生物化学与生物物理进展
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用改进的DDRT-PCR技术进行人胚差异基因筛选

Differential Expression Analysis of the Gene in Human Early Embryos Using a Modified DDRT-PCR

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中文摘要：

介绍一种从不同类型细胞或不同生长状态细胞中分离差异表达基因的快速高效mRNA差异显示技术，其特点是利用Ready-To-Go RT-PCR反应珠和Ready-To-Go RAPD分析珠进行mRNA差异显示分析，使取样步骤降至最低程度，减少了潜在的取样误差和外源DNA污染，并确保每次反应的高度重复性.通过银染测序胶分析差异显示的cDNA带,便于DNA回收和进一步克隆.用此方法分析人胚发育早期不同阶段基因的差异表达，选用6条随机引物对3、4和5周龄人胚进行mRNA差异显示分析，从2 000多条带中共分离出14个差异产物，经二次扩增及反向RNA印迹确证其中6个片段为发育不同阶段差异表达基因.

英文摘要：

PCR based method for differential display of eukaryotic mRNA has been designed to isolate differential expressed genes in various cell types or under different growing conditions. A modified method for mRNA differential display originally developed by Sokolov was employed and optimized here. This procedure, based on the application of Ready-To-Go RT-PCR beads and Ready-To-Go RAPD beads, minimized pipetting steps, decreased the potential for pipetting errors, reduced the risk of contaminating and ensured greater reproducibility between reactions. Distinct cDNA bands can be observed by silver-staining 6% sequencing urea gel and easily excised and recovered for further use in cloning. The stage-specific genes in the developing human embryos were analyzed with six sets of arbitrary primers using this modified DDRT-PCR from 3-, 4- and 5-week-old human embryos. About 14 bands containing differential fragments were obtained from sliver-straining 6% polyacrylamide gel, six of which were proved to be developmental related genes by RNA rev-Northern hybridization using [α-^{32}P] dCTP labeled first strand cDNA as probes.

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