Thesis progression

Thesis title: Development and Optimization of Advanced Genetic Techniques for Pathogen Detection

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1. Introduction

Currently, the diagnosis of bacterial infections primary relies on conventional culture techniques, which may be amplification by PCR and subsequent sequencing of the 16S rDNA gene using the Sanger method for more precise identification. Accurate estimation of microbial load plays role in the diagnosis of infectious diseases and in guiding appropriate therapeutic interventions. However, although conventional culture-based methods provide valuable clinical insights, time-consuming and their effectiveness is limited by the inability to cultivate all microorganisms, particularly fastidious or non-culturable species. The identification of bacteria begins with morphological characterization, colony morphology, gram staining, and cellular structure are analyzed to determine preliminary classifications. This step is followed by biochemical assays, which assess metabolic and enzymatic activity to differentiate bacterial species based on their phenotypic traits. MALDI-TOF mass spectrometry, which analyzes protein mass spectra, has largely replaced traditional biochemical tests for the precise and rapid identification of microorganisms. By leveraging protein profiling, it compares unique spectra to extensive microbial databases, offering high accuracy and efficiency (1).

The 16S ribosomal RNA (rRNA) gene, found in all bacterial and archaeal genomes, is an ideal target for bacterial identification (2). This gene encodes the rRNA component of the ribosome, essential for protein synthesis, and contains both variable and conserved regions (3). While the variable regions provide the necessary sequence diversity to distinguish between different species or even strains, the conserved regions enable the design of universal primers that can amplify the 16S rRNA gene across a wide range of bacteria (4).

Sanger sequencing, introduced in 1977, marked a significant milestone in sequencing history and quickly became the most common method for DNA sequencing due to its high base-pair accuracy (5). This method, based on chain-termination principles, comprises stepwise synthesis and detection of individual bases, delivering dependable findings for single-gene targets in isolated samples (6).

In this study, we analyzed data from Molecular diagnostic Unit in Srinagarind Hospital, Khon Kaen University, Thailand between 2022 – 2025 to determine a standardized approach to data analysis and established effective analysis workflow. We provide critical insights into the effectiveness and suitability of methods for microbial profiling in clinical diagnostic and research environments, by evaluating their performance in terms of sequencing depth, error rates, and practical applicability.

2. Objective

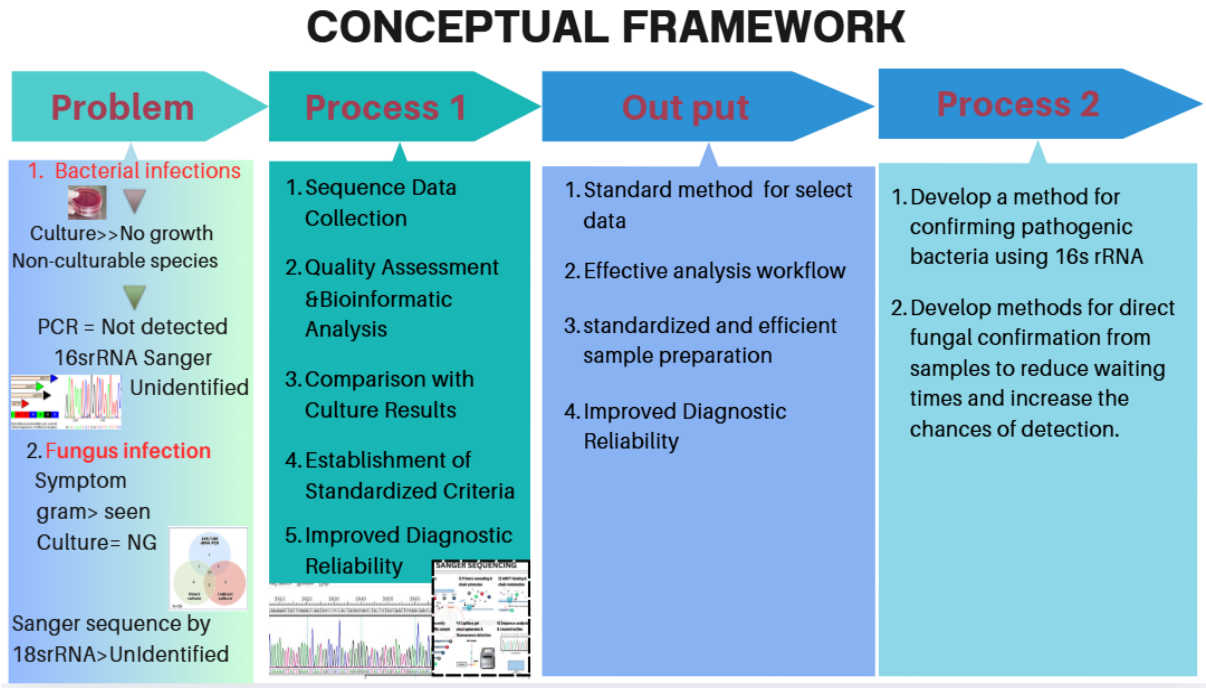
2.1 To analyze test results leading to standardized outcomes.

