Quantitative Real-Time Polymerase Chain Reaction: A Rapid and Accurate Titer Determination of Recombinant Baculovirus

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Abstract

The baculovirus expression system has been increasingly popular among the production of recombinant proteins in insect cells. Hence, this elucidates the necessity for accurate titration of viral stocks in order to warrant the efficiency and reproducibility of various works involving the utilization of baculoviruses. Several traditional methods for the virus titration were deemed laborious and time-consuming. In this experiment, quantitative Real-Time Polymerase Chain Reaction (qRT-PCR), an alternative method for rapid and accurate titer determination of recombinant baculovirus stocks was explored. Primers and probes were custom-designed for the amplification of the AcMNPV envelope glycoprotein gp64 gene. Whilst plaque assays, a traditional method, for the titer determination of recombinant baculoviruses corresponding to those amplified in qRT-PCR, were carried out. The mean quantities obtained from the qRT-PCR and the titers determined by plaque assay were employed to derive a linear regression plot illustrating the relationship between the resultant mean quantities and titrated virus dilutions. Titers of recombinant baculovirus was then rapidly determined from the linear regression line obtained. It was observed that the titers determined by plaque assay and qRT-PCR are comparable. However, to further assess the robustness of linear regression curve, future experiments similar to those described above can be carried out.

Introduction

As the applications of recombinant baculoviruses continues to diversify, the need for accurate and rapid quantification of virus titers becomes increasingly important. However, traditional methods are deemed laborious, time-consuming and error-prone, and require expertise in cell culture and virus handling. In order to address these difficulties, several alternative methods has been discussed and proposed in prior studies. Albeit the effort of these innovations, the problems arose when performing large numbers of baculoviral titrations have yet to diminish. Therefore, quantitative Real-Time Polymerase Chain Reaction (qRT-PCR), an alternative method, which offers an accelerated and less complicated way for accurate titer determination of recombinant baculovirus stocks, was further explored in this study.

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**Materials and Methods**

qRT-PCR was conducted to amplify AcMPNV gp64 gene of various recombinant baculovirus stocks. On the other hand, a series of plaque assay has been carried out to determine the titers of the corresponding recombinant baculovirus stocks that are amplified. After which mean starting quantities were obtained from the standard curve of qRT-PCR relating cycle threshold (C\text{T}) and mean starting quantities. Subsequently, titers were extrapolated, using the resultant mean starting quantities, from a linear regression curve derived from preliminary study conducted previously. The titers determined by plaque assay and qRT-PCR are then being compared using statistical analysis. Lastly, a new linear regression plot is constructed relating the mean starting quantities obtained from the qRT-PCR conducted in this study and plaque assay titers, with increased sample size, as compared to the preliminary study, and improving the accuracy of the linear regression plot.

**Results and Discussions**

It was observed that, in general, there are slight variations, which may be due to experimental errors, between the titers determined by plaque assay and qRT-PCR. A strong correlation between titers determined by both methods was inferred using statistical analysis. This may substantiate the assumption that titer quantification of baculovirus using qRT-PCR is comparable to plaque assay and, hence, the validity of qRT-PCR to be an efficient and reproducible method.

In qRT-PCR, *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) envelope glycoprotein gp64 gene was chosen as the target for amplification because it is specific to baculovirus AcMNPV and is responsible for coding a viral envelope fusion protein that is vital for receptor binding and membrane fusion in the event of viral entry.

Viral DNA was extracted and purified using columns from as it has been previously shown in prior studies that column extraction of viral DNA provides consistent yields, which is essential for reproducible qRT-PCR experiments

Probes used were labeled with 5’-FAM (6-carboxy-fluorescein) and a non-fluorescent dark quencher, 3’-BHQ1 (Black Hole Quencher). BHQ1 non-fluorescent dark quencher was observed from the preliminary study that it exhibits higher thermal stability and hybridization specificity as compared to TAMRA (6-carboxy-tetramethylrodamine), a commonly used quencher for Taqman® probes.

In order to warrant a more accurate and efficient titer quantification, it was proposed in this experiment that log mean starting quantities, instead of cycle threshold (C\text{T}) values, should be used as it offers a more consistent and accurate data between different PCR machines and reagents. Additionally, as suggested by prior studies that accurate virus
titration qRT-PCR was dependent on the age of virus to be titrated and hence younger virus stocks is preferred.

In this study, it was demonstrated that qRT-PCR can be applied for accurate and rapid quantification of recombinant baculovirus. It is apparent especially when this technique is employed for quantification of large number of recombinant baculovirus samples. Moreover, less time is required form 4-7 days for plaque assay to few hours for qRT-PCR to titrate virus stocks. Hence, qRT-PCR can be employed in future applications for convenience and accelerated virus titration.

References


