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基于DPO引物特异性检测小肠结肠炎耶尔森氏菌的PCR方法

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DPO-based PCR method for specific detection of Yersinia enterocolitica

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摘要 参考文献 相关文章

摘要 目的 引入一种设计简易、特异性强、退火温度范围宽的双启动寡核苷酸引物(dual-priming oligonucleotide, DPO)设计, 建立基于DPO引物特异性检测小肠结肠炎耶尔森氏菌的PCR方法。方法 以小肠结肠炎耶尔森氏菌16S-23S rRNA基因为靶基因, 设计一对DPO引物, 经过PCR反应体系优化, 建立小肠结肠炎耶尔森氏菌DPO-PCR检测方法。测定了检测灵敏度, 以常规PCR方法作为参照, 分析DPO-PCR方法的特异性及退火温度。结果 建立的小肠结肠炎耶尔森氏菌DPO-PCR检测方法的灵敏度为1.43×10^{2} CFU/mL;与常规PCR方法相比, DPO-PCR方法在49～69 ℃退火温度范围内均能保持高效率扩增;特异性强, 所测试17种病原菌中, 仅小肠结肠炎耶尔森氏菌为阳性结果, 且无非特异性扩增。结论 DPO-PCR方法不需要对引物参数特别是退火温度进行优化, 特异性强, 为致病微生物的快速准确检测提供了新方法。

关键词: 小肠结肠炎耶尔森氏菌 16S-23S rRNA DPO-PCR

Abstract: Dual-priming oligonucleotide (DPO), with characteristics of simple design, high specificity and annealing temperature insensitivity,

was introduced to develop a DPO-based PCR assay for detection of Y. enterocolitica. A pair of DPO primers was designed based on 16S-23S rRNA of Y. enterocolitica as target gene, and the DPO-PCR assay for detection of Y. enterocolitica was established by following optimization operation of PCR reaction system. Sensitivity of the assay was determined and its specificity and annealing temperature insensitivity were analyzed using conventional PCR as a reference. Results showed that the sensitivity of the DPO-PCR assay was 1.43×10^{2} CFU/mL. Compared to conventional PCR, the DPO-PCR assay can efficiently amplify the target gene in the annealing temperature range from 49 to 69 ℃. The specificity of the assay was evaluated using 17 bacterial strains and only Y. enterocolitica was in positive result, and no nonspecific amplification was observed, showing high specificity. The DPO-PCR assay provided a new way for rapid and accurate detection of pathogens.

Keywords： Yersinia enterocolitica　16S-23S rRNA　DPO-PCR

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