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论著

苦瓜MAP30基因功能性片段的克隆和表达

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摘要 背景与目的： MAP30是一种具有抗肿瘤、抗HIV和抗病毒等功能的蛋白。通过克隆和表达该蛋白的核心片段，用于进一步研究基因功能和结构域之间的关系。 材料与方法：设计了两对特异性的引物，通过PCR技术从苦瓜总DNA中分别扩增出两段含有成熟MAP30蛋白基因的功能性片段T8_S194和T8_N263， 然后将目标基因克隆到原核表达载体pET_28a上， 构建成带有C端6His_tag的融合表达载体，经测序鉴定后用CaCl2介导的化学转化法转化到E. coli RosettaTM(DE3)pLysS中。利用PCR筛选出阳性克隆，经IPTG诱导工程菌表达出重组蛋白。通过Western blot对重组蛋白进行分析。 结果： 含T8_S194和T8_N286基因片段的两个表达载体构建成功，它们的表达重组蛋白能与兔抗His_tag多克隆抗体发生特异性反应。结论：构建的两个含目的基因的表达载体均能在E. coli RosettaTM(DE3)pLysS中表达出预期的重组蛋白，为进一步研究这两个重组蛋白打下了基础。

关键词 苦瓜； 克隆； 原核表达； MAP30； 活性片段

Cloning and Prokaryotic Expression for Biologically Active Fragments of MAP30 Gene in Escherichia coli

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Abstract BACKGROUND & AIM: MAP30 (Momordica anti_HIV protein of 30 kDa) is a 30 kDa, a single_stranded ribosome inactivating protein purified from the fruits and seeds of bitter melon (Momordica charantia). In order to fully understand the relationship between gene function and domain of MAP30, the active gene fragment of this protein must be cloned and expressed precedingly. MATERIALS AND METHODS: Two PCR primers, one for amplifying coding gene for T8_N263 amino acids of mature MAP30 (large fragment), the other for amplifying coding gene for T8_S194 amino acids of mature MAP30 (small fragment) were designed firstly. The objective genes were amplified by PCR from the DNA of bitter melon, and then cloned into the prokaryotic expression vector pET_28a to construct the expression vectors, containing 6 His_tag in C_terminal. After sequencing analysis, the vectors were transformed into E. coli RosettaTM(DE3)pLysS by calcium chloride transformation method to obtain the recombinants respectively. The recombinants were induced by IPTG to express the recombinant proteins, which were analyzed by Western blot. RESULTS: Western blot analysis indicated that the recombinant proteins demonstrated antigenicity to rabbit anti_His_tag polyclona antibody. CONCLUSION: The result demonstrated that both of the two constructed expression vectors could express the expected recombinant proteins in E. coli RosettaTM(DE3)pLysS.

Keywords bitter melon； cloning； prokaryotic expression； MAP30； active fragment

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