癌变.畸变.突变 ISSN: 1004-616x CN:44-1063/R 2009; 21(4) :263-267

论著

带TAP标签的癌蛋白SET真核载体的构建及其表达

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收稿日期 2009-2-11 修回日期 2009-3-30 网络版发布日期:

摘要 背景与目的: 为研究三氯乙烯诱导的差异蛋白SET在肝细胞L-02中的相互作用,构建带串联亲和纯化(tandem affinity purification,TAP)标签融合表达的真核表达载体pcDNA3.1/SET-TAP。 材料与方法: 从L-02肝细胞中提取总RNA,采用RT-PCR法扩增SET基因,从质粒中扩增得到TAP基因,采用重叠PCR法将基因SET与TAP连接成SET-TAP,双酶切后纯化序列定向克隆至pcDNA3.1/zeo(+)并转化E.coli DH 5α ,取阳性克隆进行酶切和测序鉴定,重组质粒瞬时转染L-02肝细胞进行表达,Real Time-PCR和Western blotting检测融合蛋白的表达。 结果: 经双酶切和DNA测序鉴定,证实pcDNA3.1/SET-TAP真核表达载体构建成功,将该载体转染L-02细胞后,融合蛋白在肝细胞中获得高效表达。 结论: 该结果为研究SET在三氯乙烯致肝细胞毒性的蛋白质相互作用以及三氯乙烯致机体损伤的机制奠定了基础。

关键词 三氯乙烯 癌蛋白SET 重叠PCR 串联亲和纯化 融合表达

Construction of the Eukaryotic Expression Vector Carrying Tandem Affinity Purification Tag for Oncoprotein SET and Its Expression in L-02 Liver Cells

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Abstract BACKGROUND AND AIM: To construct the vector pcDNA3.1/SET-TAP carrying tandem affinity purification tag for the differentially expressed protein SET which can be used to further study protein-protein interactions of SET in L-02 liver cells. MATERIALS AND METHODS: Total RNA was extracted from L-02 liver cells: the open reading frame of SET was isolated by using RT-PCR and the TAP gene was amplified from plasmid.Adopting overlap PCR to construct the fusion gene(SET-TAP) through a chimeric primer,then the fusion gene and pcDNA3.1/zeo(+) were digested by EcoRⅠand XhoⅠ.The recombinant vector pcDNA3.1/SET-TAP was constructed through T4 ligase for 16 h at 16 ℃:and then transformed into E.coli DH 5α.After the sequence was confirmed by using double enzyme digestion and sequence analysis:L-02 liver cells were transiently transfected with recombinant vector via Lipofectamine 2000.The expression of fusion protein was preliminary detected by using real-time PCR and western blotting. RESULTS: Results from double enzyme digestion and sequencing showed that the fusion gene was correctly inserted into the vector pcDNA3.1/zeo(+).The pcDNA3.1/SET-TAP could transcribe and express the fusion protein effciently in L-02 liver cells verified by real-time PCR and western blotting. CONCLUSION: The recombinant vector pcDNA3.1/SET-TAP was successfully constructed,laying the foundation to further study the protein-protein interactions of oncoprotein SET in L-02 liver cells treated with trichloroethylene.

Keywords trichloroethylene oncoprotein SET overlap PCR fusion expression tandem affinity purification

DOI

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