

乳牛脊椎畸形複合症檢測法之開發

廖仁寶 陳若菁 蔡新興 蕭宗法 吳明哲

行政院農業委員會畜產試驗所

脊椎畸形複合症(CVM)為一種單基因隱性遺傳疾病，在 2000 年首度發現於丹麥，此種遺傳缺陷常見於荷蘭種乳牛。目前常用的檢測方法有對偶基因專一 PCR 法(AS-PCR)與引子引入限制酶切點 PCR 法(PCR-PIRA)。本研究嘗試開發三種不同的檢測法，用以篩檢乳牛族群之 CVM 基因型。第一種為 DNA 定序法，在基因序列中設計適當引子，使 PCR 產物包含 CVM 變異點，將 PCR 產物純化後，進行定序以判斷其基因型。第二種為設計適當引子對，使 PCR 產物包含變異點，以定量 PCR 進行基因型檢測。第三種則為應用 MS-PCR 的方式設計三種引子，將正常型設計成長為 110 bp，突變型設計長為之 130 bp 片段，經由簡易之 PCR 機器與膠體電泳即可進行基因型判別。以上三種方式皆可成功檢測 CVM 基因型，但若考量檢測成本時，則以 MS-PCR 法最為可行。

關鍵語：乳牛、脊椎畸形複合症、檢測

DEVELOPMENT OF IDENTIFICATION METHODS FOR BOVINE COMPLEX VERTEBRAL MALFORMATION

R. B. Liaw, J. C. Chen, S. S. Tsay, T. F. Shiao and M. C. Wu

Livestock Research Institute, Council of Agriculture, Executive Yuan

Complex vertebral malformation (CVM) is a monogenic recessive genetic disorder which was first discovered in Denmark in 2000. Currently, two assay methods including allele-specific polymerase chain reaction (ASPCR) and polymerase chain reaction–primer introduced restriction analysis (PCR-PIRA) are used to identify CVM. In this study, we tried to develop three assay methods to identify CVM. First, DNA sequencing for the amplified DNA fragment covered CVM variation site was employed to genotype CVM. Second, the DNA fragment covered CVM variation site was amplified by specific primers and examined by real-time PCR. Third, a MS-PCR based method was invented to genotype CVM. A 110-bp PCR fragment represents normal type; a 130-bp PCR fragment represents mutant type. Moreover, a carrier contains 110-bp and 130-bp PCR fragments simultaneously. The CVM genotype were able to be identified well by those three methods mentioned above. However, it is recommended to use MS-PCR method to detect CVM based on cost effectiveness.

Key Words: Dairy cow, Complex vertebral malformation, Identification