

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.)

| | |
|-----------------------------|---------------------------|
| Affiliation/Position | Universiti Sains Malaysia |
| Name | Aini Hasanah Abd Mutalib |

| |
|--|
| 1. Country/location of visit |
| Kyoto, Japan |
| 2. Research project |
| Genome Science Course |
| 3. Date (departing from/returning to Japan) |
| 1 st June 2015 until 8 th June 2015 |
| 4. Main host researcher and affiliation |
| 1) Dr Eiji Inoue (Assistant Professor, Graduate School of Science, Kyoto University, Japan) 2) Mr Tomoyuki Tajima (PhD Student, Lab of Human Evolution Studies, Kyoto University) 3) Mr Yokoyama (PhD Student, Lab of Human Evolution Studies, 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 |
| <p>In the Genome training course, I was place in the Monkey Genome Team. Our team’s aim is to understand the way to analyze the DNA which was extracted from the faecal samples that we obtained from Japanese macaques in Yakushima Island. This training course conducted the sex identification and individual identification of the Japanese macaques DNA.</p> <p>Our first day kicked off with the Extraction of DNA. We managed to extract the DNA from 23 samples from unknown Japanese macaques. Inoue-san with his student assistants had showed us how to conduct the DNA extraction. There were many steps that needed to be done carefully and meticulously to avoid contamination from occurring. We started by extracting the DNA by using QIAamp DNA stool mini kit.</p> <p>On second day, we learned how to do the sex identification from the DNA samples by using electrophoresis method. To identify the sex of the individuals, we prepared the primers, PCR mixture and Template DNA. We carried out the PCR reaction for 1 hour. Next, for the electrophoresis procedure, 1.5% agarose gel was prepared. 60 ml of 1X TAE and 0.9 agarose were measured, melt in microwave, and added with water to restore its original volume. After that, it was being cooled down to 60° C. The mixture was poured into a removable gel casting tray on a casting gate, and the comb was set. It took 30 minutes for the gel to be hardened, then a small volume of 1 X TAE was added and the comb was removed.</p> <p>The tank was filled with 1X TAE and the gel was set. A 1 µl dye was mixed with GelRed and 5µl of PCR product on Parafilm. The mixture was load to the gel in the well. A 1 µl dye was mixed with</p> |

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.)

GelRed and 5 μ l of size marker. All the mixture was loaded to the gel in the well. We ran the electrophoresis at 100V for 15 minutes to 20 minutes. The gel was put on the UV Trans illuminator and photos were taken.

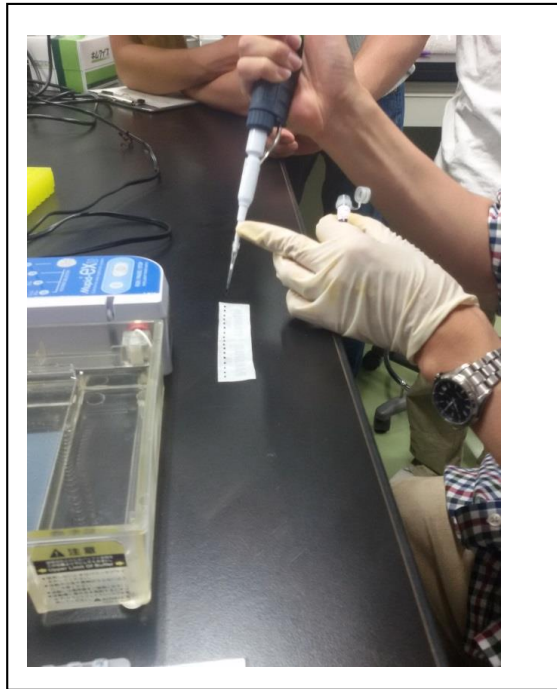


Figure 1: The GelRed dye marker was mixed with the DNA and moved to the DNA well



Figure 2: The electrophoresis gel

During this activity, I have learnt on how to differentiate male individual with female individual. There are two bands, which are 200 and 500. Male individuals would have its DNA located at both 200 and 500 bands, while female DNA only have 1 (500 band). In the picture below (source: Sugiura,2012), individual 2, 3, 6 were female individual, while the rest were male.

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.)

c

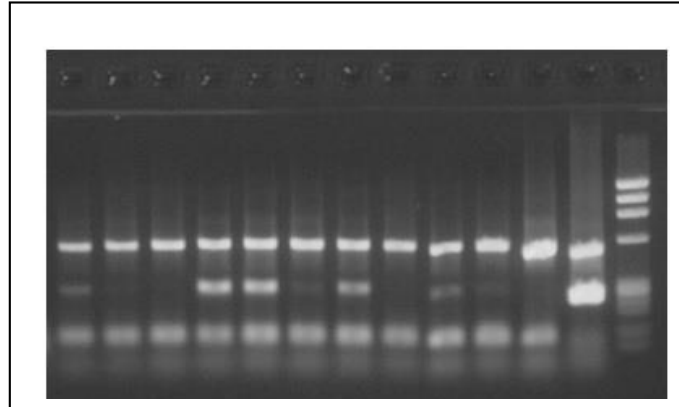


Figure 3: Result from the UV Trans illuminator device.

