microbial detection in laboratory settings have been described [4,7]. However, such prototypes require extensive additional engineering to fully address real-world applications and thus, as noted previously [4], few phage-based diagnostics have been successfully commercialized.

To achieve commercially viable bacteriophage-based diagnostics that are enrichment-free, rapid, sensitive, and specific in real-world situations, key engineering breakthroughs are needed [4]. Synthetic biology provides tools that can tackle these challenges by enabling phage engineering at an accelerated rate. For approaches using reporter phages, these technologies include high-throughput and reliable genetic engineering of environmentally isolated phages, strong and consistent expression of efficient reporter genes, and close integration of reporters with sensitive detectors (Figure 1B). A variety of advanced DNA manipulation techniques, including isothermal Gibson assembly [8], yeast-based recombining [9], and whole-genome synthesis are now available for modifying phage genomes. These strategies have enabled the engineering of environmental phage chassis at a much more rapid and reliable pace than previously possible. This is important to ensure that broad libraries of synthetic phages can be constructed, such that the ensuing product has sufficient host range to cover the desired target bacteria. Furthermore, strong and consistent expression of reporter genes is needed to meet product performance specifications across a variety of settings. Recent advances in synthetic biology can be applied towards this goal, including predictive tools for designing ribosome-binding sites, strong promoters that are insulated from contextual effects, and ribozymes or other RNA-based strategies that enhance the robustness of translational efficiency. Reporters can be further mutagenized or mined from genomic databases to improve signal output. Moreover, optimizing hardware and software for sensitive detection and tightly integrating these with the assay itself can reduce performance requirements for the reporter phages. For affinity-based

phage assays, synthetic biology can enable accelerated mutagenesis and assembly of phage tail libraries; an approach leveraged by companies such as AvidBiotics for therapeutic applications [10]. For phage assays that use qPCR or other molecular techniques to detect phage amplification, inserting optimized synthetic tags or multiple tag copies into phage genomes could further improve sensitivity and specificity.

In addition to the phage-based technology described here, synthetic biology can enable a broader generation of diagnostics through the improvement of biological sensors, construction of signal processing circuits, and integration of diagnostics into novel host chassis [11]. For example, whole-cell biosensors for *in vivo* or environmental sensing can be equipped with novel protein or RNA-based sensors mined from metagenomic sequence databases and realized via DNA synthesis. These biosensors could be designed to detect a wide range of environmental signals, including quorum-sensing molecules that can indicate the presence of pathogens, heavy metals, explosives, and others. These live-cell sensors can be diversified and optimized using directed evolution through technologies such as multiplex automated genome engineering and phage-assisted continuous evolution. Sensors could then be interfaced with processing circuits that perform digital logic, implement memory, and integrate signals for long-term stable recording of the inputs [12]. This would enable the collection of the engineered strains at a later time for interrogation. We envision that implementing these synthetic-biology-enabled capabilities in bacteriophages or other host chassis will enable a new suite of diagnostics with enhanced performance. These diagnostics may be ultimately find use in real-world environmental, industrial, clinical, and *in vivo* sensing applications.

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