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大鼠Nogo受体基因RNA干扰慢病毒载体的构建与鉴定 点此下载全文

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摘要:

目的:构建大鼠Nogo受体(Nogo receptor, NgR)基因RNA干扰慢病毒表达载体, 并观察其对293T细胞感染效率. 方法: 应用基因工程技术, 首先构建携带目的基因siNgR199的慢病毒穿梭质粒表达载体, 然后使用慢病毒包装质粒混合物和构建好的慢病毒穿梭质粒共转染293T细胞, 转染48 h后收集上清离心过滤后冰浴保存, 最后进行病毒滴度测定, 与标准病毒液分别感染293T细胞, 按MOI值分为5个梯度:1、3、5、10、20, 以确定病毒滴度及适合感染的MOI值. 其中采用PCR方法对重组载体进行鉴定, 利用绿色荧光蛋白作为报告基因, 对病毒滴度和感染效率进行检测. 结果: 酶切鉴定及PCR结果与病毒载体的预期结果一致, 病毒滴度达1×108 ifu/ml, 适合感染的MOI值为3. 结论: 应用基因工程技术, 成功构建了大鼠siNgR199慢病毒表达载体, 为应用于治疗脊髓损伤后轴突再生创造了条件.

关键词: 慢病毒 Nogo受体 RNA干扰

Construction and identification of a recombinant lentivirus harboring RNAi targeting rat Nogo receptor gene Download Fulltext

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Fund Project: Supported by National Natural Science Foundation of China (30471757), the Program for Changjiang Scholars and Innovative Research Team, and the Program for Outstanding Academic Leaders in Shanghai.

Abstract:

Objectire: To construct a recombinant lentivirus harboring RNAi sequence targeting rat nogo receptor gene and to observe its infection efficiency of 293T cells. Methods: Lentivirus shuttle plasmid containing siNgR199 cDNA was constructed by gene engineering and was used to transfect 293T cells in the presence of packaging plasmids. Forty-eight hours later the supernatant was collected and the titer of virus was determined. The recombinant lentivirus and the standard lentivirus were used to transfect 293T cells at 1 MOI, 3 MOI, 5 MOI, 10 MOI and 20 MOI. Polymerase chain reaction (PCR) was used to detect the recombinant vectort enhanced green fluorescent protein (EGFP) expression was used to determine the titer and the infection rate of the recombinant lentivirus under fluorescent microscope. Results: Restriction endonuclease and PCR analysis confirmed that the siNgR199 cDNA was successfully inserted into the lentivirus vector. The titer of the recombinant lentivirus harboring siNgR199 was 1 ×10^8 ifu/ml and the best MOI was 3. Conclusion- The recombinant lentivirus containing siNgR199 gene has been successfully constructed, which lays a foundation for future axon regeneration in treatment of spinal cord injury

Keywords: lentivirus Nogo receptor RNA interfering

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