研究论文

基于RNA杂交的马铃薯纺锤块茎类病毒检测芯片

谷宇 1,3 , 杜志游 2 , 郎秋蕾 2 , 陈集双 2 , 何农跃 3

- 1. 南京中医药大学基础医学院生物学与生物化学系, 南京 210029;
- 2. 浙江大学微生物分子生物学实验室, 杭州 310029;
- 3. 东南大学生物电子学国家重点实验室,南京 210096

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报道了一种检测植物类病毒RNA的新方法──RNA杂交芯片技术,即将cDNA芯片技术与RNA斑点杂交技<mark>▶浏览反馈信息</mark> 术相结合, 将马铃薯样品的总RNA直接固定在玻片上, 用荧光标记制备检测马铃薯纺锤块茎类病毒(PSTVd)的特异 探针, 探针与芯片杂交后分析杂交信号以确定相应的样品有无PSTVd侵染. 参照膜杂交的方法, 确定了RNA芯片的 制备条件,并用以检测了马铃薯样品的PSTVd侵染情况,检测结果与RT-PCR结果相符,阳性产物经克隆测序证实为 PSTVd.

关键词 RNA芯片杂交 斑点杂交 cDNA芯片 马铃薯纺锤块基类病毒(PSTVd)

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Detection of Potato Spindle Tuber Viroid Using RNA Hybrid. ized Chips

GU Yu^{1,3}, DU Zhi-You², LANG Qiu-Lei², CHEN Ji-Shuang², HE Nong-Yue³

- 1. Department of Biology and Biochemistry, School of Basic Medical Science, Nanjing University of Traditional Medicine, Nanjing 210029, China;
- 2. Laboratory of Microbial Molecular Biology, Zhejiang University, Hangzhou 310029, China;
- 3. State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, China

Abstract

Potato diseases caused by potato spindle tuber viroid(PSTVd) bring about substantial losses i n agricultural production. So developing a high throughput, rapid, sensitive and specific detect ion method is necessary. This article reports a new method to detect plant viroid——RNA glass slide hybridization. This method combines dot blot hybridization technique with cDNA microarr ay technique. We could spot a number of RNA samples extracted from new leaves of potatoes onto a silanated glass slide directly and then perform the hybridization on the slide with Cy5-I abeled probe of PSTVd prepared by PCR. Analyzing the results of hybridization, we could asce rtain which sample is infected by PSTVd. The factors affecting the results were optimized and used to test six potato samples with PSTVd-specific probes. The developed method was used to detect two samples which infected by PSTVd. These results were in good accordance with t hose from the RT-PCR approach and the samples are proved to be PSTVd by cloning and sequ encing. So it is an accurate and reliable method for detecting plant viroid and the first report f or the diagnosis of PSTVd using dot blot hybridization and RNA glass side hybridization with fl uorescently labeled probe.

Key words RNA glass slide hybridization Dot blot hybrid ization cDNA microarray Potato spindle t uber viroid(PSTVd)

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- 谷宇
- 杜志游
- 郎秋蕾
- 陈集双
- 何农跃

DOI:

通讯作者 何农跃 nyhe1958@163.com