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苦瓜MAP30基因原核表达载体的构建和PCR快速检测重组子的研究

Construction of Prokaryotic Expression Vector for MAP30 Gene and Study of PCR Methods for Rapid Identification of Recombinant

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摘要

根据已报道的序列，把苦瓜(Momordica charantia) MAP30基因成功克隆到原核表达载体pET28a（+）中，并对PCR快速检测阳性克隆进行了研究。结果表明，直接用菌落、菌液、酚氯仿处理过的菌液,以及提取的质粒进行PCR都可以成功地筛选阳性菌落。其中，酚氯仿处理过的菌液PCR与质粒PCR的结果最接近，而且比质粒PCR简单，因此可作为方便可靠的阳性克隆筛选的新方法。Abstract: Based on the sequence reported by Lee-Huang,S ， we cloned the MAP30 gene of Momordica charantia(balsam pear) into a prokaryotic expression vector pET28a（+）. A method by using PCR for rapid identification of positive clone was developed. Result showed this screening method can be used to detect positive colonies from samples of bacterial, purified plasmid, liquid culture ,and liquid culture treated with mixture of phenol/Chloroform .The result from liquid-culture- treated- PCR (LCT-PCR) is very close to that of by plasmid-PCR. LCT-PCR is reliable and much easier to used than plasmid-PCR, therefore the LCT-PCR can be used for clone screening during the molecular cloning.

关键词 MAP30 PCR 克隆子筛选 苦瓜 Key word MAP30 PCR identification cloning balsam pear

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