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论著

线粒体细胞色素C氧化酶RNAi慢病毒载体的构建

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摘要 目的：通过构建携带细胞色素C氧化酶基因的RNAi慢病毒载体，获得可供转染的滴度，为下一步研究该基因缺陷在真核细胞中的影响提供物质基础。 方法： 根据线粒体细胞色素C氧化酶设计的两条互补的单链寡核苷酸退火后形成双链，插入到pENTR/U6质粒缺口末端，连接在质粒上生成含RNAi盒的pENTR/U6载体；通过重组作用将pENTR/U6载体的RNAi盒重组到pLenti6/BLOCK-iT-Dest 载体上,构建含U6启动子、靶序列和Pol III终止子表达框的MTCOX-I shRNA表达重组体；经脂质体导入293FT细胞，包装成慢病毒，收集病毒上清并检测其滴度。Western blotting检测干扰后细胞内线粒体细胞色素C氧化酶I亚基的表达。 结果： 将目的序列成功连接到载体上，并经测序分析证实载体构建成功；成功包装成高滴度的慢病毒。Western blotting检测结果证实构建的MTCOX-I shRNA表达重组体可显著抑制线粒体细胞色素C氧化酶I亚基的表达。 结论： 成功构建了携带细胞色素C氧化酶基因的RNAi慢病毒载体。

关键词 基因； 线粒体； 重组； 慢病毒属； 载体； 细胞色素C氧化酶

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The construction of lentivirus-mediated RNAi vector containing cytochrome C oxidase

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Abstract

<FONT face=Verdana>AIM: To construct a recombinant lentivirus RNAi vector carrying cytochrome C oxidase gene to obtain the titer of the lentiviral stock for investigation of the expression in the eukaryotic cell and the affection of the COX gene silencing in the eukaryotic cells. METHODS: According to the DNA of the cytochrome C oxidase gene, we designed and synthesized complementary single-strand DNA oligos, annealed the single-stranded oligos to generate a ds oligo, cloned the ds oligo into pENTR/U6 to obtain an entry clone; An LR recombination reaction was performed between the pENTR/U6 entry construct and pLenti6/BLOCK-iT-Dest to generate expression construct, the 293FT cell line was cotransfected with pLenti6/BLOCK-iT expression construct, and the viral packaging mix, viral supernatant was harvested to determine the titer. RESULTS: The DNA sequence of interest clone to the vector was constructd to generate an entry clone and an expression clone successfully, which were proved by sequence determination. A vector producing cell line 293FT was established， and the titer for transfection was obtained. Western blotting analysis demonstrated that COX shRNA expression construction could suppress the expression of MTCOX-I. CONCLUSION: A lentivirus RNAi vector containing cytochrome C oxidase gene was successfully constructed.</FONT>

Key words Genes Mitochondria Recombinant Lentivirus Vectors Cytochrome-C oxidase

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