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人脑内一含有ACP样结构域新基因的发现

Exploring a New Gene Containing ACP Like Domain in Human Brain and Expression It in *E. coli*

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中文摘要:

为寻找脑内新基因,以正常成人全脑cDNA为模板,采用锚定PCR方法进行扩增,将经琼脂糖DNA电泳鉴定获得的一约1 200 bp大小的特异性条带回收,并克隆入T easy载体.用310 Genetic Analyzer进行自动测序.所得序列进行生物信息学分析: BLAST相似性分析结果证明所得序列为新序列,读框分析表明,该序列中存在一完整编码区,编码含357个氨基酸的蛋白质.ProDom软件分析发现其含有酰基携带蛋白(ACP)样结构域.随后,经3′RACE法克隆到该基因的全长cDNA,其全长为2 024 bp,染色体定位在14q11.2,含有16个外显子,15个内含子,该基因已登录到GenBank.经设计编码区引物,从T easy载体扩增出编码区后再克隆入pGEX-4T1表达载体,经异丙基硫代-D-乳糖苷(IPTG)化学诱导表达.其编码区克隆入pGEX-4T1表达载体后,转入JM109宿主菌,经IPTG诱导已得到表达.点杂交及RNA印迹表明,该基因在正常成人脑内广泛高表达.

英文摘要:

To look for new genes from human brain, get a fragment was obtained using adaptor primer and 3' anchor polymerase chain reaction (PCR) with the human adult whole brain cDNA as template. The fragment was cloned into T easy vector and automatically sequenced with 310 Genetic An alyzer. Later the whole length cDNA of this novel gene was got with the method of 3' rapid amplification of cDNA end (RACE). The whole length of cDNA of this novel gene is 2 024 bp. Chromsome location is at 14q11.2 including 16 extrons and 15 introns. After scanning the sequence against GenBank it is proved that the sequence is a new one. ORF analysis showed that there is a complete coding region in it, it can interprate a protein containing 357 amino acid residules. ProDom analysis result showed that there is an acyl carrier protein (ACP) like domain in it. The gene was banked into GenBank. Then, a pare of primers were designed and were used to amplify the coding region and cloned into pGEX-4T1 expressing vector to express it in E. coli. The Dot blotting and Northern blot showed that this novel gene is highly expressed in the normal adult human brain.

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