2.2 To establish a standard method for checking the raw data sequence result before analysis.

3. Hypothesis

We hypothesize that the lack of an effective analysis workflow contributes to suboptimal outcomes and limits the overall effectiveness of the results. Furthermore, the implementation of clearly defined criteria for sequence data quality assessment can provide insight into its practical utility and strength.

4. Conceptual framework



5. Anticipated outcomes

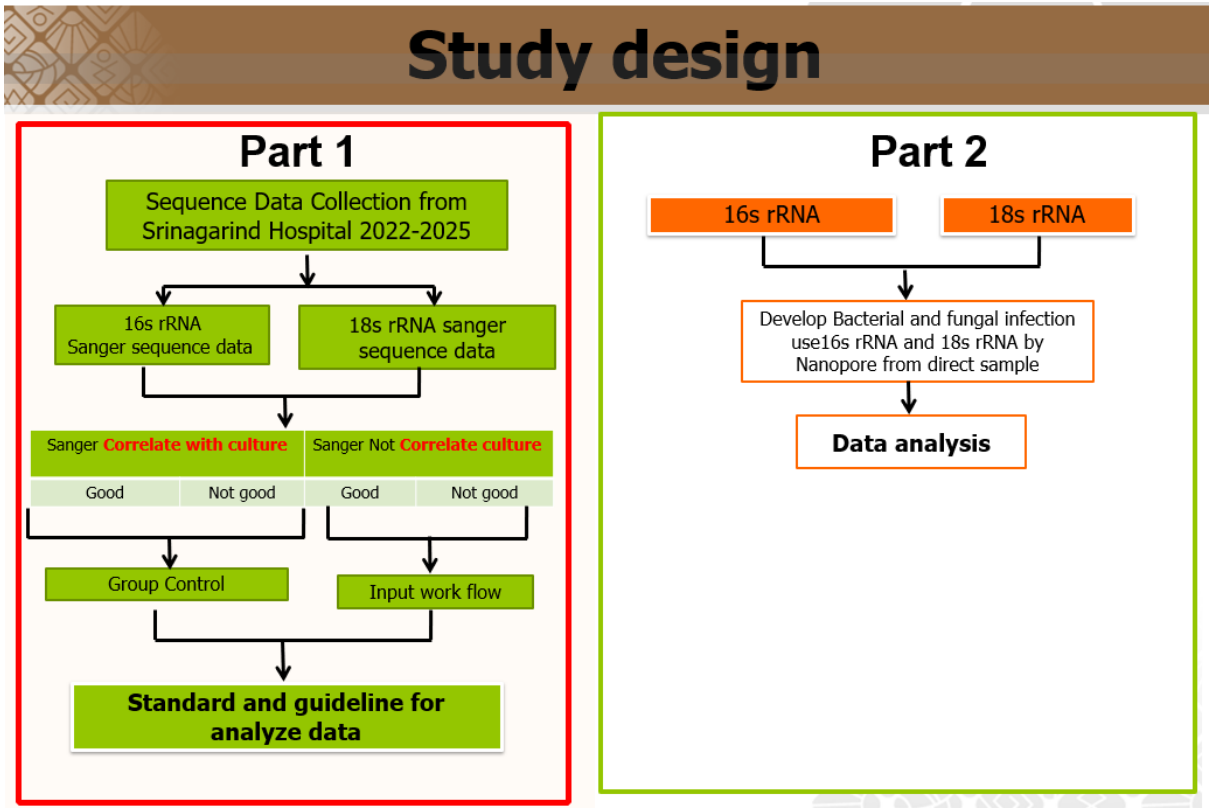
5.1 The analysis is expected to find a standard method for analyzing biomolecular data.

5.2 The gold is to develop standardized and efficient sample preparation methods for biomolecular analysis.

5.3 It can analyze in-depth biomolecular data and communicate it to the team, ensuring consistent work standards.

6. Materials and methods

6.1 The study design of this research



7. References

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