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用RACE结合cDNA文库筛选的方法获取新的锌指蛋白基因 Isolation of New Zinc Finger Genes through cDNA Library Screening Combined with RACE

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摘要　大多数有重要功能的蛋白质都含相应的由保守氨基酸顺序组成的功能结构域。本文首先根据蛋白质功能结构域保守氨基酸序列设计简并引物，用PCR方法扩增出基因EST序列，再利用改进的快速扩增cDNA末端（RACE）方法从cDNA文库中扩增出基因非同源部位，然后以非同源序列为探针，筛选cDNA文库。利用此方法成功地从人骨髓cDNA文库中克隆到几个编码锌指蛋白并代表原有EST的新的全长cDNA。这一策略也应适用于筛选编码具有其他序列保守性功能结构域蛋白的基因。

Abstract:Most of the important functionally proteins contain the corresponding function domains that consist of conserved amino acid sequences. The study provided a method to identify novel genes that encode proteins containing important functionally domains with conserved sequences. First, primers were designed according to the sequence of the cDNA library vector and the ESTs that have been obtained by reverse PCR and degenerate primers encoding Zinc finger domain. The cDNA library DNA was used as template for PCR amplification. The amplified fragment that contains nonhomologous sequences of the cDNA was inserted into pGEM-T easy vector. The fragment was recovered and used as a probe for screening the cDNA library. Several cDNAs with full length that encode proteins with Zinc finger domain and represent the original ESTs have been successfully cloned from a human bone marrow cDNA library. This strategy can also be used in screening genes that encode proteins containing differential function domains with conserved sequences.

关键词　基因克隆　快速扩增cDNA末端（RACE）　非同源DNA序列　cDNA文库筛选　Key words　gene cloning　rapid amplification of cDNA end (RACE)　nonhomologous DNA sequences　cDNA library screening

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