（Abroad・Domestic）Internship report form（Student） 2017/ 11/ 28

(Year/Month/Day)

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| Name | Wallaya Phongphaew |
| Laboratory | Division of Molecular Pathobiology |
| Year (Grade) | D4 |
| Internship institution | Duke-National University of Singapore (Duke-NUS) Medical School, Singapore |
| Internship period | Internship period: Oct 9th, 2017 – Nov 3rd, 2017<br>(Departure Date from Sapporo: 10/07/2017, Arrival Date in Sapporo: 11/05/2017) |
| Purpose | • To build up the connection for future collaborative research with Prof. Eng Eong Ooi after going back to my university in Thailand<br>• To gain practical experience and knowledge in the research of flaviviruses in aspect of clinical epidemiology and development of effective vaccine |

This report should be submitted within 2 weeks after you return to Japan.

- The reason why you chose this institute

The Duke-National University of Singapore (Duke-NUS) Medical School will provide me further collaboration for my future research in the flavivirus field when I go back to Thailand. The emerging and infectious diseases program is one of the main programs of Duke-NUS. Prof. Eng Eong Ooi has been the deputy director of this program. His research is focusing on the immunity and pathogenesis of Dengue virus which is an important infectious disease in tropical countries, including Singapore. This might be helpful for figuring out of my current and future research in flavivirus field.

Prof. Eng Eong was invited to the 15th leading seminar of our graduate school and had a lecture about a co-receptor for antibody-dependent Dengue virus infection. After his lecture, he kindly gave me for valuable suggestions for my research. He is an expert in the flavivirus field, especially for Dengue and Zika viruses. His laboratory is very active and has established many articles in high impact factor journals in collaboration with the Singapore National Hospital.

- Result of the activity （about 800 words、provide photos, tables and figures that clearly show the activities during the period）

Duke-NUS is the collaborative research institute between of the Duke

University and the National University of Singapore under the agreement since 2005 to accelerate the biomedical science. The Programme of Emerging Infectious Disease (PEID) is one of the programs provided by Duke-NUS Medical School. The OEE (Ooi Eng Eong) laboratory supervised by Professor Eng Eong is mainly focusing on Dengue, Zika and Yellow fever in aspect of clinical diagnosis and development of vaccine.

I have spent first week of my internship to attend and complete the compulsory online modules involving in laboratory safety training and biomedical responsible conduct of research according to the rule of Duke-NUS medical School.

During this internship, I had a chance to join the research related to generating and characterization of mutant Dengue virus type 1 (DENV-1) and DENV-2. Previously, the mutant of DENV-2 composed of a single mutation of nucleotide in prM (the detail of mutation needs to be confidential because of research ethics) was synthesized in his laboratory. However, characterization of the mutant DENV-2 has not determined yet. Therefore, I was assigned to characterize the infectivity of the synthesized mutant DENV-2. The mutant DENV-2 virus was recovered from the supernatants of HEK-293T cells transfected with the mutant DENV-2 plasmids, and then propagated in mosquito cells line, C6/36. Infectivity of the mutant DENV-2 was examined in baby hamster kidney cells, BHK-21 in comparison with wild-type DENV-2 by measurement of viral titers and their genomes using plaque assay and qRT-PCR, respectively. The plaque assay revealed that the prM mutant DENV-2 caused reduction of viral titer and the size of plaques was smaller compared to that of the wild type. The internship period was limited, therefore I had not enough time to obtain results of the qRT-PCR assay.

DENV-2 was the major serotype circulating in Singapore; however, the trend of DENV serotype has been changed from DENV-2 to DENV-1 in 2013. Based on sequence analysis, some mutations were found in the genome of DENV-1 isolated from 2013 compared to parental DENV-1 serotype isolated before 2006 to 2012. The single mutation of nucleotide located in the 5'-untranslated region (UTR) has been suspected for conversion of DENV serotype in Singapore. I have tried to examine whether the mutation in the 5'-UTR facilitates replication or infectivity of DENV-1. I attempted to generate the mutant DENV-1: however, the mutant DENV-1 has not been generated because of the limited time.


Figure 1: Duke-NUS building

Figure 2: the members of the OEE laboratory

The total stay in Singapore was 25 days and the daily activities are summarized as the below table.

| | Mon | Tue | Wed | Thu | Fri |
|---|---|---|---|---|---|
| Oct 9th – 13th | Laboratory introduction | Attending at the online modules and evaluation for safety training and research ethics | | | Propagation of Dengue virus in C6/36 cells |
| Oct 16th – 20th | - Propagation of Dengue virus in C6/36 cells<br>- Amplification of DENV DNA fragments and molecular cloning | | National Holiday | Plaque assay to measure the virus titers | Amplification of DENV DNA fragments to generate the mutant DENV-1 |
| Oct 23rd – 27th | -Research presentation | Amplification of DENV DNA fragments and molecular cloning | | Obtaining of plaque assay results | |
| Oct 30th – Nov 3rd | - Characterization of mutant DENV-2<br>1. Infection of mutant DENV-2 in BHK cells and harvested at 6, 24 and 48 hour post infection, followed by RNA isolation and cDNA synthesis for quantitative real-time PCR<br>2. The supernatants form infected cells collected from 6, 24 and 48 post infection were applied for plaque assay to measure and compare the viral titer.<br>- Amplification of DENV DNA fragments and molecular cloning to generate mutant DENV-1 | | | | Quantitative real-time PCR |

- Saturday and Sunday are holiday

- What do you think the positive impact of the activity will have on your further career path?

Even though the experiment I have done in Singapore was using similar skill and methods I have learned in Japan, it is still good opportunity for me to have an experience at the different laboratory culture and academic style.

As I previously described in my internship application, I have obtained the position as a lectured in Faculty of Veterinary Medicine, Kasetsart University, Thailand. Research activity is one of mandatory duty of the lecturers in Thailand. This internship provided me the research network in the Singapore. I hope that I may have a chance to collaborate with the OEE lab and the PEID to produce some good researches in near future. The number of research significantly impacts for getting promote or higher the position. This internship provides me a good chance to build up strong connection to facilitate my research in future.

Furthermore, this internship gave me the experience in academic style at the different laboratory. I may apply what I have learned either from the Hokkaido University or the Duke-NUS to set up my own laboratory or working in my home country after graduation.

- Advice for your junior fellows

The travel insurance is very important. Please do not forget to buy and bring the insurance all the time when you are travelling. During my internship, my health condition was not good and I went to hospital presented on the list in the travel insurance. The total expense was fully covered by the insurance.

Please check the requirement of compulsory document. Even though the visa application is not required for some nationality, some compulsory documents, for example temporary working pass, are still needed for the institute. The procedure for application might take long time. Early application is necessary.

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| Approval of supervisor | Institution・Official title・Name<br>Division of Molecular Pathobiology, Professor Hirofumi Sawa |

※1 Send the electronic file to the Leading School section, International Affairs Office
※2 Attach a copy certificate of the content of internship activity that is prepared by the counterpart at the internship institution (any form with a signature of the counterpart).
※3 The Steering Committee of the Leading Program will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.

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