I also used this opportunity to learn about individual identification too. It can be done by using genotyping procedure. For genotyping procedure, we amplified DNAs by using QIAGEN multiplex PCR kit. 190 μ l of water is added to the PCR product, and the reagents were mixed in the tube. 9.5 μ l of the mixture of Hi-Di and size standards were added into 96 well plates. 1 μ l of samples were added after PCR and diluted by water. The samples were heated for 5 minutes at 95°C, and chilled in a box. We put the PCR products in a sequencer (3130), and ran the sequencer.

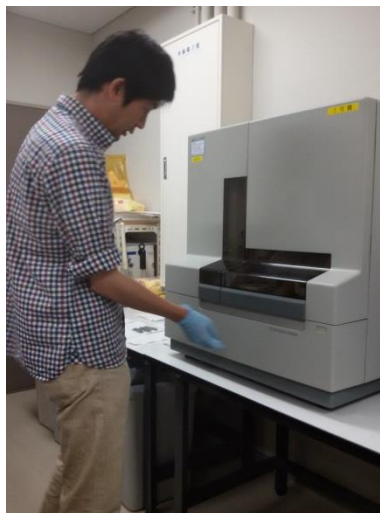


Figure 4: Inoue-san showed how sequencer worked at the lab

We determined the alleles by using the Peak Scanner software. It is again, a tedious procedure since we have to observe these alleles based on 16 markers that we were given. Examples of alleles were shown in the figure below.

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.)

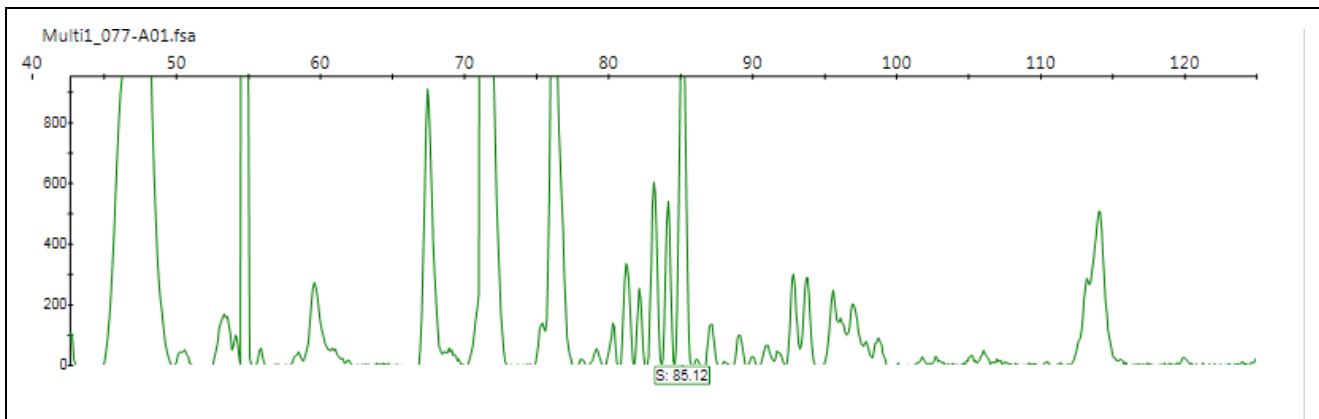


Figure 5: Alleles that we observed in individual 077.

After we determine the genotypes, we put the data in the excel sheet with GenAIEx Format 6.3. This format is an excel-based software and was useful for microsatellite analysis.

In this procedure, there were 15 known samples (that were used as baseline) and 23 unknown samples. After the analysis, we found that overall, there were 20 individuals. There was no significant difference between the macaques based on sex, but male adult macaques showed lower ratio of intact seeds compared to female. Adult also tend to show lower ratio in producing intact seeds in their faeces, based on analysis according to the age group. However, this statistic analysis has no significant correlation.

We found that Inokuma, an adult male showed lower significant different compared to among other individuals in producing intact seeds in his faeces. Almost all individuals seemed not to destroy as much seeds as Inokuma. However, the number of samples were small and period of study was rather short. Perhaps given longer period of sampling and analysis, more comprehensive findings can be found.

Through this experience, I have developed a clear vision on how to cope with genome procedures, which will be of great help in the future conservation of primates. Individual recognition of Japanese macaques might be cryptic and we need to spend much time when the number of the group is large. So, non-invasive genetic technique is useful to ensure their sex and individuals can be known easily. In conclusion, combination of field and genome analysis can provide more comprehensive and clearer result.

We produced a poster entitled “Integrating field and Genome analysis: Finding the seed dispersal among Japanese macaques” during the 4th International Primatology and Wildlife Symposium.

6. Others

I would like to express my deepest appreciation to Dr Inoue for teaching me a lot on the genome procedure and explained it thoroughly. As I seldom did lab work before, it was hard for me to cope with the labs but Inoue-san patiently had showed friends and I on steps by steps procedure of genome analysis. I also am thankful to the research assistants, Yokoyama-san and Tajima-san that had taught me a lot. Thank you to my teammates too: Makiko, Hiroko, Emi, Himori and Hino for their efforts and help through out the lab work.

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.)



Figure 6: Our Monkey team with the poster