論文内容の要旨

Dissertation Abstract

論文題目 Detection and Prevention of the Influenza Virus
（インフルエンザウイルスの検出と防御）

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The extensive application of DNA amplification technologies is dependent on the development of inexpensive and simplified detection and amplification systems. Cell lysis and subsequent release of genomic DNA is an ongoing dilemma for molecular biological techniques. In most cases, technologies like PCR and other amplification techniques require DNA extraction and purification steps. The Smart Amplification Process Version 2 (SmartAmp2) is an isothermal and integrated DNA amplification method that precludes the need for time consuming sample preparation for the rapid detection of single nucleotide polymorphisms (SNP), mutations, and other DNA targets. In addition, DNA amplification directly from whole blood is beneficial and lessens the risk of cross-contamination. Traditional SmartAmp2 assays entail two steps and require an alkali pretreatment step at 98ºC prior to the 60ºC run. To make SmartAmp2 truly isothermal and to simplify DNA

amplification, we hereby introduce the SmartAmp Isothermal Lysis Buffer (SIL-B), a newly developed chaotropic lysis buffer that enables the simultaneous recovery and denaturation of genomic material directly from whole blood at a uniform 60ºC. The improved method for isolating nucleic acids from whole blood is a critical milestone in making SmartAmp2 truly isothermal from start to finish at one temperature, increasing its potential to be routinely used in field point-of-care diagnostics. Furthermore, pretreatment with SIL-B enables the PCR amplification of genomic material directly from whole blood. This technological development may have various applications including the potential diagnosis of point mutation detection of clinical viral samples.

Vaccination is the primary form of protection from influenza virus infection. The recently developed replication-incompetent PB2-knockout (PB2-KO) influenza virus that possesses a reporter gene (the green fluorescent protein gene) in the coding region of the PB2 segment replicated to high titers in PB2-expressing, but not unmodified, cells, suggesting its potential safety and feasibility as a vaccine. I tested its efficacy in a murine model. The levels of IgG and IgA antibodies against influenza virus in sera, nasal washes, and bronchoalveolar lavage of mice immunized with the PB2-KO virus were higher than those induced by a conventional inactivated vaccine. All PB2-KO virus-immunized mice survived a challenge with lethal doses of influenza virus. Moreover, importantly, mice immunized with the PB2-KO virus produced antibodies against the reporter protein, suggesting that the PB2-KO virus has potential as a multivalent vaccine to combat infection with not only influenza virus but also of other pathogens. In summary, consistent resistance

to lethal infections, modest viral titers in organ homogenates, lack of visible illness or pathology in mice, feasibility in vaccine preparation and administration, and its ability to trigger an immune response without inducing adverse reactions, are factors that strongly indicate that PB2-KO(GFP) is a safe and efficacious multivalent vaccine candidate.