遗传 2004 26 (5): 589-593 ISSN: 0379-4172 CN: 11-5450/R

应用PCR技术对先天性长QT综合征KCNQ1 基因进行定点突变的研究

PCR Site-Directed Mutagenesis of Long QT Syndrome KCNQ1 Gene in vitro

李 伟, 杨钧国,任法鑫,康彩练,张守焰LI Wei,YANG Jun-Guo,REN Fa-Xin, KANG Cai-Lian, ZHANG Shou-Yan

华中科技大学同济医学院心血管病研究所，武汉430022Institute of Cardiology, Tongji Medical College, Huazhong Science and Technology University, Wuhan 430022, China

收稿日期 修回日期 网络版发布日期 接受日期

摘要

利用聚合酶链反应（PCR）技术对长QT综合征（LQTS）KCNQ1基因进行定点突变的研究。首先设计两对引物（包含预定的突变），通过3轮PCR扩增，扩增出含有所需突变位点的片段，然后将片段克隆入T载体中，通过酶切连接的方法将突变点引入到pIRES2-EGFP-KCNQ1中，随后用Effectene转染试剂介导转染HEK293细胞。结果在真核表达载体pIRES2-EGFP-KCNQ1基础上获得了KCNQ1 cDNA C934T的突变体，测序表明在序列中发生了预期的突变。将含突变点的pIRES2-EGFP-KCNQ1转染HEK293细胞后，在荧光显微镜下观察到被转染的HEK293细胞发出绿色荧光，表明含突变点的pIRES2-EGFP-KCNQ1得到了表达。Abstract: To study PCR site-directed mutagenesis of long QT syndrome KCNQ1 gene in vitro. The site-directed mutagenesis of LQTS gene KCNQ1 was made by PCR. Two sets of primers were designed according to the sequence of KCNQ1 cDNA, and mismatch was introduced into primers. Mutagenesis was performed in a three-step PCR. The amplified fragments from the third PCR which contained the mutation site were subcloned into the T-vecor PCR2.1.Then the fragments containing the mutation site was obtained from PCR2.1 with restriction enzyme digestion and was inserted into the same restriction site of pIRES2-EGFP-KCNQ1. With Effectene Transfection Reagent, pIRES2-EGFP-KCNQ1 was transfected into HEK293 cell. The sequencing analysis showed that the mutation site was correct. Mutation from T to C in 934 site of KCNQ1 cDNA was found. Under the fluorescence microscope, the green fluorescence was spread in the transfected HEK293 cell, meaning the pIRES2-EGFP-KCNQ1 containing the mutation site was expressed correctly.

关键词 长QT综合征 KCNQ1 PCR 定点突变 Key words long QT syndrome KCNQ1 PCR site-directed mutagenesis

分类号

扩展功能

本文信息

Supporting info

PDF(0KB)

[HTML全文](0KB)

参考文献

服务与反馈

把本文推荐给朋友

加入我的书架

加入引用管理器

复制索引

Email Alert

文章反馈

浏览反馈信息

相关信息

本刊中 包含“长QT综合征”的相关文章

本文作者相关文章

- 李 伟
- 杨钧国
- 任法鑫
- 康彩练
- 张守焰LI Wei
- YANG Jun-Guo
- REN Fa-Xin
- KANG Cai-Lian
- ZHANG Shou-Yan

Abstract

Key words

DOI:

通讯作者