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畜牧—研究报告

牛胎儿成纤维细胞的分离培养及转染线虫ω-3脂肪酸去饱和酶基因

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摘要:

为了为核移植法制作转线虫ω-3脂肪酸去饱和酶基因克隆牛提供供体细胞，体外分离培养牛胎儿成纤维细胞并进行了线虫ω-3脂肪酸去饱和酶基因转染。首先采用组织块贴壁法分离培养牛胎儿成纤维细胞，线虫ω-3脂肪酸去饱和酶基因表达载体以脂质法介导转染牛胎儿成纤维细胞，通过G418筛选获得稳定转染的细胞克隆，PCR 鉴定外源基因在细胞基因组中的整合，对阳性克隆进行传代2-3次后利用RT-PCR检测外源基因的转录。结果表明，组织块贴壁后7天可获取原代成纤维细胞，基因转染后利用G418筛选9天即可获得转基因细胞，对转基因细胞进行传代，PCR 检测显示CMV启动子和ω-3脂肪酸去饱和酶基因整合到细胞基因组中，RT-PCR检测显示ω-3脂肪酸去饱和酶基因在转基因传代细胞中得到有效转录。本研究为下一步通过核移植方法获得转基因肉牛提供了条件。

关键词: 脂质体

In vitro Culture of Bovine fetal Fibroblast Cells and Caenorhabditis elegans ω-3 Fatty Acid Desaturase Gene Transfection

Abstract:

In order to offer doner cells for bovine transgenic cloning by nuclear transfering, fibroblast cells were isolated by attaching tissue explants from bovine fetal. The plasmid containing genes of Caenorhabditis elegans ω-3 fatty acid desaturase Gene was transfected into bovine fetal fibroblast by Lipofectmine, Cell clones were obtained after screening by G418. The recombinant of extrogenous DNA was identified by polymerase chain reaction，the positive colonies were maintained in culture medium containing G418 for 2-3 passages. the transcription of extrogenous DNA was identified by reverse transcription-polymerase chain reaction. Results showed that primary bovine fibroblast cells were isolated from the tissue cultured for 7 day，and the transgenic cells were obtained after G418 selection for 9d. Identification of the transgene in the cell clones was examined by PCR and the exogenous DNA (CMV promoter and Caenorhabditis elegans ω-3 fatty acid desaturase gene) had been integrated into genome. The transgenic cell line could transcript the Caenorhabditis elegans ω-3 fatty acid desaturase DNA efficiently.after passage These results have paved the way to obtain the new transgenic bovine by nuclear transfer in the future.

Keywords: lipofectmine

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2．井乐刚 赵新淮.复合维生素脂质体的制备[J]. 中国农学通报, 2011,27(第4期2月): 330-333