即時恆溫環式核酸增幅法應用於豬第二型環狀病毒之基因型區別診斷

豬瘟研究組
王 羣 助理研究員

摘要

豬第二型環狀病毒(porcine circovirus type 2; PCV2)是一直徑 17 毫微米(nm)、不具封套、並具有單股環狀 DNA 之病毒，目前該病毒可分為二基因型，PCV2a 以及 PCV2b。 目前許多研究報告顯示環式恆溫增幅法(loop-mediated isothermal amplification; LAMP)是具有高效率、快速和容易操作之診斷方法。利用二組或三組之引子對，可以檢測出樣品中微生物核酸。在這次研究中，主要設計二組環式恆溫增幅法之引子對，並採用即時檢測系統來檢測田間病材之豬第二型環狀病毒核酸。為了解引子對之特異性，總共收集 19 株 PCV2a 病毒株以及 28 株 PCV2b 病毒株加以測試。檢測結果發現引子具有高特異性，並沒有出現交叉反應。在引子對敏感性測試方面，引子對之敏感度可達 10 copy number / reaction。上述結果顯示環式恆溫增幅法可作為一精準、敏感和快速的豬第二型環狀病毒診斷方法。

Differential and Real-time Detection of Genotypes Porcine Circovirus Type 2 (PCV2) by Loop-Mediated Isothermal Amplification Assay (LAMP)

Chun Wang

Abstract

Porcine circovirus type 2 (PCV2) is a 17 nm in diameter, non-enveloped virus, and contains a single–stranded circular DNA. PCV2 can be divided into two genotypes, type 2a (PCV2a) and type 2b (PCV2b). Studies indicated that loop-mediated isothermal amplification (LAMP) is a specific, efficiency, and easy method to detect nucleic acid of various microorganisms in field samples by using two or three sets LAMP primer. In this study, we aim to design two specific primer sets and develop a real-time LAMP system for detection and differential of PCV2a and PCV2b in field samples. To determine the diagnostic specificity of the two genotypes LAMP assays, a total of 19 PCV2a isolates and 28 PCV2b isolates were collected and tested. The analytical specificity for the two genotypes of PCV2 identified that only target gene could be detected, respectively, no cross-reaction were observed using two LAMP assays. The analytical sensitivity of LAMP for the PCV2a and PCV2b were 10 copy number / reaction for each genotype of positive recombination plasmid, respectively, and the sensitivity limit of PCV2a and PCV2b These results indicated that the PCV2a and PCV2b could be detected and accurately distinguished using two genotypes of PCV2 LAMP assays with real-time monitored. The LAMP is a specific, sensitive and quick diagnostic method for detection and differentiation of PCV2a and PCV2b.