

報告番号	※	第	号
------	---	---	---

主論文の要旨

Development of novel method for rapid and accurate
detection of viral infection
論文題目 (ウイルス感染の迅速な検出に関する新規手法の開発)

氏名 DOYSABAS Karla Cristine Callano

論文内容の要旨

Adenosine 5'-triphosphate (ATP), the major energy currency of the cell, is involved in many cellular processes including the viral life cycle and can be used as an indicator of early signs of cytopathic effect (CPE). In this study, we demonstrated that CPE can be analyzed using a FRET-based ATP probe named ATeam. An ATeam probe was ligated into the pMXs-IRES-Puro to generate pMXs-ATeam-IRES-puro, which was transfected to BHK-21 cells to produce BHK-ATeam cells. The BHK-ATeam cells were infected with selected viruses and subsequently fixed with 4% PFA to inactivate the virus. The fixed cells were then examined under confocal microscope, and the Venus/cyan fluorescent protein (CFP) ratios were measured manually or automatically using the developed ImageJ and Python program set. Results revealed that as early as 3 h, virally infected cells showed a significantly different Venus/CFP ratio compared to the mock-infected cells. The ATeam technology is therefore useful in determining early signs of ATP-based CPE as early as 3 h without morphology-based CPE by light microscopy and enables high throughput determination of the presence of microorganisms in neglected samples stored in laboratories.