

# 登革熱病毒 2 型 PL046 非結構性蛋白

## NS2A、NS2B、NS4A 及 NS4B 之功能性研究

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### 中文摘要

登革熱病毒屬於黃質病毒科(*Flaviviridae*)黃質病毒屬(*Flavivirus*)。此屬病毒會造成許多人類的感染性疾病。在登革熱病毒產生蛋白質的過程，會先產生單一個蛋白質轉譯區，再切割成 10 個獨立的蛋白質。其中，NS2A、NS2B、NS4A 及 NS4B 是較小的非結構性蛋白，具有疏水的特性，因此可能為和細胞膜有關的蛋白質。這四種蛋白質中，只有 NS2B 已知會參與蛋白切割酶反應，而 NS2A、NS4A、NS4B 尚未被鑑定出確切的生化功能。為研究登革熱病毒此四蛋白質的結構與功能，首先必須建構及表現此四種基因並對這四種蛋白質做功能性測試。本研究嘗試將這四種蛋白質在大腸桿菌和哺乳類細胞中，以加上 HA-His (influenza hemagglutinin-polyhistidine protein) 的融合蛋白(fusion protein)形式表現。為了在大腸桿菌表現系統中表現此四種蛋白質，遂將 NS2A、2B、4A 及 4B 的轉錄區域構築在修飾過的 pET-30a(+) 表現載體——pETΔ5T 上。NS2B、4A 及 4B 的 HA-His 融合蛋白可在大腸桿菌中，以西方墨點法測得。NS2A 的轉錄產物則可以北方墨點法測得。NS2B、4A、4B 在 BHK-21 以及 NS2A 在 293T 細胞中表現，也可以西方墨點法偵測。溶斑試驗(plaque assay)的結果顯示：表現 NS2B 的細胞經登革熱病毒感染後，會形成較對照組小的溶斑，而表現 NS4A 的細胞則會產生較大的溶斑。

# **Functional Expression of Nonstructural Proteins NS2A, NS2B, NS4A, and NS4B of Dengue Virus Type 2 PL046 Strain**

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## **Abstract**

Dengue virus is a member of *flaviviridae*, which cause many infectious diseases of human. Dengue virus encodes 10 proteins in a single open reading frame. Among them, NS2A, 2B, 4A and 4B are small non-structural proteins exhibiting hydrophobicity profiles, suggesting their role as membrane-associated proteins. NS2B has been suggested to involve in protease activity while specific biological functions for NS2A, 4A and 4B have not been identified. With the interest to study the structure and function of these four proteins, I attempt to achieve cloning, expression, and functional assay of these four genes. Their proteins were expressed as HA-His (influenza hemagglutinin-polyhistidine protein) fusion proteins in *E. coli* and mammalian cells. For the expression in *E. coli*, the coding region of NS2A, 2B, 4A and 4B were successfully cloned into a modified pET-30a(+) vector, pETΔ5T. Recombinant proteins of NS2B, 4A and 4B fused with HA-His tag could be detected in *E. coli* by Western blotting analysis. Whereas, the transcripts of NS2A were detected by Northern blot. Expressions of NS2B, 4A, and 4B in BHK-21 and NS2A expressed in 293T cells were also confirmed by Western technique. The results of plaque assay suggested that cells expressing NS2B formed smaller plaques than negative control while cells expressing NS4A formed larger plaques.

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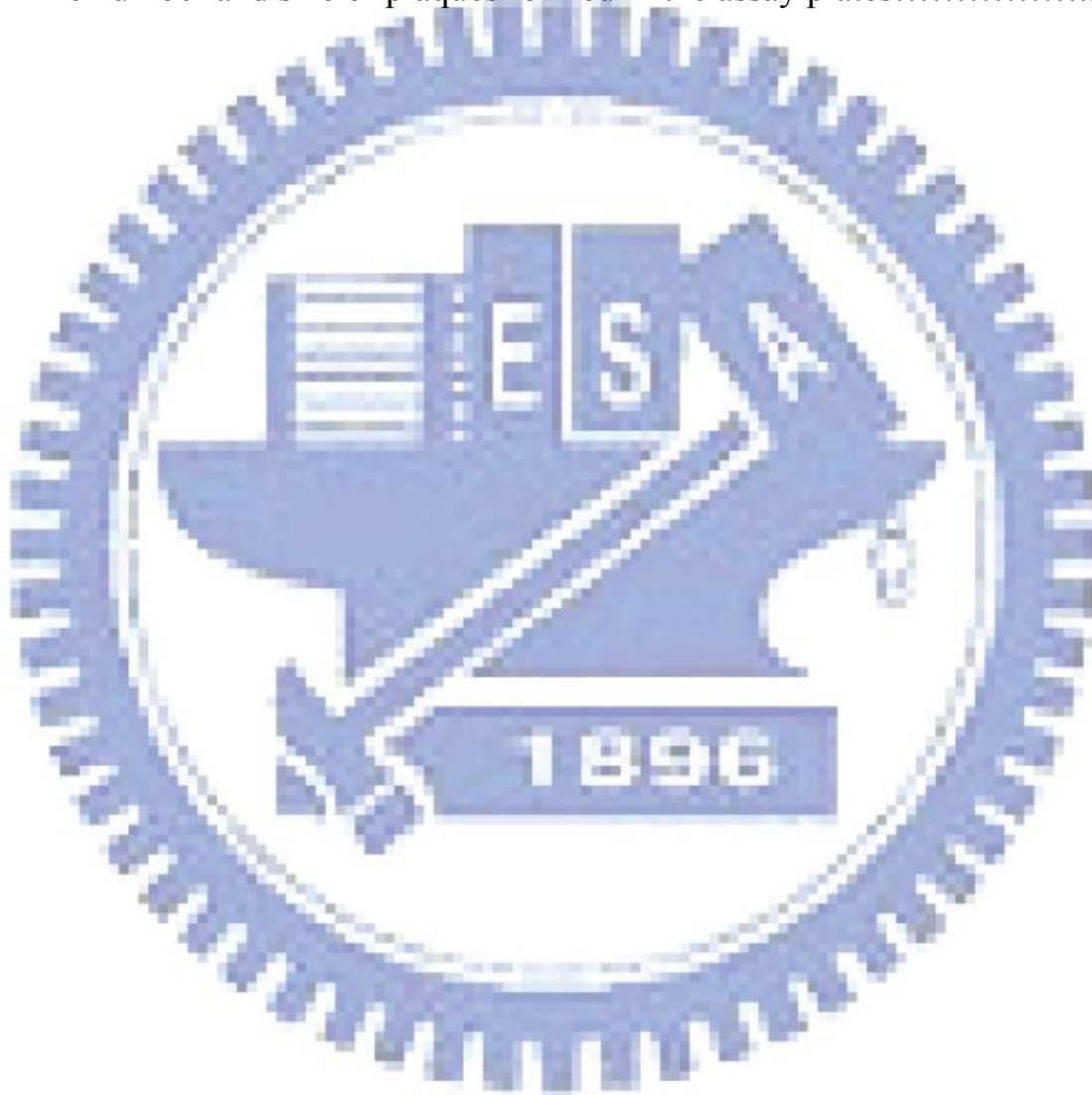
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