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技术与方法

小鼠H2B-GFP真核表达载体的构建与鉴定

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摘 要 目的 构建小鼠H2B-绿色荧光蛋白（GFP）真核表达载体，为动态观察小鼠细胞中染色体的形态变化，进一步研究小鼠耐药细胞中双微体（DMs）的形成机制提供有效的分子工具。方法 利用RT-PCR的方法获得小鼠H2B cDNA，以羧基端插入pcDNA3.1/CT-GFP-TOPO载体上，构建H2B-GFP真核表达载体，经菌液PCR、酶切及DNA测序鉴定插入片段大小、方向及序列的正确性，提取质粒转染小鼠胚胎成纤维细胞系NIH3T3进行鉴定。结果 经菌液PCR、酶切和测序，证明小鼠H2B-GFP真核表达载体含有大小、方向及序列正确的H2B cDNA片段，转染NIH3T3后在细胞核中表达。结论 作者成功构建了同时携带有G418筛选位点及多酶切位点的小鼠H2B-GFP真核表达载体，为其在小鼠体外培养细胞中的表达奠定了基础。

关键词 H2B 绿色荧光蛋白 真核表达载体 小鼠

分类号

Construction and identification of mouse H2B-GFP eukaryotic expressing vector

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Abstract Objective To construct a fluorescent eukaryotic expression vector coding mouse H2B gene. Methods The mouse H2B cDNA was amplified by RT-PCR from the total RNA and inserted into pcDNA3.1/CT-GFP-TOPO vector. The structure of the recombinant vector has been verified by PCR amplification, restriction analysis and sequencing. Then the reporting vector was transfectted into mouse fibroblast cell line NIH3T3. Result The recombinant fluorescent expression vector coding mouse H2B gene was correctly constructed in size, orientation and sequence, and which was expressed in the nucleus of NIH3T3. Conclusion The recombinant mouse H2B-GFP expression vector has been successfully constructed, which can help to study the biochemical role of H2B and chromosomes morphologic varietion.

Key words H2B green fluorescent protein eukaryotic expressing vector mouse

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