#### 研究论文

转基因玉米的多重PCR-毛细管电泳-激光诱导荧光检测方法研究

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采用三重PCR反应,同时扩增CaMV 35S启动子、 hsp70 intron1和CryIA(b)基因之间序列以及Inve rtase基因, 扩增产物用无胶筛分毛细管电泳-激光诱导荧光检测, 从而建立了多重PCR-毛细管电泳-激光诱导荧光 快速检测转基因玉米的新方法. 对影响多重PCR扩增和毛细管电泳的因素进行了优化. 在优化的条件下, 本方法可 以同时检测转基因玉米样品中3种外源基因. 经序列测试证实, 三重PCR 扩增产物的序列与原基因完全一致, 表明 扩增结果可靠. 该方法能检出0.05% MON810转基因玉米成分, 远低于欧盟对转基因食品规定标识的质量分数阈 ▶浏览反馈信息 值(1%). 该方法对玉米及其制品的检测结果与实时荧光PCR方法的检测结果一致, 与传统的琼脂糖凝胶电泳法相 比, 具有特异性高\, 快速及灵敏等优点, 适用于玉米中转基因成分以及转基因玉米MON810品系的快速筛选、 鉴 定和检测, 能满足我国实施转基因食品标签法规的要求.

关键词 多重PCR 无胶筛分毛细管电泳 激光诱导荧光检测 转基因玉米 分类号 0657

Rapid and Sensitive Analysis of Genetically Modified Maize. by Multiplex PCR and Capillary Electrophoresis with Laserinduced Fluorescence Detection

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Abstract A rapid method for detection of genetically modified maize by multiplex polymerase ch ain reaction(PCR) followed by capillary electrophoresis with laser-induced fluorescence was de veloped. The triplex PCR procedure was applied to simultaneously amplify CaMV 35S promoter and the junction between hsp70 intron 1 and CryIA(b) gene as well as Invertase gene. The a mplified products were analyzed by capillary sieving electrophoresis with laser-induced fluores cence detection. The parameters of multiplex PCR and capillary sieving electrophoresis were o ptimized. Under the optimal conditions, the proposed method was able to simultaneously det ect the three heterogenous genes existing in genetically modified maize. The amplified DNA fr agments were sequenced and showed the good agreement with original gene, which indicate d the reliability of the amplification reaction. The limit of detection was 0.05%, which is much l ower than 1% threshold for labeling genetically modified food required by the European Com munity. There was a good agreement in the determined results of maize and its products by u sing this method and real-time quantitative PCR method. Compared with conventional agaros e gel electrophoresis, the proposed method offered a more specific and sensitive procedure t o rapid screening, identification and detection of genetically modified maize.

Key words Multiplex polymerase chain reaction Capillary sieving electrophoresis Laser-induced fluor escence detection Genetically modified maize

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