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Design and Optimization of a Mycoplasma Detection Assay

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ABSTRACT

Mycoplasma are among the smallest free living microorganisms. These bacteria grow slowly, lack a rigid cell wall and are not eliminated by filter sterilization methods used in tissue culture. Mycoplasma infection affects biochemical and genetic aspects of cultured cells, resulting in experimental inconsistency. Therefore, it is necessary to establish routine testing for mycoplasma contamination in tissue culture laboratories.

Our goal is to develop a reliable and cost-effective test for mycoplasma in cell culture based on established methods found in literature. We first cloned and sequenced a PCR product from a commercial mycoplasma detection kit. Sequencing revealed the 16s rRNA as the target for mycoplasma detection; we confirmed this target by conducting a literature search.

PCR primers were designed using 16s rRNA gene as a target. We set-up reactions and optimized conditions for the real-time PCR assay to detect the target and confirmed amplicon size with agarose gel electrophoresis. We identified that 56ºC was the best temperature for the PCR and found that agarose gel electrophoresis was a better detection method because it identified the size to confirm the proper product.

The primers we ordered to develop this assay produce the proper band; however, results of several assays have been inconsistent as sometimes a known positive sample fails to amplify. As well, in several PCR reactions the negative showed a signal. The overall reaction needs improvements to have greater reliability and to eliminate all sources of contamination.
Research is continuing results are not final.

Key Words: cell culture contamination, mycoplasma, low cost, reliable detection

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