

转Cry1Ab基因水稻分子特征及其特异性PCR检测方法

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摘要 转Cry1Ab基因水稻Bt01为一种新型的转基因水稻, 文章首先利用Southern blotting验证了外源基因Cry1Ab转入了Bt01中, 且为单拷贝, 再利用TAIL-PCR方法获得了其插入位点信息, 根据获得的Bt01的5'端插入位点序列, 设计了相应的定性定量PCR检测体系的引物及探针, 实验结果显示, 定性PCR检测体系的最低检测极限(LOD)为10个拷贝, 定量PCR检测体系的LOD为5拷贝, 最低定量极限(LOQ)为10拷贝。同时为了验证建立的定量PCR体系的准确性, 利用该体系检测已知转基因水稻Bt01含量分别为3%和0.5%的样品, 定量结果分别为2.7%和0.47%。研究结果表明, 该转化体特异性定性定量检测方法具有高度的特异性和良好的灵敏性, 为转基因水稻Bt01的身份识别和检测提供了有效的方法。

关键词: 分子特征 转基因 Cry1Ab 定性定量PCR 特异性检测

Abstract: Bt01 is a new type of rice that has been genetically modified to express Cry1Ab protein. This study confirmed that Cry1Ab was inserted into Bt01 as a single copy using Southern blotting analysis. TAIL-PCR method was further used to obtain its insertion site information. Specific PCR primers and TaqMan probes were designed based on the 5' integration junction sequence of transgenic rice Bt01. The results showed that the limit of detection (LOD) was ten copies in qualitative PCR. The quantitative PCR assay showed that the LOD was five copies, and the limit of quantification (LOQ) was ten copies. In addition, the accuracy of the established quantitative PCR was verified by detecting two samples containing 3% and 0.5% Bt01, respectively. The quantitative PCR analysis showed the results were 2.7% and 0.47%, respectively. The above results indicated that the event-specific PCR methods developed have high specificity and good sensitivity, which could be effective methods for identifying and testing the genetically modified Bt01 rice.

Keywords: molecular characteristics, transgenic, Cry1Ab, qualitative and quantitative PCR, specific detection

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

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