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用差异显示反转录PCR银染技术研究植物基因表达的差异

Analyzing Plant Gene Expression Differences Using a Silver Staining Based DDRT-PCR Protocol

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中文关键词：差异显示反转录PCR　银染　拟南芥　*ast*突变型

英文关键词：differential display reverse transcription polymerase chain reaction　silver staining　*Arabidopsis thaliana*　*ast* mutant

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中文摘要：

通过调整差异显示反转录PCR(DDRT-PCR)中总RNA、锚定引物、随机引物、cDNA和dNTP等关键试剂的用量，优化了适用于银染检测的DDRT-PCR方法.PCR扩增产物经6%变性聚丙烯酰胺凝胶垂直电泳分离后，银染能检测到多而清晰的条带.泳道中的条带数最少为40个，最多达80个，平均为60个，条带大小分布在100～900 bp范围，灵敏度为5 pg/mm^2 .此方法操作简便快速，灵敏度高，重复性好.采用这个改良的方法，分析了拟南芥野生型和ast突变型基因表达的差异.从16 000个cDNA扩增产物条带中筛选出28个差异条带.二次PCR扩增后，进一步筛选出13个差异条带，其中7个是野生型特异表达的，6个是突变型特异表达的，为进一步认识*ast*突变表型的产生机制奠定了基础.

英文摘要：

A procedure of differential display reverse transcription polymerase chain reaction (DDRT-PCR) applicable for silver staining was optimized by adjusting the amount of several critical reagents, including total RNA, anchor primer, arbitrary primer, cDNA and dNTP. The PCR amplification products were separated on 6% vertical denaturing polyacrylamide gels. Numerous and distinct bands could be detected by silver staining. The minimum number of bands in one lane was 40, the maximum was 80 and the average was 60. The range of displayed PCR products extended from about 100 base to about 900 base. The sensitivity was 5 pg/mm^2. This procedure was simple, time-saving, high sensitivity and reproducible. Based on the improved procedure, the differential gene expression were studied between immature siliques of *Arabidopsis* wide-type and *ast* mutant. From nearly 16 000 cDNA fragments analyzed, 28 differential cDNA fragments were screened. After the second PCR amplification, 13 differential cDNA fragments were identified among which 7 fragments were wild type specific and 6 fragments were *ast* mutant specific.

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