Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(Please be sure to submit this report after the trip that supported by PWS.)

	2016. 06, 04
Affiliation/Position	Universiti Sains Malaysia
Name	Nur Juliani Shafie

1. Country/location of visit

Primate Research Institute, Inuyama, Japan

2. Research project

Study on plant-insect DNA sequencing (Genome science course)

3. Date (departing from/returning to Japan)

2016. 05. 30 – 2016. 06. 03 (5 days)

4. Main host researcher and affiliation

1. Professor Takashi Hayakawa (Primate Research Institute, Kyoto University)
2. Professor Munehiro Okamoto (Primate Research Institute, Kyoto University)
3. Ms Liesbeth Frias (Primate Research Institute, Kyoto University)
4. Mr Shintaro Ishizuka (Primate Research Institute, Kyoto University)

5. Progress and results of your research/activity (You can attach extra pages if needed)

Please insert one or more pictures (to be publicly released). Below each picture, please provide a brief description.

The main purpose of this course is to conduct the DNA extraction, purification, and sequencing of figs and fig wasps collected in Yakushima Island. In plant-insect genome science course, our group was divided into three small groups (1 group for plant and 2 groups for animal tissue). I was place in plant DNA sequencing team. I'm very excited to learn DNA sequencing since I don't have any basic knowledge on molecular work. Basically, plant-insect DNA sequencing involved four main steps; (1) DNA extraction and purification for plant-animal tissue, (2) Polymerase chain reaction (PCR) process for plant-animal tissue, (3) DNA sequencing and (4) phylogenetic analyses.

On the first day, we started to extract the DNA from 8 fig leave samples using NucleoSpin Plant II (TaKaRa) and 48 insect samples using QIAamp DNA Micro Kit (QIAGEN). There is a difference in term of methods for both plant and animal tissue, especially the type of buffer that has been used. After done with the DNA extraction, the quality of purified DNA was check using Nanodrop,

After the first step (DNA extraction and purification) was done, we proceed for the second step, PCR process and we prepared the Master Mix and the DNA template. For plant tissue, the primer used were C9F (forward primer) and C9R (reverse primer), meanwhile for insect tissue, 28S-01 (forward primer) and 28SR-01 (reverse primer) were used. Then, samples were put into the PCR machine and the temperature condition was set based on the type of sample. Next, for the electrophoresis procedure, 1% agarose gel was prepared. 100 ml IXTAE buffer, 5µl gel green and 1g agar rose powder were measured and poured into a removable gel casting tray and the comb was set. We ran the electrophoresis at 100V for 20-30 minutes and the photos were taken using UV Trans illuminator.

The third step involved DNA sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit (Dye terminator/ Sanger method). After the sequencing reaction products were purified using magnetic beads (Agencourt CleanSEQ), the samples were ready to sequence. We put the samples in a sequencer (3130) and ran the sequencer.

For the last step, we have been taught how to match the DNA sequences with sequence database using BLAST and how to generate phylogenic tree using Mega6 software. We produced phylogenic trees for both fig species and fig wasp in order to identify the species of pollinator and non-pollinator of *Ficus spp* in Yakushima Island. At the end of this course, we produced a group poster entitled "Preliminary assessment of composition and variation in fig and fig wasp species on Yakushima Island, Japan and their corresponding phylogenetic relationships" during the 5th International Primatology and Wildlife seminar.

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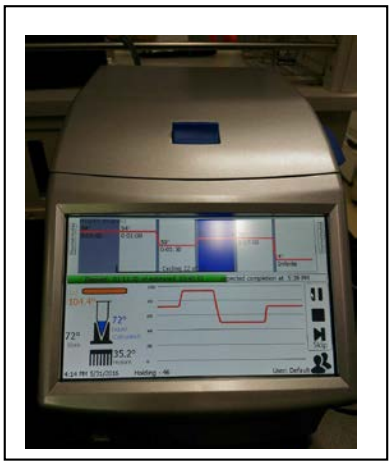

Figure 1: The Polymerase Chain Reaction (PCR) machine

Figure 2: The electrophoresis gel

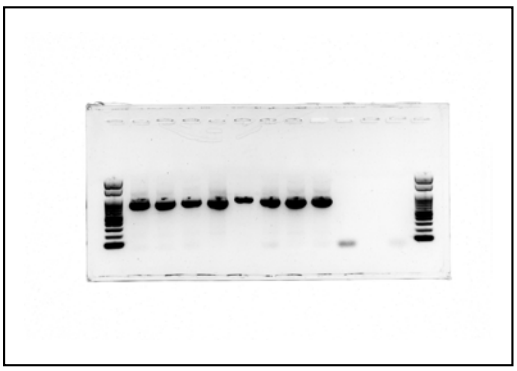

Figure 3: Result for plant samples from the UV Trans illuminator

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Figure 4: The sequencer machine in Primate Research Institute laboratory, Inuyama

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Figure 5: Me and my team members during the genome science course

6. Others

I would like to thank to all Professors; Takashi Hayakawa, Munehiro Okamoto, Goro Hanya, and both tutors; Lies and Ishizuka-san for your help during this genome science course. Thank you so much to all my teammates; Kunal, Evan, Yuka, Izumi, Gao Jie, Anna and Otto for your efforts and help during the lab work.