论著

携带mIL-12基因逆转录病毒载体的构建与表达

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摘要 目的:构建携带小鼠IL-12(mouse IL-12, mIL-12)基因逆转录病毒表达载体pLXmIL-12SN并检测其在B16F10细胞中的表达。 方法:应用限制性内切酶从载体pGCp35-IRES-p40SN中酶切p35-IRES-p40(mIL-12基因片段)及将逆转录病毒载体pLXSN切开,通过连接酶连接,通过序列测定确定p35-IRES-p40基因片段正向插入载体pLXSN中的重组子,将阳性重组子转染B16F10细胞并通过RT-PCR方法检测mIL-12基因在B16F10细胞中表达。 结果:成功地构建表达载体pLXmIL-12SN并可在mRNA水平检测到mIL-12基因在B16F10细胞中表达。 结论:载体pLXmIL-12SN的构建成功及可在B16F10细胞中表达,为mIL-12基因治疗眼部疾病的研究奠定了基础和提供了借鉴。

关键词 基因,白细胞介素-12; 载体构建; 表达

分类号 R363

Construction and expression of retroviral vector containing mIL-12 gene

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Abstract

AIM: To construct retroviral expression vector pLXmIL-12SN containing mouse IL-12 (mIL-12) gene and detect the gene expression in B16F10 cells. METHODS: Vector pGCp35-IRES-p40SN was digested to obtain p35-IRES-p40 (mIL-12 gene fragment) and retroviral vector pLXSN was digested by restriction enzyme, and then they were linked by ligase. The recombinant vector that mIL-12 gene fragment had correctly been inserted into pLXSN was identified by sequencing. The recombination vector with mIL-12 gene was transfected into B16F10 cell and was detected by RT-PCR. RESULTS: Expression vector pLXmIL-12SN was successfully constructed. mIL-12 gene was confirmed to express in B16F10 cells at the level of mRNA. CONCLUSION: Acquired vector pLXmIL-12SN and confirmed mIL-12 gene expression lays a foundation for mIL-12 gene therapy to eye diseases.

Key words Genes interleukin-12 gene Vector construction Expression

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