**構築應用於PCR試驗之可辨識陽性對照DNA**

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**摘要**

聚合酶鏈反應 (PCR) 已普遍成為傳染性疾病診斷實驗室的常規診斷技術。而偽陽性的檢驗結果為 PCR技術最嚴重的問題之一。避免偽陽性的方法，除了從試劑、設備、實驗操作區著手外，陽性對照核酸也可能是汙染的原因之一。本實驗旨在構築應用於Neospora PCR試驗之可辨識陽性對照的DNA，即兩端為Neospora引子序列，中間置換成禽流感病毒序列。實驗步驟如下：合成Neospora-AI 引子對，以欲置換的AI PCR產物為模板，進行PCR反應，PCR產物選殖至Topo載體後，即完成可辨識陽性對照的Neospora DNA核酸。為便於區別，合成之對照Neospora DNA其置換的AI序列較長，故其PCR反應產物較陽性檢體產物增加211bp，同時此產物也可以使用AI的引子再進行巢式PCR做為辨識用途。另外，以相同方法完成Parainfluenza 3-goat pox可辨識陽性對照DNA，再以 MEGAscript™ SP6套組轉錄成RNA即可。

**Constructing a differentiable positive control for PCR using plasmid DNA**

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**Abstract**

The Polymerase chain reaction (PCR) has been a routine diagnostic technique used in laboratories concerned with the detection of the etiological agents of infectious diseases. When employing this method, one of the considerations is the potential of false positive PCR results. To minimize the risk of producing false-positive results, nucleic acid positive controls are a high risk factor for contamination that need to be taken into account for, in addition to reagents, equipment, and working areas. The aim of this study was to construct a standard DNA construct as a differentiable positive control when using PCR to detect Neospora. The positive control is a plasmid inserted with a fragment composed of an inner sequence of avian influenza virus and the primer annealing regions of Neospora sequences at both ends. When the construct was employed in the Neospora-AI PCR assay, the reaction generates a product 211 bp longer than that of the normal Neospora positive PCR product. In addition, the product can be identified by nested PCR which is used for the routine identification of avian influenza viruses. The same strategy was used to construct a Parainfluenza 3-goatpox differentiable positive control DNA, followed by transcribing it into RNA with the MEGAscript™ SP6 Kit.