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.)

	2024. 11, 25
Affiliation/Position	Wildlife Research Center/M1
Name	Liu Liu

1. Country/location of visit

WRC, Kyoto

2. Research project

Genome sequencing field course

3. Date (departing from/returning to Japan)

2024/11/11-2024/11/18 (6 days)

4. Main host researcher and affiliation

Dr. Murayama, and Dr. Sato, Kyoto University WRC

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.

Day 1:
On the first day, the members began by visiting Kyoto-Gosho in the morning to collect feather samples. I was not with them the first day due to a presentation. Along the way, they observed many egrets and cormorants in the Kamogawa and stopped there on the return trip to gather additional samples. A total of 43 samples were collected, though only 18 were processed that day, along with 2 additional samples provided by the zoo.
The team extracted DNA from the basal tips of the feathers and measured DNA concentrations. They then performed PCR amplification for species identification using mitochondrial DNA, with CO1-forward as the primer. PCR conditions included 40 cycles of specific temperature phases (95°C, 55°C, and 74°C), followed by a 10-minute incubation at 74°C and cooling at 10°C overnight, in preparation for the next day's analysis.

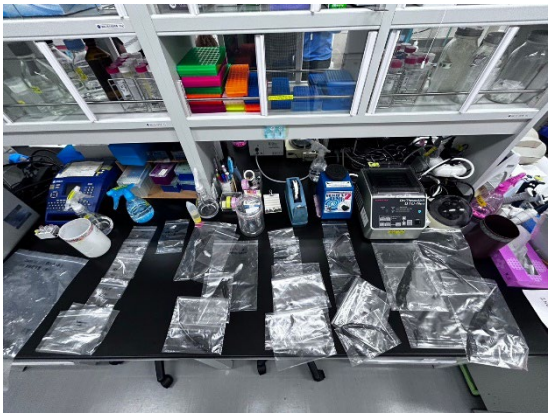

Figure 1 The feathers my member collected

Day 2:
I joint the team from day 2. We started with gel electrophoresis to check if the PCR worked. We also practiced making gels, using 1.5% TBE gels, which we found to work best. Before loading the DNA samples, we mixed them with a heavy buffer to keep them in place. Four samples failed the electrophoresis because they didn't have enough DNA, so we planned to redo them later.
Next, we purified the successful PCR products to remove extra primers and get clean DNA. After purification, we started sequencing by creating DNA fragments with a Bird F1 primer. This involved mixing the primer, BigDye reagents, and our DNA samples, then running them in the PCR machine under specific conditions. The samples were incubated at 10°C overnight for the next step.
It was quite a lot of new knowledge to me, and the experiment was very much interesting to try.

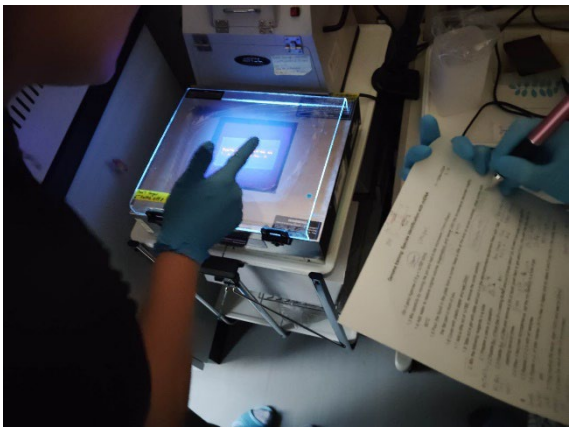

Figure 2 We are checking the result

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Day 3:
We continued the sequencing process from Day 2. We purified the DNA again using ethanol to remove extra primers and DNA, then prepared the samples for the sequencer. This included adding HDFA, heat-denaturing at 96°C, and snap-cooling on ice to keep the DNA single-stranded. We used the ABI3130xl sequencer, which runs 16 samples per cycle for 90 minutes.
The sequencer provided results as ab1 and seq files. We analyzed the ab1 files using FinchTV, looking for strong peaks over 400 base pairs for accurate species identification. For species identification, we pasted the sequences into BOLDSystems' nucleotide Blast function and considered matches with 98% similarity as accurate. Out of 20 samples, we successfully identified the species of 11, which is a good result for non-invasive feather samples.

Day 5:
My members did the continuing work on day 4. On Day 5, we focused on repeating the sex ID procedure for our samples (excluding #18-19, which were provided by Fadel). We also included control samples from a known male and female to ensure accuracy. This step aimed to verify the sex ID results for the previously failed samples.

Day 6:
We re-sequenced the already purified DNA samples (set 2). We started by running gel electrophoresis to confirm the presence of DNA, but one sample failed this step and was excluded. We then repeated the sequencing reaction using BigDye reagents and the CO1 primer. Afterward, we performed ethanol purification and gene sequencing for 8 samples. To improve accuracy, we tested two DNA volumes—1μL in one column and 3μL in another—to ensure sufficient DNA was present for successful sequencing.
The result is not perfect, but we are satisfying enough for what we have learnt. PCR and sex identification need a lot of patient, and Murayama-sensei suggest us to have a trial-and-error dairy, I think it is very much important tips not only for PCR work but also for my own research.

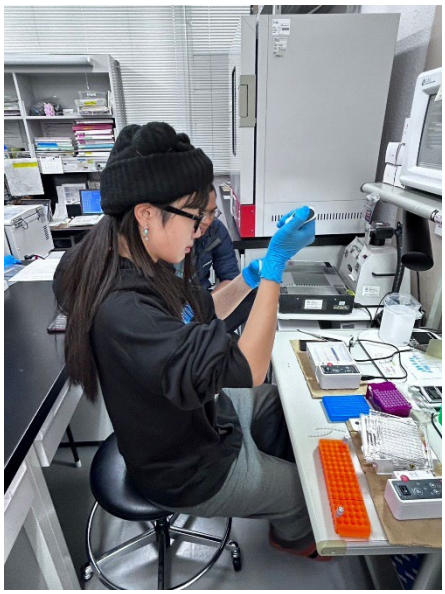

Figure 3 Me doing PCR

*Please have your mentor check your report before submitting it to [report@pws.wrc.kyoto-u.ac.jp].

6. Others