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基础研究

Smad2/3/4真核表达质粒的构建及重组蛋白表达

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摘要:

目的:构建pcDNA3.1myc-HisA-Smad2/3/4真核表达质粒，证实融合蛋白在细胞内表达。方法:以pcDNA3.1-Smad2/3和pGEX2T-Smad4质粒为模板，设计特异性引物，PCR扩增Smad2/3/4全长编码基因，亚克隆至含有pcDNA3.1myc-HisA 标签的真核表达载体中。将构建的重组质粒测序并转染到人胚胎肾细胞HEK293 中，提取细胞蛋白进行 Western blotting 检测。结果：Smad2/3/4 全长基因序列克隆到真核表达载体pcDNA3.1myc-HisA中，酶切鉴定片段为1 401、1 275和1 656 bp。Western blotting检测到融合蛋白pcDNA3.1myc-HisA-Smad2/3/4的表达。结论:成功构建pcDNA3.1myc-HisA-Smad2/3/4真核表达质粒，同时鉴定其融合蛋白的表达。

关键词： Smad；蛋白免疫印记；融合蛋白

Construction of |recombinant plasmid pcDNA3.1myc-HisA- Smad2/3/4 and its protein expression

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Abstract:

To construct the expression plasmid of pcDNA3.1myc-HisA-Smad2/3/4 and identify its fusion protein expression.Methods pcDNA3.1- Smad2/3 and pGEX2T-Smad4 were used as templates,and the special primers were designed.The Smad2/3/4 coding sequence was amplified by polymerase chain reaction (PCR) method and subcloned into pcDNA3.1myc-HisA vector. After the target region was sequenced,the plasmid was transfected into HEK293 cell line. The expression of the recombinant plasmid in HEK293 cells was detected by Western blotting.Results Smad2/3/4 was constructed into expression vector pcDNA3.1myc-HisA successfully.The lengthes of the fragments were 1 401,1 275 and 1 656 bp,and they were identified by restriction enzymes digestion.The expression of pcDNA3.1myc-HisA-Smad2/3/4 fusion protein was proved by Western blotting.Conclusion The eukaryotic expression plasmid pcDNA3.1myc-HisA-Smad2/3/4 is successfully constructed,and the expression of pcDNA3.1myc-HisA-Smad2/3/4 fusion protein is identified.

Keywords: Smad;Western blotting;fusion protein

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