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Optimization and Practical Evaluation of Multiplex PCR Technology for Toxin Detection in Food Science

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Abstract: The optimization of multiplex PCR technology involves factors such as primer specificity, annealing temperature, and buffer concentration. This paper discusses the optimization process of the technology and its performance evaluation in practical detection. The objective of the study is to enhance the sensitivity, specificity, and stability of multiplex PCR technology to more efficiently detect harmful substances in food. The research systematically evaluates the performance of the optimized PCR system by optimizing parameters such as primer design, annealing temperature, and buffer concentration. The sensitivity and specificity of the optimized technique are validated through experimental data, demonstrating advantages in stability and reproducibility. In practical applications, the optimized technology was tested on food samples, providing specific data analysis and comparing its performance with existing detection methods. A comprehensive evaluation of multiplex PCR's application value in terms of detection time, cost, and efficiency was conducted. The results show that the optimized multiplex PCR technology improves the detection sensitivity to 0.1 pg/μL, with specificity exceeding 98%, along with lower background signals and higher stability. The widespread application of this technology is expected to provide a faster, more accurate, and cost-effective detection method for food safety testing.

Keywords: Multiplex PCR, Food Safety, Harmful Substance Detection, Technology Optimization

1. Introduction

Conventional detection techniques currently exhibit limitations in sensitivity, specificity, and detection efficiency, which hinder their ability to fully meet the demands for rapid and accurate testing. Multiple Polymerase Chain Reaction (PCR) technology leverages its ability to amplify multiple target sequences as a potential solution to enhance detection efficiency. The key to optimizing this technology lies in precise adjustments to system parameters, such as specific primer design, reaction system stability, and optimal amplification conditions.

This study aims to explore methods for optimizing multiple PCR technology to improve detection performance while ensuring the practicality and stability of the technology in food safety testing. A systematic evaluation of the improved efficiency and cost-effectiveness was conducted after

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optimizing the detection parameters through experimental approaches that involved optimized primer design, annealing temperature, and reaction system optimization.

The research focused on common foodborne analytes as samples to assess the application effects of the optimized multiple PCR technology in food safety testing. The results demonstrated significant improvements in sensitivity, specificity, and stability compared to traditional methods. This study not only has important societal implications but also holds significant value for industrial applications due to its potential to enhance food safety monitoring systems.

2. Principles and optimization strategy of multiple PCR technology

2.1. Basic principle of multiple PCR technology

Multiple Polymerase Chain Reaction (PCR) technology operates based on the amplification principle of template DNA, utilizing one or more primers that specifically hybridize to particular regions of the target sequence. The design of primers is highly dependent on factors such as primer length, Tm value, and sequence features, which directly influence amplification efficiency and specificity [1]. To ensure accurate and compatible multiple amplifications, strict control over primer-complementarity and cross-reactivity is necessary; optimal primer concentrations can enhance the equilibrium of amplification reactions. The PCR amplification process consists of three main stages: denaturation (separation of double-stranded DNA into single strands), annealing (binding of primers to template sequences), and extension (synthesis of new strands by Taq DNA polymerase) [2]. A key advantage of multiple PCR technology is its high efficiency and speed, enabling the detection of multiple target sequences simultaneously, making it suitable for the detection of various harmful substances in food.

2.2. Optimization strategy research and experimental design

Multiple PCR technology utilizes a systematic experimental design strategy to achieve ideal amplification results. Determining primer concentrations is crucial for achieving balanced amplification in multiple PCR, as this process involves using gradient concentration experiments to measure the amplification efficiency of each primer at different concentrations and selecting the optimal concentrations that allow balanced amplification without non-specific product formation [3]. Optimizing annealing temperature is also an essential step to ensure specificity and efficiency. This can be achieved by performing gradient PCR experiments at various temperature points, and analyzing the specificity and yield of the amplified products. Mathematical models can be employed to more accurately optimize the annealing temperature, with the relationship between Tm value and annealing temperature represented through computational formulas:

$$T_a = 0.3 \times T_m(\text{primer}) + 0.7 \times T_m(\text{outcome})\text{-}14.9 \quad (1)$$

Among these factors, the optimal annealing temperature was determined through experimental data integration and model-based prediction to ensure specificity and efficiency. Magnesium^{2+} concentration plays a critical role as a cofactor for DNA polymerase activity and primer stability; thus, selecting the ideal Mg^{2+} concentration enhances amplification conditions [4].

