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ZNF230/荧光蛋白融合基因表达载体的构建及其在Cos细胞中的表达与定位Construction of Recombinant ZNF230/GFP Fused Plasmids and Their Expression and Cellular Localization

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摘要

为了用绿色荧光蛋白标记观察人类无精症相关基因ZNF230在Cos7细胞中的蛋白质表达及定位，用PCR方法扩增得到突变的人和小鼠mt-ZNF230和mt-znf230基因，使其3′端的终止密码TGA突变为TGG，并装入T-载体，双酶切后通过定向克隆将其与真核表达载体pEGFP-N1的绿色荧光蛋白（green fluorescence protein，GFP）基因融合，构建了ZNF230—荧光蛋白融合基因表达载体。然后经真核表达质粒－脂质体介导，导入Cos7细胞系。荧光显微镜观察显示：在空白载体pEGFP-N1转染的Cos细胞中荧光布满整个细胞，而在转染阳性载体pEGFP-ZNF230的Cos细胞中荧光主要聚集在细胞核中。表明转染的Cos细胞系能高效表达人ZNF230和小鼠znf230蛋白，ZNF基因表达的蛋白定位于细胞核内。Abstract: To use green fluorescent protein as a marker to studythe localization of the fusion protein, the mutant full length cDNAs of human ZNF230 and mouse znf230 with their stop codon TGA changed to TGG were obtained by PCR amplification., and then cloned into pGEM-Teasy vector. After the double enzyme cutting, the mutated human and mouse ZNF230(znf230) were inserted into mammalian expression plasmid pEGFP-N1.Thus we constructed the plasmid with fusion gene of ZNF230 and green fluorescent protein(GFP).Then the Cos cell was transfected with the fused gene by liposome. Fluorescence microscopy showed that green fluorescence protein expressed over the whole cell when transfected with vector pEGFP-N1.While after the transfection with pEGFP-ZNF230, the fluorescence located mainly on the nuclei of the cells. We demonstrated that the transfected Cos cell line can express human ZNF230 and mouse znf230 with high efficiency.When transfected with the constructed recombinant pEGFP-ZNF230 vector, the ZNF230 protein localizes mainly on the nucleus.

关键词 ZNF230基因 绿色荧光蛋白 基因表达定位 Cos细胞 Key word ZNF230 gene green fluorescent protein localization of gene expression Cos cell
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Abstract

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