畜牧兽医学报 » 2018, **Vol. 49** » Issue (12): 2698-2706 DOI: 10.11843/j.issn.0366-6964.2018.12.019

同时检测禽类六种病毒的金标银染可视化基因芯片的构建及初步应用

向华, 杨国淋, 曹三杰, 黄小波, 伍锐, 赵勤, 文心田*, 文翼平*

四川农业大学动物医学院, 成都 611130

Development and Application of Gold Label Silver Stain Visual Chip for Simultaneously Detecting Six Kinds of Poultry Virus Diseases

XIANG Hua, YANG Guo-lin, CAO San-jie, HUANG Xiao-bo, WU Rui, ZHAO Qin, WEN Xin-tian*, WEN Yi-ping*

College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China

摘要 图/表 参考文献(25) 相关文章 (1)

全文: PDF (27403 KB) HTML (16)

输出: BibTeX | EndNote (RIS)

摘要

本研究旨在利用金标银染显色技术，构建同时检测禽白血病病毒（ALV）、鸡马立克病病毒（MDV）、传染性法氏囊病毒（IBDV）、新城疫病毒（NDV）、禽流感病毒（AIV）、鸡传染性喉气管炎病毒（ILTV）的可视化基因芯片。利用T-A法分别克隆得到ALV-*env*、MDV-*meq*、IBDV-*vp*$_2$基因，并利用本实验室已保存的AIV-*np*、NDV-*f*、ILTV-*tk*基因序列，设计寡核苷酸探针，制备ALV-MDV-IBDV-AIV-NDV-ILTV基因芯片阵列。引入生物素标记的引物进行PCR扩增，并与芯片进行杂交银染显色，肉眼观察检测结果，并对芯片的特异性、敏感性、稳定性进行评价，以及临床初步验证。成功克隆ALV-*env*、MDV-*meq*及IBDV-*vp*$_2$共3个靶基因，测序鉴定其正确性。发现寡核苷酸探针最优使用浓度为50 μmol·L^{-1}，40℃条件下杂交2 h，Nanogold-Streptavidin链霉亲和素溶液的质量浓度为4 μg·mL^{-1}，银染5 min，可见明显的检测信号。特异性试验显示，ALV、MDV、IBDV、NDV、AIV、ILTV之间无交叉反应，且以鸡传染性支气管炎病毒为模板制备的PCR扩增标记不与ALV-MDV-IBDV-AIV-NDV-ILTV芯片杂交。芯片检测的灵敏度为1 pg·μL^{-1}。芯片在常温条件下保存60 d，在4℃条件下保存75 d仍可进行有效检测。临床病料检测结果与PCR检测结果一致。本研究成功构建同时检测ALV、MDV、IBDV、NDV、AIV和ILTV的金标银染可视化基因芯片，并确定其最佳反应条件，为临床标准化应用奠定了基础。

关键词 ： 家禽, 病毒检测, 可视化芯片, 金标银染

Abstract :

The aim of the present study was to develop a visual chip for simultaneously detecting avian leukosis virus (ALV), chicken Marek's disease virus (MDV), infectious bursal disease virus (IBDV), Newcastle disease virus (NDV), avian influenza virus (AIV), and infectious laryngotracheitis virus (ILTV) using gold label silver stain method. The gene sequences of ALV-*env*、MDV-*meq* and IBDV-*vp*$_2$ were cloned using T-A method. These sequences, also the sequences of AIV-*np*, NDV-*f*, ILTV-*tk* stored by our laboratory, were used for probes design, following which the simultaneously detecting ALV-MDV-IBDV-AIV-NDV-ILTV chip was developed. Following the PCR amplification using a pair of biotin-labeled primers, the chip was used for naked-eyes observation after hybridization and silver stain. The chip was then validated by specificity and sensitivity identification, stability evaluation, and clinical verification. The sequences of ALV-*env*, MDV-*meq*, IBDV-*vp*$_2$ were cloned successfully. The hybridization, at 40℃ for 2 h, of 50 μmol·L^{-1} oligonucleotide probes with the biotin-labeled PCR products resulted in visible silver stain signal. No cross reaction was observed among ALV, MDV, IBDV, NDV, AIV and ILTV by specificity test. The specificity of the chip was confirmed by its negative hybridization with the PCR products from infectious bronchitis virus. The minimum-does of the plasmid as PCR template was 1 pg·μL^{-1} by sensitivity test. The chip can be stored at room temperature for 60 d, or at 4℃ for 75 d according to stability test. The results of chip test consistent with that of PCR/RT-PCR method. The present study successfully developed a visual chip for simultaneously detecting ALV,MDV,IBDV,NDV,AIV and ILTV with an optimization reaction condition, which will facilitate the clinical application of the chip in the future.

Key words : avian virus diagnosis visual chip gold label silver stain

收稿日期: 2018-02-28

PACS: S858.315.3

基金资助:

禽病高通量检测技术及配套试剂与设备研究（2016YFD0500808）

通讯作者: 文心田,男,博士,教授,博导,主要从事预防兽医学研究,E-mail:xintian3211@126.com;文翼平,男,博士,硕导,主要从事预防兽医学研究,E-mail:yueliang5189@163.com

作者简介: 向华(1986-),女,四川蒲江人,博士生,主要从事动物传染病研究

服务

- 把本文推荐给朋友
- 加入我的书架
- 加入引用管理器
- E-mail Alert
- RSS

作者相关文章

- 向华
- 杨国淋
- 曹三杰
- 黄小波
- 伍锐
- 赵勤
- 文心田
- 文翼平

引用本文:
向华, 杨国淋, 曹三杰, 黄小波, 伍锐, 赵勤, 文心田, 文翼平. 同时检测禽类六种病毒的金标银染可视化基因芯片的构建及初步应用[J]. 畜牧兽医学报, 2018, 49(12): 2698-2706. XIANG Hua, YANG Guo-lin, CAO San-jie, HUANG Xiao-bo, WU Rui, ZHAO Qin, WEN Xin-tian, WEN Yi-ping. Development and Application of Gold Label Silver Stain Visual Chip for Simultaneously Detecting Six Kinds of Poultry Virus Diseases. ACTA VETERINARIA ET ZOOTECHNICA SINICA, 2018, 49(12): 2698-2706.

链接本文:

http://www.xmsyxb.com/CN/10.11843/j.issn.0366-6964.2018.12.019 **或** http://www.xmsyxb.com/CN/Y2018/V49/I12/2698

地址：北京市海淀区圆明园西路2号中国农业科学院北京畜牧兽医研究所 邮政编码：100193
Tel：010-62815987 E-mail：xmsyxb@263.net