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论著

荧光定量PCR用于重组杆状病毒鉴定及病毒滴度检测的研究

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摘要 目的：建立一种高效、简便的荧光实时定量PCR方法，用于重组杆状病毒鉴定及病毒滴度的检测。方法：利用Bac-to-Bac载体系统在昆虫细胞中构建含人IL-18基因的重组杆状病毒，收获的病毒母液以10倍梯度系列稀释后，提取病毒基因组DNA。以10倍梯度稀释的重组杆状病毒穿梭质粒（bacmid）作为标准模板，进行荧光定量PCR反应扩增IL-18基因片段并绘制标准曲线，然后以上述的重组杆状病毒基因组DNA作为模板，采用同样体系进行实时PCR反应检测，同时用琼脂糖空斑法测定病毒母液的滴度。结果：成功构建了重组杆状病毒并建立了病毒滴度的实时荧光PCR检测方法。运用标准模板进行的PCR反应显示该方法的线形范围为101-108拷贝，病毒母液的DNA拷贝浓度（vg/mL）值约为空斑检测的滴度 pfu/mL值的10倍。结论：荧光定量PCR方法可灵敏快速地鉴定重组杆状病毒，并在较大的线形范围内检测重组杆状病毒滴度，较之空斑法更准确地反映了重组杆状病毒的实际数量。

关键词 荧光定量PCR； 重组病毒鉴定； 病毒滴度

分类号 R363

Identification of recombinant baculovirus and determination of virus titer with fluorescence quantitative PCR assay

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Abstract

<FONT face=Verdana>AIM: To develop a real-time PCR assay based on TaqMan technology for the identification of recombinant baculovirus and determination of virus physical titers in Bac-to-Bac system.METHODS: The recombinant baculovirus containing human IL-18 gene was produced using Bac-to-Bac system.A 10-fold serially diluted primary viral stock was used for plaque assay and DNA extraction.Bacmid (baculovirus plasmid) was 10-fold serially diluted and served as standards.Real-time PCR amplification of the IL-18 gene was performed in triplicate for each diluted recombinant virus.At the same time,plaque assays were performed using overlay agarose method.RESULTS: The standard linear range (101 to 108 copies) for quantitation was achieved with the standard curve.We also find that the“vg/mL”titer value is generally about 10 times than“pfu/mL”titer of the same recombinant virus stock.
CONCLUSION: A TaqMan real-time PCR method is established to identify the recombinant baculovirus and determine the“vg/mL”titer of virus.The method is rapid and quantitative over a wide range of virus titers.</FONT>

Key words Fluorescence quantitative PCR Identification of recombinant baculovirus Virus titer

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