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论著

NGAL 基因5’侧翼区转录调控元件萤火虫荧光素酶报告基因表达载体的构建与鉴定

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摘要 背景与目的：构建NGAL 基因5’侧翼区转录调控元件萤火虫荧光素酶报告基因表达载体。材料与方法：以PCR法从SHEEC食管癌细胞基因组DNA中扩增NGAL基因5’侧翼转录调控区不同长度片段G0～G6(－1431～＋84)。 PCR产物经pGEM－T easy转载，再定向亚克隆插入pGL3－Basic。重组子通过特异限制性内切酶切割，琼脂糖电泳予以鉴定。结果：成功构建NGAL 基因5’侧翼区转录调控元件萤火虫荧光素酶报告基因表达载体7个，pGLB－G0～G6。结论：为借助于双荧光素酶报告基因检测系统研究确定NGAL基因5’侧翼转录调控区转录调控元件的分布特点以及转录调控元件与转录调控蛋白之间相互作用的性质提供了基本实验条件。

关键词 NGAL基因；基因表达调控；荧光素酶；报告基因；载体构建

Constructing and Identifying the Firefly Luciferase Report Gene Vectors For 5' Flanking Regulated Elements of NGAL Gene

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Abstract BACKGROUND & AIM:To construct the firefly luciferase report gene vectorsfor5'flanking regulated elements of NGAL gene. MATERIAL AND METHODS:The DNA fragments G0～G6 (-1431～＋84) of 5'flanking of NGAL gene were amplified from the genomic DNAs of esophageal cancer cell line SHEEC by using PCR. The PCR products were cloned into pGEM-Teasx vector , then directionally subcloned into pGL3-Basic vector. The recombined clones were identified by agarose gel electrophoresis after restriction endonuclease digesting. RESULTS:7 expression vectors , pGLB-G0～G6 , for 5'flanking regulated elements of NGAL gene had been constructed. CONCLUSION:These expression vectors offered the basic experimental conditions for studying the distribution characters of NGAL gene 5' flanking regulation elements and the properties of interactions between the elements and regulation proteins with the help of dual luciferase report gene assay system.

Keywords NGAL gene regulation of gene expression luciferase report gene construction vector

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