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论著

小鼠pdx-1基因真核表达载体的构建及其在胚胎干细胞中的表达

周光纪[1]；徐海伟[2△]；杨丽[2]；唐军[2]；李达兵[2]；屈纪富[3]

1 广东医学院基础部生理学教研室，广东 湛江 524023； 第三军医大学 2 生理学教研室， 3 附属西南医院急诊科， 重庆 400038

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摘要 目的： 克隆小鼠pdx-1基因，构建其真核表达载体，并在小鼠胚胎干细胞中表达，为糖尿病的细胞移植治疗奠定基础。方法： PCR扩增小鼠胰腺pdx-1基因 cDNA，酶切后和携带绿色荧光蛋白报告基因的真核表达载体pEGFP-N1重组，将pdx-1基因 cDNA片段连接到pEGFP-N1载体的多克隆位点，形成重组载体pEGFP/pdx-1，转化大肠杆菌DH5α菌株，构建成pdx-1基因真核表达载体质粒。扩增DH5α后抽提质粒DNA，Hind Ⅲ 和BamH Ⅰ酶切，电泳，DNA测序鉴定。鉴定正确的质粒DNA用脂质体包裹后转染小鼠胚胎干细胞MESPU13。结果： 从小鼠胰腺cDNA扩增出876 bp的DNA片段并成功重组到pEGFP-N1载体中。经酶切和DNA测序验证，插入载体的DNA片段为pdx-1基因，插入方向正确。重组质粒经脂质体转染胚胎干细胞MESPU13，24 h 后观察到绿色荧光蛋白报告基因和目的基因的pdx-1表达。结论： 小鼠pdx-1基因的克隆和真核表达载体构建获得成功，为进一步研究其功能奠定了基础。

关键词 基因,pdx-1 胚胎干细胞； 真核表达载体

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Construction of mouse pdx-1 gene eukaryotic expression vector and its expression in embryonic stem cells

ZHOU Guang-ji[1],XU Hai-wei[2],YANG Li[2],TANG Jun[2],LI Da-bing[2],QU Ji-fu[3]

1 Department of Physiology, Guangdong Medical College, Zhanjiang 524023, China; 2 Department of Physiology, 3 Emergency Department, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

Abstract

<FONT face=Verdana>AIM: To clone mouse pdx-1 gene and construct its eukaryotic expression vector for expression of pdx-1 in mouse embryonic stem cells.
METHODS: Mouse pdx-1 cDNA fragment was amplified with polymerase chain reaction (PCR) from mouse pancreatic cDNA. The purified fragment was recombinated with a eukaryotic expression vector carrying enhanced green fluorescent protein, pEGFP-N1. The pdx-1 cDNA fragment was inserted into the multi-clone sites of the vector to construct a new plasmid, pEGFP/pdx-1. E.colli strain DH5α was transfected with the new recombinant plasmid to expand it. Plasmid DNA extracted from the expanded DH5α was identifed by cutting with Hind Ⅲ, BamH Ⅰ nuclease and by DNA sequencing. Identified plasmid DNA was transfected into mouse embryonic stem cell line MESPU13 by carrying with liposome.
RESULTS: A 876 bp cDNA fragment was amplified from mouse pancreatic cDNA by PCR and it was inserted into the vector pEGFP-N1 correctly. The fragment was defined to be pdx-1 gene by nuclease digestion and DNA sequencing. Mouse embryonic stem cell line MESPU13 was transfected with the new recombinant plasmid DNA. The green fluorescent protein report gene and pdx-1 gene expressed in transfected mouse embryonic stem cells within 24 h.
CONCLUSION: Mouse pdx-1 gene is cloned and its recombinant eukaryotic expression vector carrying green fluorescent protein is constructed successfully. It provides a useful tool for further research on the function of pdx-1.</FONT>

Key words Genes pdx-1 Embryonic stem cells Eukaryotic expression vector

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