

实验研究

柔嫩艾美球虫杂交株F2 SO₇基因重组鸡痘病毒的构建和筛选

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摘要

目的 构建柔嫩艾美球虫 (*E. tenella*) 杂交株F2 SO₇基因重组鸡痘病毒。方法 将柔嫩艾美球虫杂交株F2 SO₇基因, 插入到以胸苷激酶 (TK) 基因为侧翼的鸡痘病毒表达载体pUTA2中的复合启动子下游, 获得重组表达质粒pUTA-SO₇。用脂质体将重组表达质粒转染鸡痘病毒 (FPV) 感染的鸡胚成纤维细胞 (CEF), 培养、收获病毒后, 用含40 mg/L 5-溴-2-脱氧尿嘧啶 (BrdU) 的培养液, 在TK基因阳性 CEF细胞中筛选培养2代, 然后用不含BrdU的培养液进行病毒噬斑纯化以筛选rFPV。结果 PCR扩增可见650 bp左右蛋白条带, 间接荧光抗体试验 (IFAT) 可见重组病毒感染细胞表面有绿色荧光物质, 蛋白质印迹分析 (Western Blotting), 在相对分子质量 (*Mr*) 36 000处有1条特异条带, 证实了重组病毒在CEF中表达了SO₇基因。结论 成功筛选出表达*E. tenella*杂交株F2 SO₇基因的重组鸡痘病毒。

关键词 [柔嫩艾美球虫](#) [SO₇](#) [重组](#) [鸡痘病毒](#)

分类号

Construction and Screening of Recombinant Fowlpox Virus Expressing *Eimeria tenella* F2 Hybrid Strain SO₇ Gene

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Abstract

Objective To Construct the recombinant fowlpox virus expressing *Eimeria tenella* F2 hybrid strain SO₇ gene. Methods A recombinant expression plasmid pUTA-SO₇ was constructed by inserting the SO₇ gene of *Eimeria tenella* F2 hybrid strain into downstream of a hybrid poxvirus promoter which was flanked by the TK gene of fowlpox virus (FPV). The constructed pUTA-SO₇ was firstly transfected into chicken embryo fibroblast cells (CEF) pre-infected with FPV strain 282E4 by using liposome, then the viruses resulted from the transfection were selected for 2 passages by culturing in CEF cells with MEM medium containing 40 mg/L 5-bromo-2-deoxy-uridine (BrdU). The selected viruses were plaque-purified in CEF cultured with MEM medium without BrdU. Results Polymerase chain reaction (PCR), indirect immunofluorescence assay and Western blotting showed that SO₇ gene was expressed in recombinant fowlpox virus. Conclusion The recombinant FPV (rFPV) expressing the SO₇ gene has been obtained.

Key words [Eimeria tenella](#) [SO₇](#) [Recombinant](#) [Fowlpox virus](#)

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