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基础研究

抑癌基因BRCA1启动子荧光素酶报告基因的构建及活性测定

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摘要:

目的:克隆人BRCA1基因的启动子,构建荧光素酶报告基因载体,并在细胞内检其活性，为其后续基因调控研究提供依据。方法:采用PCR技术,从人正常宫颈组织细胞中扩增出BRCA1启动子,插入荧光素酶报告基因载体pGL3-basic中,测序所扩增的DNA序列,将其转染入HCT 116细胞中并检测其活性。结果:酶切及基因测序方法证实所构建质粒含有pGL3-basic全序列及BRCA1启动子上游调控序列,扩增的BRCA1启动子序列正确;双报告基因实验检测荧光素酶活力表明,p53缺失的HCT116细胞中BRCA1启动子明显增加（P<0.05），构建的报告基因具有启动子活性。结论:克隆BRCA1启动子及成功构建人BRCA1启动子报告基因,可实现快速、经济和准确地克隆已知基因启动子分子和构建启动子载体的目的。

关键词: BRCA1;启动子;荧光素酶;报告基因

Construction and identification of human tumor suppressor gene BRCA1 promotor luciferase report gene vector

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Abstract:

Abstract:Objective To construct the human BRCA1 promotor luciferase report gene vector and detect its activity in cells. Methods The BRCA1 promoter from human normal cervix tissues
was amplified by PCR, and was inserted into the luciferase report gene pGL3-basic vector. The amplified DNA sequence was confirmed by sequencing and then the constructed vector was transfected into HCT116 cells to detect its activity by Premaga Dual-luciferase report gene detection system. Results The recombinant plasmid was tested by gel electrophoresis and sequencing analysis, it was proved that the plasmid included pGL3-basic DNA sequence and PRL regulating sequence.The sequencing results indicated that the amplified sequence was correct, in p53 minus HCT116 cells the number of BRCA1 promoter was increased (P<0.05), and the luciferase activity detection result demonstrated that the constructed vector had the promotor activity.Conclusion The human BRCA1 promotor luciferase report gene vector has been constructed successfully, and it will become essential material for further study on the function of BRCA1 regulation.

Keywords: BRCA1 promotor luciferase report gene

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