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利用磁珠富集马铃薯BAC文库中NBS-LRR类晚疫病抗性基因

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Isolation of NBS-LRR Late Blight Resistance Genes from Potato BAC Library by System of Magnetic Separation

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摘要 栽培马铃薯是高度杂合的四倍体作物，利用传统的基因克隆方式进行晚疫病抗性基因分离难度很大。然而，晚疫病抗性基因具有序列保守性，属于NBS-LRR类基因。本研究中，根据晚疫病抗性基因*R3a*家族的序列比对结果设计*R3a*基因家族的保守探针，并将含有*R3a*基因的BAC SH23G23部分酶切成7~11 kb DNA片段。通过结合保守探针的磁珠系统对上述7~11 kb DNA片段进行*R3a*基因分离，将磁珠富集的片段克隆到双元载体pBINPLUS上。通过阳性克隆和菌落PCR鉴定表明，含有*R3a*基因的克隆比率达到82.76%，相对于磁珠系统富集前，提高*R3a*基因比率近19倍。本研究建立了抗病基因及其同源序列的磁珠分离系统，为分离马铃薯等多倍体作物中具有保守结构的基因提供了实验基础。

关键词： 马铃薯 晚疫病 抗性基因 磁珠富集

Abstract: Cultivated potato (*Solanum tuberosum*) is an auto-tetraploid crop, which is very difficult to isolate late blight resistance genes. Nevertheless, it is discovered that there is conseved NBS-LRR domain among late blight resistance genes. In this study, conserved probes of *R3a* late blight resistance genes family were developed by alignment of sequences. And then, the DNA of BAC SH23G23 containingthe *R3a* gene was partially digested into 7–11 kb fragments. By magnetic separation system combined with conserved probes, the 7–11 kb fragments were enriched and cloned into binary vector pBINPLUS. Through identification of positive clones and colony PCR, the ratio of clones including *R3a* gene to all positive clones reached 82.76%, which was nearly 19 times higher than enrichment before. The system of magnetic separation for *R* genes and their analogs established in this study provides a new strategy for conserved domain genes cloning from polyploid crops.

Keywords: Potato Late blight Resistance genes Magnetic separation

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