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.)

	2014. 6, 13
Affiliation/Position	Human and Evolution Studies, D2
Name	Etienne-Francois Akomo Okoue

1. Country/location of visit

Kyoto, Japan

2. Research project

Metagenomics Analyis on Gut Micobiome of wild Japanese Macaques

3. Date (departing from/returning to Japan)

Ph. D student Graduate School of Science Kyoto University

4. Main host researcher and affiliation

Dr. Juichi Yamagiwa, Professor at Graduate School of Science 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.

During this training, we conducted research on Metagenomic microbiota of Japanese macacaques. From fecal samples collected in Yakushima, we extracted DNA using QIAamp DNA stool Mini kit (QIAGEN). The first PCR was carried out to amplify the partial sequence of 16S rRNA of bacterial genome. After to confirm the specific amplicons by agarose gel electrophoresis, the PCR products were purified using AMPure XP. The second PCR was carried out to add indices and adaptater sequences to be attached Miseq sequencer using Nextera XT Index Kit. Against, after to confirm the specific amplicons by agarose gel electrophoresis, PCR products were purified using AMPure XP. PCR products of all samples were quantified and mix in equal weight of each sample of same DNA barcode excluding strange products. The measurement of average size of each library using Agilent 2200 TapeStation. The libraries with with non-specific products were discard. To obtain the final library, we mixed all libraries in equal molarity. The final library was diluted by adequate concentration using 10 mM Tris-Cl (pH 8.5). After denaturation by NaOH, the final library was introduced in MiSeq to sequence the samples. All data was imported to Claident for making Demultiplexed FASTQ format. Statistical analysis were performed using R.

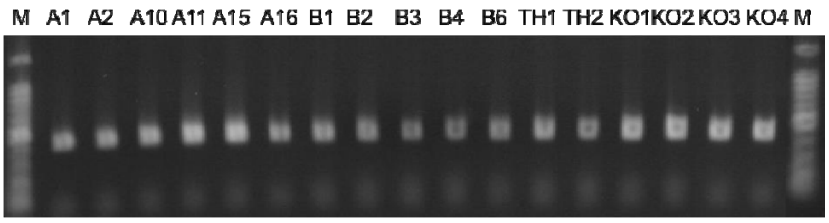

Fig. 1. Agarose gel electrophoresis result (PCR1) M: Size marker
A1 to KO4 sample identification

Fig. 2. Illumina Miseq Picture

This experimental protocol has allowed me to better know the different steps required for obtaining the final library for next generation sequencing. Through this experience, Through this experience, I developed a clear vision on how we can study duiker diets to better understand how these small frugivorous ungulates coexist in the same area.

Currently we continued to analyze the results for writing the paper for publication in academic journal.

6. Others

During this Genome training course, I appreciated a lot the cooperation between the students and the trainers

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.)