

论文

H7N9禽流感病毒重组质粒构建及应用

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摘要:

目的 构建一种含H7N9禽流感病毒全长血凝素/神经氨酸酶/基质蛋白(HA/NA/M)基因的重组质粒,为核酸检测方法提供一种通用的阳性定量标准品。方法 设计H7N9禽流感病毒HA、NA及M抗原基因全长开放阅读框的克隆引物,提取H7N9禽流感病毒总RNA后用实时荧光定量PCR获得相应片段,用3次酶切连接方法,依次插入到pGEM-T easy质粒,进行测序确认;线性化后的重组质粒用T7 RNA聚合酶进行体外转录, RNA转录产物纯化后测定浓度,用实时荧光定量PCR构建标准曲线进行验证。结果 HA、NA和M基因扩增片段大小分别约为1.7、1.3、1.1 kb,与预期相符;构建的重组质粒pGEM-HA-NA-M插入片段的测序结果与GenBank 公布序列一致;由重组质粒体外转录获得同时含有H7N9禽流感病毒HA、NA、M全长开放阅读框序列的RNA片段质量浓度为399.5 ng/μL,梯度稀释后用3种实时荧光定量PCR方法均获得了良好的标准曲线。结论 成功构建重组质粒pGEM-HA-NA-M,由此质粒体外转录获得的RNA片段可作为H7N9禽流感病毒核酸快速检测方法通用的阳性定量标准品。

关键词: H7N9禽流感病毒 血凝素 神经氨酸酶 基质蛋白 标准品

Construction and application of H7N9 avian influenza virus gene recombinant plasmid pGEM-HA-NA-M

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Abstract:

Objective To construct a combinant plasmid of full length HA/NA/M gene of H7N9 avian influenza virus and to provide quantitative reference for pathogen detection. Methods According to specific sequence of HA/NA/M gene of H7N9 avian influenza virus, the primers were designed and synthesized. Total RNA extracted from H7N9 avian influenza virus and the cDNA of HA/NA/M were cloned by reverse transcription PCR(RT-PCR) and inserted into pGEM-T easy vector after three times of restriction enzyme assay. The linearized plasmids were used to transcript RNA *in vitro* by T7 RNA polymerases, then the products were purified and diluted to a series of standard concentrations of cRNA which was used as standard quantitative template of real-time fluorescence quantitative RT-PCR method. Results The amplified fragment by RT-PCR was of expected size and its sequence was in concordance with that published on GenBank. The cRNA including full-length HA/NA/M was obtained by *in vitro* transcription with the recombinant plasmid and the mass concentration was 399.5 ng/μl. The cRNA were diluted to precise quantification copy number, which were proved by real-time RT-PCR amplification. Conclusion The combinant plasmid pGEM-HA-NA-M was constructed successfully and *in vitro* transcription products of the plasmid can be used as a quantitative reference for the rapid detection of nucleic acid of H7N9 avian influenza virus.

Keywords: H7N9 avian influenza virus hemagglutinin neuraminidase matrix protein standard substance

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