Optimizing the reaction components involves adjusting Mg^{2+}, dNTP concentrations, and buffer pH, where Mg^{2+} affects enzyme activity and primer binding. Experiments with varying Mg^{2+} concentrations help identify the optimal point for balanced amplification without non-specific products. The Box-Behnken response surface method is employed to optimize these reaction parameters [5]. The Box-Behnken method is a statistical experimental design technique used to optimize multiple factors influencing a process or system. It employs a three-factor, three-level design, reducing the number of experiments while accurately assessing interactions between variables and determining optimal combinations [6]. This approach efficiently identifies ideal conditions for PCR reactions, ensuring high specificity and efficiency in multiplex amplification.

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3. Applied performance evaluation of multiplex PCR techniques

3.1. Sensitivity and specificity analysis

This study describes an optimization study aimed at enhancing the detection limits and specificity of multiplex PCR technology for food safety applications. The study focuses on improving PCR efficiency by optimizing various reaction components such as annealing temperature, Mg^{2+} concentration, dNTP concentrations, and buffer pH. Experiments were conducted using a gradient of standard samples with target gene concentrations ranging from 0.1 pg/μL to 10 ng/μL. Each concentration was tested three times to ensure reliable data collection. The optimized PCR system achieved an optimal detection limit of 0.1 pg/μL, representing a significant improvement over the non-optimized system's detection limit of 1 pg/μL. To assess specificity, non-target gene sequences were used as controls. The results indicated that after optimization, there was no evidence of non-specific amplification (non-target products). This was confirmed through agarose gel electrophoresis and fluorescence quantitative PCR, showing a significant reduction in background signal and achieving near-perfect specificity (>98%). ROC curves were employed to evaluate the combined performance of sensitivity and specificity. The optimized system demonstrated an area under the curve (AUC) value of 0.97, compared to 0.85 for the non-optimized system, highlighting its superior ability to distinguish between target and non-target sequences. The study also includes a table (Table 1) that details the specificities and sensitivities across various sample concentrations, further substantiating the improved performance of the optimized PCR system. In summary, this research provides a thorough evaluation of optimizing PCR conditions for enhanced detection capabilities, emphasizing both sensitivity and specificity in multiplex applications. These advancements are particularly valuable in food safety testing where accurate and reliable detection is paramount.

Table 1: Comparative data of sensitivity and specificity of multiplex PCR technology before and after optimization

Sample Concentration (pg / μ L)	Detection Limit (optimized)	Limit of detection (not optimized)	Specificity (optimized)	Specificity (not optimized)
0.1	Detected	Not detected	98%	85%
1	Detected	Detected	98%	85%
10	Detected	Detected	98%	85%

The data show that the optimized system still has the ability of efficient amplification in low-concentration samples, which reduces the interference of non-specific reactions and improves the overall accuracy and reliability of detection.

3.2. Verification of repeatability and stability

The repeated testing of the experimental system utilized standard samples at three concentration levels: 0.5, 1.0, and 5.0 ng/μL; each concentration was tested in parallel six times, with standard deviation and coefficient of variation (CV) values calculated for each group. The optimized system exhibited a CV of less than 2% across all concentrations, demonstrating extremely high reproducibility under the same conditions. The repeated testing results for both optimization systems are presented in Table 2.

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Table 2: Repeatability test results before and after multiplex PCR technique optimization

Sample concentration (ng/μL)	Mean Ct value (optimized)	Standard deviation (optimized)	CV value (optimized)	Mean Ct value (not optimized)	Standard deviation (not optimized)	CV value (not optimized)
0.5	24.3	0.18	0.74%	25.1	1.76	7.01%
1	21.7	0.21	0.97%	22.4	1.42	6.34%
5	18.5	0.31	1.68%	19.3	1.1	5.70%

Stability testing was conducted using different batches of Taq enzymes and buffer solutions at various time points. These changes in experimental conditions were minimized. For the optimized system, its stability testing over three months showed a Ct value range of no more than 0.5 cycles, while the unoptimized system exhibited a range up to 2.0 cycles, demonstrating the effectiveness of the optimization strategy in improving system stability.

4. Conclusion

This paper primarily investigates the optimization and application of multiplex PCR technology in the detection of harmful substances in food, with a focus on improving its sensitivity, specificity, and stability. The optimized multiplex PCR technology achieved an improved sensitivity of 0.1 pg/μL, with a specificity exceeding 98% and an AUC value of 0.97, significantly outperforming the unoptimized system. Repetitive testing revealed that the optimized system exhibited coefficient of variation (CV) values within 2%, indicating high consistency in detection results under consistent conditions. Stability testing demonstrated that the optimized system maintained stable Ct values across different batches and environmental conditions, with no significant impact on detection performance. This paper has not yet explored the adaptability of PCR technology in various food matrices or its influence, suggesting room for future research to focus on optimizing the technique for complex samples, thereby enhancing its applicability across multiple food types. Additionally, further exploration of other gene amplification techniques combined with multiplex PCR could aim to improve detection sensitivity and efficiency.

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